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REVIEW ARTICLE

Precision hydrogel environments for advanced microbial culture and patterning

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Abstract

Hydrogel materials and scaffolds have emerged as transformative tools in biological research by offering precise control over cell viability, metabolism, and productivity. Their compatibility with three-dimensional (3D) bioprinting and patterning technologies enables the precise and reproducible organization of living components, facilitating novel experimental paradigms across diverse disciplines. Although most 3D hydrogel research has emphasized mammalian cell applications, particularly in tissue engineering, there is a growing body of research applying these technologies to study, manipulate, and harness a variety of microorganisms, such as bacteria. This review explores the latest advances in microbial hydrogel encapsulation, focusing on material selection and patterning methods designed to preserve microbial viability and function. We compare the distinct requirements and challenges of culturing microorganisms in hydrogels versus mammalian systems and highlight recent breakthroughs in bacterial bioprinting that are advancing microbiological research, paving the way for current and emerging applications in various areas, including oral health. By synthesizing current knowledge and identifying promising future directions, this review underscores the potential of microbial hydrogel culture as a versatile platform for investigating microbial communities, probing bacterial–material interactions, and engineering living materials with applications in human health and environmental systems.

Keywords: Bacteria–material interaction; Bacterial bioprinting; Microbial hydrogel culture; Microbial living materials; Three-dimensional bioprinting

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1. Introduction

Bacteria and biofilms have been extensively studied due to their omnipresence in nature, critical roles in human health, and potential for both beneficial applications and harmful effects across diverse industries and ecosystems.[1,2] Microbial culture systems are fundamental tools that facilitate the study of microbial behavior under controlled experimental conditions, enabling investigations into interspecies interactions and antimicrobial resistance.[3] These systems are also utilized to harness microbial

metabolism for the production of valuable bioproducts, or for bioremediation applications that address environmental contamination.[4,5] Advances in synthetic biology are further expanding the frontiers of engineered microbial systems, enabling tailored applications in therapeutic and industrial contexts.[6]

Traditional microbial culture methods, generally consisting of planktonic growth in liquid media and two-dimensional (2D) surface cultivation, remain widely adopted throughout the research community due to their simplicity and ease of use. However, these methods fall short in replicating the complex, three-dimensional (3D) microenvironments that organisms naturally inhabit, such as porous biofilm structures or tissue-like matrices.[7] In contrast, 3D culture techniques, including microbial immobilization within hydrogels and other 3D matrices, offer significant advantages.

Hydrogels are 3D, cross-linked polymer networks that absorb and retain large volumes of aqueous fluid while preserving structural integrity. Their mechanical behavior—including tunable stiffness and viscoelasticity (i.e., the time-dependent response under stress)—can be precisely engineered, enabling these materials to mimic the microenvironments of native tissues and microbial biofilms.[8,9] Recent studies show that embedding bacterial populations within hydrogel-based 3D matrices enhances the physiological relevance of *in vitro* models, better replicating natural environments and facilitating more accurate behaviors, such as regulated growth, intercellular signaling, metabolic activity, and functional outcomes.[10,11] Moreover, 3D biofilm models, particularly those generated via bioprinting, exhibit markedly greater antimicrobial resistance compared with traditional 2D cultures, thereby better recapitulating *in vivo* conditions.[12] Figure 1 illustrates the applications and associated drawbacks of planktonic and 2D plate-based microbial cultures, contrasting these methods with the potential advantages of 3D and hydrogel-based cultures and biofilms.

Moreover, growing evidence shows that bacterial behavior is fundamentally altered by physical confinement within 3D matrices.[7] These environments introduce diffusion limitations and structural constraints that cannot be replicated in homogeneous liquid or surface cultures. By embedding microbes within tailored scaffolds—particularly hydrogels—researchers not only create environments that reflect biological reality more accurately but also protect microbial cells from environmental stresses. This physiologically relevant protection is crucial in a

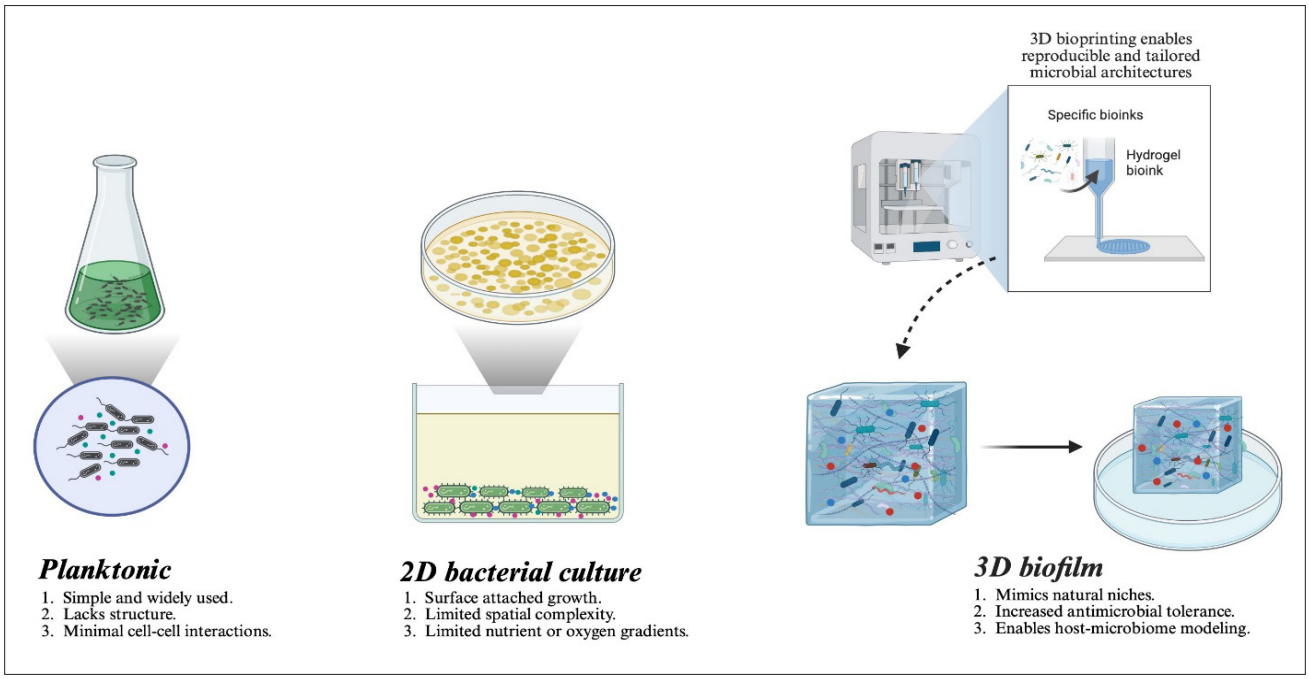

Figure 1. Comparison of microbial culture methods. Planktonic cultures are simple and widely used but lack structure and cell–cell interactions. 2D surface-attached cultures allow microbial growth on agar or plastic but oversimplify natural biofilms as they lack spatial complexity and nutrient or oxygen gradients. In contrast, 3D hydrogel-based biofilms mimic natural niches by providing structural confinement, nutrient and oxygen gradients, and enhanced microbial interactions, resulting in increased antimicrobial tolerance and enabling host–microbe modeling. Created in BioRender. Klein, C. (2025) https://BioRender.com/0y4ljkp. Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional.

variety of applications, including fundamental microbial studies and antimicrobial evaluation, providing superior relevance to biofilms, a predominant form of microbial existence in which bacterial cells can exhibit up to a 1,000-fold greater antibiotic tolerance than their planktonic counterparts.[13] The precision and reproducibility of 3D bioprinting also support the construction of functional microbial architectures optimized for research, industrial, or environmental applications.

This review aims to examine the evolution of hydrogel-based bacterial culture systems, assessing how material selection, fabrication techniques, and bioprinting strategies provide tailored levels of spatial and metabolic control over microbial communities. We synthesize advances in 3D bacterial culture methodologies, highlighting how precision, from macroscale encapsulation for bioproduction to microscale patterning for single-cell analysis, enables applications ranging from scalable bioremediation and engineered living materials (ELMs) to advanced models for studying biofilm dynamics and host–microbe interactions. Finally, we discuss current limitations and future directions, with a focus on opportunities for innovation in microbiology applications, such as oral research, where hydrogel-based systems offer unique potential to mimic complex natural biogeography.

2. Literature methodology

We adopted a narrative-review framework[14] to explore recent advances in 3D hydrogel platforms for microbial culture and manipulation. A narrative review was deemed most appropriate because the heterogeneity of study designs, materials, and microbial targets rendered a formal systematic review unsuitable. This approach provides the flexibility needed to integrate diverse methodologies and conceptual developments in a coherent, interpretive synthesis.

A comprehensive literature search was conducted for English-language, peer-reviewed articles published from January 2010 to July 2025. We utilized PubMed, Web of Science, and Google Scholar, incorporating both controlled vocabulary and free-text terms, such as "microbial hydrogel culture," "bacterial bioprinting," and "microbial living materials." This strategy balanced sensitivity and specificity, ensuring coverage of key publications while aligning with best practices in narrative review methodology.

Studies were considered eligible if they met all of the following criteria: (i) reported original empirical data; (ii) developed, characterized, or applied hydrogel-based constructs for microbial culture; and (iii) employed 3D bioprinting or comparable spatial patterning techniques. The scope of analysis regarding printed constructs was limited to 3D bioprinting applications utilizing cell-laden bioinks designed to support microbial viability and function. Studies describing 3D printing with acellular materials ("biomaterial printing") were excluded, although the term "3D printing" is sometimes used in the literature to refer to both approaches. Two reviewers independently screened titles and abstracts, resolving conflicts through discussion before proceeding to full-text review. This dual-review process, though within a narrative context, reinforces transparency and rigor.

For each included study, we extracted the following key details: microbial species, hydrogel composition (e.g., natural vs. synthetic), immobilization or patterning technique, 3D bioprinting modality used, intended function, and application domain (e.g., biosensing, bioproduction, or bioremediation). These data were organized thematically to enable comparative analysis of natural versus synthetic hydrogels, crosslinking chemistries, and printing strategies, with a particular focus on how material and architectural choices influence microbial viability, spatial distribution, and functional output. Given the narrative nature of this review, we did not conduct a meta-analysis. Instead, we synthesized methodological innovations and persistent challenges, structured by application domains to guide future research directions.

