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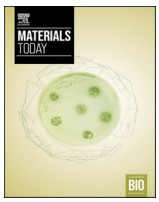

Microenvironment-sensitive hydrogels as promising drug delivery systems for co-encapsulating microbial homeostasis probiotics and anti-inflammatory drugs to treat periodontitis

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ABSTRACT

Developing and utilizing effective local antimicrobial agents can help treat periodontitis while minimizing the risks associated with systemic antibiotic use. Recent studies have shown that the mucosal adhesion properties of hydrogels can play a potential role in the treatment of periodontitis. The hydrogel can improve the contact and retention time of drugs in the periodontal pocket. Through the adhesion of mucosa, it interacts with the mucin coating surface of epithelium and teeth to form a specific interface force. The hydrogel exhibits strong mucosal adhesion (adhesion strength: 5–6 N/cm^2) and prolonged retention in periodontal pockets (≥6 h), enabling sustained drug release through dynamic sol-gel transitions triggered by pH and reactive oxygen species (ROS). This design overcomes the limitations of poor mechanical stability in conventional formulations. The dynamic balance of oral microbiota plays an important role in maintaining oral health. Probiotics, by colonizing the oral cavity, transform the infected site from an environment rich in inflammatory cytokines to a more benign environment, inhibit harmful pathogenic microorganisms, and contribute to overall health. Microenvironment sensitive hydrogels can perform dynamic sol gel transformation in situ, and can accurately control drug release when exposed to various stimuli (such as temperature change, light, pH change, reactive oxygen species, etc.). Oral probiotics and anti-inflammatory drugs are encapsulated in hydrogels to inhibit the proliferation and adhesion of oral pathogens by planting in the mouth and producing metabolites, effectively preventing and treating oral diseases.

1. Introduction

Periodontitis is a prevalent chronic inflammatory oral disease that affects over 700 million people globally, accounting for approximately 10–15 % of the adult population worldwide [1]. According to data from the Global Burden of Disease Study, the prevalence of periodontitis significantly increases with age, affecting more than 50 % of individuals aged 65 years and older [2]. As a multifactorial disease, periodontitis is not merely a localized oral health issue but is also closely associated with a wide range of systemic health conditions, including cardiovascular diseases, diabetes, and respiratory disorders [3,4]. Studies have shown that the chronic inflammatory response in periodontitis can extend beyond the oral cavity, disseminating pro-inflammatory mediators such as IL-1 (Interleukin-1), IL-6 (Interleukin-6), and TNF-α (Tumor Necrosis Factor-α) to distant organs and tissues, thereby inducing a state of systemic inflammation that elevates the risk of atherosclerosis and other

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cardiovascular diseases [5,6] (see Fig. 1).

In recent years, there has been a growing focus on the relationship between oral microbiota dysbiosis and systemic health. Under normal conditions, the diversity and homeostasis of the oral microbiome play a crucial role in maintaining host health. However, when oral microbial balance is disrupted, pathogenic bacteria such as *Porphyromonas gingivalis* and *Tannerella forsythia* can dominate, exacerbating both local and systemic inflammatory responses [7]. This microbial dysbiosis has been found to not only drive the progression of periodontitis but also significantly contribute to the development of atherosclerosis and other cardiovascular conditions [8]. Innovative therapeutic strategies targeting the modulation of the oral microbiome have emerged as promising approaches to manage periodontitis and its systemic complications. Traditional antibiotic treatments face limitations in addressing microbial dysbiosis and inflammatory responses, including issues of antibiotic resistance and the disruption of beneficial microbial communities. As a result, there is increasing interest in leveraging the synergistic effects of probiotics and anti-inflammatory agents to restore microbial homeostasis and modulate immune responses, thereby reducing the risk of systemic inflammation and related diseases [9]. In particular, the in situ hydrogel containing drug-loaded nanomedicine can be adhered to the infected site to treat periodontitis. The nanoparticles loaded with anti-inflammatory drugs and probiotics were co encapsulated into the in situ hydrogel, releasing probiotics and drug loaded nanoparticles (Fig. 2.). At this time, probiotics colonize in the oral microbiota environment, playing a role in regulating the microbiota. The drugs are slowly released in nanoparticles, playing an anti-inflammatory role and having great potential for development in the treatment of chronic periodontitis. The use of biodegradable, biocompatible, and controllable nanomaterials to encapsulate anti-inflammatory drugs have excellent drug sustained release effects, enabling targeted drug delivery and therapy. Moreover, the rigidity of hydrogel can be improved after carrying nanomaterial preparation, and the inherent shortcomings of poor mechanical properties of hydrogel can be overcome.

