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REVIEW ARTICLE

Injectable Macroporous Cryogels for Minimally Invasive Drug Delivery and Tissue Engineering

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Abstract: Cryogels are a distinct class of polymeric biomaterials synthesized through cryogelation at sub-zero temperatures, resulting in a unique interconnected macroporous architecture. Unlike conventional hydrogels, these scaffolds exhibit exceptional shape-memory properties, mechanical robustness, and rapid swelling kinetics, enabling them to withstand reversible deformation. These attributes facilitate the development of injectable scaffolds that can be delivered via minimally invasive procedures through standard hypodermic needles, subsequently regaining their original geometry in situ. The current state of knowledge regarding the fabrication principles, physicochemical characterization, and biomedical utility of injectable cryogels are discussed in this paper. The cryotropic gelation mechanism, where the critical role of ice crystals as porogens and the non-frozen liquid microphase in defining the scaffold's microstructure are discussed in this review. Cryogels have several important applications including oncology and regenerative medicine.

Keywords: Cryogelation; Shape-memory scaffolds; Minimally invasive delivery; Immunotherapy; Non-frozen liquid microphase.

1. Introduction

The development of three-dimensional (3D) scaffolds that mimic the extracellular matrix (ECM) is a cornerstone of modern biomedical engineering. Hydrophilic polymer networks, or hydrogels, have been extensively employed for this purpose due to their high-water content and biocompatibility. However, traditional hydrogels, typically formed at room temperature, possess a nanoporous or microporous structure (pore size < 10 μm) which often restricts the convective transport of nutrients and prevents the deep infiltration of host cells [1]. Moreover, these materials are generally brittle or permanently formed, requiring invasive surgical implantation or limiting their use to liquid precursors that gel in situ, which carries the risk of leakage and off-target polymerization [2].

Table 1. Comparison of Conventional Hydrogels and Macroporous Cryogels

Feature	Conventional Hydrogels	Macroporous Cryogels
Pore Size	Nanoporous to Microporous (< 10 μm)	Macroporous (10 – 200 μm)
Pore Structure	Closed, often isolated pores	Open, highly interconnected channels
Mass Transport	Diffusive (slow)	Convective and Diffusive (fast)
Mechanical Properties	Often brittle or permanently shaped	Elastic, sponge-like, shape-memory
Injectability	Liquid precursors (sol-gel in situ) or shear-thinning	Preformed solid scaffolds (reversible compression)
Cell Infiltration	Limited to surface or requires encapsulation	Deep infiltration and migration possible
Swelling Kinetics	Slow (diffusion-controlled)	Rapid (capillary-driven)

To address these limitations, cryogels macroporous hydrogels formed at sub-zero temperatures have emerged as a superior alternative. Fabricated via cryotropic gelation, these materials possess a sponge-like architecture with interconnected pores ranging

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from 10 to 200 μm [3]. This structure confers unique mechanical properties, most notably "shape memory," which allows fully formed cryogels to be compressed, injected through a narrow-gauge needle, and autonomously recover their original shape upon hydration [4]. This capability combines the minimally invasive nature of injectable fluids with the structural integrity and localized retention of preformed implants.

Current research focuses heavily on optimizing the fabrication parameters to tailor pore geometry and mechanical resilience for specific tissues. From delivering immunotherapeutic agents to serving as niches for stem cell differentiation, injectable cryogels are redefining the paradigms of drug delivery and tissue regeneration [5].

2. Principles of Cryogelation

The synthesis of cryogels is governed by the principles of cryotropic gelation, a process distinct from traditional sol-gel transitions. It exploits the solvent crystallization phase separation to template the polymer network.

2.1. The Cryotropic Gelation Mechanism

The process initiates when a precursor solution containing monomers or polymers and cross-linking agents is cooled below the solvent's freezing point (typically water). As the temperature decreases, pure solvent crystallizes into ice, acting as a solid porogen. This crystallization expels solutes (monomers, initiators, and polymers) into a non-frozen liquid microphase (NFLM) [6].

