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# Synthetic control of living cells by intracellular polymerization

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## Abstract

An emerging cellular engineering method creates synthetic polymer matrices inside cells. By contrast with classical genetic, enzymatic, or radioactive techniques, this materials-based approach introduces non-natural polymers inside cells, thus modifying cellular states and functionalities. Here, we cover various materials and chemistries that have been exploited to create intracellular polymer matrices. In addition, we discuss emergent cellular properties due to the intracellular polymerization, including nonreplicating but active metabolism, maintenance of membrane integrity, and resistance to environmental stressors. We also discuss past work and future opportunities for developing and applying synthetic cells that contain intracellular polymers. The materials-based approach will usher in new applications of synthetic cells for broad biotechnological applications.

## Biomedical applications of engineered cells

Engineered cells are broadly adopted in cell therapy, tissue engineering, synthetic biology, and other biomedical applications. To bestow cells with the desired functionality and maintenance control, eukaryotic and prokaryotic cells have commonly been modified using genetic, chemical, and radioactive techniques [1–4]. By contrast with altering **endogenous** (see Glossary) biological components and signaling, integrating synthetic polymers into cellular cytosols creates new flexibility and opportunities in cellular engineering.

The various types of polymerization chemistries can be categorized as chain-growth or step-growth polymerizations. In chain-growth polymerization, free radicals or radical initiators transform a **monomer** into a reactive intermediate compound, which then **crosslinks** with other reactive intermediates for polymer chain propagation. Step-growth polymerization relies on reactions between distinctive functional groups on different monomers, thus involving multiple monomer species for cross-reactive bonding [5]. These polymerization

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Declaration of interests

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chemistries are frequently used for creating scaffolds in biomolecular separation and cellular encapsulation. While there have been extensive studies on enhancing the **biocompatibility** of these polymerization chemistries for biomedical applications [6,7], these chemistries have only recently been introduced into the cytosolic domain either through direct chemical permeation or by membrane disruption (Figure 1, Box 1, Table 1). For instance, membrane-diffusible hydrogel precursors have been proposed for enzyme-mediated formation of hydrogels inside *Escherichia coli* [8]. These **hydrogels** form a network that inhibits the growth of the *E. coli* cells. Direct cellular uptake of hydrogel monomers has also been demonstrated to induce intracellular photopolymerization in specific intracellular domains of living mammalian cells, enabling *in situ* formation of fluorescent polymers and nanoparticles [4]. In parallel, membrane disruption by a freeze–thaw process has also been applied to introduce light-mediated radical polymerization chemistry into the intracellular domain of eukaryotic and prokaryotic cells [9,10], enabling biomembrane and functional preservation while modulating cellular metabolic activities. In addition, carrier-mediated chemical delivery introduces complex polymerizing chemicals into living cells both *in vitro* and *in vivo* [11,12], leading to new strategies for reversible cellular modulation and anticancer treatments.

Efforts to introduce polymer chemistries into living organisms have demonstrated induction of **intracellular polymerization** via photochemistry [1,4,9,13,14], click chemistry [6,11], oxidative reaction [12], and enzyme-mediated monomer activation [8,15]. These techniques open broad possibilities for cellular engineering and biomaterials development, and at the same time they draw curiosity on the functional and biological interplays between artificial polymers and living cellular systems from the scientific community. While early studies on intracellular polymerization have focused primarily on synthetic designs and proof-of-principle examinations, scientists and engineers have taken pioneering and divergent explorations towards modulating, enhancing, and maintaining cellular properties across different cell types. These divergent studies highlight the vast biomedical potential of this emerging technique and can be categorized into two distinct directions: (i) modulating cellular states of living systems, and (ii) creating inanimate cell-mimicking biomaterials. Given the versatile chemical designs and applications associated with intracellular polymerization, this review highlights recent studies and advances in the field with separate discussions for eukaryotic and prokaryotic cell modifications. To distinguish from the broader literature that discusses intracellular condensate formation based on noncovalent interactions [16–25], this review focuses on covalent-chemistry-based polymerization strategies, which we anticipate to offer added control and functionalities to cellular engineering given the specificity, flexibility, and stability of covalent chemistries. Emerging intracellular polymerization chemistries and novel biomedical applications are discussed.

## Intracellular application of biomaterials in eukaryotic cells

Among studies with eukaryotic cells, efforts to induce intracellular polymerization have given rise to a multitude of polymerization chemistries and cellular infusion approaches. Eukaryotic cellular polymerization has been applied in two distinctive directions: modulation of living cells, and cellular fixation. In the first, intracellular polymerization

retains or modifies cellular processes. In the second, intracellular polymerization fixates cellular interior to stabilize plasma membranes. The following section discusses advances in these areas (Figure 2).

## Modulation of living cells by intracellular polymerization

The feasibility of inducing polymerization inside living cells was questioned initially by Geng and coworkers [4], who examined whether the complex intracellular environment may be permissible for covalent polymerization chemistries. The authors demonstrated that a free-radical photopolymerization process can be carried out inside HeLa cells by incubating live cells with a variety of monomers in the presence of I2959 photoinitiator prior to ultraviolet (UV) bombardment. The study showed successful polymerization of the aforementioned monomers, and fluorescent polymers prepared by intracellular polymerization were verified through long-term fluorescence observation. The approach presents a potential strategy for live cell tracking, which has broad utility in regenerative medicine and adoptive cell therapy for monitoring cellular distribution and survival (Figure 2A). The authors noted that cellular viability can be influenced by polymerization parameters, including the monomer type, concentrations, incubation conditions, and photoactivation settings. HeLa cells polymerized under 50 mM N-(2-hydroxypropyl) methacrylamide (HPMA) exhibited reduced migratory capability and lower actin polymerization at the cellular boundaries. This phenomenon may be attributed to increased **molecular crowding** following intracellular polymerization, and its impact on alternative cell types remains to be explored. The study highlights that improved control over the **spatiotemporal** contexts of intracellular polymerization may present new possibilities for manipulating cellular behavior.

