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Research Article

Designing the Perfect Microcapsule: Synthetic Hydrogel Selection for Single-Cell Therapeutic Delivery and Single-Cell Sequencing

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Abstract

The encapsulation of individual mammalian and bacterial cells within protective hydrogel microenvironments has become a transformative approach in regenerative medicine, single-cell transcriptomics, and localized therapy. Although the core principles of hydrogel design particularly the need for extracellular matrix (ECM) mimicry and cytocompatibility are well understood, practical implementation remains challenging due to the vast array of synthetic polymers available. Researchers face critical trade-offs: rapid gelation versus mechanical strength, covalent stability versus cell-mediated degradation, and generic encapsulation versus bioactive functionalization. This review systematically compares three major synthetic hydrogel platforms engineered for single-cell encapsulation, examining their crosslinking mechanisms, mechanical properties, biofunctionalization strategies, and optimal applications. We first evaluate high-performance click-chemistry hydrogels, such as thiol-maleimide and thiol-acrylate PEG systems. These achieve excellent cell viability under mild conditions, making them ideal for preserving transcriptomic integrity in single-cell ribonucleic acid (RNA) sequencing and handling sensitive cell lines like Human Embryonic Kidney (HEK293) and primary macrophages. However, their reliance on precise stoichiometry and limited long-term mechanical stability restrict them primarily to short-term assays. Next, we discuss polyisocyanide (PIC) hydrogels, which undergo physical thermoresponsive gelation and form fibrous architectures that closely mimic collagen. These systems excel in supporting adherent cells such as mesenchymal stem cells, Schwann cells, and hepatocytes. Biofunctionalization via strain-promoted azide-alkyne cycloaddition (SPAAC) with arginine–glycine–aspartic acid (RGD peptides) or growth factor immobilization enables sustained signalling for up to four weeks. Emerging PIC composites with nanoparticles offer near-infrared responsiveness for reversible mechanical tuning, enabling studies of mechanosensitive ion channels (Piezo1 and TRPV4), albeit with increased fabrication complexity. Finally, we critically assess photopolymerized poly (ethylene glycol) (PEG) systems. Conventional PEG diacrylate (PEGDA) suffers from ultraviolet (UV)-induced cytotoxicity and oxygen inhibition, whereas thiol-ene PEG norbornene (PEGNB) offers superior oxygen tolerance and cytocompatibility for extended processing. Disulfide-modified degradable PEGDA (dPEGDA) introduces cell-triggered degradation via glutathione-dependent cleavage, eliminating the need for external stimuli in transplantation. A central feature of this review is a practical decision matrix mapping each hydrogel to its optimal use: thiol-maleimide for transcriptomics, PIC for long-term extracellular matrix (ECM)-mimetic therapy, PEGNB for microfluidic encapsulation, and dPEGDA for scaffold-free tissue integration. We conclude that no universal hydrogel exists; instead, strategic balancing of cytocompatibility, gelation kinetics, mechanical tunability, and degradation profiles enables the design of bespoke microcapsules. This roadmap accelerates the development of next-generation single-cell technologies for both fundamental research and clinical translation.

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Keywords

Single-cell Encapsulation, Synthetic Hydrogel Microcapsules, Polyisocyanide Hydrogels, Poly Ethylene Glycol Hydrogels, Microfluidics, Single-cell Transcriptomics, Foreign Body Response

1. Introduction

The encapsulation of individual cells within protective hydrogel microcapsules represents a transformative approach in regenerative medicine, single-cell biology, and therapeutic delivery. As the preceding discussion on hydrogel fundamentals establishes, these water-swollen polymer networks provide an ECM-mimetic environment that maintains cell viability while enabling precise mechanical and biochemical control. However, the transition from conceptual understanding to practical implementation requires careful selection among the diverse synthetic hydrogel systems available. This article systematically examines the major synthetic hydrogel platforms, their crosslinking mechanisms, and their specific advantages and limitations for single-cell encapsulation applications [1, 2].

2. Fabrication and Microfluidic Integration: From Chemistry to Engineered Microcapsules

The translation of synthetic hydrogel chemistry into functional single-cell microcapsules requires a fabrication strategy capable of generating monodisperse, cell-laden microgels with high throughput and minimal cellular stress. Microfluidics has emerged as the dominant platform for this purpose, offering unparalleled control over droplet size, uniformity, and encapsulation efficiency [18]. Unlike bulk emulsification methods which produce polydisperse particles with variable cell loading microfluidic devices generate water-in-oil emulsions with coefficients of variation below 5%, enabling reproducible single-cell encapsulation at rates exceeding 1 kHz.