This review aims to explore the potential of a novel therapeutic strategy involving the co-delivery of probiotics and anti-inflammatory drugs within a hydrogel matrix, specifically designed to restore oral microbial homeostasis and alleviate systemic inflammation in the context of periodontitis. By systematically evaluating the latest research findings, we seek to highlight the role of this innovative approach in modulating the oral microbiome and periodontitis, ultimately laying the groundwork for future multidisciplinary research and clinical applications.

2. Current anti-inflammatory strategies for periodontitis

2.1. Inflammatory response in periodontitis and systemic impact

Periodontitis is a chronic inflammatory disease triggered by oral microbiota, characterized by the destruction of gingival tissue, formation of periodontal pockets, and alveolar bone resorption. Numerous studies have demonstrated that periodontitis is not merely a localized condition but can also induce a systemic inflammatory response [10]. The central mechanisms of these inflammatory responses involve the release of pro-inflammatory mediators, such as IL-1, IL-6, and TNF-α, which can spread to other parts of the body through the bloodstream [11]. The local microenvironment of periodontitis is characterized by macroscopic changes such as pH reduction and increased reactive oxygen species (ROS) and the oral cavity maintains a temperature of approximately 37 °C, which can effectively leverage the sensitivity of smart hydrogels to these microenvironmental factors. This enhances drug adherence and release specifically in the periodontal region, thereby promoting the treatment of periodontitis and potentially other related conditions.

Periodontitis severity is clinically categorized based on attachment loss and radiographic bone destruction, with calculus formation being a hallmark of advanced stages. Initial biofilm accumulation (stage I-II) responds to antimicrobial therapies like chlorhexidine rinses (reducing plaque index by 38 %, but calcified calculus (stage III-IV) requires ultrasonic scaling due to its mineralized hydroxyapatite structure (200–400 μm thickness) that resists chemical penetration [12]. While antimicrobials effectively reduce early-stage biofilm virulence (e.g., 72 % porphyromonas gingivalis suppression [12]), their efficacy diminishes against mature calculus. Mechanical debridement remains indispensable, achieving 89 % calculus removal rates versus 23 % with chemical agents alone. [12]. Post-scaling adjunctive therapies (e.g., localized antibiotic fibers) demonstrate synergistic effects, reducing recurrence rates by 41 % compared to monotherapy [12]. This aligns with current classification systems emphasizing calculus as a key diagnostic criterion for intervention escalation [12].

Systemic inflammation acts as a crucial link between periodontitis and systemic diseases. Pro-inflammatory mediators activate endothelial cells, promote monocyte adhesion and infiltration, and increase vascular permeability, thereby accelerating the development of atherosclerosis [13]. Research has shown that patients with periodontitis have significantly elevated serum levels of C-reactive protein (CRP) and other inflammatory markers, further confirming the connection between periodontitis and systemic inflammation [14].

Moreover, recent studies have demonstrated that nitric oxide (NO) exhibits a unique affinity for ROS [14], leading to interactive processes that generate nitrogen-activated compounds with significant