In an aspirational work aimed at mimicking the protective, reversible stasis state exhibited by certain animals under extreme conditions, intracellular polymerization by click chemistry was proposed for reversible inhibition of cellular activities [11]. Click chemistry has long been adopted as a versatile conjugation approach in complex media conditions [26,27], yet performing click chemistry inside mammalian cells often meets with limited success, with poor intracellular ligand delivery being identified as a limiting factor [6]. To facilitate click chemistry ligand delivery, Macdougall and colleagues adopted lipofectamine for sequential delivery of dibenzylcyclooctyne (DBCO)-functionalized, polyethylene glycol (PEG)-based macromers and azide group. Upon lipofectamine-mediated delivery, the two macromers underwent strain-promoted azide-aklyne cycloaddition for crosslinking, resulting in hydrogel-mediated molecular crowding that slows down cellular processes and imitates a stasis-like state. Polymerized cells were shown to enter a quiescent state following the onset of polymerization as cellular proliferation, protein translation, and cellular motility were markedly reduced. The authors further embedded a photolabile nitrobenzyl moiety to the PEG macromer, which facilitated polymer degradation upon exposure to UV light. As compared with cells polymerized with nondegradable macromers, those polymerized with degradable macromers had a reduction in cytosolic viscosity and regained migratory capability upon light exposure. The study demonstrates reversible modulation of live cell mechanics and provides a novel strategy for cell and tissue preservation (Figure 2B).

A cell-specific environmental cue has also been adopted as a trigger for targeted intracellular polymerization towards anticancer treatment *in vivo*. Leveraging the increased oxidative stress (reactive oxygen species, ROS) commonly associated with the tumor microenvironment and cancer cells [28], Dai *et al.* developed a nanoparticle reservoir of organotellurides (Te-O) to achieve cancer-cell-specific oxidative polymerization [12]. The nanoparticle reservoir enables intravenous delivery, and in the presence of cancer-specific ROS, tellurides are oxidized for oxidative polymerization. The resulting Te-O polymer initiates a positive feedback loop by inhibiting selenoprotein-associated antioxidant processes, thereby leading to higher oxidizing polymerization and ROS-related damage and cell death. Notably, the polymerization process was not activated by the lower basal ROS level of normal cells. The study demonstrates selective intracellular polymerization based on environmental cues in the cytosol, highlighting chemistry designs for stimuli-responsive cellular modulation and therapeutics development (Figure 2C).

The examples showcase varying degrees of cellular modulation by intracellular polymerization. The behavior of polymerized cells ranges from being robustly viable, succumbing to a quiescent state, or undergoing apoptosis. The differing effects of intracellular polymers point to important nuances behind the specific context of each study. Biocompatibility of polymerization chemistry, degree of polymer crosslinking, and specific cell type can all contribute to the fate of polymerized cells, prompting continuing research towards tunable polymerization chemistries and in-depth mechanistic examination of polymer networks in the cytosol.

## Cellular fixation by intracellular polymerization

In addition to altering the cellular processes of living systems, intracellular polymerization has been adopted to create inanimate, cell-like biomaterials for various biomedical applications. In a pioneering work by Lin *et al.* [9], intracellular photopolymerization was applied to induce rapid cytosolic immobilization and creation of cell-like biomaterial constructs. By contrast with typical cellular fixation techniques based on chemical crosslinking, photoactivated radical polymerization of PEG diacrylate (PEG-DA) induces hydrogel-mediated solidification of cellular cytosol without disrupting the cell membrane components (Figure 2D). The speedy nature of the polymerization process enabled the preservation of cellular morphology, surface membrane ruffles, and intracellular actin structures, while the rigidity of the system was controlled by the input PEG concentration. Membrane ligands on the polymer-fixated cells underwent clustering during biological engagement events, and biomimetic functionalities were demonstrated via virus-induced agglutination and cellular engagement with lymphocytes possessing cognate receptors. The polymerization approach was adopted to facilitate membrane proteome study as the solidification and segregation of cellular cytosol from the plasma membrane provided an efficient way to isolate membrane proteins for identification [29]. Altogether, cellular fixation by intracellular polymerization was demonstrated as a unique approach to maintain the dynamic functionality of the plasma membrane, providing a novel cell-mimicking system for various biomedical applications.

The therapeutic applicability of polymer-fixated cells was further demonstrated by Lin *et al.* in a study that polymerized primary dendritic cells (DCs) for antigen-specific T-cell expansion [30]. The study refined the intracellular polymerization process using a membrane-permeable PEG-DA (550 Da), and the obviation of free-thaw treatment significantly improved the applicability of the technique. Polymerization of human primary DCs using the direct monomer permeation process yielded a recovery rate of >80%, and the polymer-fixated DCs addressed the cumbersome maintenance requirement of live DCs in adoptive T-cell therapy. Unlike live DCs that require stringent **cryopreservation**, gelated DCs could be preserved by **lyophilization** and retain their T-cell-activating capability upon reconstitution (Figure 2E). The robust stability of the polymerized cells further enabled their subsequent modification with cytokine-bearing carriers for immunoengineering designs, and an interleukin 2 (IL2)-bearing polymerized cellular spheroid was constructed for T-cell enhancement. In addition, intravenous injection of the polymerized DCs was shown to be well tolerated in mice, reinforcing the safety and *in vivo* applicability of the biomaterial.