3. Materials and techniques for increased microbial culture precision

Advancements in hydrogel fabrication and processing have enhanced our ability to encapsulate and culture diverse microorganisms with control over their microenvironment and behavior. The selection of hydrogel materials and additional chemical groups, as well as material modifications and patterning techniques, play a critical role in determining bioactivity and microbial compatibility, making material choice and tailoring critical for the development of precision 3D microbial materials.[15,16]

While hydrogel-based culture and bioprinting are more established for mammalian cells, primarily for tissue engineering, their application to microbes presents a distinct set of requirements and challenges. Mammalian cell systems demand tailored conditions to mimic physiological environments, including strict temperature control and high biocompatibility to support delicate cell membranes and behaviors like cell binding.[17] In some cases, to encapsulate mammalian cells, biomaterial systems must also exhibit degradation kinetics to facilitate cell growth[18] and extracellular matrix (ECM) production, as well as sophisticated porosity for nutrient diffusion and eventual vascularization.[17,19,20] The properties, requirements, and

challenges of specific mammalian cell patterning methods and the materials they utilize are also extensively discussed in recent reviews.[20–24]

In contrast, microbial encapsulation leverages the inherent robustness conferred by bacterial cell walls and protective behaviors, such as spore formation,[25] which allows for a wider range of processing parameters, such as higher extrusion temperatures or freeze-drying.[26,27] However, this robustness introduces unique design constraints. The rapid growth rate of bacteria necessitates bioinks and containment strategies that prevent overgrowth and structural degradation, while also addressing the challenge of ensuring effective physical containment to prevent environmental contamination or unintended pathogenesis.[28,29] Interestingly, despite these divergent requirements, the base hydrogel materials (e.g., alginate, gelatin methacryloyl [GelMA]) are often similar; the key differences lie in the processing methods and the exploitation of microbial attributes, such as integrating self-secreted extracellular polymeric substances to enhance printability and the biomimicry of natural biofilms.[30,31]

Both naturally derived and synthetic hydrogel materials can be modified through crosslinking, pore generation, and 3D patterning on different length scales. These modifications affect nutrient and bioproduct transport, microbial architecture, and potential interspecies interactions, which must be tailored to optimize microbial viability, metabolic activity, and functional output.[27] The foundation of material choice and advantages of microstructural control and spatial patterning in increasing the precision of microbial cultures are illustrated in Figure 2.

3.1. Hydrogel materials

A variety of hydrogel materials and precursors are available for integrating microbial species for applications in culture or for harnessing different microbial functions. Hydrogels can be derived from natural or synthetic sources and subsequently modified by the addition of different functional groups to support microbial growth and function within the matrices. A summary of the most commonly used hydrogel materials for cellular encapsulation and bioinks is provided in Table 1.

Natural hydrogels such as collagen, gelatin,[32,33] alginate,[34] hyaluronic acid, and bacterial cellulose[35] are advantageous for microbial culture and bioprinting applications due to their ability to emulate the conditions of ECM and tissue environments, supporting microbial growth, and function. Alginate- and gelatin-based hydrogel materials comprise the most studied and utilized natural hydrogels for bioprinting applications. Alginate is widely used in extrusion-based bioprinting systems due to its ability to quickly crosslink and stabilize printed filaments upon contact with calcium compounds, such as calcium chloride. This rapid gelation mechanism allows for excellent shape

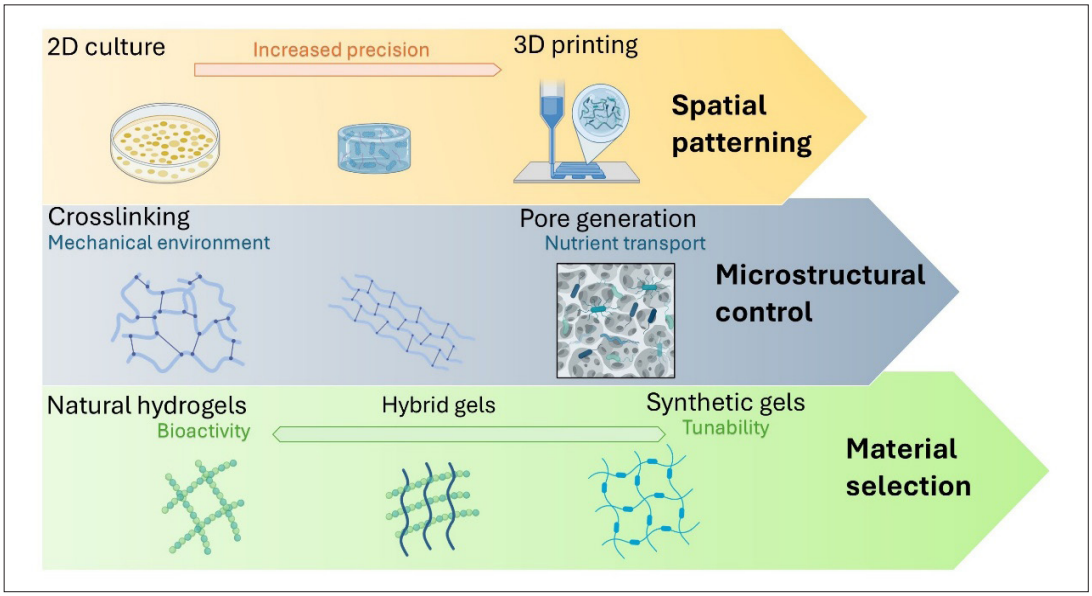

Figure 2. Schematic illustration of material modifications and their impact on microbial culture effects. Material selection forms the foundation, balancing overall tunability and biocompatibility of hydrogels for culture, whereas crosslinking and pore generation methods provide versatility in microbial microenvironments and nutrient transport. These features enable precise hydrogel culture and 3D patterning, offering advantages in microbial study and manipulation compared to 2D and planktonic cultures. Created in BioRender. Elias, J. (2025) https://BioRender.com/2qe2f6y. Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional.

fidelity during printing processes. One of the alginate's most valuable contributions to the bioprinting field is its effectiveness for mammalian cell encapsulation. The mild gelation conditions required for alginate crosslinking can maintain cell viability and long-term cell survival, allowing cells to be mixed with the alginate solution prior to printing and subsequently encapsulated within the hydrogel matrix after crosslinking. The alginate matrix can also be utilized for the delivery of growth factors and other bioactive components, creating microenvironments that can direct and enhance specific cellular responses.[36,37] However, unlike other natural bioink materials, such as collagen, fibrin, or gelatin, which contain integrin-binding sites, alginate lacks the functional groups and recognition sites that allow for direct cell attachment, requiring chemical modifications, or combinations with other natural polymers to supplement its bioactive capabilities.

Gelatin represents a versatile biomaterial derived from the denatured form of collagen, obtained through partial hydrolysis. Like collagen, gelatin can be readily obtained from animal tissues, typically bovine or porcine sources, providing advantages for its biomaterial uses. Despite undergoing denaturation, gelatin maintains similar advantages to its parent molecule, most notably the presence of the Arg-Gly-Asp (RGD) peptide sequence, which promotes robust cell interactions.[32,33] The most distinctive characteristic of gelatin for bioprinting applications is its unique thermo-responsive behavior—existing in gel form at low temperatures and undergoing a transition to a liquid state at higher temperatures above 37 °C. When cooled below approximately 25 °C, gelatin molecules create a physically crosslinked network that imparts gel-like properties. This temperature-dependent phase transition offers significant advantages for bioprinting workflows, as at reduced temperatures, it exhibits suitable viscosity and structural stability for extrusion and shape maintenance during the printing process. This allows for the creation of complex structures with good resolution and fidelity.

The thermo-responsive nature of gelatin also presents challenges. Due to its dissolution into a liquid state at physiological temperatures (37 °C), modification through crosslinking or combination with other biomaterials is needed for gelatin to be utilized effectively in bioprinting applications requiring long-term structural stability. Chemical crosslinkers such as glutaraldehyde, genipin, or carbodiimides (e.g., *N*-ethyl-*N*'-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide) are used to stabilize gelatin structures through the formation of covalent bonds between amino groups on adjacent gelatin chains.[38] Photocrosslinking represents another widely employed approach for gelatin stabilization. This typically involves the modification of gelatin with photosensitive groups, such as in GelMA, which contains methacryloyl groups grafted onto the gelatin backbone. This approach enables precise spatial and temporal control over the crosslinking process through exposure to ultraviolet (UV) or visible light, allowing for complex structuring of the material during bioprinting.

Materials such as collagen, a major component of many mammalian tissues,[39] or bacterial cellulose, which is secreted and can be isolated from natural microbial sources,[40] provide advantages of emulating physical properties, nutrient transport conditions, and functional groups experienced by microbes in their natural environments of growth and function. While naturally derived hydrogel materials carry many advantages, they may suffer drawbacks such as poorer reproducibility[41] and lower mechanical properties compared to their synthetic counterparts,[21,42] thereby requiring chemical modifications to improve their properties for ideal microbial interactions.

Synthetic hydrogels, such as polyethylene glycol (PEG), polyacrylamide, polyvinyl alcohol (PVA),[43,44] and poloxamers (also known by the trade name Pluronic) are less widely used than natural-based hydrogels in many studies,[21] but continue to provide advantages such as tunability, batch-to-batch consistency, and the ability to functionalize with a wide variety of chemical groups to customize bioactive properties and microbial interactions.

Polyethylene glycol has emerged as one of the most widely used synthetic polymers in biomedical devices and applications, establishing itself as a versatile platform technology in tissue engineering, drug delivery, and bioprinting. The use of PEG stems from its unique combination of chemical simplicity, biocompatibility, and versatility for functionalization. One unique advantage of PEG is its ability to be highly customized with a variety of proteins and bioactive polymers to create tailored biomaterials with specific functionalities. The terminal hydroxyl groups of PEG molecules serve as reactive sites that can be readily modified, enabling the attachment of bioactive molecules, including growth factors, cell adhesion peptides, enzymes, and other functional proteins that can direct cellular behavior within engineered tissues.[45] PEG can be tailored for different protein adsorption and bioactive properties through conjugation or variation of chemistry, providing exceptional control over cell–material interactions. A significant benefit of PEG in bioprinting applications is its ability to be modified with groups such as diacrylate and methacrylate to crosslink and modify mechanical properties. These functional end groups transform PEG into photocrosslinkable macromers, such as poly(ethylene glycol) diacrylate (PEGDA) or poly(ethylene glycol) dimethacrylate.[46] These modified PEG molecules

can undergo rapid polymerization with photocrosslinking, forming covalently crosslinked hydrogel networks with tunable mechanical properties.

Pluronic is a triblock copolymer with a central hydrophobic poly(propylene oxide) block flanked by two hydrophilic poly(ethylene oxide) blocks. This amphiphilic structure enables unique phase behavior and self-assembly properties that have been utilized across a spectrum of biomedical applications, from drug delivery systems to tissue engineering scaffolds and, more recently, bacterial culture platforms. The versatility of Pluronic stems from the ability to precisely control the molecular weight and ratio of the hydrophilic to hydrophobic blocks during synthesis. This control allows for the creation of numerous Pluronic variants, each offering distinct properties optimized for specific applications. Pluronic is often used in combination with other hydrogels, such as PEG for drug delivery[47] or natural materials like collagen or hyaluronic acid for cell culture,[48] creating hybrid systems that leverage the complementary advantages of each component. These composite approaches address some of the limitations of pure Pluronic systems, such as their relatively weak mechanical properties and rapid dissolution under dilute conditions. Pluronic possesses thermoreversible gelation properties that provide initial structural stability, but these physical hydrogels can dissolve over time under physiological conditions, thereby limiting their application in longer-term implants or sustained delivery systems. To address this limitation, Pluronic can be modified through enzymatic crosslinking[49] or photocrosslinking methods[50] to enhance mechanical strength, creating more stable networks with prolonged persistence *in vivo*.