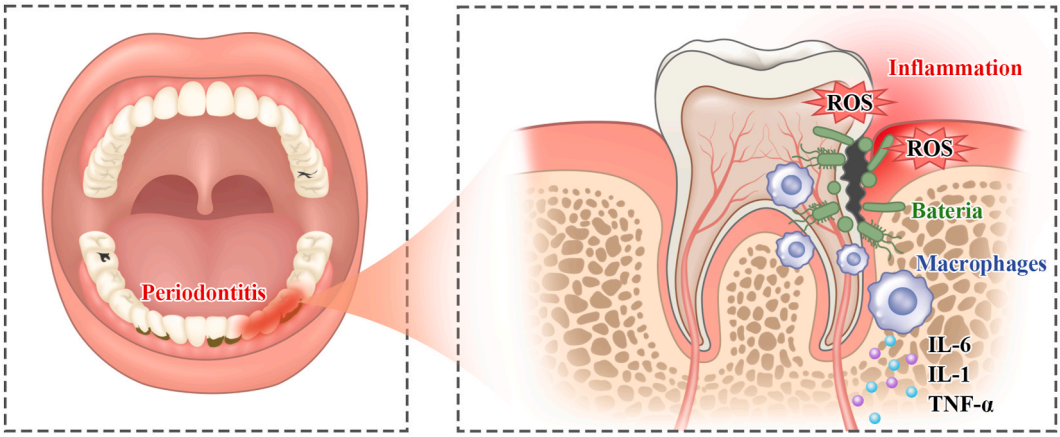


Fig. 1. Schematic illustration of the chronic inflammatory response in periodontitis.

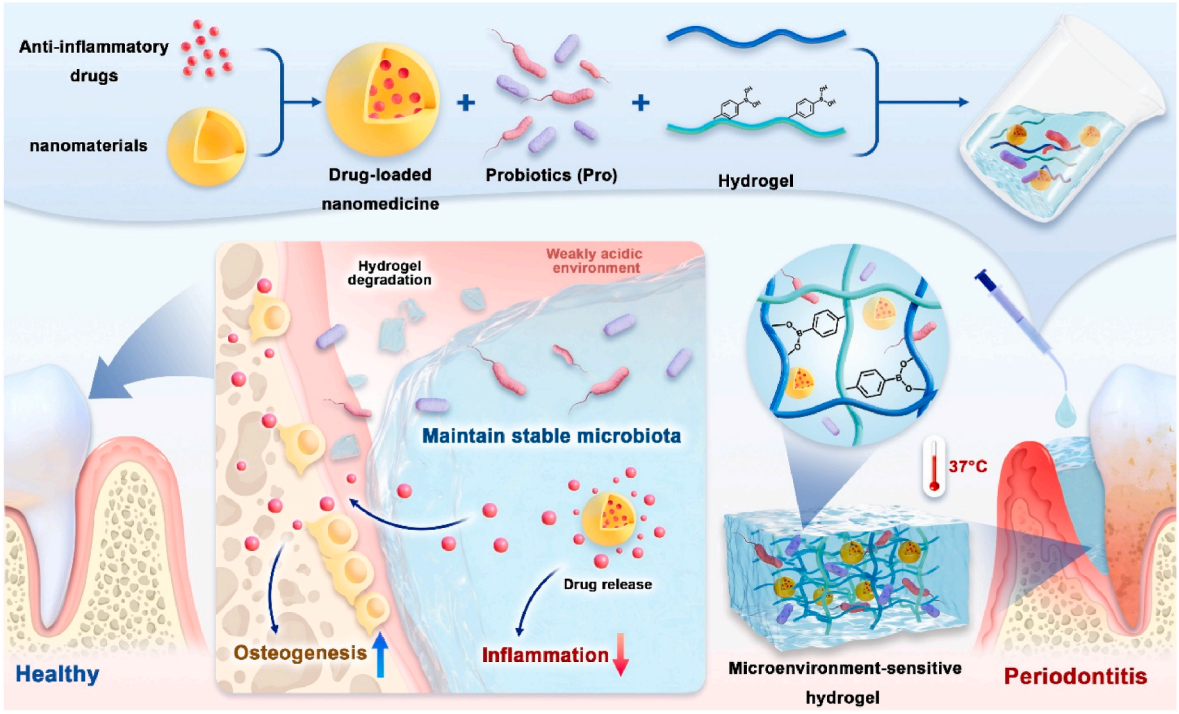


Fig. 2. Schematic illustration of co-delivery of drug-carrying nanomaterials and probiotics using microenvironment-sensitive hydrogels for the treatment of periodontitis.