Exploration of the intracellular polymerization approach has also led to the fixation of confluent **cellular monolayers** for the preparation of **biomimetic** devices amenable to tissue engineering. In a study examining feeder layer engineering, Chien *et al.* demonstrated that intracellularly polymerized cellular monolayers retain their biomimetic feature for 180 days without cellular detachment. The polymerized monolayers also preserve the protein presentation, membrane fluidity, and cellular topology of live-cell monolayers [31]. The biomimetic feeder layer was shown to sustain the expansion of murine and human induced pluripotent stem cells. Notably, due to their lack of nutrient consumption or metabolite production, the polymerized monolayers maintained higher media quality and enhanced stem cell expansion as compared with live feeder cells. The modularity of the polymerized feeder layer approach was further pitched for tailored substrate design towards tissue engineering, and the study showed that HeLa cells (a fast-growing, immortalized human cell line) could be completely fixated by the intracellular polymerization process for stem-cell maintenance, offering a xeno-free, genetically modifiable option for feeder engineering (Figure 2F).

The biomimetic nature of polymer-fixated cells has further been adopted to create devices that can engage and detect pathogenic organisms and molecules. In recognition of the broad number of pathogen-binding molecules on immune cell surfaces, Gui *et al.* developed a smart pathogen detector using polymerized macrophages for early pathogen detection [32] (Figure 2G). The system takes advantage of multiple membrane-bound pathogen receptors, particularly mannose receptors, to facilitate direct capture of Gram-negative and Gram-positive bacteria in serum and other body fluids. To enable pathogen detection, the polymerized macrophages were further conjugated with DNAzyme, which generates fluorescent signals in the presence of bacterial DNA. Of note, presumably due to bacteria enrichment as a result of the polymerized macrophage capture, the pathogen detector had a limit of detection as low as 500 CFU/ml, which is significantly more sensitive than common DNAzyme-based methods [32]. In addition, the authors demonstrated a clever adoption of the polymerized macrophage for pore-forming toxin detection by incorporating a membrane-impermeable propidium iodide, which stains the cellular interior in the

presence of membrane-disrupting agents. The system effectively detected the presence of staphylococcal α-hemolysin in the sputum samples of pneumonia patients, highlighting robustness and functional ability for different clinical needs.

The modularity of polymer-fixated cells has also spawned new formulation designs for ingestible therapeutics. Towards modulating murine microbiota for the treatment of inflammatory bowel disease (IBD), Wang *et al.* attached probiotics to the surface of intracellularly hydrogelated peritoneal macrophages for gastrointestinal delivery. The formulation leverages two key features of the hydrogelated macrophages to enhance colitis treatment: (i) adhesion molecules on macrophage surfaces for retention at the gastrointestinal epithelium, and (ii) cytokine sequestration by membrane-bound ligands for anti-inflammatory functions. The biomimetic delivery and cytokine neutralization concepts stem from prior literature of cell-membrane-coated carriers that derive cell-like functions from plasma membrane functionalization [33–35], and the study suggests that intracellular polymerization offers functional and logistical advantages as the technique bypasses membrane derivation and carrier coating processes. The ingestible, hydrogelated macrophages enhanced retention of probiotics in the gut, reduced IBD progression, and altered the intestinal microbiota. Altogether, the study provides a new biomedical application of polymer-fixated cells.

## Intracellular application of biomaterials in prokaryotic cells

Prokaryotes, particularly bacteria, have been extensively researched for their potential applications in medical therapy [36,37], and they can be engineered to perform complex computations, sense and respond to environmental cues, and contain custom-designed intracellular architecture. Despite promising preclinical results in testing therapeutic microbes, significant hurdles still need to be addressed for broad clinical translation. These concerns include biological safety, **biocontainment** issues, and improved efficacy [36,38–40]. To address these issues, prokaryotes can be genetically modified and combined with biomaterials to form biocomposites with unique features for biomedical applications [41].

When engineering hybrid bacterial–material systems, many options exist for biological and material-based components, and the selection of these components collectively dictates the resulting functionality of the engineered biocomposites [18,42]. These hybrid bacterial-material systems offer several advantages, including predictable functions, increased tolerance to certain environmental stressors, and ease of engineering [14]. In this context, extracellular and intracellular polymerization approaches have been adopted to generate bacterial–material systems. In extracellular polymerization approaches, numerous polymers have been utilized to create coating layers on different types of bacteria. For example, bacteria can serve as a template to synthesize polymers exhibiting aggregation-induced emission characteristics for self-selective killing of the bacteria templates without inducing antimicrobial resistance [43]. Another extracellular–material approach uses a layer-by-layer technique to coat gold and silver nanoparticles on the surface of bacterial cells, enabling the acquisition of **surface-enhanced Raman scattering (SERS) spectra** [44]. We refer the readers to a recent review paper on the extracellular application of biomaterials in prokaryotic cells [42].

## Bacterial growth inhibition by intracellular polymerization

By comparison with eukaryotic cells, intracellular polymerization of prokaryotic cells has been less well explored (Figure 3), and few reports have examined the implication of prokaryotic cellular modification by introducing synthetic materials into the cellular cytosols. In the early 2000s, Zhang *et al.* introduced small **precursor** molecules inside a bacterium that used an enzyme to catalyze the precursor conversion into a hydrogelator that **self-assembled** into nanofibers [8]. The authors demonstrated that subsequent intracellular hydrogelation of these nanofibers effectively inhibited bacterial growth. The study selected $C_{10}H_7CH_2C(O)$-$_L$-Phe-$_L$-Phe as the precursor molecule where the Phe-Phe motif has been previously shown to self-assemble in water [45]. Upon phosphorylation at its C terminus with tyrosine phosphate ($C_{10}H_7CH_2C(O)$-$_L$-Phe-$_L$-Phe-Tyr-$(PO(OH)_2)$), the peptide becomes labile to tyrosine phosphatase enzyme cleavage. The end product – $C_{10}H_7CH_2C(O)$-$_L$-Phe-$_L$-Phe-Tyr – readily self-assembles into nanofibers (Figure 3A), resulting in intrabacterial hydrogelation. This study demonstrated enzyme-mediated activation of intracellular hydrogelation, which offers flexible hydrogel formation via enzyme expression. The tyrosine phosphatase-based hydrogelation approach has thus far been demonstrated only in *E. coli* strains [8], and it is unknown whether the same enzyme would be innately present in other strains. Precursor molecule redesign may thus be needed to accommodate specific enzyme profiles in other prokaryotes.