One overall drawback of these synthetic hydrogel materials is the lack of inherent biocompatibility and bioactive groups possessed by natural ECM materials,[24,51] thereby requiring modification to influence and enhance the behavior of microbial cells in certain circumstances.[21] The integration of multiple material types into hybrid hydrogels presents a powerful strategy to overcome the limitations of single-component systems, enhancing control over the microbial microenvironment. By synergistically combining natural and synthetic polymers, these hybrids merge the biofunctionality and biocompatibility of natural materials with the tunable mechanical properties and enhanced structural fidelity of synthetic networks.[11] This approach enables the creation of scaffolds that present the chemical cues of native tissue while offering independent adjustment of mechanical stiffness and degradation kinetics, which are critical for directing microbial growth and biofilm formation. For example, Denton *et al.*[11] utilized a gelatin–PEGDA hybrid hydrogel to model tonsillitis biofilms, leveraging gelatin's biocompatibility and cell-adhesive motifs while utilizing the PEGDA network to compensate for gelatin's characteristically low mechanical strength to achieve a stable and biomimetic 3D culture environment. Hybrid hydrogel formation also presents the opportunity for direct incorporation of microbial-derived components, such as secreted polysaccharides[52] or curli fibers, into synthetic or natural hydrogel backbones. This leverages the enhanced functionality of natural biofilm matrices while retaining the versatility and precision offered by tailored polymer chemistry for microbial manipulation and culture.[52]

3.2. Hydrogel modifications for increased precision

In addition to material choice, hydrogels can be engineered to better support and manipulate microbial growth and behavior. Microbial behavior, biogeography, and function are linked to characteristics such as local architecture and confinement, chemical signals, and nutrient transport, many of which are dictated by the natural hydrogels and substrates in *in vivo* environments of microbial growth. In *in vitro* microbial culture models, these characteristics can be manipulated by varying the hydrogel's chemical composition, porosity, and pore architecture, as well as by patterning at micro- and macroscales, thereby achieving additional advantages for hydrogel-based cultures.

3.2.1. Crosslinking strategies

Crosslinking is a fundamental strategy for tailoring the mechanical and functional properties of hydrogels, particularly in bioinks and microbial encapsulation systems, where structural integrity and stability are critical. Crosslinking is essential for the gelation and robustness of hydrogels, enabling the increased precision and tunability of physical and chemical properties that allow for more precise microbial manipulation.[62] Modulating hydrogel crosslinking density provides a fundamental mechanism for tailoring the network mesh size, or spaces within the crosslinked hydrogel network, which dictates scaffold permeability.[63,64] This permeability is critical for microbial applications, as it regulates the inward diffusion of nutrients, the outward diffusion of microbial metabolites, and the physical confinement or controlled release of bacterial cells within the engineered matrix.

The selection of a hydrogel crosslinking strategy is critical for the precision, stability, and biocompatibility of microbial culture platforms. Physical crosslinking, driven by hydrogen bonding, ionic interactions, or hydrophobic forces, offers significant advantages for sensitive microbes, primarily due to its mild, reagent-free gelation conditions that preserve biocompatibility. Methods such as ionic crosslinking of alginate with calcium ions are widely adopted for microbial encapsulation due to their rapid, gentle, and effective stabilization.[65] However, these methods

Table 1. Summary of hydrogel materials used for cellular encapsulation

Hydrogel material	Concentration range	Primary advantages	Primary limitations	References
Alginate	1–3% w/v	• Easy ionic crosslinking with divalent cations. • Excellent biocompatibility. • Biodegradable and tunable mechanical properties. • Inexpensive and readily available. • Versatile in composite formulations.	• Lacks bioactive components. • Requires surface modification (RGD peptide conjugation) for cell adhesion promotion. • Moderate mechanical properties when used alone.	[51,54]
Collagen	0.8–4 mg/mL	• Predominant structural protein in mammalian ECM. • Superior *in vitro* and *in vivo* biocompatibility. • Tissue-matching physicochemical properties. • Promotes cell attachment and tissue-specific differentiation. • Supports intercellular communication.	• Variable printability across different crosslinking methods. • Mechanical properties highly dependent on formulation. • Requires careful crosslinking strategy to avoid cell toxicity. • Batch-to-batch variability from natural sources.	[42,51,55]
Gelatin (modified GelMA)	2–10% w/v	• Biodegradable with low antigenicity. • Contains intrinsic RGD cell adhesion motifs. • Low cost and ease of processing. • Thermosensitive gelation at physiological temperatures. • Accessible active groups for chemical modification.	• Low mechanical properties without reinforcement. • Limited structural rigidity in standalone application. • Mechanical strength decreases during the culture period. • Requires dual crosslinking procedures (physical + ionic/chemical).	[42,56]
HA	0.5–2% w/v	• Naturally occurs in mammalian ECM. • Excellent biocompatibility and biodegradability. • Bioresorbable in physiological environments. • High porosity facilitates nutrient diffusion. • Maintains hydrated microenvironment for wound healing.	• Poor cell adhesion without functionalization. • Low water solubility stability leads to degradation. • Requires a combination with other polymers for functional applications.	[51]
Chitosan	0.5–2% w/v	• Biodegradable *in vivo*. • Antibacterial and wound healing properties. • Positively charged (promotes cell adhesion). • Versatile gel-forming capability.	• Poor mechanical properties when used alone. • Limited standalone applications. • Requires a combination formulations. • Variable bioactivity depending on the deacetylation degree.	[42,57]
Gellan gum	0.5–2% w/v (in composites: 0.5–1.5% combined)	• FDA-approved food additive (low toxicity profile). • Fine processability and tunable mechanical properties. • Improved rheological properties in composite bioinks. • Promotes cell proliferation (higher concentrations benefit cells). • Rheology tunable via ionic interactions.	• Limited mechanical properties when used alone. • Requires a combination with other polymers (GelMA, alginate). • Ionic crosslinking may affect cell viability if not optimized. • Concentration-dependent behavior.	[42]
Fibrin	2–10 mg/mL	• Natural ECM component. • Excellent biocompatibility. • Supports cell attachment and differentiation. • Biodegradable through enzymatic pathways.	• High mechanical variability between batches. • Rapid degradation in physiological conditions. • Difficult to standardize for clinical translation. • Limited printability without modification.	[42,58]

Table 1. Summary of hydrogel materials used for cellular encapsulation

Hydrogel material	Concentration range	Primary advantages	Primary limitations	References
Matrigel	0.5–30 μg/mL	• Contains laminin, collagen, and entactin proteins. • Includes endogenous peptides and growth factors. • Promotes cell growth and adhesion.	• Poor mechanical strength. • Potential immunogenicity concerns. • Decreasing scaffold degradability with increased Matrigel concentration.	[42]
Silk fibroin	1–5% w/v	• Abundant natural polymer (silkworm/spider sources). • Excellent mechanical properties and biocompatibility. • Controllable degradability. • Shear thinning properties ideal for extrusion printing. • Physical crosslinking possible.	• Low viscosity (limited printability alone). • Frequent clogging during extrusion printing. • Requires bulking agents or composite formulation. • Requires crosslinking support in most applications.	[42,59]
Dextran (with HA)	Variable (1–3% combined with HA)	• Nontoxic polysaccharide. • Hydrophilic nature. • Biodegradable in mammalian tissues (via dextranase). • Tunable viscoelastic properties. • Useful for semi- interpenetrating polymer network structures.	• Limited mechanical properties when used alone. • Requires a combination with other biomaterials (e.g., HA). • Dextranase-mediated degradation may be unpredictable *in vivo*.	[42,60]
PEG	10–20 wt%	• Mechanical and biochemical properties can be precisely tailored through chemical modification. • Can be rapidly crosslinked via light-based methods • Can be formulated to achieve robust, stable, and elastic networks	• Lacks inherent cell-adhesive motifs and biological cues • Cell viability is highly dependent on crosslinking parameters • Unmodified PEG is often unsuitable for bioprinting, requiring chemical derivatization	[51,61]
Poly(vinyl alcohol)	10–40 wt% (optimal 30–35 wt%)	• Synthetic, nontoxic, and biocompatible. • Low cost and easy production. • Excellent water solubility and hydrophilicity. • Good thermal stability. • Autonomous self-healing capability (freezing/thaw method). • Highly tunable mechanical and physical properties. • Low immunogenicity.	• Limited mechanical properties at lower concentrations. • Crystallinity-dependent behavior affects chain mobility. • Concentration-dependent self- healing efficiency. • Lower fracture stress compared to the original hydrogel (72% recovery at 48 h) • Air bubble formation in solution preparation.	[43]

Abbreviations: ECM, Extracellular matrix; FDA, Food and Drug Administration; GelMA, Gelatin methacryloyl; HA, Hyaluronic acid; PEG, Polyethylene glycol.

suffer from inherent limitations for high-precision applications: they yield networks with weak mechanical strength, exhibit instability under physiological conditions, and offer minimal spatial control over gelation. While the responsive nature of these gels (e.g., to temperature[66] or pH) allows for dynamic tunability, the formation of crosslinks is diffusion-limited, which prevents high-resolution patterning without advanced techniques like light-directed ion release.[67]

In contrast, chemical crosslinking strategies provide the robust, tunable, and mechanically stable networks required for advanced microbial manipulation.[68] Among these, photopolymerization is particularly favored due to its exceptional spatiotemporal precision, enabling the fabrication of constructs with defined architectures and controlled gelation kinetics through photopatterning. This capability is especially important for programming microbial biogeography and creating complex cocultures. However, this enhanced control introduces new biocompatibility challenges. UV light can be antimicrobial or cytotoxic,[69] thereby necessitating the use of visible light systems and compatible photoinitiators like riboflavin[70] to preserve microbial viability. Furthermore, conventional free-radical polymerization is susceptible to oxygen inhibition and network heterogeneity, which can compromise structural fidelity and reproducibility.[71] These drawbacks can be mitigated through additives such as

oxygen scavengers[72] or next-generation chemistries, such as thiol-ene click reactions[71,73] and mixed-mode systems,[45] which offer improved reaction uniformity, reduced oxygen sensitivity, and greater cytocompatibility. The evolution of chemical crosslinking, in addition to physical methods, can expand precision techniques in microbial hydrogel culture, enabling the fabrication of complex living materials with defined functions.

These emerging advanced chemistries also offer tunable mechanical properties and stimuli-responsive behavior for controlled microbial release, nutrient diffusion, metabolite transport, and drug delivery.[74] To further optimize hydrogel performance, hybrid crosslinking strategies[75,76] can be leveraged for increased customization in microbial cocultures and tissue engineering applications. Versatile hybrid crosslinking techniques go hand in hand with material choice as well, for example, the combination of weaker physical crosslinks of alginate gels and the stronger covalent crosslinks of acrylamide gels explored by Sun *et al*.,[77] creating tough hydrogels with tailored mechanical properties that mitigate drawbacks of poor mechanical stability, such as microbial release from matrices and cell death.