antimicrobial properties [15,16]. This presents promising implications for the healing of infected wounds. Unlike other antimicrobial agents, NO can directly eliminate bacteria by impairing protein functionality, fragmenting DNA, and inducing lipid peroxidation, without generating antibiotic resistance [17,18]. More importantly, NO acts as a membrane-disruptive molecule, facilitating the breakdown of biofilms through the resolution of c-di-GMP, thereby enhancing bacterial motility while reducing adhesion and extracellular matrix production, ultimately disrupting the bacterial membrane [19,20]. In the context of periodontal disease treatment, Zeng et al. 2024 utilized a poly--ε-caprolactone and guanidinated-poly-ε-lysine (PCL-PLG) composite loaded with chlorhexidine (CHX) [21]. Upon exposure to infected sites, the PCL-PLG@CHX construct allows effective accumulation of its arginine group on the negatively charged surface of bacterial membranes. This enables ROS-meditiated NO generation, which not only promotes deep penetration into bacterial membranes but also functions as a signaling molecule to disrupt biofilms. The subsequent release of CHX enhances antimicrobial efficacy through synergistic action with NO. Yuanqi Chen et al. 2025 developed a multifunctional therapeutic platform for treating oral mucosal inflammation through the self-assembly of guanidine-functionalized dendritic peptides (Arg-EA-SA) and encapsulation of diallyl trisulfide (DATS) [22]. The surface-rich in DATS@Arg-EA-SA effectively neutralizes ROS, generates NO within the wound environment, disrupts bacterial membranes, and achieves highly effective bacterial killing, demonstrating outcomes comparable to the traditional antibiotic penicillin.

2.2. Oral microenvironment features and current challenges in periodontal therapy

The oral microenvironment is defined by its dynamic physicochemical gradients, enzymatic activity, and polymicrobial interactions. These factors create a niche where pathogenic biofilms like *Porphyromonas gingivalis* thrive, driving dysbiosis and immune dysregulation [23,24]. Current therapeutic limitations include: (1) Targeted delivery barriers: systemic antibiotics poorly penetrate calcified calculus (<25 % efficacy), while topical agents face rapid clearance due to saliva flow and masticatory forces. (2) Microbiome complexity: restoring microbial equilibrium requires precise modulation of competing species, as broad-spectrum antimicrobials disrupt beneficial flora. (3) Inflammation-immune crosstalk: chronic inflammation induces oxidative stress (ROS levels 3 × baseline, impairing tissue regeneration and perpetuating dysbiosis; (4) Clinical translation hurdles: hydrogel-based therapies face challenges in probiotic viability (50 % activity loss during storage), patient compliance with repeated applications, and heterogeneous treatment responses due to genetic/epigenetic variability. Future advancements must integrate multi-responsive hydrogels with biofilm-disrupting nanomaterials and personalized microbial consortia to overcome these barriers [24–27].

2.3. Current anti-inflammatory strategies for periodontitis

Periodontal treatment strategies encompass three interconnected approaches: direct antimicrobial therapy, host modulation, and biologic interventions, each addressing distinct aspects of disease pathology.

2.3.1. Antimicrobial therapy

Localized treatments such as minocycline gel and chlorhexidine rinses target pathogenic biofilms (e.g., *Porphyromonas gingivalis*) within periodontal pockets, minimizing systemic exposure. However, prolonged use disrupts microbial symbiosis and risks antibiotic resistance [28]. Systemic antibiotics (e.g., doxycycline, metronidazole) remain effective for aggressive periodontitis but incur gut dysbiosis and gastrointestinal toxicity [29]. Emerging modalities like photodynamic therapy (methylene blue + laser) selectively eliminate pathogens while preserving commensals, though clinical validation in periodontitis is ongoing [30].

2.3.2. Host modulation therapies (HMT)

These strategies mitigate immune-mediated tissue destruction. MMP (Matrix metalloproteinase) inhibitors (e.g., subantimicrobial doxycycline) preserve collagen by blocking MMP-8/13 activity, combining

efficacy with safety [31]. Pathway inhibitors (e.g., Nuclear factor kappa-B/Janus kinase Signal transducer and activator of transcription, NF-κB/JAK-STAT) suppress inflammatory cascades (IL-6, TNF-α), while SIRT1 (Silent information regulation factor 1) activators like resveratrol enhance antioxidant defenses. Limitations include modest monotherapy effects and the need for adjunctive mechanical debridement [32].