## Engineering nonreplicating but active bacteria by intracellular polymerization

Around the same time, Brockstedt *et al.* described a new approach for bacteria modulation that combines genetic engineering with incorporation of a photosensitive psoralen (S-59) molecule [1]. The authors first deleted genes for nucleotide excision repair (uvrAB), rendering bacteria more sensitive to UV light-induced psoralen crosslinking with pyrimidine bases of DNA and RNA [46]. The crosslinking process yielded **killed but metabolically active (KBMA)** bacteria and was pitched as a novel vaccine system for effector T-cell induction. The researchers tested *Listeria monocytogenes* using this concept, and showed that the genetic and chemical modulation approach effectively halted the proliferation of the bacteria. More importantly, metabolic activity was preserved in the KBMA bacteria, which remained capable of synthesizing cell wall and escaping from phagolysosomes of dendritic cells via pore-forming toxin secretion (Figure 3B). Administration of the KBMA bacteria as a vaccine candidate elicited strong T-cell induction and conferred robust protection against viral and bacterial challenges, although humoral and memory responses from the KBMA vector were not reported in the study. The psoralen-based intracellular polymerization has been applied for vaccine development against several human pathogenic bacteria, including *Edwardsiella tarda* [47], *Salmonella typhimurium* [48], and *Bacillus anthracis* [49]. For cancer treatment, KBMA bacillus Calmette–Guérin (BCG) has been proposed as a safer alternative to live BCG for stimulating the immune system against cancer cells [50].

Efforts to develop nonreplicative but metabolic active vectors for synthetic biology research culminated in a recent work on **Cyborg cells** [14], in which the authors crosslinked hydrogel monomers in bacterial cytosols in a fashion analogous to constructing prosthetic skeletons. Adopting PEG-DA-based photochemistry, the authors explored a broad range of crosslinking conditions with varying PEG-DA concentrations and UV bombardment

durations that resulted in different phenotypic alterations. Under optimal crosslinking conditions, the polymerized bacteria – *E. coli* BL21 (DE3) – denoted as Cyborg cells, became nonreplicative but preserved their metabolic activity. Transmission electron microscopy (TEM) examination unveiled synthetic polymeric networks inside Cyborg cells, which presumably constrained the bacteria from cell division but remained sufficiently porous for protein translation and metabolic functions. The Cyborg cells showed no measurable growth for over 6 days, and yet they exhibited quantifiable metabolic activity for up to 3 days and remained amenable to inducible gene expression. The polymerized bacteria also acquired non-native functionalities as they became resistant to multiple stressors, such as a hyper-oxidative environment containing a lethal dose of hydrogen peroxide, cell-wall targeting antibiotic D-cycloserine, and high pH media (>pH 7) (Figure 3C). Functional preservation of Cyborg cells was also demonstrated through Invasin-mediated invasion of cancer-derived cell lines SH-SY5Y (neuroblastoma) and HeLa (adenocarcinoma) cells. This work illustrated a comprehensive profiling of intracellular hydrogelation in prokaryotic systems. In addition, it characterizes responsive structures such as the cell membrane, and protein expressions of the modified cells, which is valuable for future translation work to other nonmodel systems and applications. Overall, this novel paradigm in developing semi-living bacteria opens exploration in intracellular polymeric networks for cell control of biomolecular functions and interactions with host environments and cells.

## Concluding remarks and future perspectives

Intracellular polymer hydrogelation has ushered in a fundamentally different method for cellular engineering, and the diverse literature on the topic highlights broad relevance in chemistry, biotechnology, and cell biology. Current approaches introduce polymer monomers into living cells, followed by polymerization driven by intracellular enzymes, chemicals, or light. Although the intracellular-material-based methods are in their infancy compared with traditional cellular engineering methods, they have demonstrated unique and superior features. For eukaryotes, the intracellular polymerization approach has resulted in mammalian cells that are ‘fixed’ but maintain their membrane activity. Furthermore, it has been used as a reversible method to control mammalian cell state. For prokaryotes, intracellular hydrogelation has resulted in bacteria that do not divide but remain metabolically active. The results represent a significant leap forward compared with prior approaches that rely on species-specific genetic-based approaches to drive prokaryotes into nonreplicating states. The intracellular hydrogelation approach has also demonstrated promising applications in vaccination, anticancer therapy, and biosensing.

As an early-stage technology, questions remain in basic understanding, technology refinement, and practical applications of intracellular polymerization. One fundamental question is how intracellular polymers interact with cellular components. It remains unclear which cellular components (lipids, proteins, RNA, metabolites) interact directly with intracellular hydrogels (see Outstanding questions). While recent work by Contreras-Llano *et al.* unveiled the proteomic changes of hydrogelated bacteria for the first time [10], the mechanistic link between hydrogel and subsequent proteome and genome changes has not been fully elucidated. The question could be addressed by quantifying changes in metabolic flux and gene expression due to intracellular hydrogelation. Coupling this with

prior proteomics and transcriptomics of cells could provide a macro-view of the effects of intracellular gelation. Single-molecule imaging could also provide molecular insights at the nanometer scale regarding molecular interactions between intracellular polymers and cellular components. High-throughput genetic studies could also be used to interrogate molecular pathways that are affected by the intracellular hydrogelation. In addition, most work studies only hydrogelated cells within a short time frame, likely because of technical limitations. As a result, it remains unclear whether the hydrogelated cells can evolve after an extended duration.