3.2.2. Pore generation techniques

The manipulation of hydrogel porosity—specifically pore size, distribution, and interconnectivity—serves as a critical design parameter for directing microbial behavior and function within ELMs. Precisely tuned pore architectures govern essential dynamics, such as nutrient diffusion, metabolic waste removal, microbial motility, and cell–cell communication,[78] each of which directly influences the efficacy of applications ranging from bioproduction to biofilm modeling. Creating and modifying microstructures in hydrogels is also essential for mimicking the complexity of native ECM or biofilms, where mechanical properties and nutrient transport affect cell behavior.[79]

Several established techniques enable the creation of porous hydrogel structures, each offering distinct advantages and limitations for microbial culture applications.[80] Salt templating is a widely adopted approach in which polymer or hydrogel precursors undergo crosslinking around a salt template, followed by salt leaching from the matrix to generate defined pore structures.[81] Alternative porogens, including hydrogel microbeads[82] and other sacrificial materials, can substitute for traditional salts in this process. This method provides control over pore size and offers good compatibility with cells and microorganisms. However, salt templating faces challenges in achieving complete porogen removal and generally produces discontinuous pore structures that may limit efficient nutrient transport throughout the scaffold.

Gas foaming involves the bubbling or generation of gas bubbles within polymer precursors during polymerization. Various agents facilitate this process, including carbonates, nitrates, or solid foaming agents such as carbon dioxide (CO_2).[80] This technique is a rapid and inexpensive method with minimal dependence on porogen extraction procedures; however, gas foaming provides limited control over pore size and distribution, potentially resulting in inconsistent pore architectures.[83] This lack of precision makes it less suitable for applications requiring predictable microbial infiltration or structured community formation.

Emulsion templating employs emulsions composed of two or more immiscible liquids, where a pore-generating internal phase becomes dispersed within an external or continuous phase that subsequently undergoes crosslinking.[84] This approach is effective in generating high porosity levels and good pore interconnectivity, creating well-connected pore networks favorable for cellular communication and nutrient transport.[85] However, emulsion templating suffers from inadequate control over pore size parameters and complications arising when organic solvents interfere with living biological components, potentially compromising cell viability and function. Similar mechanisms exploiting liquid–liquid phase separation have been utilized for hybrids of resilin-like polypeptide and PEG in hydrogels, in which phase separation combined with photocrosslinking enables more precise control of microstructure and polymer domain size based on timing.[86] This control enhances properties such as cell viability and can be used to localize cells and microbes preferentially around specific domains.[86]

The selection of a pore generation strategy is therefore a trade-off between architectural control, biocompatibility, and practicality,[87] and additional techniques such as combinations of methods or leveraging additive manufacturing[80] can pave the way for next-generation scaffolds with hierarchically structured porosity, offering spatial command over microbial microenvironments. Dong *et al*.[88] utilized tailorable hydrogel networks in this fashion to extend expansion microscopy analyses and evaluate microbial interactions at a single-cell level, providing increased precision and control for endpoint microbial analyses. Additionally, previous studies have exploited swelling of an acrylamide-based hydrogel network due to electrostatic interactions to expand aggregated cells in biofilm communities, increasing resolution of individual microbial components,[88] and enabling quantification of adhesive interactions between cells.[88,89] These techniques reveal the importance of hydrogel microstructural control in evaluating interactions within and between microbes in communities.

3.3. 3D patterning methods

In addition to parameters of hydrogel material selection, crosslinking and chemical modification, and pore customization, hydrogel patterning can also be manipulated to precisely modify and assess microbial positioning, proximity, and metabolic behavior. Traditional 2D and planktonic methods offer limited to no control over bacterial positioning and biofilm architectures; however, encapsulation and immobilization in hydrogel matrices allow for both precision in spatial patterning and tunability in chemical and physical properties to manipulate and enhance microbial behavior.[90] The use of conventional molds and hydrogel patterning remains prominent for the integration of living cells with hydrogel matrices due to their cost-effectiveness, high throughput, and material versatility. Simple molding, utilizing basic geometries like cylindrical,[19,91] is particularly valuable for high-throughput *in vitro* studies where reproducibility and scalability are critical. For more sophisticated applications, micromolding techniques bridge the gap between simplicity and precision; by leveraging templates fabricated via photolithography, they enable the creation of hydrogel structures with microscale features that more accurately emulate complex physiological environments while maintaining compatibility with a wide range of polymers and allowing gentle crosslinking conditions to ensure cell viability.[92,93]

Moreover, additive manufacturing and 3D bioprinting methods have emerged to provide additional capabilities for microbial patterning and customization, each with its own associated benefits and drawbacks. Prominent 3D bioprinting techniques of vat polymerization, inkjet, and extrusion-based printing are illustrated in Figure 3, and a list of selected hydrogel patterning methods for structural control and corresponding potential applications based on precision is provided in Table 2.

Extrusion-based printing serves as a versatile workhorse for patterning microbial communities within hydrogels,[20] extending spatial control and precision from simple encapsulation to defined macroscale structures. It is one of the most widely used methods for bioprinting[34] and among the first to be harnessed for biological applications.[94] Extrusion-based printing can be carried out through a variety of approaches and is generally executed by the extrusion of viscous hydrogel materials with air pressure, known as pneumatic printing, or by mechanical force with

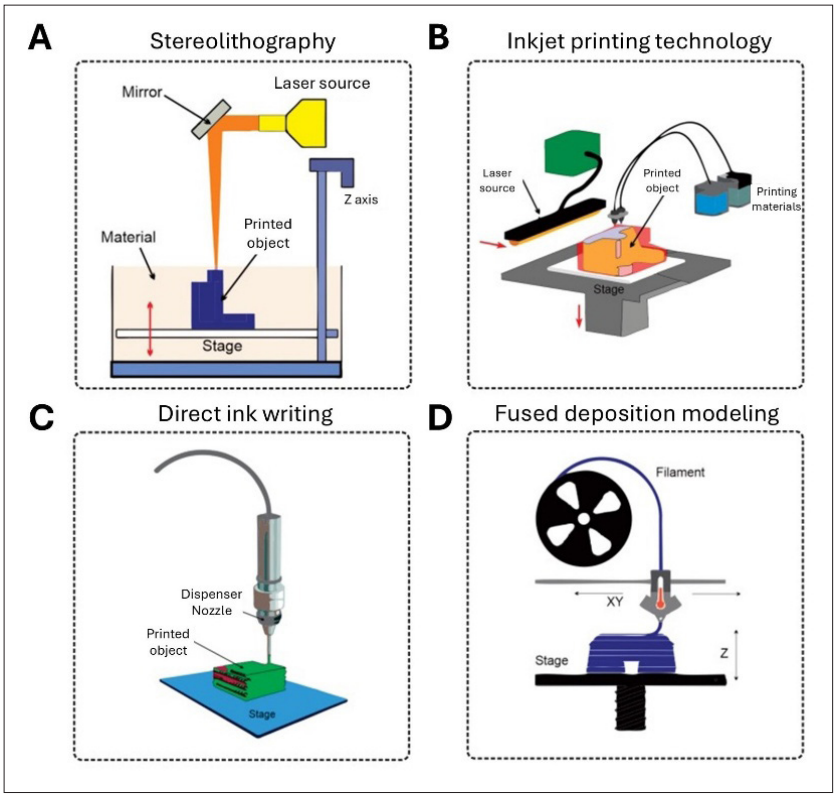

Figure 3. Schematic illustration of various 3D printing techniques, including light-based methods, such as (A) stereolithography and (B) inkjet printing technology, as well as extrusion methods, such as (C) direct ink writing and (D) fused deposition modeling. Reprinted with permission.[19]

Table 2. Examples of hydrogel patterning techniques and applications based on the precision level

Precision level	Macroscale encapsulation (>1 mm)	Mesoscale patterning (100 μm)	Microscale patterning (100 nm–1 μm)
Techniques	Hydrogel molding, simple gel casting.	Extrusion bioprinting, inkjet printing, spin coating.	Digital light processing, stereolithography, multiphoton lithography.
Applications	• Bulk bioproduction (e.g., biofuels, bioplastics). • Whole-cell biosensing and diagnostics. • Therapeutic probiotic delivery. • Bioremediation.	• Structured biofilm and antimicrobial resistance studies. • Synthetic microbial ecology and coculture. • Engineered living materials with logic functions.	• Single-cell analysis and confinement. • High-resolution quorum-sensing studies. • Decoding interspecies communication. • Precision biomolecule gradient formation.

pistons or screws.[95] Modifications through sacrificial and freeform printing, which use support baths[96,97] or "fugitive" inks to form stable complex networks,[98–102] or coaxial printing, where multiple streams of bioink are deposited in concentric rings, allow materials and components to be deposited within or enveloping one another in printed filaments.[103]

A major challenge in extrusion bioprinting lies in the inherent trade-off between structural printability and cell viability. While high viscosity bioinks are essential for achieving construct stability and shape fidelity, the shear forces and extrusion pressures required to deposit them can compromise the integrity of encapsulated cells. Methods and modifications, such as in situ crosslinking during bioprinting, serve to address these challenges and strike a balance between spatial precision and material versatility, preserving suitable printability while providing tunable, biocompatible environments for microorganisms.[104] In situ crosslinking methods, which stabilize hydrogels through photocrosslinking during the printing process and prior to deposition, enable precise patterning of nonviscous inks, mitigating shear forces on cells to preserve viability while also maintaining compatibility with a wider range of materials to support and influence cell and microbe microenvironments. These methods and techniques enable the patterning of hydrogels and the printing of bioinks that are traditionally outside of the criteria for cell and microbe support, expanding the range of possibilities for the study and manipulation of microbial species.[105] One main disadvantage of extrusion processes for some applications is the relatively low resolution (approximately 100 μm) of extruded filaments,[95] thereby limiting the resolution and size of components that can be patterned for applications at various length scales.

While extrusion printing is effective at creating robust structures, achieving higher-resolution patterning requires alternative methods; inkjet bioprinting addresses this need by employing precise droplet deposition to control microbial placement at a finer scale.[106] The resolution of inkjet bioprinting, characterized by droplet diameters in the tens of microns and volumes under 100 pL, is most commonly achieved through either thermal or piezoelectric actuation to deposit bioinks in precise, predefined patterns.[22,23] The precision in droplet patterning enables control over properties, such as porosity and pore distribution, and it allows for the embedding of various biological factors for additional material tailoring. Although its droplet-based approach allows for greater spatial patterning than extrusion, inkjet printing suffers from poor structural stability in the resulting constructs and presents challenges for maintaining cell viability due to shear stresses and the small volumes involved.[107] Jet-based bioprinting generally demands low-viscosity, low-cell-density bioinks that can rapidly solidify after printing to ensure structural integrity. This requirement for fast gelation also limits the selection of suitable hydrogels and necessitates strategies to mitigate challenges like cell sedimentation prior to fixation.