2.3.3. Biologic agents

Advanced biologics directly neutralize key mediators. Anti-TNF-α monoclonal antibodies (e.g., adalimumab) reduce inflammatory infiltration, while TSLP (Thymic stromal lymphopoietin) inhibitors (e.g., tezepelumab) show promise in suppressing Th2 (Type 2 helper T cells)-driven inflammation. B-cell modulators (e.g., belimumab) attenuate autoantibody production, relevant in autoimmune-linked periodontitis. Challenges include high costs, infection risks (e.g., herpes zoster), and insufficient periodontitis-specific trials [33].

2.3.4. Challenges and future directions

Current limitations span microbial resistance (local/systemic antibiotics), transient efficacy of HMTs (Histone Methyltransferases), and biologics' cost-to-benefit imbalance. Future integration of multi-modal strategies—combining biofilm-disrupting agents, immunomodulators, and patient-specific microbiomic restoration—may optimize outcomes while addressing the dynamic interplay between dysbiosis and host immunity [31].

3. Development and application of probiotics

3.1. Criteria for probiotic selection and screening process

In the development of probiotics, selecting the appropriate strains is crucial. First and foremost, probiotics must exhibit safety, being capable of surviving and functioning within the host without causing adverse reactions. Additionally, the functionality of probiotics is a key criterion, including their benefits to host health, such as modulating the immune system, enhancing digestion, and inhibiting pathogenic microorganisms [34]. The current screening process for probiotics typically involves laboratory cultivation, in vitro assays, and animal model studies, followed by clinical trials to validate their efficacy and safety [35]. For example, *Streptococcus salivarius* M18 has been identified as an oral probiotic with a positive impact on oral health, as evidenced by its safety profile in clinical evaluations [36]. Overall, the selection criteria and screening process for probiotics must comprehensively consider factors such as safety, functionality, and clinical validation to ensure their effectiveness and safety in practical applications.

The broad-spectrum antimicrobial agents often indiscriminately eliminate both pathogens and beneficial commensals due to shared bacterial targets. For instance, periodontal pathogens like *Porphyromonas gingivalis* and oral probiotics such as *Streptococcus sanguinis* both rely on conserved metabolic pathways (e.g., peptidoglycan synthesis), making selective eradication challenging [37,38]. This non-specificity disrupts the oral microenvironment by reducing microbial diversity (over 700 bacterial species in healthy individuals [37] and enabling dysbiosis. Transient bacteremia caused by periodontal pathogens further exacerbates systemic risks, as oral bacteria like *Streptococcus* spp. can translocate into circulation even during routine activities like toothbrushing [38,39].

3.2. Role of probiotics in modulating the oral microbiome

Probiotics play a significant role in the regulation of the oral microbiome. The microbial community within the oral cavity directly influences oral health, with imbalances potentially leading to conditions such as dental caries and periodontal disease. Studies have shown that specific probiotics can inhibit the growth of harmful bacteria while promoting the activity of beneficial microbes, thereby maintaining the balance of the oral microbiota [4]. For instance, certain strains of *Lactic acid bacteria* and *Streptococci* have been demonstrated to suppress the growth of pathogenic bacteria and support the proliferation of beneficial microbes within the oral cavity [40]. Furthermore, probiotics can enhance the stability and diversity of the oral microbiome by producing antimicrobial substances and modulating the host immune response [41]. Thus, the application of probiotics presents a novel strategy for promoting oral health by maintaining a balanced and resilient microbiome.

3.3. Potential of probiotics in disease prevention and treatment

Probiotics have shown substantial potential in the prevention and treatment of various diseases. Emerging probiotics such as *Lactobacillus reuteri* MG5346 exhibit dual therapeutic effects by modulating host immunity and microbial ecology. Experimental studies demonstrated that *L. reuteri* MG5346 suppresses osteoclastogenesis via downregulating RANKL (Receptor Activator of Nuclear Factor-κ B Ligand)/OPG (osteoclastogenesis inhibitory factor) ratio (by 35 %) in periodontal tissues, thereby inhibiting alveolar bone resorption [42]. Although no significant improvement in bone loss was observed in rat models, its ability to reduce inflammatory biomarkers highlights its potential as an adjunct therapy for periodontitis.

