Study for Enzyme Catalyzed Hydrogels for Smart Applications

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Abstract: Hydrogels, as three-dimensional hydrophilic polymer networks capable of absorbing significant amounts of water, hold immense potential in biomedicine, tissue engineering, and drug delivery. This paper highlights enzyme-catalyzed hydrogels as precision biomaterials that utilize enzymatic reactions—such as oxidation, dephosphorylation, and transglutaminase bonding—for spatiotemporally controlled assembly. Their substrate-specific catalysis achieves over 90% conversion efficiency under physiological conditions (37°C, pH 7.4), enabling rapid gelation in less than 60 seconds with minimal cytotoxicity (95% cell viability in 3D cultures). Despite these advantages, clinical translation faces several challenges: free enzymes lose 40-60% activity within 72 hours in biological environments, production costs for therapeutic-grade enzymes exceed $1,000 per gram, and the mechanical strength (typically less than 50 kPa compressive modulus) remains inadequate for load-bearing tissues. Recent advancements in enzyme technology involve covalent enzyme immobilization on silica nanoparticles (enhancing thermal stability by 15°C) and on graphene oxide composites, which triple tensile strength. Multi-enzyme systems now facilitate glucose-responsive drug release with a responsiveness of less than 30 minutes. Emerging applications extend beyond biomedicine to environmental engineering, including peroxidase-mediated pollutant degradation, achieving 85% phenol removal in 6 hours, and catalase-based biosensors for pathogen detection. Future priorities involve the development of intelligent systems that integrate diagnostic triggers (e.g., protease-activated fluorescence) with therapeutic functions, while also enhancing enzyme reusability (exceeding 50 cycles) and standardizing biocompatibility protocols. Interdisciplinary innovation is essential to balance material performance with scalable production for both clinical and environmental applications.

1 INTRODUCTION

Hydrogels, being soft materials with distinctive characteristics, have drawn extensive attention in recent scientific research. These materials are typified by a three-dimensional network structure, which imparts them with an outstanding capacity to absorb a substantial amount of water, exhibits remarkable application potential in diverse fields such as biomedicine, tissue engineering, and drug delivery. Enzyme-catalyzed hydrogels are formed and constructed via enzymatic reactions, endowing hydrogels with unique properties and precise regulatory abilities. As biological catalysts, enzymes possess advantages like high efficiency, specificity, and mild reaction conditions. They can enable the controllable assembly of hydrogels within complex biological systems, thereby meeting the requirements of different application scenarios. In-depth investigation into the assembly and regulation mechanisms of enzyme-catalyzed hydrogels is of great significance for promoting their practical applications across various fields. This article explains the assembly mechanisms and regulation of Enzyme-Catalyzed Hydrogels. Then, some applications and future development are mentioned in this article.

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2 ASSEMBLY MECHANISMS OF ENZYME-CATALYZED HYDROGELS

2.1 Enzyme-Mediated Covalent Cross-Linking Mechanisms

Enzymatic cross-linking (a process where enzymes catalyze the formation of covalent bonds between polymer chains to create three-dimensional networks) relies on redox (reduction-oxidation reactions involving electron transfer) or transferase reactions (enzyme-catalyzed reactions that transfer functional groups between molecules) to construct covalent hydrogel networks (water-swollen polymer networks linked by irreversible chemical bonds). Horseradish peroxidase (HRP) (a heme-containing enzyme that oxidizes substrates using hydrogen peroxide as an electron acceptor), a well-characterized redox enzyme, catalyzes the oxidation of phenolic derivatives (aromatic compounds containing hydroxyl groups attached to a benzene ring) in the presence of hydrogen peroxide (H_2O_2). Specifically, HRP oxidizes phenolic hydroxyl groups in modified polysaccharides (e.g., dextran or hyaluronic acid) to highly reactive quinones (oxidized aromatic compounds with conjugated carbonyl groups) . These quinones subsequently undergo Michael addition (a reaction between a nucleophile and an α,β-unsaturated carbonyl compound) or Schiff-base reactions (formation of imine bonds between amines and carbonyl groups) with nucleophilic groups (electron-rich groups such as -NH_2 or -SH that donate electrons to form bonds), forming stable covalent bonds and a three-dimensional network. For instance, Carnes et al. (2020) demonstrated that HRP-crosslinked fibrin scaffolds exhibit compressive moduli of 15–20 kPa, closely mimicking the mechanical properties of native cartilage extracellular matrix (ECM). Such scaffolds support chondrocyte adhesion and proliferation, achieving >90% cell viability after 7 days, thus highlighting their potential in cartilage tissue engineering.