For applications demanding a higher degree of spatial fidelity, light-based printing techniques transcend the resolution limits of droplet-based methods to pattern microbes with up to single-cell precision.[108] Stereolithography and digital light processing printing achieve superior spatial resolution, down to approximately 10 μm,[109,110] by using focused light to photopolymerize hydrogels with micrometer-scale precision, enabling increased control over the patterning of various microenvironments. While light-based printing methods offer the advantage of high resolutions, they have limited use in some other areas of bioprinting. The need for photocurable inks limits material versatility for constructs, and vat polymerization methods have limited capabilities for creating cocultured environments with spatial specificity. To mitigate the drawbacks of some bioprinting methods, combinations of approaches can be used to take advantage of the benefits of multiple processes, such

as the combination of digital light processing and direct ink writing.[111]

This progression in patterning technologies, from the community-level architecture of extrusion-based methods to the droplet-level control of inkjet, and to the near-cellular resolution of light-based printing, provides a toolkit for researchers to select the appropriate level of precision required to interrogate and manipulate the microbial world.

4. 3D hydrogel culture applications and advancements

Applications and advancements in the field of microbial hydrogel culture and encapsulation, including hybrid microbial–hydrogel materials, can be categorized into four main areas of study: bioproduction, bioremediation, responsive and sensing materials, and fundamental microbial study, with different levels of spatial precision and microbial manipulation tailored to enhance each application beyond planktonic and 2D culture methods. Within this review, applications are categorized based on the characteristic scale of microbial manipulation: macroscale (>1 mm), mesoscale (100 μm–1 mm), and microscale (<100 μm). Techniques within the four main application categories are presented to correlate their achievable resolution with the level of control required for specific microbial studies. An overview of hydrogel microbial culture applications, based on technique and precision, is shown in Table 3.

4.1. Bioproduction applications from the macroscale

Microbial biosynthesis has long been harnessed for producing high-value compounds, including antimicrobials,[133] bioplastics,[134] and biofuels.[135] While traditional bioreactors remain the standard for industrial-scale fermentation due to their high volumetric productivity,[136] hydrogel-based culture systems are increasingly recognized for their ability to enhance and control microbial metabolism through tailored material environments. These systems offer superior regulation of nutrient diffusion, product retention, and cell viability, enabling more efficient and stable bioproduction processes. Although scalability often prioritizes lower-resolution methods like extrusion bioprinting, precision in material design—from polymer selection to spatial patterning—is critical for optimizing microbial function and coculture synergies in ELMs.

Smith and Francis[137] highlighted the strategic use of hydrogels to manipulate microbial metabolism and interspecies interactions. They developed a hydrogel-based coculture system using the *Synechococcus elongatus* cscB strain, a cyanobacterium engineered to secrete sucrose under osmotic stress, providing an alternative sucrose source for other organisms. By tailoring the swelling of poly(sodium acrylate) hydrogels encapsulating *S. elongatus* cscB, they controlled osmotic stress and subsequent sucrose production. This system was applied in a coculture with *Azotobacter vinelandii* AV3, an ammonia-secreting bacterium capable of producing biopolymers like polyhydroxybutyrate. The bulk hydrogel encapsulation enhanced the bioproduction of *A. vinelandii* AV3, demonstrating the advantages of this approach for mutually dependent cocultures. Their results highlight how hydrogel encapsulation allows metabolic conditions of one species to be modified and optimized without disrupting the other, offering a promising strategy for engineered microbial systems and sustained multispecies bioproduction.

Beyond metabolic tuning in bulk hydrogels and constructs, hydrogels provide physical protection that stabilizes microbial biocatalysts. Johnston *et al.*[90] demonstrated these advantages of hydrogel preservation with increased spatial precision by printing programmed *Escherichia coli* and yeast cells in F127 dimethacrylate and inducing the production of "high-value" products, such as 2,3-butanediol, levodopa, and antibiotics. These printed cell-laden hydrogels maintained similar levels of production even after lyophilization and rehydration, demonstrating increased stability due to microbial incorporation into bioink matrices. Integrating ECM material produced by microbes into hydrogel matrices can provide additional reinforcement to biofilm-inspired environments, enhancing resistance to both antibiotics and chemical dissolution of the hydrogels.[30] This reinforcement was harnessed and demonstrated by Schmieden *et al.*,[31] in which extrusion printing of *E. coli* in alginate-based gels provided space and conditions for biofilm-forming CsgA protein secretion. The patterned biofilms with microbial secretion exhibited increased protection and linkage of the microbial cells even under conditions where the alginate matrix was later dissolved. This spatial control, patterning, and induction of natural microbial biofilms, though limited in resolution, demonstrates promise in scalability for living structured materials for a variety of applications harnessing natural bioproducts. This approach was advanced by Duraj-Thatte *et al.*,[138] who printed *E. coli* through extrusion-based methods using their own ECM as a bioink. Secreted CsgA nanofibers were collected and printed into predefined structures, and incorporated *E. coli* were programmed for various applications, including secretion of an anticancer drug or sequestration of bisphenol A,[138] highlighting how precision in material sourcing and printing can enable multifunctional living systems.

Table 3. Comparative overview of microbial immobilization in hydrogel architectures

Application	Microorganism	Immobilization technique	Material	Purpose	Refs..
Study of microbial dynamics and biocatalysis	*Pseudomonas aeruginosa*	Photolithography (micro-3D printing)	Photocrosslinked gelatin	Real-time study of quorum-sensing communication	[113]
	Escherichia coli	Extrusion-based bioprinting	2% agarose and 2% alginate	Spatially organize bacteria for controlled growth, communication, and guided chemotaxis	[114]
	Escherichia coli, Lactobacillus rhamnosus GG.	Extrusion-based bioprinting (continuous chaotic bioprinting)	Alginate	Build microenvironments to study interface-driven competition and cooperation	[115]
	Soil microparticles	Laser bioprinting	Hyaluronic acid-based gel	Use laser engineering of microbial systems to recover rare uncultivable soil microbes with minimal habitat disruption	[116]
Bioremediation and biosensing	*Synechococcus sp.* PCC 7002	Extrusion-based bioprinting with ionic crosslinking	Alginate–methylcellulose hydrogel with sea sand	Bioprinted scaffolds incorporating cyanobacteria to induce calcium carbonate formation for sustainable construction	[117]
	Escherichia coli, *Photobacterium kishitanii*	Digital light processing and volumetric printing	Photopolymerizable methacrylated hyaluronic acid and Pluronic F-127	Light-based printing of bacteria for autonomous chemical sensing	[118]
	Geobacillus stearothermophilus, *Bacillus atrophaeus*	Thermal inkjet printing	Guar gum	Colorimetric temperature indicator	[119]
	Escherichia coli, *Caulobacter crescentus*	Projection microstereolithography (SLAM bioprinting)	PEGDA	Enhance the sequestration and sensing of rare earth elements and uranium	[120]
	Escherichia coli	Extrusion-based bioprinting	Alginate	Determine how biofilm matrix composition enhances bioremediation resilience	[121]
	Chlorella zofingiensis	Extrusion-based bioprinting + tannic acid coating	Alginate and PEGDA	Confine microalgae in retrievable 3D-printed hydrogels to enable antibiotic degradation with leakage control	[122]
	Chlorella vulgaris, *Bacillus subtilis*	Extrusion-based bioprinting	Hyaluronic acid with methacrylate groups and cysteine–phenylalanine dipeptides	Create reusable, printable dual-network bioinks that sustain bioremediation	[123]
	Pseudomonas aeruginosa	Electro-assisted bioprinting of hydrogel beads	Alginate, silk fibroin methacryloyl	Create an interwoven-network bioink for stable cell immobilization and pollutant removal	[124]
	Synechococcus elongatus	Extrusion-based bioprinting (direct ink writing)	Alginate	Enable stimulus-responsive dye degradation with adjustable cell death	[125]
	Escherichia coli	Hydrogel encapsulation (alginate core–shell capsules)	Alginate	Biosensing platform for diagnosing dextran–sodium sulfate-induced colitis	[28]

Table 3. Comparative overview of microbial immobilization in hydrogel architectures

Application	Microorganism	Immobilization technique	Material	Purpose	Refs..
Bioproduction	*Saccharomyces cerevisiae*	Extrusion-based bioprinting (direct ink writing)	PEGDA	Enhance ethanol catalysis by boosting glucose and carbon dioxide production through engineered porous 3D geometries	[26]
	Escherichia coli	Extrusion-based bioprinting	Alginate	Demonstrate a proof-of-concept for low-cost, sustainable bacterial material fabrication using simple 3D printing	[126]
	Escherichia coli, Saccharomyces cerevisiae (mono- and cocultures)	Extrusion-based bioprinting	F127–bisurethane methacrylate	Enable freeze-dried, extrusion-printed compartments to preserve and reactivate microbes for on-demand biosynthesis of levodopa or 2,3-butanediol	[90]
	Lactobacillus reuteri	Encapsulation of bacteria in microspheres, + photocrosslinking	Methacrylate-modified hyaluronic acid bulk hydrogel incorporating methacrylated gelatin microspheres	Living hydrogel scaffold enhances wound healing via bacterial secretion of antibacterial agents	[127]
Living materials	*Bacillus subtilis*	Extrusion Bioprinting and microencapsulation	TasA amyloid biofilms	Programmable, self-renewing living materials that merge catalytic functionality with structural integrity	[128]
	Pseudomonas putida, *Acetobacter xylinum*	Extrusion-based bioprinting (direct ink writing)	Hyaluronic acid, κ-carrageenan, and fumed silica (Flink).	Enhance bacterial metabolism and growth to enable cellulose production for biomedical applications or phenol degradation for bioremediation	[129]
	Caulobacter crescentus	Hydrogel culture with self-assembled protein matrix	Engineered BUD fusion displayed via RsaA	Engineer bacteria to display and secrete a self-assembling protein matrix for tunable living materials	[130]
	Escherichia coli	Autonomous secretion and surface-guided assembly of engineered curli fibrils.	Amyloid curli fibrils (engineered CsgA variants) forming a hierarchical protein matrix	Integration of bacteria into multiscale assemblies for functional composite materials	[131]
	Bacillus subtilis	Extrusion-based bioprinting	Agarose hydrogel	Embed spores in printed hydrogel to enable long-term storage and on-demand germination for sensing/actuation	[132]
	Escherichia coli	Extrusion-based bioprinting	Alginate	Combine 3D printing and inducible curli expression to create stable, spatially patterned biofilm-inspired materials	[31]
	Shewanella oneidensis MR-1	Extrusion-based bioprinting (direct ink writing)	Alginate and cellulose	Create a functional living anode with improved bioelectrochemical performance	[133]

Abbreviations: 3D, Three-dimensional; PEGDA, Polyethylene glycol dimethacrylate.