For potential applications, there remain challenges in controlling the underlying chemical processes to ensure the purity and **homogeneity** of hydrogelated cells. An enhanced understanding of intracellular polymer–biomolecule interactions will help to pinpoint critical chemistry changes required to achieve homogeneous intracellular polymerization. Furthermore, reporters of intracellular hydrogelation and subsequent cellular phenotypes could be further improved for fidelity and accuracy. New material chemistry could be exploited to enhance the infusion of hydrogelation components into cells and the subsequent hydrogelation. Ensuring the high quality of hydrogelated cells is critical for scaling up the technology for broad adoption and applications.

The quality requirement is especially needed when translating the technology for human applications, including vaccination, anticancer drugs, and microbiome modulation.

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## Glossary

**Biocompatibility**
not eliciting toxicity in biological systems.

**Biocontainment**
the concept of preventing the unintended spread of living entities.

**Biomimetic**
artificial methods, mechanisms, and materials that mimic natural systems.

**Cellular monolayer**
a single layer of cells growing next to each other on a surface.

**Crosslink**
a chemical bond that is formed between monomers in a complex polymeric structure.

**Cryopreservation**
the process of freezing cells, materials, tissues, or organs for extended preservation.

**Cyborg cell**
the integration of synthetic polymers within living cells.

**Endogenous**
processes or components that originate from or exist within the living system.

**Homogeneity**
the state of being equally similar in terms of size, composition, and structural features.

**Hydrogel**
three-dimensional networks of hydrophilic or hydrophobic polymer chains.

**Intracellular polymerization**
the process of polymerization that occurs inside cells.

**Killed but metabolic active (KBMA)**
KBMA bacteria are nonviable or dead bacteria with retained metabolic functions.

**Lyophilization**
the process of freeze-drying cells, materials, and tissues by freezing them and removing the ice under low pressure and temperature.

**Molecular crowding**
a high density of molecules occupying a significant cell volume.

**Monomer**
structurally simple molecules that can react with other molecules to form more complex and larger structures (polymers).

**Precursor**
a compound or a molecule that mediates the formation of another molecule, without it being integrated into the final product.

**Self-assemble**
spontaneous formation of an organized and ordered structure from single components, molecules, or polymers.

**Spatiotemporal**
space and time changes of cellular processes.

**Surface-enhanced Raman scattering (SERS) spectra**
a spectroscopic technique that enhances the Raman scattering signals of molecules absorbed by or in close proximity to certain surfaces.

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## Highlights

- Integrating synthetic polymers into cellular cytosol offers new opportunities in cellular engineering, enabling precise modification of cellular states and functionalities, and unlocking new avenues for developing biomimetic materials.
- Intracellular polymerization of eukaryotic cells enables new design principles for anticancer treatment, live cell tracking, immunoengineering, regenerative medicine, and pathogen detection.
- Polymerized prokaryotic cells can be bestowed with desirable properties – including nonreplicating but metabolically active, enhanced cell-membrane integrity, and increased environmental stress resistance – for synthetic biology studies.
- The ability to control cellular state and generate robust cell-like biomaterials by intracellular polymerization has broad biomedical applicability in fundamental and translational research.

**Box 1.**

## Polymerization techniques

Polymerization is a chemical process that links small molecular units, called monomers, to form long chains known as polymers. In biomedicine, polymerization creates biocompatible materials for drug delivery and tissue engineering. In hydrogel formation, polymerization is crucial in constructing three-dimensional networks with high water content, enabling their use in wound healing and controlled drug release. The main polymerization techniques are listed as follows.

### Photopolymerization

Photopolymerization chemistries involve light-directed radical polymerization through light-sensitive compounds called photoinitiators. Light absorption produces free-radical molecules that can then cleave C–C, C–Cl, and C–S bonds to produce chain reaction sites to nucleate from and connect to other radical/reactive monomers or chains. Common reaction monomers include acrylated polyethylene glycol (PEG), and acetophenone molecules. Due to their rapid reaction time and biocompatibility, these chemistries have been extensively explored in hydrogel formation [54].

### Click chemistry

Click chemistry has been explored as a biorthogonal polymerization scheme with nontoxic and nonreactive intermediates. As another route for intracellular polymerization, the chemistry derives from a copper-based C–C bond formation involving Diels–Alder, azide–alkyne cycloaddition, and thiol–ene reactions. Although traditionally including copper catalysis, recent advances towards non-toxic EDTA purification or copper-free hydrogels have adapted click chemistry to be suitable for biocompatible hydrogel formation with fast reaction rates and selectivity in aqueous phase [55].

### Oxidative polymerization

Through oxidative polymerization, passive bond formation can occur within the moderately reducing environment of the intracellular space. Due to displaced charges on oxidative species and hydroxyl groups, H-bond formation can gradually occur in solution, forming highly viscoelastic hydrogels. In addition to high biocompatibility, this polymerization route requires minimal external stimulation, and monomers can readily be introduced through the cell membranes [56].

### Enzymatic polymerization

Native enzymatic reactions such as actin polymerization and nanofiber formation are common in multiple cell types and are involved in various biochemical and biomechanical applications. Directed by enzymatic cleavage and activation of a precursor molecule into a hydrogelator, self-assembly can occur rapidly *in vivo* under physiological conditions. Moreover, polymerization depends on specific enzymes (such as phosphatase), which can be cell-specific and couple polymerization rates with cell states or protein concentration [57].

### Outstanding questions

- Polymerization is characterized by the degree of crosslinking. How can intracellular polymerization be further refined to confer precise control of polymer crosslinking inside cells?
- Intracellular molecular crowding and confinement are the bases of the unique functionalities of polymerized cells. How can molecular crowding be defined relative to the degree of polymerization to precisely engineer polymerized cells?
- What high-throughput strategies can be employed to optimize the intracellular polymerization of synthetic cells for specific application objectives?
- What are the prospects and challenges in translating the ‘Cyborg cell’ concept to other cell types?