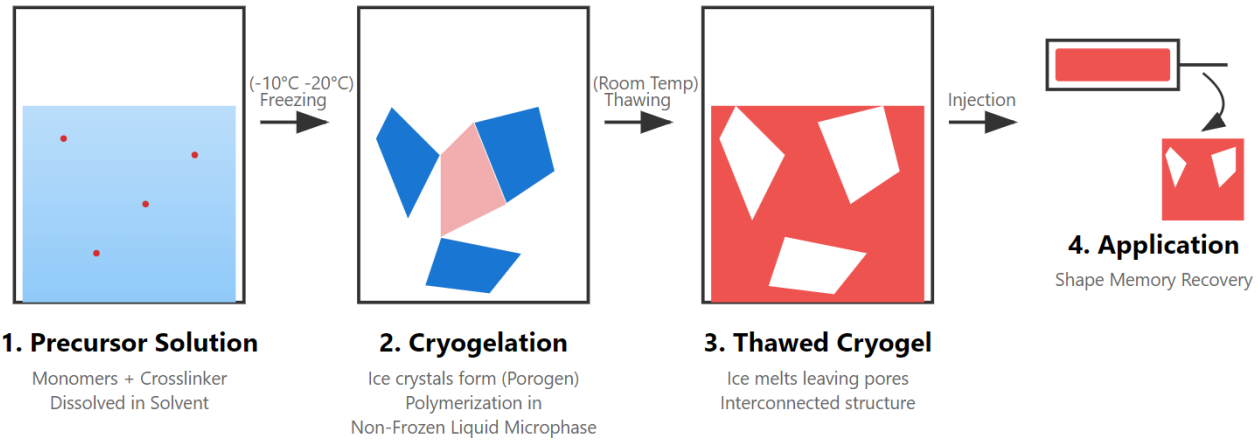

Figure 1. Mechanism of Cryotropic Gelation

2.1.1. The Non-Frozen Liquid Microphase (NFLM)

The NFLM is the critical reaction locus in cryogelation. Despite the macroscopic bulk appearing frozen, a small volume of liquid remains unfrozen due to the cryoscopic concentration of solutes. In this microphase, the concentration of reactants can increase by orders of magnitude compared to the initial solution [7]. This "cryoconcentration" effect accelerates reaction kinetics, allowing polymerization and cross-linking to proceed efficiently even at sub-zero temperatures.

2.1.2. Pore Formation and Morphology

As the polymer network forms within the NFLM, it encases the growing ice crystals. Once the reaction is complete and the system is thawed, the ice melts, leaving behind empty voids that mirror the crystal geometry. The result is an open-cellular structure with high interconnectivity [8]. The morphology of these pores is dictated by the freezing method:

- Directional Freezing: Produces anisotropic, channel-like pores aligned with the temperature gradient.
- Isotropic Freezing: Produces randomly oriented pores, typical for bulk injectable cryogels.

2.2. Critical Processing Parameters

The structural properties of cryogels are highly sensitive to fabrication conditions.

2.2.1. Temperature

Lower temperatures (e.g., -80°C) lead to rapid nucleation, creating smaller, more numerous ice crystals and consequently smaller pores. Warmer sub-zero temperatures (e.g., -10°C to -20°C) favor the growth of larger crystals, resulting in larger macropores ideal for cell migration [9].

2.2.2. Cooling Rate

Slow cooling rates allow for larger crystal growth (larger pores), while flash-freezing creates dense, microporous structures that may lack the requisite permeability for cell infiltration.

2.2.3. Precursor Concentration

Higher polymer concentrations increase the wall thickness and density of the cryogel, improving mechanical strength but potentially reducing total porosity [10].