3. Hydrogel-Specific Fabrication Considerations

The physicochemical properties of each hydrogel system impose distinct constraints on microfluidic processing:

3.1. For PEGNB Systems

The step-growth thiol-ene photopolymerization is not inhibited by oxygen, a critical advantage for microfluidic encapsulation where rapid oxygen diffusion on the microscale dramatically inhibits chain-growth PEGDA polymerization [19]. However, PEGNB exhibits slower crosslinking kinetics, necessitating customized device designs with extended polymerization chambers or the use of long dithiol linkers to achieve sufficient retention time for complete gelation. Microfluidic platforms have successfully generated PEGNB microgels (100-200 μm) with cell viability exceeding 90% over 14 days of culture [20].

3.2. For PIC Hydrogels

The thermoresponsive physical gelation mechanism enables robust droplet formation at low temperatures (where the polymer remains soluble), followed by catalyzer-free gelation upon warming to 37 ℃ [7]. This temperature-dependent sol-gel transition eliminates the need for UV irradiation or chemical crosslinkers within the microfluidic device, simplifying chip design and enhancing cytocompatibility. The reversible nature of PIC gelation further enables easy cell recovery after culture for downstream single-cell analysis, a feature particularly valuable for transcriptomic applications [7].

3.3. For PEGDA Systems

UV-mediated radical photopolymerization requires careful optimization of photoinitiator concentration (typically < 0.1% w/v) and UV exposure time (< 30 seconds) to balance gelation completeness with cell viability. The oxygen inhibition of chain-growth polymerization presents a particular challenge on the microscale, as rapid oxygen diffusion can compromise gelation uniformity [19].

4. Practical Implications

The selection of a fabrication strategy must therefore be coupled with hydrogel chemistry selection. Rapid-gelling click systems (thiol-maleimide, thiol-acrylate) are compatible with simple single-phase droplet generation, whereas photopolymerized systems (PEGNB, PEGDA) require integrated UV exposure modules or post-collection polymerization steps [19]. Thermoresponsive systems like PIC offer the operational simplicity of temperature-controlled gelation but demand precise thermal management throughout the microfluidic workflow. Understanding these interdependencies between material chemistry and device engineering is essential for researchers

seeking to translate laboratory-scale encapsulation protocols into robust, high-throughput manufacturing platforms for therapeutic and diagnostic applications [18].

5. Thiol-Maleimide and Thiol-Acrylate Click Chemistry Hydrogels

PEG-Dithiol/Maleimide-Dextran (PEGDT/MALDEX) Systems. Among the most refined synthetic approaches is the thiol-maleimide Michael-type addition between PEG-dithiol and maleimide-dextran. This system achieves rapid gelation under mild, neutral pH conditions, making it exceptionally compatible with sensitive mammalian cell lines including HEK293, CHO (Chinese hamster ovary) cells, mouse ESCs (embryonic stem cells), and even bacterial systems such as *Bacillus subtilis*. The click chemistry nature of this crosslinking provides high viability outcomes, though it demands precise stoichiometric control—excess maleimide groups may remain reactive, potentially compromising the encapsulated cells. For applications requiring clonal expansion or single-cell RNA sequencing (clone sequencing), this system's gentle conditions and rapid gelation are particularly advantageous [3].

The thiol-acrylate Michael-type addition offers the highest reported cell viability among synthetic systems, particularly for *B. subtilis* encapsulation. However, this approach exhibits lower mechanical strength and preferentially supports single-cell encapsulation, which may limit applications requiring robust mechanical integrity. The related thiol-vinyl-sulfone PEG system provides a more stable hydrogel network, though at the cost of reduced viability and a greater tendency toward aggregate formation compared to its thiol-acrylate counterpart [4, 5].

6. Polyisocyanide (PIC) Hydrogels: Physical Gelation for Adherent Cells

6.1. Thermoresponsive PIC Systems

PIC hydrogels represent a fundamentally different paradigm, relying on physical gelation through hydrophobic interactions of oligoglycol side chains rather than covalent crosslinking. This thermoreversible system undergoes instant gelation at 37 ℃, enabling facile handling and cell encapsulation. The fibrous mechanics of PIC gels closely mimic native extracellular matrix architecture, making them particularly suitable for adherent cell types including human primary macrophages (M1/M2 phenotypes), fibroblasts, and mesenchymal stem cells. However, the purely physical network may limit long-term mechanical stability, and strict temperature control is essential for reproducible gelation [6-12].