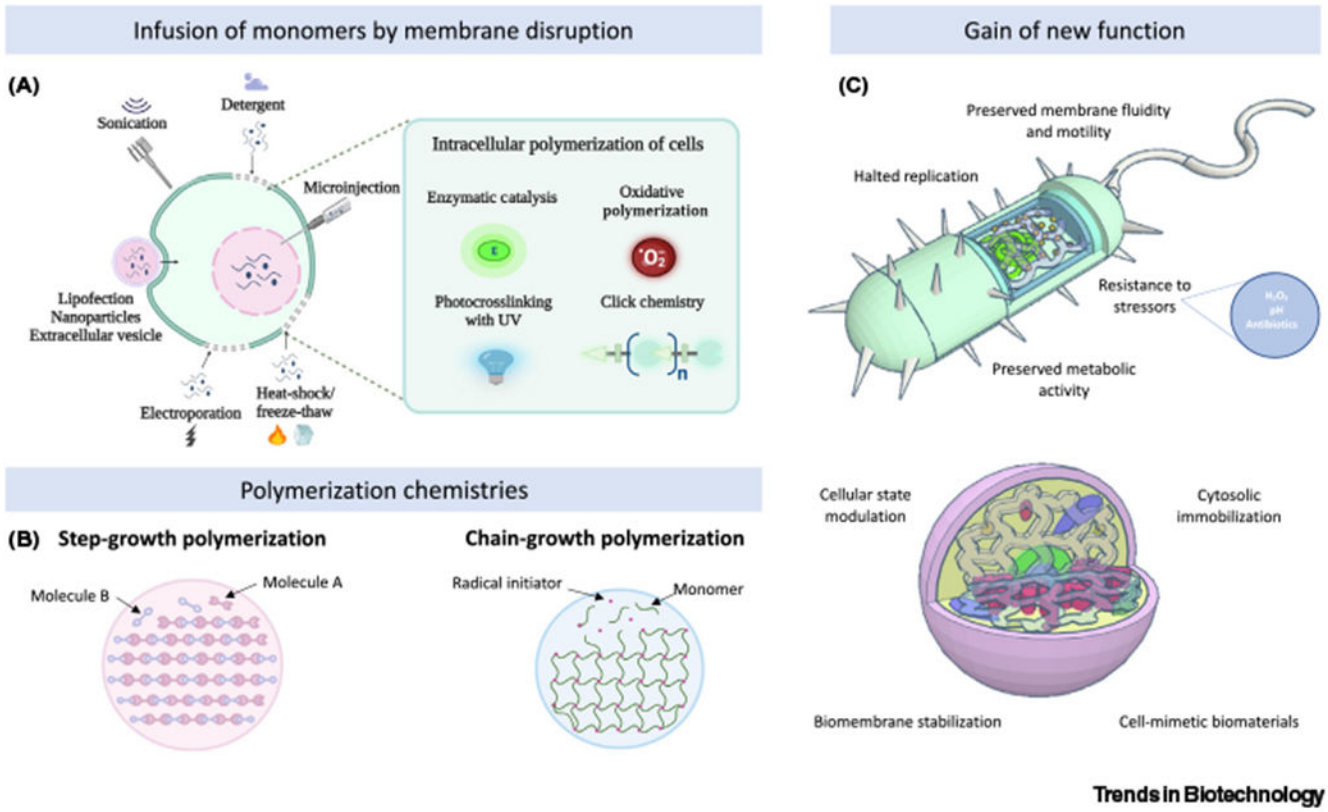


**Figure 1. Schematic illustration of the intracellular polymerization approaches.**

(A) Schematic of polymer infusion and intracellular polymerization techniques. Various chemicals can be introduced into cellular cytosols through direct membrane permeation, membrane disruption techniques, or delivery carriers. Induction of intracellular polymerization can be mediated by enzyme-mediated activation, oxidative reaction, photoactivated crosslinking, or click chemistry. (B) Schematic of polymerization chemistries – including step-growth and chain-growth polymerization – that can be carried out intracellularly. (C) Schematic of the intracellular polymerization in eukaryotic and prokaryotic cells, and the resulting gain of new functions. Polymerization in prokaryotic cells can suppress proliferation, preserve metabolic activity and functionality, and confer stressor resistance. Polymerization of eukaryotic cells can lead to cellular state modulation, biomembrane stabilization, and generation of inanimate biomimetic materials.