Table 2. Influence of Processing Parameters on Cryogel Properties

Fabrication Parameter	Condition Change	Effect on Pore Architecture	Effect on Mechanical Properties
Freezing Temperature	Lower (e.g., -80°C vs -10°C)	Smaller pores due to rapid nucleation	Higher wall density, stiffer scaffold
Cooling Rate	Faster Cooling	Smaller, less connected pores	Increased brittleness
Precursor Concentration	Increased	Thicker pore walls, lower total porosity	Increased compressive modulus (stiffer)
Cross-linking Density	Increased	Slightly smaller pores	Higher elasticity, slower degradation
Thawing Rate	Slow vs. Fast	Minimal effect on structure	Minimal effect

3. Classification of Cryogel Precursors

The versatility of cryogels lies in the wide range of polymers that can be subjected to cryogelation. These are broadly categorized into synthetic and natural polymers.

3.1. Synthetic Polymers

Synthetic polymers offer batch-to-batch consistency and precise control over mechanical properties and degradation rates.

3.1.1. Poly(ethylene glycol) (PEG)

PEG is widely utilized due to its immunocompatibility and resistance to protein adsorption ("stealth" properties). PEG-diacrylate (PEGDA) cryogels can be synthesized via free-radical polymerization. While biologically inert, they are often functionalized with RGD peptide sequences to promote cell adhesion [11].

3.1.2. Poly(vinyl alcohol) (PVA)

PVA cryogels are unique as they can be formed through physical cross-linking (hydrogen bonding and crystallite formation) via repeated freeze-thaw cycles, avoiding toxic chemical cross-linkers. These cryogels exhibit high elasticity and fatigue resistance, making them suitable for cartilaginous tissue mimics [12].

3.1.3. Poly(acrylamide) (PAAm) and Derivatives

Polyacrylamide derivatives are frequently used in fundamental studies of cryogelation due to their robust polymerization kinetics. However, due to the non-biodegradability of the carbon backbone, their clinical use is limited to permanent implants or external applications [13].

3.2. Natural Polymers

Biopolymers are inherently bioactive, often containing signals that promote cell interaction and enzymatic biodegradation.

3.2.1. Polysaccharides (Alginate, Chitosan, Hyaluronic Acid)

- Alginate: Derived from brown algae, alginate cryogels are often cross-linked using calcium ions or chemically modified (e.g., methacrylated alginate) for covalent bonding. They are particularly useful for bone tissue engineering [14].
- Chitosan: A cationic polymer derived from chitin, chitosan exhibits inherent antimicrobial and hemostatic properties. Chitosan cryogels are excellent candidates for wound dressing and hemorrhage control [15].
- Hyaluronic Acid (HA): A primary component of the ECM, HA is critical for cell signaling. HA-based cryogels, often cross-linked with glycidyl methacrylate (HAGM) or adipic acid dihydrazide, support angiogenesis and soft tissue repair [16].

3.2.2. Proteins (Gelatin, Collagen)

Gelatin, the hydrolyzed form of collagen, retains RGD cell-binding motifs. Methacrylated gelatin (GelMA) is a popular precursor for cryogels, balancing biological activity with tunable mechanical strength via photo-crosslinking or redox initiation [17].

Table 3. Common Polymers Used for Injectable Cryogel Fabrication

Polymer Class	Precursor Examples	Cross-linking Mechanism	Key Biological Attributes
Synthetic	Poly(ethylene glycol) (PEG)	Free-radical polymerization (photochemical/redox)	Non-immunogenic ("stealth"), antifouling, tunable mechanics
	Poly(vinyl alcohol) (PVA)	Physical (crystallite formation via freeze-thaw)	High elasticity, fatigue resistance, biocompatible
	Poly(acrylamide) (PAAm)	Chemical (BIS cross-linker)	Robust kinetics (mostly for in vitro/sensing use)
Natural	Alginate	Ionic (Ca^{2+}) or Chemical (EDC/NHS, Methacrylation)	Osteoconductive, biodegradable, abundant
	Chitosan	Chemical (Glutaraldehyde) or Physical	Hemostatic, antimicrobial, mucoadhesive
	Gelatin / GelMA	Enzymatic (Transglutaminase) or Photo-crosslinking	Contains RGD motifs (cell adhesion), biodegradable
	Hyaluronic Acid (HA)	Chemical (BDDE, ADH, Methacrylation)	Promotes angiogenesis, non-inflammatory

4. Physicochemical and Mechanical Characterization

The suitability of cryogels for injectable applications is determined by specific physicochemical attributes.