The scope of microbial bioproduction extends beyond *E. coli*, a popular model organism for analysis and manipulation of ECM production, to include specialized species whose innate biosynthetic capabilities are enhanced by 3D patterning. *Streptococcus zooepidemicus* was encapsulated in GelMA-based gels and induced to produce hyaluronic acid (HA).[139] Previous research described this production as an example of a "3D-printed bioreactor," noting that scaffold orientation and geometry could also be manipulated to affect HA yield. *Bacillus subtilis* is also utilized for living materials in addition to its study as a model of biofilm formation. These bacteria are attractive due to being "generally regarded as safe," and the amyloid fibers that form biofilms of *B. subtilis* can be harnessed for adhesion to host tissues and antibiotic resistance.[126] The versatility of printed biofilms containing these amyloid fibers was demonstrated by Huang *et al.*,[127] where *B. subtilis* was programmed to secrete biofilm-forming proteins with varied functional properties based on customized domains. These tunable patterned biofilms represent an added advantage over planktonic or free microbial cells in applications, such as drug delivery, where greater precision in protein and biomolecule release is needed. Qian *et al.*[26] utilized freeze-dried and printed cells of Baker's yeast (*Saccharomyces cerevisiae*) with nanocellulose to act as a biocatalyst for the metabolism of glucose. The 3D-printed lattice structures showed improved efficiency over bulk structures, demonstrating the advantages of combining 3D patterning with biofilm technologies.

Hydrogel culture and bioprinting have significantly advanced microbial bioproduction by enabling the precise spatial organization of microbial cells and cocultures. This controlled physical placement, achieved through methods ranging from bulk encapsulation to extrusion-based patterning, creates tailored microenvironments that enhance metabolic efficiency and the yield of target bioproducts and biopolymers. A primary limitation, however, is the inherent trade-off between precision and scalability; in some cases, the specialized materials and fabrication processes required for these tailored environments incur high costs and material usage that are not yet practical for large-scale, low-value commodity production. Future progress hinges on developing more biomaterial-efficient and cost-effective fabrication strategies, which would dramatically expand the economic viability of these platforms, particularly for the synthesis of high-value compounds. Overall, these advances highlight a shift in microbial bioproduction from large-scale, unstructured fermentation toward spatially and materially optimized systems where hydrogel design dictates microbial output.

4.2. Multiscale microbial control for bioremediation

Engineered living materials are redefining decontamination by embedding pollutant-degrading microorganisms in 3D-printed hydrogels that capture metals, detoxify organics, and even mineralize CO_2. A persistent bottleneck, however, is achieving the dual requirement of mechanical strength and biocompatibility, without which the resident biocatalysts cannot function effectively. 3D-printed, engineered biofilms offer a versatile platform for environmental remediation: they can support bioremediation, selectively capture rare-earth elements (REE) and heavy metals, strip assimilable organic carbon from water, and be incorporated into wastewater-treatment systems.[30]

Balasubramanian *et al.*[120] 3D-printed four-layer stripes (approximately 550 μm) of curli$^+$/cellulose$^+$ *E. coli* encapsulated in a calcium alginate hydrogel bioink. After 1 week, the constructs developed approximately 300–400 μm anoxic cores, withstood 1% Virkon S—a broad-spectrum disinfectant—with only a two-log viability loss, and fully recovered their geometry after manual folding or twisting, offering the mechanical resilience demanded by in-situ bioremediation reactors and turbulent wastewater streams.[120] In contrast to native *E. coli*, which successfully mineralizes aromatic acids, genetically engineered derivatives can degrade complex hydrocarbons and immobilize Cu^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, and atrazine.[141] Incorporating these genetically engineered strains within the printed scaffold would thus broaden its catalytic repertoire, accelerate pollutant turnover, and sustain performance even under high shear, continuous-flow treatment regimens.[120]

Using projection microstereolithography (SLAM), Dubbin *et al.*[119] photopolymerized a 15% PEGDA bioresin layer by layer; the projected light polymerizes the resin into a hydrogel, providing a supportive matrix for the immobilized bacteria and achieving 10 μm z-resolution. Grid-printed films expressing lanthanide-binding peptides halve soluble neodymium in 5 min ($T_{1/2}$), whereas cube controls required 120 min—a kinetic edge that accelerates REE recovery, which is vital for clean energy supply chains.[119] A companion Pphyt-GFP *Caulobacter crescentus* biosensor emitted strong fluorescence, providing a cost-effective in situ biosensor for environmental uranium levels that are otherwise challenging to detect.[119] Together, SLAM-printed, cell-laden hydrogel films fuse rapid REE capture with responsive uranium sensing for next-generation bioremediation materials.[119]

Jiang *et al.*[122] extrusion-bioprinted microalgae hydrogel networks (MHN@TA) by photocrosslinking a PEGDA/sodium alginate bioink laden with *Chlorella zofingiensis*

and then coating the lattice with tannic acid, which generates a semipermeable membrane that prevents cells from escaping. This tannic acid layer limited algal leakage to ≤0.7% over 7 days and allowed the construct to be recovered and reused without loss of integrity.[121] In batch tests, MHN@TA removed 99.3% of 100 mg/L tetracycline within 72 h and maintained >95% efficiency even at 400 mg/L, outperforming suspended cultures.[121] By coupling high-performance antibiotic degradation with robust containment and recyclability, this matrix offers a durable and sustainable solution for treating pharmaceutical wastewater.[121]

Additive manufacturing yielded a dual-network hydrogel from methacrylated, cysteine–phenylalanine-modified HA; UV photopolymerization further stiffened the self-supportive lattice architecture.[122] Compartmentalized *B. subtilis* and *Chlorella vulgaris* synergistically degraded 70% methyl orange and 40% acrylamide (textile dye pollutants) within 12 h, converting CO_2 that algae photosynthetically returned to O_2, thereby sustaining bacterial respiration.[122] The living material therefore fast-tracks pollutant mineralization while maintaining biosafety and recyclability.[122] Wang *et al.*[123] developed an interpenetrating 1.5% sodium alginate/20% silk-methacryloyl hydrogel beads (1.5SA/20SilMA-S-Ca) that withstand 300 mg/L sodium and preserve porosity after prolonged use. They selected this formulation to assess pollutant control. Beads entrapped *Pseudomonas aeruginosa* PAO1—an aerobic denitrifier capable of using nitrate or nitrite as terminal electron acceptors.[123] In synthetic wastewater, they outperformed free bacteria, cutting total nitrogen by 74.4% in 12 h with negligible nitrate accumulation and delivering faster COD decline.[123] The mechanically robust matrix thus couples efficient aerobic denitrification with bacterial immobilization, offering a reusable platform for high-salinity wastewater treatment.[123]

With construction generating 39% of global CO_2 emissions, Reinhardt *et al.*[116] bioprinted *Synechococcus* PCC 7002 into ELMs by extrusion of alginate–methylcellulose hydrogels. Besides photosynthetic uptake, the cyanobacteria fix carbon through microbially induced carbonate precipitation, depositing calcium carbonate that acts as a biocement within the printed lattice.[116] Extrusion-based bioprinting yields self-supporting scaffolds with sustained cell viability while enhancing compressive strength as mineralization proceeds.[117] This demonstrates a scalable living biomaterial that sequesters atmospheric CO_2 while forming environmentally friendly construction components.[116]

In line with cyanobacterial research, Datta *et al.*[124] modified an ELM by 3D-printing an alginate hydrogel scaffold with *Synechococcus elongatus* genetically engineered to overexpress CotA laccase for chemical decontamination. Encapsulated bacteria stayed viable, photosynthetically active, and released laccase that oxidized and removed the textile dye indigo carmine, showing efficient bioremediation.[124] The printed construct also integrated a theophylline-triggered "kill switch" for biocontainment.[124] This research shows how additive manufacturing can program responsive, self-sustaining ELMs for practical pollutant management. Together, these next-generation materials illustrate how judicious bioink design and microbial engineering can convert environmental liabilities into self-sustaining detoxification platforms. Continued advances in scaffold chemistry, genetic safeguards, and reactor integration should soon translate their laboratory innovations into solid, ready-to-use solutions for the most stubborn water-quality challenges.[140]

4.3. Programmable living materials: High-resolution responses from macro- to microscale

Microbial 3D bioprinting methods have enabled the creation of improved living materials, or combinations of cells and biologically engineered matrices.[90] Some notable advances include the incorporation of bacteria into soft robotic devices, as demonstrated by Justus *et al.*,[141] where engineered bacteria are incorporated for the detection of environmental signals or chemicals, and the bacterial responses are converted into electronic signals for robotic gripping abilities.[141] Methods that utilize 2D bacterial culture on different substrates, or the patterning of planktonic bacteria in molds or channels, have proved transformative for the secretion of products or the execution of functions in response to tailored conditions.[142] Embedding these microbes and systems in man-made or natural hydrogels provides additional versatility in 3D patterning and precision for the detection of environmental factors or the display of chemical or mechanical signals.

For systemic therapeutic delivery and diagnostics, macroscale encapsulation strategies prioritize functional precision over spatial resolution. This approach has proved significant for therapeutic applications, providing protection from environmental factors while enabling precision in the detection and treatment of physiological issues. Aghlara-Fotovat *et al.*[28] demonstrated the diagnostic potential of hydrogel-encapsulated bacteria for detecting inflammatory conditions, specifically colitis. They encapsulated engineered *E. coli*—designed to sense thiosulfate, a biomarker of gut inflammation—within ionically crosslinked alginate hydrogel capsules, which

maintained structural integrity and bacterial viability. Upon oral delivery to rat models, the encapsulated bacteria successfully detected colitis, as evidenced by the activation of fluorescent reporters upon exposure to inflammatory conditions. Notably, the hydrogel system enabled higher bacterial retrieval and detection rates compared to free-floating bacteria, enhancing diagnostic reliability. This hydrogel platform provides an advantage for microbial diagnostics by offering a stable, targeted approach to monitor inflammation, potentially extending to other diseases that are difficult to assess through conventional methods.[28]

Similarly, Ming *et al.*[126] developed an innovative probiotic-loaded hydrogel system to enhance wound healing. They encapsulated *Lactobacillus reuteri* within gelatin hydrogel microparticles, which were then incorporated into a photopolymerizable methacrylated HA precursor, forming an injectable living wound dressing. The hydrogels were stabilized via photocrosslinking to ensure microbial growth, viability, and retention within the wound site. The hydrogel matrix provided a dual function: protecting *L. reuteri* from environmental stresses and challenges from the immune system while also facilitating its antibacterial function through sustained lactic acid and antimicrobial secretion, combating pathogenic bacteria. Hydrogel encapsulation also prevented bacterial escape into surrounding tissues, thereby mitigating potential risks. Their study highlights the promise of probiotic–hydrogel hybrid therapies for tissue regeneration and infection control in wound care applications.