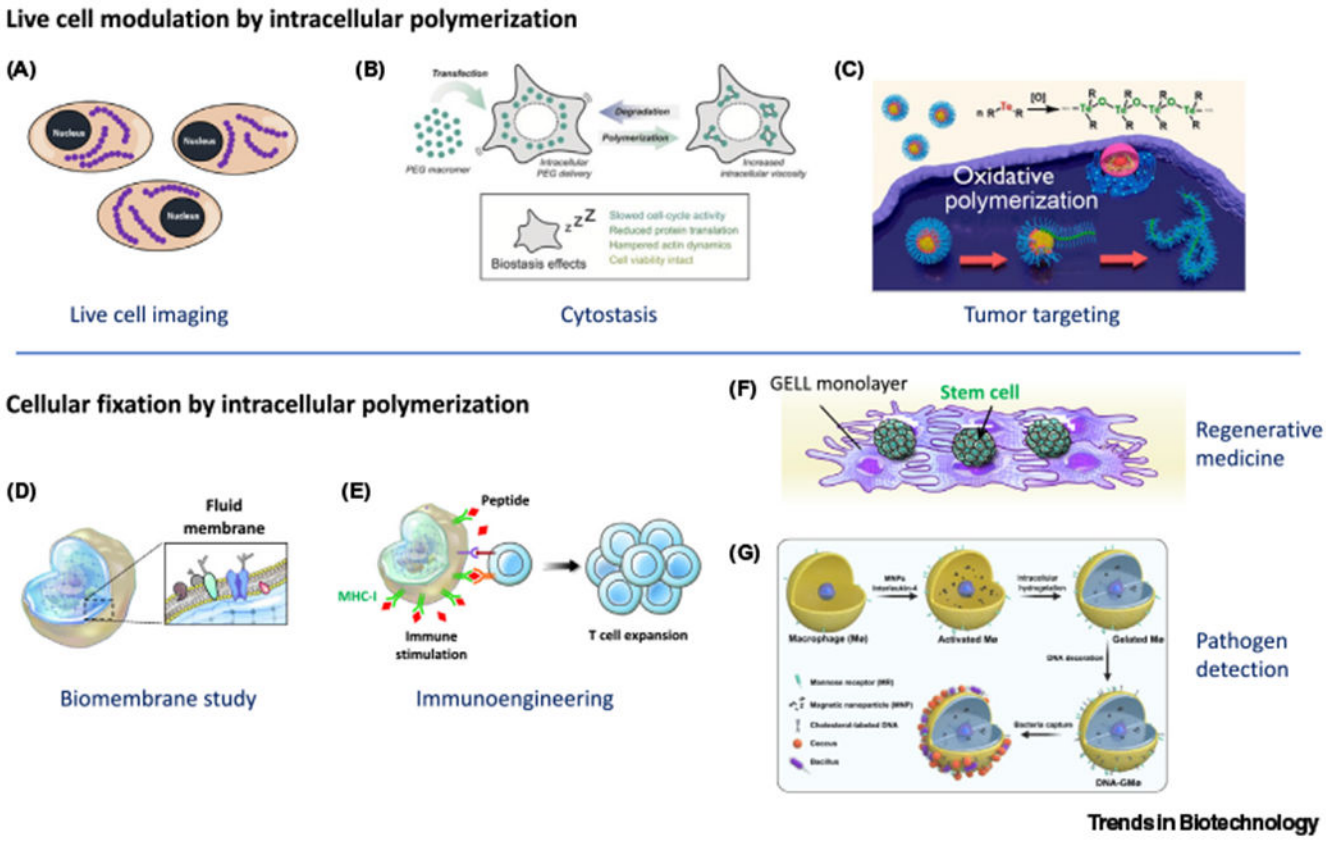


**Figure 2. Intracellular polymerization of eukaryotic cells.**

(A) Intracellular polymerization of fluorescent polymers has been achieved in living cells, offering long-term tracking potential for living-cell therapeutics in regenerative medicine and adoptive cell therapy [4]. (B) Reversible polymer crosslinking inside living cells is proposed as a potential strategy for induction of cytostasis. Cells present a stasis phenotype when polymerized and exit stasis otherwise. Image reproduced with permission from [11]. (C) Cancer-cell-specific oxidative polymerization of organotellurides (Te-O) delivered via a nanoparticle reservoir. This novel intracellular polymerization approach inhibits cancer cells. Image reproduced with permission from [12]. (D) Cellular fixation via rapid intracellular polymerization preserves biomembrane features, including membrane fluidity, protein mobility, surface ruffles, and lipid orders. Image reproduced with permission from [9]. (E) Intracellularly hydrogelated human primary dendritic cells (DCs) provides an off-the-shelf solution for antigen-specific T-cell expansion and anticancer adoptive cell therapy. Image reproduced with permission from [30]. (F) Intracellularly polymerized cellular monolayers (GELL) provide a biomimetic feeder layer for the expansion of murine and human induced pluripotent stem cells. Image reproduced with permission from [31]. (G) Intracellular polymerization of macrophages conjugated with DNAzyme. The DNAzyme allows subsequent capture of bacteria on the outer surface of the macrophages. The hybrid cellular construct enables sensitive bacterial pathogen detection. Image reproduced with permission from [32].