4.1. Pore Architecture and Interconnectivity

The defining feature of cryogels is their interconnected macroporosity (typically >75% porosity). Techniques such as Micro-Computed Tomography (Micro-CT) and Scanning Electron Microscopy (SEM) are used to quantify pore diameter (ranging from 10–200 μm) and wall thickness. High interconnectivity is essential for the "sponge" effect allowing water to be rapidly squeezed out during injection and reabsorbed upon release [18].

4.2. Mechanical Resilience

Injectability relies on the material's ability to withstand high shear and compressive strain without fracture.

4.2.1. Shape Memory

Cryogels can be compressed to 90% of their volume and recover their original shape almost instantaneously. This is attributed to the reversible collapse of the macropores and the elasticity of the polymer walls [19].

4.2.2. Syringe Injectability

The force required to inject a cryogel depends on the compression ratio and the needle gauge (typically 16G–18G). The interconnected pores allow water to efflux during compression, reducing the hydraulic resistance, while the polymer skeleton folds rather than fractures [20].

4.3. Swelling Kinetics and Mass Transport

Unlike nanoporous hydrogels which swell via diffusion (slow), cryogels swell via capillary action (fast).

4.3.1. Swelling Ratio

$Q = (\{W_{swollen} - W_{dry}\}) / W_{dry}$. Cryogels often exhibit Q values between 10 and 50 depending on the cross-linking density.

4.3.2. Permeability

The mass transport capability is described by Darcy's Law. The high permeability (K) of cryogels ensures that oxygen and nutrients can reach cells located in the center of the scaffold, preventing the necrotic core formation often observed in bulk hydrogels [21].

4.4. Biodegradation

For regenerative applications, the degradation rate of the cryogel must match the rate of new tissue formation. Degradation occurs via hydrolysis (for synthetic polyesters) or enzymatic cleavage (for biopolymers like collagenase-mediated degradation of gelatin). By adjusting the cross-linking density or blending slow-degrading synthetic polymers with fast-degrading natural polymers, the lifespan of the scaffold can be tuned from weeks to months [22].

Table 4. Characterization Techniques for Injectable Cryogels

Property	Analytical Technique	Purpose of Analysis
Morphology	Scanning Electron Microscopy (SEM)	Visualize pore shape, wall texture, and interconnectivity
	Micro-Computed Tomography (μ-CT)	3D reconstruction to calculate pore volume and distribution
Swelling Behavior	Gravimetric Analysis	Determine Swelling Ratio (Q) and equilibrium water content
Mechanical Strength	Unconfined Compression Test	Measure Young's modulus, compressive strength, and shape recovery
Injectability	Syringe Force Gauge Test	Quantify force required to inject scaffold through various needle gauges
Degradation	In vitro Hydrolysis / Enzymatic Assay	Monitor mass loss over time in simulated physiological fluids
Biocompatibility	MTT / Live-Dead Assays	Assess cytotoxicity and cell viability within the scaffold

5. Biomedical Applications

The unique combination of macroporosity, mechanical durability, and injectability has positioned cryogels as a transformative tool across a spectrum of clinical disciplines. Unlike conventional hydrogels that often act merely as passive carriers, the open-cellular

architecture of cryogels allows them to actively integrate with host tissue, facilitating cellular infiltration and vascularization. This section details their application in key therapeutic areas.