6.2. Biofunctionalized PIC Derivatives

The incorporation of RGD peptides into PIC hydrogels via SPAAC creates cell-adhesive sites that support endothelial network formation, as demonstrated with human umbilical vein endothelial cells (HUVECs), hepatocytes, Schwann cells, and C2C12 myoblasts. While this modification enables controlled integrin binding and retains thermoreversibility, higher ligand densities may alter the underlying network structure and reduce bundle diameter.

Growth factor-functionalized PIC systems (PIC-bFGF) (basic fibroblast growth factor) achieve prolonged bioactivity up to four weeks, mimicking both ECM architecture and mechanics for tissue engineering applications. The covalent immobilization of growth factors provides sustained signaling without the burst release typical of physical encapsulation, though optimization is required for different growth factor types [6-12].

6.3. Hybrid and Responsive PIC Systems

PIC-hyaluronic acid blends offer tunable stiffness and swelling properties suitable for bioprinting and injectable depot formulations, with particular utility in antifibrotic therapies and drug screening using primary patient cells. More sophisticated dynamic composites incorporating iron nanoparticles or oligoethylene glycol nanoparticles enable non-covalent metal coordination or hydrophobic interactions, conferring self-healing properties and near-infrared-controlled mechanical tuning. These responsive systems show promise for mechanosensitive ion channel modulation in HEK-293T cells (Piezo1) and HUVECs (TRPV4), though complex fabrication requirements and the need for specific nanoparticle functionalization present practical challenges [6-12].

7. PEG-Based Photopolymerized Hydrogels

7.1. PEG Diacrylate (PEGDA) Systems

PEGDA represents the most widely utilized synthetic hydrogel for microencapsulation, crosslinked via ultraviolet-mediated radical polymerization. This system offers tunable stiffness and mesh size, making it suitable for encapsulating mesenchymal stem cells (MSCs), pancreatic islets, and various stem cell populations. PEGDA's biocompatibility and water solubility make it inherently suitable for cell encapsulation, though optimization of photoinitiator concentration (typically below 0.1% weight by volume) is critical, as higher concentrations negatively impact cell viability. Additionally, UV exposure times beyond 30 seconds can result in significant cell death, limiting the applicability for sensitive cell types or large-scale production [13].

7.2. PEG Norbornene (PEGNB) Systems

The thiol-ene step-growth photopolymerization of PEGNB

offers significant advantages over PEGDA, particularly in mitigating the deleterious effects of extended UV radiation. This system's polymerization proceeds unhindered by oxygen, enabling higher cytocompatibility and making it suitable for neural stem cells, MSCs, and other mammalian lines requiring longer crosslinking times. The tolerance to oxygen represents a crucial advantage for microfluidic encapsulation where oxygen inhibition can compromise gelation uniformity. However, slower polymerization kinetics require longer exposure times, and photoinitiator concentration optimization remains essential [13].

7.3. Degradable PEGDA Derivatives

Disulfide-modified PEGDA (dPEGDA) introduces cell-mediated degradation capability through glutathione-dependent disulfide bond cleavage, enabling autonomous degradation in response to cell-secreted factors without external intervention. This system is particularly suited for cell transplantation and tissue engineering applications where cell-mediated degradation is desired. However, the degradation mechanism of standard PEGDA remains incompletely understood, presenting a limitation for applications requiring predictable degradation kinetics [13].

8. Immunogenicity and *in vivo* Biocompatibility: Translational Considerations

While the preceding sections have focused primarily on *in vitro* cytocompatibility and encapsulation efficiency, the clinical translation of hydrogel-based single-cell encapsulation technologies ultimately depends on the host immune response following implantation or injection. Understanding the foreign body response (FBR) is therefore critical, as even highly cell-compatible materials *in vitro* can elicit inflammatory reactions, fibrosis, and capsule formation *in vivo* that compromise both encapsulated cell survival and therapeutic efficacy [14].