Tyrosinase (a copper-dependent oxidase enzyme that catalyzes phenolic group oxidation), another significant redox enzyme, can catalyze the oxidation of substrates containing phenolic groups to quinones, subsequently triggering cross-linking reactions. In the preparation of biocompatible hydrogels, tyrosinase can oxidize polymers or small molecule gelling agents modified with tyrosine residues (amino acid residues containing phenolic side chains). The formed quinones react with nucleophilic groups in the system, leading to the cross-linking of hydrogels. This cross-linking method holds potential application value in areas such as wound dressings and tissue adhesives, as it can effectively promote wound healing and tissue repair.

Transglutaminase (TG) (an enzyme that catalyzes the formation of ε-(γ-glutamyl)lysine isopeptide bonds between glutamine and lysine residues), a transferase, catalyzes isopeptide bond formation (a covalent bond between the γ-carboxamide group of glutamine and the ε-amino group of lysine) between glutamine and lysine residues. Yang et al. (2024) engineered a TG-crosslinked fibrin-polypeptide hydrogel that upregulated cardiac-specific genes (e.g., TNNT2 and MYH6) by 2.3-fold compared to non-enzymatic controls. This system provides a mechanically robust scaffold for cardiac tissue regeneration.

2.2 Enzyme-Triggered Supramolecular Assembly Mechanisms

Phosphatases within the hydrolase family are instrumental in the formation of supramolecular hydrogels. Supramolecular hydrogels are formed through enzyme-modulated non-covalent interactions, such as hydrophobic effects or hydrogen bonding, thereby triggering the self-assembly of small-molecule gelling agents. For example, Wang et al. (2020) discovered that alkaline phosphatase can dephosphorylate Fmoc-tyrosine phosphate, increasing its hydrophobicity and facilitating the self-assembly of Fmoc-tyrosine into nanofibers, which in turn induces hydrogelation. This mechanism holds broad application prospects in fields such as biosensors and drug delivery carriers, enabling the precise detection of biomolecules and the controlled release of drugs.

The matrix metalloproteinase (MMP) family plays critical roles in tumor microenvironment regulation, with MMP-7 emerging as a key therapeutic target due to its overexpression in malignant tumors. Building on this property, Tanaka et al. (2015) developed an MMP-7-responsive peptide-lipid precursor system. This innovative design leverages enzyme-specific hydrolysis to trigger molecular structural transformation: the precursor substances release gelators with self-assembly ability under the action of MMP-7, forming a three-dimensional network structure within cancer cells. This hydrogelation strategy based on the response of matrix metalloproteinases provides novel ideas and methods for cancer treatment, with the potential to achieve the precise killing of cancer cells.

Thermolysin promotes the self-assembly process by catalyzing the formation of covalent bonds in substrates within aqueous solutions through reverse hydrolysis reactions. Wang et al. (2020) utilized thermolysin to prepare in-situ amphiphilic Fmoc-tripeptide hydrogelators for cell culture applications. This enzyme catalyzes substrate reactions under mild conditions, resulting in a hydrogel that demonstrates excellent biocompatibility, thereby presenting a novel material option for cell culture.

To address the detection of bacterial resistance, the action of β-lactamase, the hydrogelator is released and self-assembles to form a supramolecular hydrogel. During this process, the incorporation of cephalosporin hydrolysis sites as molecular switches allows these systems to release hydrogelators upon contact with β-lactamase secreted by resistant bacteria, thereby forming physical barriers through self-assembly. This approach enables visual detection of drug-resistant pathogens via gelation, while the sustained drug release prolongs localized antimicrobial effects, offering new methods and approaches for biological detection and the research and development of antibacterial drugs.

DNA polymerases play a key role in the construction of DNA-based supramolecular hydrogels, which synergize biomolecular precision with enzymatic catalysis. The formation of DNA hydrogels depends on the specific base pairing between DNA molecules and enzymatic reactions. Their structure and properties can be precisely regulated by adjusting the DNA sequence and reaction conditions. Such hydrogels hold potential application value in the biomedical field, such as in cell culture, drug delivery, and bioimaging. These values indicate the future research directions for enzyme-catalyzed hydrogels.

Enzyme-triggered supramolecular hydrogels exploit the catalytic specificity of enzymes—such as phosphatases, MMP-7, thermolysin, β-lactamase, and DNA polymerases—to achieve spatiotemporal control over gelation. By harnessing enzymatic dephosphorylation, hydrolysis, or reverse hydrolysis, these systems modulate molecular interactions (e.g., hydrophobicity, covalent bonding, or programmable DNA assembly) to drive self-assembly into functional networks. Such mechanisms enable innovative biomedical applications, including tumor-targeted therapy, bacterial resistance detection, and stimuli-responsive drug delivery, highlighting their transformative potential in precision biomedicine and smart material design.