5.1. Wound Healing and Skin Regeneration

The treatment of deep, non-healing cutaneous wounds requires scaffolds that can manage exudate, prevent infection, and promote re-epithelialization. Injectable cryogels address these needs through their high absorptive capacity and ability to conform to irregular wound geometries. Upon application, the macroporous structure draws in wound exudates, reducing the risk of maceration while maintaining a moist microenvironment conducive to healing.

Recent advancements have focused on functionalizing these scaffolds with antimicrobial agents to combat multidrug-resistant bacteria. For instance, chitosan-based cryogels inherently possess bacteriostatic properties due to their cationic charge, which disrupts bacterial cell membranes. To further enhance healing, researchers have developed bilayered cryogel systems mimicking the skin's anatomy: a dense upper layer to prevent bacterial ingress and moisture loss, and a porous lower layer to support fibroblast migration and collagen deposition. In vivo studies utilizing rabbit models have demonstrated that such composite cryogels can accelerate wound closure and promote hair follicle regeneration significantly faster than standard gauze dressings [23]. Furthermore, the incorporation of bioactive molecules such as epidermal growth factor (EGF) within the cryogel matrix has been shown to stimulate angiogenesis, a critical step in restoring tissue viability in chronic diabetic ulcers [24].

5.2. Cancer Immunotherapy and Vaccine Delivery

One of the most promising frontiers for injectable cryogels is in the field of cancer immunotherapy. Traditional cancer vaccines often fail due to the rapid dispersion of antigens and the lack of a sustained immune-activating environment. Cryogels serve as an artificial immune niche that recruits and educates the host's immune cells.

These scaffolds are typically loaded with tumor-associated antigens (TAAs) and adjuvants like granulocyte-macrophage colony-stimulating factor (GM-CSF) and cytosine-phosphoguanosine (CpG) oligonucleotides. Upon injection into the subcutaneous space, the macroporous structure allows dendritic cells (DCs) to infiltrate the scaffold deeply. Within this "training ground," DCs encounter the high concentration of antigens and adjuvants, become activated, and subsequently migrate to the draining lymph nodes to prime cytotoxic T-lymphocytes against the tumor [25]. Studies involving melanoma models have indicated that cryogel-based vaccines induce a potent and durable immune response, significantly inhibiting tumor growth and preventing recurrence compared to bolus injections of the same agents. This localized delivery strategy minimizes systemic toxicity, a common drawback of conventional chemotherapy and cytokine treatments [26].