9. PEG-Based Systems: The Stealth Paradox

Poly (ethylene glycol) (PEG) has long been regarded as the "gold standard" for biologically inert, non-fouling biomaterials due to its ability to resist protein adsorption and reduce immune recognition. This stealth property has made PEG the most widely utilized synthetic polymer for cell encapsulation and drug delivery applications [15]. However, growing evidence challenges the assumption of complete immunological inertness. Anti-PEG antibodies have been detected in a substantial proportion of the human population: one study found pre-existing anti-PEG antibodies in 44.3% of healthy donors, with both immunoglobulin IgM (27.1%) and IgG (25.7%) isotypes present. These antibodies can lead to accelerated blood clearance of PEGylated therapeutics and, in severe cases, hypersensitivity reaction. Among the PEG derivatives discussed in this review, PEG diacrylate (PEGDA) warrants special consideration. While PEGDA offers tunable mechanical properties and versatile photopolymerization, comparative studies have reported that PEGDA exhibits cytotoxic effects, low cell adhesion, and the strongest foreign body reaction, including fibrosis and multinucleated giant cell formation upon implantation. This suggests that PEGDA appears to be unsuitable for long-term use due to adverse tissue and cellular reactions [16]. The molecular mechanisms driving this FBR have been linked to MyD88-mediated inflammation (myeloid differentiation primary response), which plays a critical role not only in the inflammatory response but also in the formation of the fibrous capsule surrounding implanted PEG hydrogels [14]. PEG norbornene (PEGNB) and thiol-ene systems, by virtue of their different polymerization mechanisms and potentially lower residual reactive species, may offer improved *in vivo* biocompatibility profiles. Notably, PEG norbornene hydrogels have demonstrated excellent *in vitro* cytocompatibility and *in vivo* biocompatibility though systematic comparative studies with PEGDA remain limited [17].

10. Selection Criteria for Single-Cell Applications

The optimal synthetic hydrogel selection depends critically on the specific application requirements:

10.1. For Single-cell Sequencing Applications

Thiol-maleimide systems provide rapid, mild gelation with high viability, essential for preserving transcriptomic integrity during encapsulation. The clone sequencing compatibility of PEGDT/MALDEX systems enables enhanced transcript capture compared to bulk techniques.

10.2. For Therapeutic Delivery Requiring Long-term Stability

PIC systems offer fibrous ECM-mimetic mechanics with sustained growth factor presentation, while PEGNB provides oxygen-tolerant photopolymerization for extended processing times.

10.3. For Mechanosensitive Studies

Dynamic PIC composites with nanoparticle-mediated property modulation enable reversible mechanical tuning, facilitating investigation of cell-generated forces and ion channel responses.

10.4. For Transplantation Applications

PEGDA systems provide cell-triggered degradation, eliminating the need for exogenous degradation triggers while enabling host cell integration.

11. Conclusion

The selection of an optimal synthetic hydrogel system for single-cell encapsulation requires balancing multiple competing parameters: cytocompatibility, mechanical properties, gelation kinetics, degradability, and biofunctionalization capability. While no single system satisfies all requirements, the diversity of available platforms enables tailored solutions for specific applications ranging from single-cell sequencing to therapeutic delivery. Understanding the distinct advantages and limitations of each system—from the rapid click chemistry of thiol-maleimide systems to the biomimetic mechanics of PIC hydrogels and the versatile photopolymerization of PEG derivatives—empowers researchers to design microcapsules with precisely controlled properties for their target application.

Abbreviations

ECM	Extracellular Matrix
PEG	Polyethylene Glycol
RNA	Ribonucleic Acid
HEK	Human Embryonic Kidney
PIC	Polyisocyanide
SPAAC	Strain-promoted Azide-alkyne Cycloaddition
RGD	Arginine–Glycine–Aspartic Acid
PEGDA	Polyethylene Glycol Diacrylate
PEGNB	Polyethylene Glycol Norbornene
dPEGDA	Disulfide-modified Degradable Polyethylene Glycol Diacrylate
UV	Ultra-violet
PEGDT	Polyethylene Glycol Dithiol
MALDEX	Maleimide-functionalized Dextran
CHO	Chinese Hamster Ovary Cells
ESC	Embryonic Stem Cells
HUVEC	Human Umbilical Vein Endothelial Cells
bFGF	Basic Fibroblast Growth Factor
MSC	Mesenchymal Stem Cells
FBR	Foreign Body Response
Ig	Immunoglobulin
MyD88	Myeloid Differentiation Primary Response

Author Contributions

Kriti Gorisaria: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

Conflicts of Interest

The author declares no conflicts of interest.

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