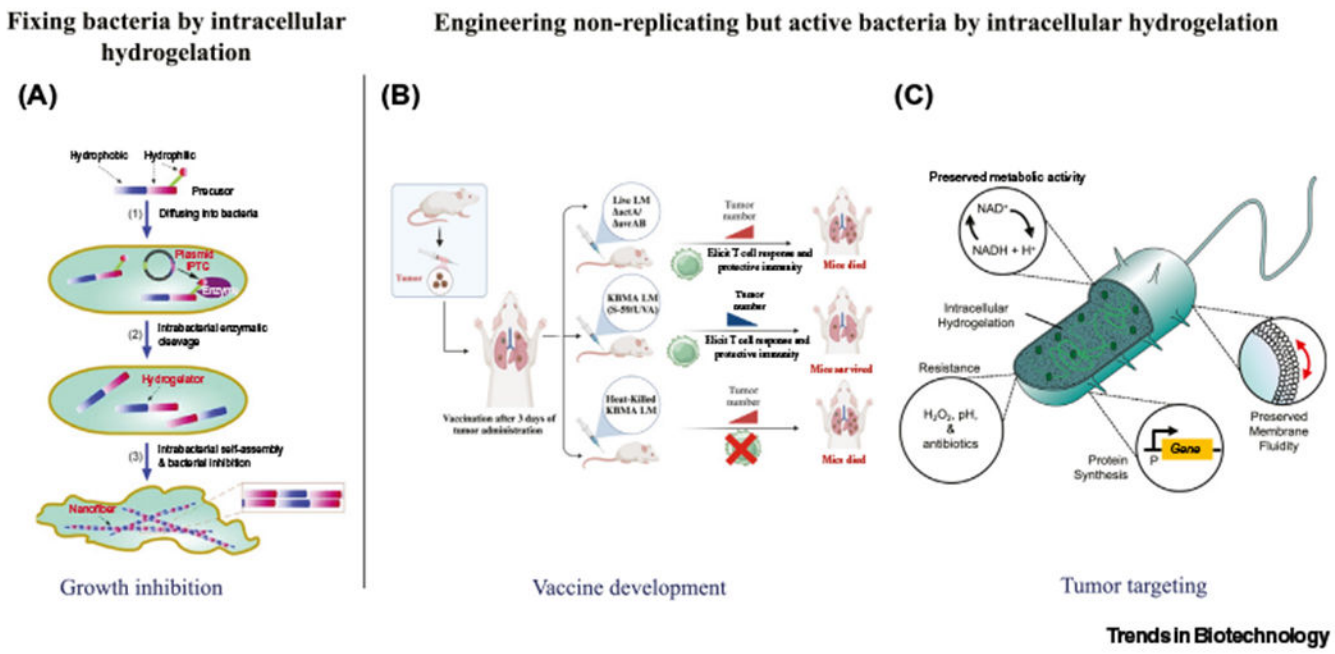


**Figure 3. Intracellular polymerization of prokaryotic cells.**

(A) Diffusion of small-molecule hydrogelator into *Escherichia coli* followed by intrabacterial enzymatic cleavage induces nanofiber self-assembly and bacterial growth inhibition. This early example of intra-bacteria polymerization establishes the feasibility of the approach. Image reproduced with permission from [8]. (B) Intracellular crosslinking of psoralen by ultraviolet light (S-59/UVA) in *Listeria monocytogenes*. Vaccination of mice with these modified bacteria provides therapeutic benefit in a mouse lung cancer model and induces effector T-cell responses. Live LM actA/uvrAB: nucleotide excision repair mutants of *L. monocytogenes* by removing the uvrAB genes with deleted actA. Abbreviation: KBMA, killed but metabolically active. Image adapted from [1]. (C) Cyborg cells prepared from intracellular hydrogelation preserve essential cellular functions (such as metabolic and protein-synthesis activities), maintain membrane fluidity, and gain new resistance to environmental stressors. The properties are exploited to create Cyborg cells that can invade cancer cells. Image reproduced with permission from [14].

**Table 1.**

Intracellular polymerization in eukaryotic and prokaryotic systems.

| **Eukaryotic systems** | | | | |
|---|---|---|---|---|
| **Material components** | **Strains tested** | **Phenotype and genotype** | **Applications** | **Refs** |
| PEG-DA | HeLa, avian erythrocytes, dendritic cell line | Cellular fixation with retention of membrane fluidity, membrane ruffles, cytoskeletal features, protein mobility, and surface functionality | Membrane protein analysis, biomembrane studies, examination of cell–cell and cell–pathogen interactions, and intravenous delivery | [9] |
| PEG-DA/PLGA-COOH | Murine and human primary dendritic cells | Cellular fixation preserving membrane-bound lymphocyte activation signals for T-cell expansion. | Long-term storage of DC-derived product and antigen-specific T-cell expansion for adoptive cell therapy | [30] |
| PEG-DA | Primary mouse embryonic fibroblasts and HeLa | Fixation of cellular monolayer preserving membrane fluidity, surface topology, and protein functionalities | Establishment of nonmetabolizing feeder substrate and xeno-free stem-cell expansion | [31] |
| PEG-DA | RAW264 murine macrophage cell line | Cellular fixation preserving surface mannose receptors and other pathogen-binding molecules | Functionalization with DNAzyme for sensitive pathogen detection with an LOD of 500 CFU/mL; pore-forming toxin analysis | [32] |
| PEG-DA | Peritoneal macrophage | Cellular fixation preserving membrane-bound ligands for toxin sequestration | Gastrointestinal delivery of probiotics for microbiota modulation and colitis treatment | [51] |
| HPMA/4-styrenesulfonate/4-vinylalanine | HeLa | Viable and proliferating cell population, decreased DNA synthesis, increased stiffness/elongation | Modulatory control of cell stiffness, actin bundling activity, and motility; tracking application for live cell therapeutics | [4] |
| Acryloyl-X, SE (6-((acryloyl) amino)hexanoic acid, succinimidyl ester-guanine | HeLa, HEK 293 | Viable cells with reduced cytosolic transcripts and bound RNA in polymerized scaffold | RNA imaging with temporospatial control | [52] |
| Elastin-like peptides | MCF-7, SH-SY5Y | Induced apoptosis in cancers | Topologically controlled molecular aggregation tracking, cancer therapeutics | [15] |
| Acrylate-functionalized monomers/tyrosine residues | Yeast cells | Maintenance of complete yeast cell metabolism for 134 h following polymerization | Functional modulation of yeast cells | [53] |
| Azide-Tat peptides | HUVECS, OVCAR5 | Viable cells with reduced proliferation | Cell-targeting copper-based click chemistry polymerization | [6] |
| DBCO/Azide-PEG DBCO/Azide-PEG | MCF10A, C2C12, HS68 | Reversible retardation of cell proliferation, DNA and protein synthesis 120 h following polymerization | Reversible stasis induction in living cells. | [11] |
| Te-O | LO2 and HepG2 cell lines | Increased intracellular reactive oxygen species (ROS) and selective polymerization in ROS-abundant cells | Cancer-cell apoptosis and *in vivo* anticancer treatment | [12] |
| **Prokaryotic systems** | | | | |
| Psoralen–DNA | *Listeria monocytogenes*, *Escherichia coli* MG1655 | *uvrAB* (nucleotide excision repair) mutant for restricted growth, and variable protein expression | Vaccine production in *Listeria* variants through nondividing but metabolically active bacteria | [1,13] |
| $C_{10}H_7CH_2C(O)$-LFLFY-$(PO(OH)_2)$ | *Escherichia coli* BL21 | Reduced cell growth due to increased membrane rigidity | Restriction of cell proliferation, and application in enzyme-coupled hydrogelation | [8] |
| PEG-DA | *Escherichia coli* BL21DE3, Nissle 1917 | Inhibited division while maintaining 70% of metabolic activity. | Production of nondividing but metabolically active bacteria, cancer invasion/targeting | [14] |

| **Eukaryotic systems** | | | | |
|---|---|---|---|---|
| **Material components** | **Strains tested** | **Phenotype and genotype** | **Applications** | **Refs** |
| | | Upregulated protein homeostasis gene expression | | |