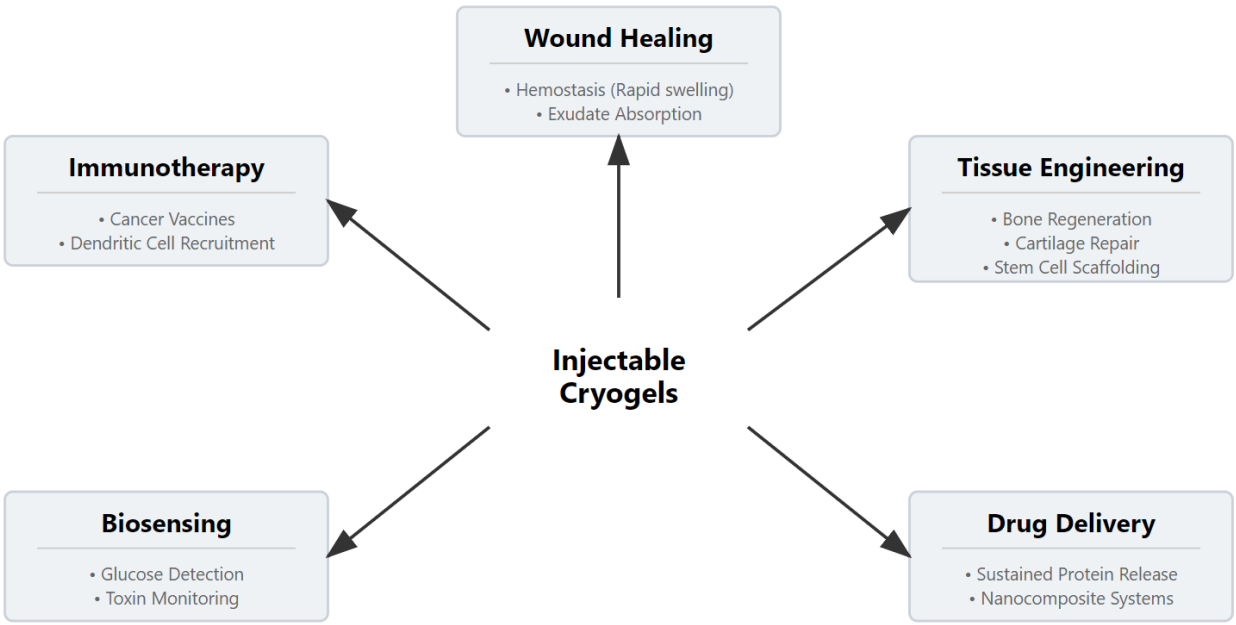

Figure 2. Biomedical Applications of Injectable Cryogels

5.3. Tissue Engineering and Regenerative Medicine

The restoration of functional tissue requires a scaffold that not only fills the defect but also creates a permissive environment for stem cell differentiation and matrix remodeling.

5.3.1. Bone Regeneration

Bone defects, particularly those with irregular shapes or in load-bearing areas, present a significant challenge for rigid preformed implants. Injectable cryogels offer a solution by conforming to the defect cavity and providing immediate mechanical stability via their shape-memory expansion. Alginate and gelatin cryogels have been engineered to deliver osteoinductive factors such as Bone Morphogenetic Protein-2 (BMP-2) or to carry mesenchymal stem cells (MSCs). The interconnected porosity facilitates the ingress of osteoblasts and the subsequent mineralization of the matrix. Recent innovations include the incorporation of mineral phases, such as hydroxyapatite or bioactive glass nanoparticles, within the polymer network. These nanocomposite cryogels not only enhance the compressive modulus of the scaffold to match native bone but also provide a continuous supply of calcium and phosphate ions to fuel osteogenesis [27].

5.3.2. Cartilage Repair

Cartilage possesses limited intrinsic healing capacity due to its avascular nature. Injectable cryogels provide a distinct advantage in cartilage repair by serving as a vehicle for chondrocytes or MSCs while delivering chondrogenic factors like Transforming Growth Factor-β3 (TGF-β3). Hyaluronic acid-based cryogels are particularly favored for this application as they mimic the sulfated glycosaminoglycans found in native cartilage ECM. The elasticity of these cryogels allows them to withstand the dynamic cyclic loading of joints without permanent deformation. Research has shown that maintaining the rounded morphology of chondrocytes within the macropores is essential for preventing dedifferentiation into fibroblasts, ensuring the production of high-quality hyaline cartilage rather than inferior fibrocartilage [28].

5.4. Hemostasis and Emergency Trauma Care

Uncontrolled hemorrhage remains a leading cause of preventable death in trauma cases. Injectable cryogels have emerged as rapid-response hemostatic agents capable of treating deep, penetrating wounds that are not amenable to external compression (non-compressible hemorrhage).

The mechanism of action is twofold: physical and biological. Physically, the shape-memory cryogel can be injected into a wound tract where it rapidly expands, exerting internal pressure on severed vessels to mechanically stanch bleeding. Biologically, the highly porous surface area promotes the rapid absorption of plasma, concentrating platelets and clotting factors to accelerate the coagulation cascade [29]. Materials such as quaternized chitosan or shape-memory gelatin cryogels have demonstrated the ability to stop lethal bleeding in liver and femoral artery injury models in under two minutes. Unlike granular hemostats which can be difficult to remove and may cause embolisms, monolithic cryogels remain intact and can be easily retrieved during subsequent surgical debridement [30].