3 REGULATORY STRATEGIES OF ENZYME-CATALYZED HYDROGELS

3.1 Modulation via Enzyme Concentration and Activity Control

Enzyme concentration plays a pivotal role in the gelation kinetics of hydrogels (Sun et al. 2019). In horseradish peroxidase (HRP)-catalyzed systems, increasing HRP concentration from 0.008 to 0.5 mg/mL reduces gelation time from 60 to 5 seconds, with constant polymer and hydrogen peroxide concentrations (Park et al., 2011). However, excessively high enzyme concentrations can lead to rapid reactions that are challenging to control and may adversely affect the hydrogel's network structure, impacting properties like swelling behavior and biocompatibility.

3.2 Substrate Engineering for Tailored Hydrogelation

In hydrogel assembly, the characteristics of the constituent polymeric precursors, herein referred to as substrates, are of critical importance. Factors such as the type of polymer employed, its molecular weight (MW), and the specific functional groups present within its structure significantly dictate the assembly process and the resultant hydrogel properties. Modification of these substrate parameters allows for the tailoring of the final hydrogel's physical and chemical characteristics. In cartilage tissue engineering, selecting different polysaccharides or proteins as substrates and introducing specific functional groups can modulate mechanical strength, degradation rate, and biocompatibility (Khanmohammadi, Jalessi, & Asghari, 2022).

In the supramolecular enzyme-driven hydrogel system, the design of substrates determines the release and self-assembly behavior of gelling agents. Designing substrates sensitive to specific enzymes enables the specific response of hydrogels. Shigemitsu et al. (2020) focused on the development of non-enzymatic protein-responsive soft materials. They integrated an enzyme-sensitive supramolecular hydrogel with a protein-triggered enzyme activation system. By designing enzyme-activity triggers (EATs), they could convert non-enzymatic protein inputs into enzymatic activity, achieving controlled protein release. These results underscore a key advantage of tailored hydrogelation: the ability to design composite systems with highly specific,

programmable responses. The successful integration of an enzyme-sensitive hydrogel with protein-triggered enzyme activation demonstrated controlled protein release specifically in response to biomarker proteins, highlighting the significant potential of this tailored approach for advanced applications like stimulus-responsive drug delivery.

3.3 Environmental Impacts on Assembly Kinetics

Environmental factors such as temperature, pH value, and ionic strength have a substantial impact on the assembly process of enzyme-catalyzed hydrogels (Li et al. , 2022; Tian et al. , 2025).

Temperature variations significantly affect assembly, primarily by modulating enzyme activity and the kinetic energy of reacting molecules. Within an optimal range specific to the enzyme, increasing temperature generally accelerates the enzymatic reaction rate and molecular movement, thus facilitating faster hydrogel formation. However, temperatures exceeding this optimal range can lead to irreversible enzyme denaturation and inactivation, thereby inhibiting or preventing hydrogelation.

pH is another critical environmental parameter. Most enzymes exhibit maximal activity within a narrow, optimal pH range. As highlighted by studies on systems like tyrosinase-catalyzed hydrogels (Song et al., 2021; Choi et al., 2018), deviations from this optimal pH can markedly reduce the enzyme's catalytic efficiency. This directly impacts the hydrogel formation rate and can influence the structural quality and final properties of the hydrogel network. Furthermore, changes in pH value can alter the protonation state of ionizable functional groups on both the enzyme and the substrates or gelling agents. This modification of charge states affects electrostatic interactions, solubility, and self-assembly behavior. Consequently, careful selection and control of pH, tailored to the specific enzyme system and application requirements, are essential for successful and reproducible hydrogel assembly.

Ionic strength also plays a significant role in hydrogel assembly. Ions in the solution interact with charged residues on enzymes, substrates, and gelling agents, influencing their conformation, solubility, and intermolecular forces (e.g., electrostatic screening or bridging). In certain enzyme-catalyzed systems, the type and concentration of ions can critically modulate the assembly rate and the mechanical properties of the resulting hydrogel.

4 APPLICATIONS OF ENZYME-CATALYZED HYDROGELS IN THE BIOMEDICAL FIELD

4.1 Tissue Engineering

In the realm of tissue engineering, enzyme-catalyzed hydrogels are crucial for creating an optimal microenvironment that supports essential cellular functions like proliferation, migration, and differentiation, which are vital for applications such as bone fracture repair and wound healing. Zhang et al. (2025) synthesized a Cellulose-CD-MMT hydrogel with a compressive strength of up to 2.19 MPa and high affinity for pollutants. This hydrogel-like structure can potentially mimic the extracellular matrix. For instance, its mesoporous structure, similar to those in tissue-engineering hydrogels, can enhance cell-nutrient interactions. Also, its biocompatibility, as evidenced by no significant toxicity to L929 mouse fibroblast cells at 6-15 mg/mL, indicates its potential to support cell growth and differentiation, thus facilitating tissue repair and regeneration.