Notable studies involving the manipulation of *E. coli* include its printing in various patterns through extrusion-based methods and its induction to form curli fibers, a natural component of *E. coli* biofilm in ECM.[31,125] Biofilm components, such as curli, play significant roles in bacterial adhesion and pathogenesis[143]; therefore, studying these factors in reproducible environments is of critical importance. 3D bioprinting and hydrogel culture methods enhance control over biofilm characteristics, such as composition and microbial density, which are essential for tunable responses and induction of diverse behaviors, such as biomolecule production.[31] Liu *et al.*[144] developed a Pluronic F127 diacrylate-based material system containing programmed *E. coli* bacteria. This system combined chemicals and chemical signals that act as inputs, with cells, which act as outputs for logic gate functions. These materials and microbial systems have also demonstrated potential for decision-making functions, spatiotemporal patterning, and wearable devices that can exhibit fluorescence in response to various stimuli.[144]

At the highest level of integration, microscale synthesis techniques harness microbial activity to directly constitute or modify the material itself. Reinhardt *et al.*[116] also utilized cyanobacteria to modify and strengthen hydrogel matrices. *Synechococcus sp.* bacteria were incorporated into alginate–methylcellulose gels containing sea sand, and the bacteria were induced to mineralize the matrices with calcium carbonate mineral. The bacteria-incorporated matrices displayed maintained viability and higher compressive strength than normal matrices, showing potential for "living building materials" that can be used for construction purposes. Schaffner *et al.*[128] demonstrated the utility of bioprinted living materials for both bioremediation and biomedical resource production. They encapsulated *Pseudomonas putida,* which can degrade harmful environmental products, or *Acetobacter xylinum,* which can fabricate bacterial cellulose for a variety of biomedical applications, into HA-based hydrogels via extrusion-based printing. The hydrogel encapsulation provided distinct advantages over free or planktonic bacteria in each application, as immobilization of bacteria in biocompatible gel matrices preserved the microbes for sustained, programmable bioconversion while preventing bacterial overgrowth.[128]

Engineered living materials exemplify the transformative potential of hydrogel-based microbial culture, leveraging spatial patterning and encapsulation to create sophisticated biosensing and responsive systems. The protective hydrogel matrix enables precise processing of environmental inputs and microbial outputs at customizable resolutions, while simultaneously safeguarding the encapsulated microbes. A central challenge is achieving simultaneous control over two critical material properties: the tailored diffusion of molecular signals to ensure rapid sensor response, and robust containment of microbial cells to prevent environmental release. Promisingly, recent advances in material design and advanced crosslinking strategies demonstrate that these competing requirements can be reconciled through meticulous system engineering. Overcoming this hurdle will be pivotal for unlocking the full potential of hydrogel-based living materials in real-world applications, from environmental monitoring to smart therapeutic devices.

4.4. High-precision methods for fundamental microbial study

Hydrogel materials have become valuable tools in fundamental microbial research, providing the reproducibility, customizability, and biomimetic properties necessary to reconstruct and probe complex microbial behaviors from community-level interactions to single-cell functions.[145] By offering increased tunable control over

the physical and chemical microenvironment, hydrogel-based platforms enable researchers to tailor experimental systems with varying levels of precision to address specific biological questions, moving beyond the limitations of traditional planktonic or agar-based cultures.

Extrusion-based bioprinting has emerged as a versatile method for investigating clinically relevant microbial community behaviors, such as biofilm formation and antibiotic resistance. Ning *et al.*[12] compared 2D and 3D microbial cultures using extrusion-based hydrogel printing methods, noting that antibiotic treatments developed for planktonic cultures can become ineffective once the bacteria form biofilms. They employed alginate hydrogels to maintain long-term cultures of clinically relevant pathogens, including *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus*. Antimicrobial susceptibility testing revealed significantly enhanced resistance in 3D-printed *S. aureus* and *E. coli* biofilms compared to their 2D counterparts, better mirroring the resilience observed in clinical infections. This highlights crucial advantages of hydrogels and 3D bioprinting for microbial study, emphasizing that biofilm properties can be altered when embedded in the host ECM.[12]

Multispecies evaluations using advanced hydrogel formulation techniques have also enabled recapitulation of more complex environments and conditions relevant to natural tissues. Zheng *et al.*[146] developed an engineered microbiota culture platform using multilayered PEGDA-based hydrogels fabricated via spin coating—a technique that deposits uniform polymer films by spinning precursor solutions at high speeds—to isolate and culture gut bacteria. This system maintained microbial diversity and composition comparable to natural gut microbiota, offering improved reproducibility over conventional liquid cultures. Additionally, the hydrogel-based platform supported coculture with host cells, enabling a more physiologically relevant *in vitro* model. They leveraged this system to evaluate the efficacy of chemotherapeutic drugs, with results closely mirroring *in vivo* outcomes. Furthermore, the platform allowed for the assessment of patient-derived gut microbiome influences on drug response, facilitating tailored therapeutic adjustments, including dose optimization or combination with cytokines like granulocyte–macrophage colony-stimulating factor to enhance treatment efficacy.[146]

Aliyazdi *et al.*[147] also demonstrated the use of combined microbial hydrogel cultures and host cells by creating a controlled, bioprinted model to investigate host–pathogen interactions. They engineered *E. coli* MG1655 into custom lattice-patterned biofilms (Figure 4B), recapitulating the large-scale architecture (sometimes exceeding ≈1,000 μm) observed in natural environments. They successfully cultured these printed biofilms on monolayers of human bronchial epithelial cells while preserving host cell viability and function.[147] Such approaches, which simultaneously meet the stringent biocompatibility requirements of mammalian cells and the structural demands of microbial bioprinting, represent a significant step forward. Establishing design principles for using 3D bioprinting to achieve increased control and reproducibility in *in vitro* infection models enables the direct investigation of the interplay between living host tissues and structured bacterial communities, providing a valuable tool for personalized medicine and drug development.

The ability to control microbial arrangement within hydrogels enables direct investigation of social behaviors and biogeographical effects. Ceballos-Gonzalez *et al.*[115] utilized extrusion-based microbial printing with modifications to bioink flow and bacterial spatial arrangements to evaluate the social behavior of bacteria with respect to biogeography and local environments. Their use of a "continuous chaotic bacterial bioprinting" method (Figure 4A) enabled the creation of various patterns and organizations incorporating different *E. coli* strains within extruded filaments. The bacterial communities were analyzed with fluorescent microscopy and scanning electron microscopy, and they highlighted that increased physical intimacy and interfacial contact between strains disrupted bacterial community stability.

Three-dimensional bioprinting and patterning methods, such as photolithography, allow for the highest spatial precision for microbial spatial manipulation, which provides a key advantage for analyzing bacterial communities and gaining insight into fundamental single-cell level properties, such as quorum sensing[148] and antibacterial resistance.[149] The multiphoton lithography-based "micro-3D printing" methods utilized by Connell *et al.*[149,150] further enhance the benefits of high resolution in creating 3D structures and enable the entrapment of single bacterial cells to probe properties of bacterial communities in response to community size (Figure 4). They noted that the behavior of small bacterial clusters in confinement is crucial for assessing the social behaviors of bacteria and their potential roles in infections.[150] These high-resolution micro-3D printing methods were shown to be effective in combination with analytical methods such as scanning electrochemical microscopy to map chemical signals and evaluate quorum-sensing relationships with respect to microbial colony size and spatial arrangements.[112]

Lithography-based micro-3D printing methods were utilized for multispecies analysis, exploiting the ability to confine multiple bacterial populations in arrangements

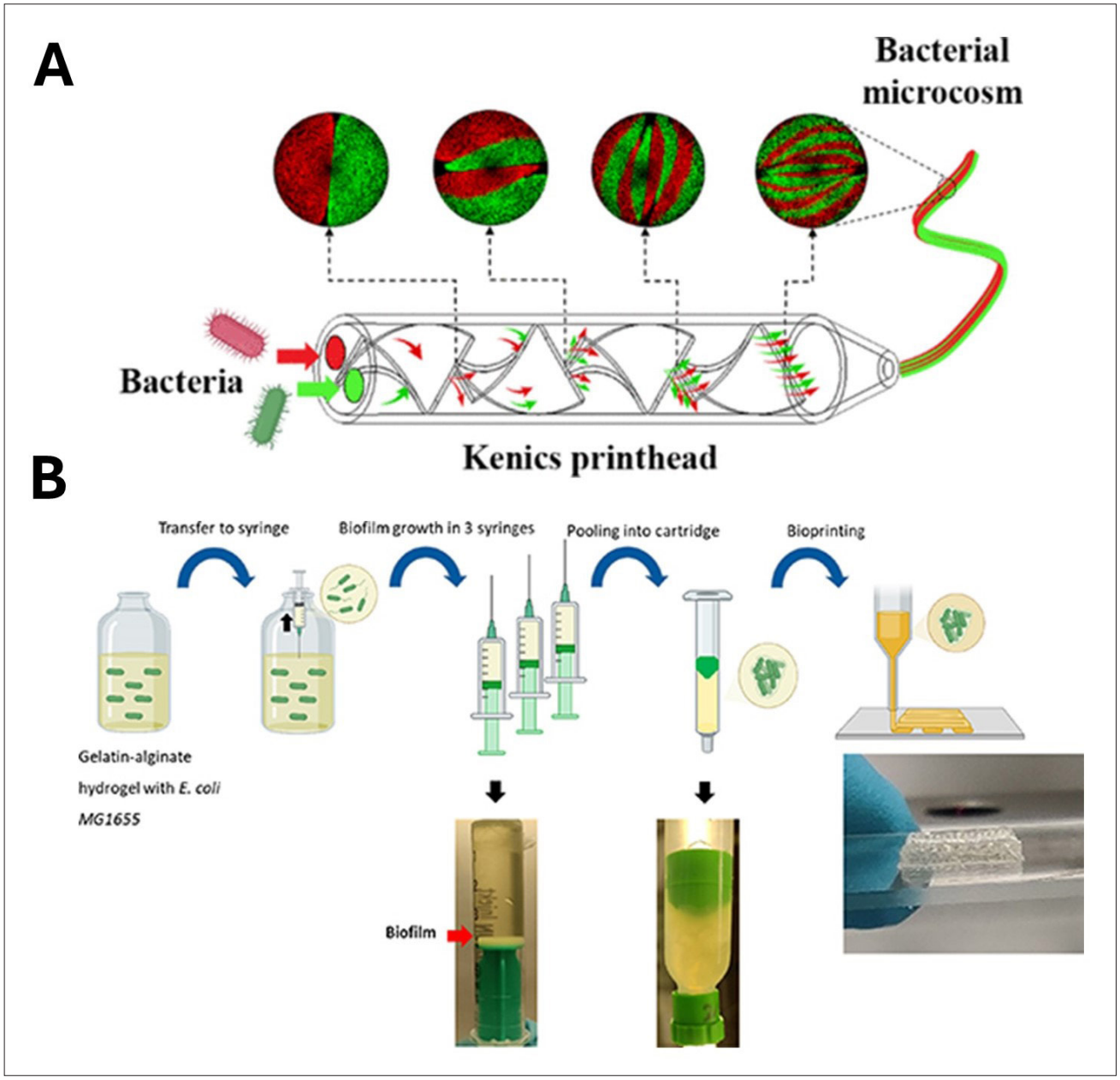

Figure 4. Illustrations of microbial bioprinting and encapsulation strategies at different precision levels. (A) Multilamellar bacterial patterns generated via continuous chaotic bacterial bioprinting to analyze interactions between *Escherichia coli* strains (image adapted with permission from Ceballos-González *et al.*[114] Copyright © 2021, American Chemical Society). (B) Schematic workflow of bioink preparation and bioprinting process of *E. coli* MG1655 biofilms for printing on mammalian cell monolayers. Inoculated gelatin–alginate-based hydrogels are loaded into syringes for growth, pooled, and printed with the capability for postprinting crosslinking. Reprinted from.[147] Copyright.