5.5. Controlled Delivery of Biomacromolecules

The delivery of therapeutic proteins, such as antibodies and growth factors, is often hindered by their rapid enzymatic degradation and short half-life in vivo. While hydrogels have been used for this purpose, the release is often dominated by a rapid "burst" followed by negligible sustained release. Composite cryogel systems address this by incorporating drug-loaded nanoparticles or liposomes within the macroporous walls. This hierarchical structure provides dual protection for the protein payload. For example, researchers have developed "plum-pudding" morphologies where protein-loaded nanogels are embedded within the bulk cryogel matrix. This design allows for the sustained release of vascular endothelial growth factor (VEGF) over several weeks, promoting stable neovascularization rather than the leaky vessels associated with bolus VEGF administration [31]. Moreover, the open pore structure prevents the accumulation of acidic degradation products often seen in bulk degrading polyesters, thereby preserving the bioactivity of pH-sensitive proteins [32].

5.6. Biosensing and Diagnostic Platforms

Beyond therapeutics, injectable cryogels function as effective matrices for in vivo biosensing. Their macroporosity ensures unrestricted diffusion of analytes from the surrounding tissue fluid to the sensor elements embedded within the gel. Conductive cryogels, often synthesized by incorporating conductive polymers like polypyrrole or carbon nanotubes, serve as flexible electrodes for monitoring physiological signals or detecting biomarkers. For instance, an injectable enzymatic glucose sensor based on an alginate cryogel scaffold demonstrated high sensitivity and a rapid response time due to the efficient mass transport of glucose

through the pores. Similarly, DNA-functionalized cryogels have been utilized to detect specific toxins or metabolic byproducts, with the porous structure amplifying the signal by providing a large surface area for ligand-target interactions. These "soft" electronics offer a biocompatible interface with tissue, reducing the foreign body response that typically compromises the longevity of rigid implanted sensors [33, 34].

Table 5. Biomedical Applications of Injectable Cryogels

Application Domain	Cryogel Composition	Active Cargo / Therapeutic	Therapeutic Outcome	Reference
Cancer Immunotherapy	Alginate-RGD	GM-CSF, CpG-ODN, Tumor Antigens	Recruitment and activation of dendritic cells; tumor regression	[26]
Bone Regeneration	Gelatin / Alginate	BMP-2, Mesenchymal Stem Cells (MSCs)	Enhanced mineralization and osteogenic differentiation	[27]
Wound Healing	Chitosan / Gelatin	EGF, Antimicrobial Nanoparticles	Rapid hemostasis, infection control, accelerated closure	[29]
Protein Delivery	Alginate-composite	VEGF-loaded PLGA nanoparticles	Sustained release of bioactive VEGF; neovascularization	[31]
Biosensing	Alginate-Conductive	Glucose Oxidase (enzyme)	Real-time, sensitive electrochemical detection of glucose	[33]

6. Conclusion

Injectable macroporous cryogels are a significant evolution in biomaterials science, due to the mechanical integrity of preformed implants and the minimally invasive delivery of injectable fluids. Their defining characteristic the ability to undergo extreme reversible deformation allows for the delivery of large, pre-seeded scaffolds through narrow-gauge needles, a capability unmatched by traditional hydrogels. Despite these promising advances, several challenges must be addressed to facilitate clinical translation. First, the degradation kinetics of cryogels must be precisely tuned to synchronize with tissue regeneration rates; a scaffold that degrades too quickly may lead to mechanical failure, while one that persists too long may hinder tissue integration. Second, achieving zero-order release profiles for small-molecule drugs remains difficult due to the high diffusivity of the macroporous network, necessitating further development of nanocomposite strategies. Finally, the sterilization of preformed cryogels without compromising their shape-memory properties or bioactivity requires standardized protocols.

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