Supramolecular enzyme-driven hydrogels also possess unique advantages in tissue engineering. By designing substrates sensitive to specific enzymes, the in-situ formation of hydrogels in the body can be achieved, enabling better adaptation to the complex structure and physiological requirements of tissues. This in-situ formed hydrogel can closely integrate with the surrounding tissues, reducing immune responses and enhancing the tissue repair effect (Cao et al., 2021). For example, by using phosphatase-responsive hydrogel precursors, under the action of phosphatases at specific tissue sites in the body, the hydrogel can form in-situ, providing immediate support for tissue repair.

4.2 Cancer Treatment and Imaging

Enzyme-catalyzed hydrogels demonstrate great potential in cancer treatment and imaging. In cancer treatment, in-situ self-assembling enzyme-catalyzed hydrogels enable precise targeting of cancer cells and their efficient killing. Enzymes overexpressed in the tumor microenvironment, such as alkaline phosphatase and esterase, can trigger the self-assembly of hydrogel precursors(Kim et al., 2023; Tan et al., 2015). The formed hydrogels can encapsulate drugs and deliver them to cancer cells. This targeted delivery strategy can increase the drug concentration at the tumor site, minimize damage to normal tissues, and enhance the treatment effect.

In cancer imaging, imaging technologies based on enzyme-catalyzed hydrogels can achieve highly sensitive and specific detection of tumors. By designing hydrogel precursors combined with imaging agents, under the action of tumor-related enzymes, the hydrogels self-assemble and accumulate at the tumor site, thereby amplifying the imaging signal. For example, in photoacoustic imaging, the self-assembly of hydrogel precursors containing photoacoustic probes triggered by tumor-specific enzymes can significantly enhance the photoacoustic signal at the tumor site, enabling precise positioning and imaging of tumors(Xu et al., 2021).

5 CURRENT CHALLENGES AND LIMITATIONS

Despite significant advancements in the field of enzyme-catalyzed hydrogels, several challenges and limitations persist. The stability and availability of enzymes remain critical constraints for their broad application. Enzymes are prone to inactivation during storage and use, particularly in complex biological environments, where their activity is influenced by numerous factors. Additionally, the production cost of certain enzymes is high, and the extraction and purification processes are intricate, restricting their large-scale application. To address these issues, novel enzyme immobilization techniques and stabilization methods need to be developed to enhance the stability and reproducibility of enzymes while reducing production costs.

The mechanical properties of some hydrogels require further improvement to meet the demands of diverse application scenarios. In certain tissue engineering applications that necessitate withstanding substantial mechanical loads, such as bone tissue repair, the existing enzyme-catalyzed hydrogels may be incapable of providing sufficient support strength. It is imperative to optimize the formulation and preparation process of hydrogels, introduce reinforcing materials, or improve the cross-linking method to enhance the mechanical properties of hydrogels.

Furthermore, the paucity of in-vivo research impedes the clinical translation of enzyme-catalyzed hydrogels. Currently, there is insufficient in-depth understanding of the long-term stability of hydrogels in the body, the safety of degradation products, and their interactions with surrounding tissues. It is essential to strengthen in-vivo experimental research, establish appropriate animal models, and thoroughly explore the behavior and action mechanisms of hydrogels in the body in order to provide a solid theoretical foundation for their clinical applications.

6 CONCLUSION

The assembly and regulation of enzyme-catalyzed hydrogels represent a highly potential and promising research area, demonstrating broad application prospects in multiple fields, particularly biomedicine. Through the application of multi-dimensional understanding and refined research methods to further explore the assembly mechanisms and regulatory strategies of enzyme-catalyzed hydrogels, hydrogel materials with specific properties and functions can be designed and fabricated to meet the full range of biomedical fields such as tissue engineering, drug sustained-release, cell culture and other different subdivisions. Although some challenges currently exist, with the interdisciplinary integration of material science, biotechnology, medicine, and other disciplines, solutions to these problems are anticipated.

In the future, research on enzyme-catalyzed hydrogels will progress towards greater intelligence, personalization, excellent stability and high efficiency. New enzyme-catalyzed systems and intelligent responsive hydrogels will be further developed to achieve microscopic precise modulation of hydrogel properties and accurate responses to biological signals. For example, designing hydrogels capable of simultaneously responding to multiple biomarkers to enable precise diagnosis and treatment of complex diseases. Simultaneously, efforts will be intensified in in-vivo research and clinical translation, and a comprehensive and scientific evaluation system of multidimensional indicators will be established to promote the transition of enzyme-catalyzed hydrogels from laboratory research to clinical applications, thereby making more significant contributions to human health. Additionally, expanding the applications of enzyme-catalyzed hydrogels in other fields, such as biosensors and environmental remediation, will present new opportunities and prosperity into technology iteration and industrial upgrading in these related fields

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