that are physically separated but can chemically communicate for analysis.[149] These methods revealed that the presence of *P. aeruginosa* in localized shells was able to lower *S. aureus* susceptibility to antibiotic agents, demonstrating how precision hydrogel patterning can decode complex interspecies interactions and their role in infection resilience.[149]

Hydrogel-based platforms provide a critical advancement for microbial and biomedical studies by recapitulating tissue-like mechanical and transport properties, enabling the investigation of host–microbe interactions and phenotypes—such as antimicrobial resistance—that are poorly represented in traditional 2D or planktonic cultures. This capacity to tailor 3D

microenvironments offers a more physiologically relevant context for analyzing dynamic microbial behaviors. A primary limitation hindering the full potential of these systems is the current lack of standardized bioink formulations and fabrication parameters, which compromises reproducibility and cross-study comparisons. Establishing such standards is a crucial step, as it would significantly enhance the translational relevance of these models for both fundamental research into single-cell microbial dynamics and applied biomedical screening.

The studies encompassing these main applications highlight a central theme: the level of spatial and material control offered by hydrogel systems can be strategically matched to the biological or mechanistic question at hand. As shown in Figure 5, advancements in microbial research enabled by hydrogel-based culture and 3D patterning are categorized by fabrication technique and associated levels of spatial precision. Whether using extrusion printing for community-level biofilm models or lithography for single-cell signaling dynamics, hydrogel-based design provides a versatile and powerful framework for advancing microbial science.

5. Limitations and future perspectives

The advancement of microbial bioprinting and hydrogel encapsulation holds significant promise for biomedical research, sustainable bioproduction, and environmental applications. However, its development and adoption currently lag behind that of mammalian cell bioprinting and hydrogel culture due to distinct technical and conceptual challenges. A primary limitation is the perceived lack of clinical urgency; unlike mammalian tissues, microbial cultures often do not require complex ECM-mimetic hydrogels for basic survival, given their inherent robustness. This reduces incentives to develop sophisticated bioinks for microbes outside specialized applications in which replicating native biofilm microenvironments with precise chemical gradients, spatial organization, and host interactions is essential.

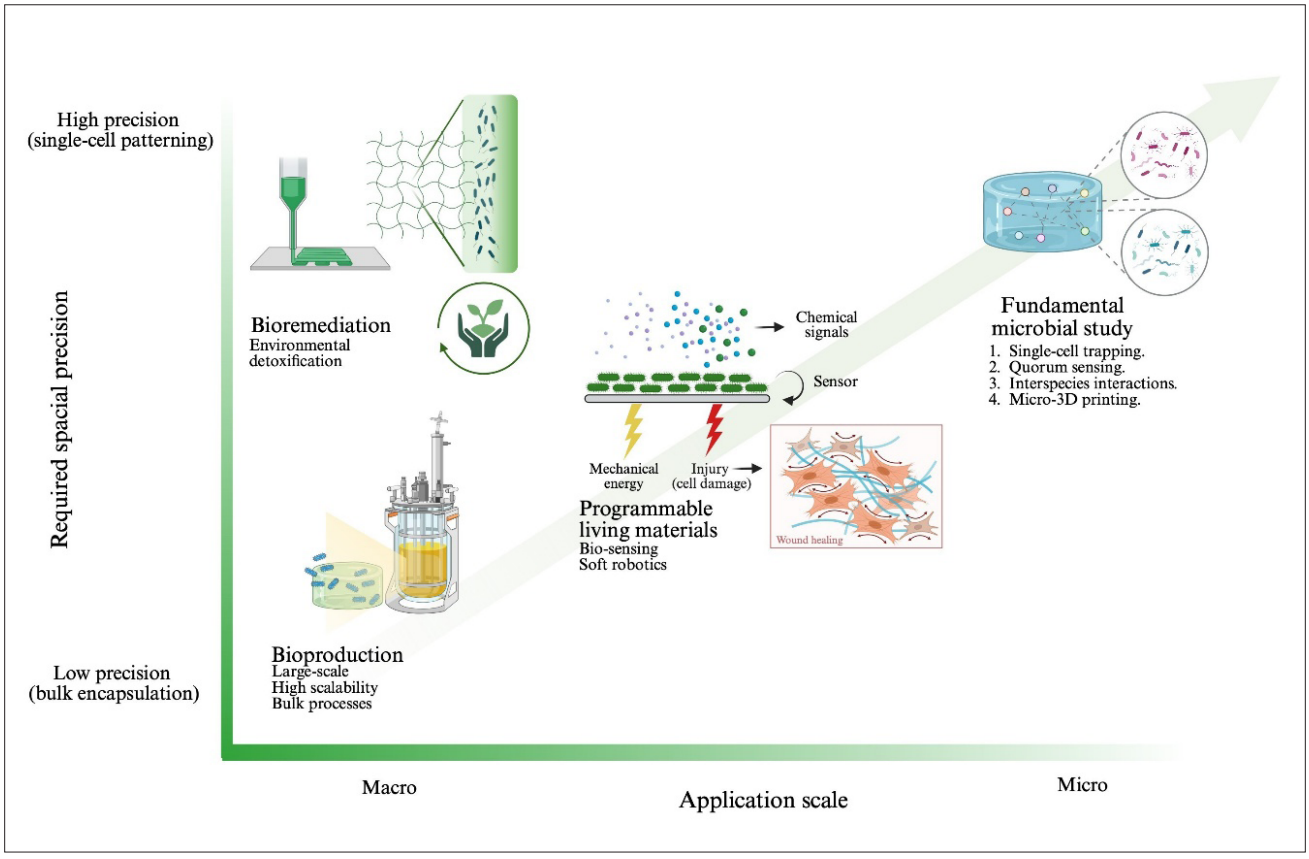

Figure 5. Conceptual applications map showing how spatial precision requirements correspond to application scale in microbial bioprinting. The figure highlights that the required level of precision should be matched to the intended application: macroscale bulk encapsulation for bioproduction and bioremediation, and microscale single-cell patterning for programmable living materials and fundamental microbial studies. Created in BioRender. Klein, C. UDD, D. (2025) https://BioRender.com/y6mmky5. Abbreviation: 3D, Three-dimensional.

Nevertheless, the unique benefits of increased spatial and metabolic control continue to drive innovation in the field. Hydrogel-based systems enable precise manipulation of microbial ecosystems, allowing researchers to dictate cell positioning, control nutrient diffusion, engineer chemical gradients, and stabilize cocultures—capabilities largely unattainable with traditional liquid cultures or growth on 2D substrates. These advantages are critical for applications, such as synthetic ecology, advanced bioremediation, and personalized medicine, where community structure and function directly determine system performance.

With the four main microbial culture applications—bioproduction, bioremediation, responsive programmable materials, and fundamental microbial study—in mind, there are crucial future developments that can be pursued to overcome current limitations and broaden applicability. Current and next-generation hydrogels can be engineered to respond to microbial activity and environmental changes, such as pH conditions and metabolite secretion. Such materials could adapt to their physical properties or release antimicrobials/nutrients in real-time, enabling closed-loop control of microbial growth and function. This is particularly relevant for oral or gut microbiome applications, where dynamic conditions require biomimetic adaptability.

While high-resolution techniques, such as lithography offer single-cell precision, scaling these methods for industrial or clinical use remains a challenge. Future work must bridge the gap between resolution and scalability, potentially through hybrid printing (e.g., combining extrusion for structure with light-based or inkjet patterning for surface functionalization) or the development of rapid, continuous fabrication processes. With respect to tissue engineering applications, hybrid bioprinting that combines multiple techniques and material types has demonstrated the potential to incorporate complex, multifunctional tissue models into single constructs.[151,152] These strategies may also enable the creation of hierarchically structured microbial constructs where critical functions, such as nutrient transport, waste removal, and product secretion, are engineered directly into the architecture. This approach provides a pathway to scale functional complexity[152] beyond increasing size, thereby enhancing the production capabilities and practical viability of ELMs.

Establishing standardized bioink formulations and printing parameters for microbial systems remains an essential priority in the field. Establishing benchmarks for viability, function, and stability postprinting is essential for reproducibility and comparison across studies. Versatility in hydrogel materials and fabrication methods also allows for advanced in situ monitoring techniques, such as embedded sensors for metabolic tracking or compatibility with advanced imaging, which are needed to validate microbial activity within 3D constructs over time.

For biomedical applications, future platforms must also improve the integration between printed microbial communities and host tissues, such as oral or gut microbiome components within dynamic and interconnected physical and chemical conditions. A critical design parameter will be replicating the microscale architecture of native tissues. For instance, successful integration in the oral cavity requires recapitulating the precise pore sizes (often in the low micrometer range[153]), as well as chemical gradients of pH, oxygen, and nutrients, and topographical features that dictate microbial colonization and host–microbe interactions. By engineering hydrogels with enhanced detail and precision, we can create more predictive models for oral biofilm formation and develop effective antimicrobial or probiotic delivery systems that function within the complex *in vivo* landscape.

Emerging technologies, such as stimulus-responsive materials that adapt to oral pH changes or microbial secretion, exemplify the potential for intelligent hydrogel systems to operate in dynamic environments. By focusing on these future directions, microbial hydrogel culture can transition from a niche tool to a robust platform for programming microbial communities with precision, enabling breakthroughs in medicine, biotechnology, and beyond.

6. Conclusion

The encapsulation and culture of microbes within hydrogels have emerged as a powerful platform in microbial research, offering precise control over 3D spatial organization, nutrient transport, and coculture conditions. This review highlights the advantages of 3D microbial culture that stem from key hydrogel properties, including material composition, crosslinking methods, porosity, and 3D architecture. These characteristics collectively enhance microbial metabolism, provide protective immobilization, and enable the creation of tailored microenvironments. By tuning these parameters, hydrogel–microbial systems have found use in a diverse range of applications, from advanced studies of microbial behavior and programmable responsive materials to optimized bioproduction and bioremediation strategies. Looking ahead, continued improvements in hydrogel fabrication, such as improved 3D bioprinting strategies and dynamic crosslinking, will further expand these capabilities, enabling more accurate replication of complex microbial niches, such as oral microbiomes, and accelerating translational research in medicine, biotechnology, and environmental science.

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Conflict of interest

The authors declare they have no competing interests.

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Consent for publication

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Availability of data

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