Research indicates that probiotics can modulate the gut microbiota to reduce the risk of conditions such as inflammatory bowel disease and allergic disorders [43]. Clinical studies have validated the role of probiotics in improving gut health, alleviating inflammatory responses, and even aiding recovery in certain medical conditions [44]. Moreover, the potential of probiotics in cancer prevention and therapy has attracted increasing attention, with evidence suggesting that probiotics may inhibit tumor growth and progression by modulating the immune system and gut microbiota [45]. Collectively, the multifunctional roles of probiotics in disease prevention and treatment highlight their promising prospects in clinical applications.

3.4. Role of probiotics in anti-inflammatory and immune modulation

The anti-inflammatory and immune-modulatory effects of probiotics have garnered significant attention. Research suggests that probiotics (see Table 1) can regulate the host immune response through various mechanisms, including the activation of immune cells and the enhancement of anti-inflammatory cytokine production [46] by targeting the TLR4 (Toll Like Receptor 4)/MyD88 (Myeloid Differentiation factor 88)/NF-*κB* signaling axis. For instance, *Lactobacillus rhamnosus* GG-derived extracellular vesicles (LGG-EVs) suppress MyD88 phosphorylation and subsequent NF-κB nuclear translocation, thereby reducing NLRP3 (NOD-like receptor thermal protein domain associated protein 3) inflammasome activation and IL-1β secretion in colitis models [47]. Similarly, short-chain fatty acids (SCFAs) produced by Bifidobacterium longum inhibit histone deacetylase (HDAC), leading to enhanced acetylation of the TLR4 promoter and transcriptional downregulation [48]. These mechanisms collectively reduce pro-inflammatory cytokines (e.g., TNF-α, IL-6) and mitigate periodontal tissue damage.

For example, specific probiotic strains have been shown to reduce systemic inflammation by modulating the gut microbiota, thereby mitigating the risk of chronic diseases [49]. Additionally, probiotics can strengthen the host immune defense by improving gut barrier function and limiting the entry of harmful substances into the bloodstream [50]. They modulate immune responses through metabolite-mediated epigenetic reprogramming. Butyrate-producing strains like *Bifidobacterium longum* inhibit HDAC (Histone deacetylase) activity, enhancing histone acetylation at anti-inflammatory gene promoters (e.g., IL-10) while suppressing NF-κB p65 deacetylation to reduce TNF-α and IL-6 transcription. Concurrently, folate from *Lactobacillus reuteri* induces DNA hypomethylation at Treg-specific FoxP3 sites, amplifying JAK-STAT inhibitors'

Table 1
Summary of key probiotic strains, functional roles, screening criteria, and delivery strategies.

Probiotic Strain	Classification	Key Functions	Screening Criteria	Clinical Applications	Delivery Carrier	References
Streptococcus salivarius M18	Classical	Inhibits *P. gingivalis*, reduces dental plaque biofilm	Safety, colonization efficiency	Caries prevention, oral microbiome modulation	Mucoadhesive chitosan films	[36]
Lactobacillus rhamnosus GG	Classical	Suppresses TNF-α/IL-6, enhances mucosal barrier	Anti-inflammatory efficacy, gut stability	Periodontitis, IBD	Alginate-Ca^{2+} hydrogel microspheres	[91]
Lactic acid bacteria	Classical	Produces bacteriocins, inhibits *Tannerella forsythia*	Pathogen inhibition, acid tolerance	Oral dysbiosis correction	pH-sensitive pectin microgels	[40]
Bifidobacterium longum	Classical	Modulates gut-oral axis via SCFAs, reduces systemic inflammation	Immune modulation, acid resistance	Periodontitis-associated cardiovascular risks	Dual-network alginate/chitosan hydrogels	[9]
Lactobacillus reuteri	Classical	Secretes reuterin (antimicrobial), inhibits NF-κB	ROS scavenging, mucosal adhesion	Chronic periodontitis, gingival repair	Thermosensitive P407 in situ hydrogels	[92]
Streptococcus thermophilus	Emerging	Competes for adhesion sites, stabilizes oral pH	Adhesion capacity, pH tolerance	Prevention of oral biofilm formation	Electrosprayed cellulose nanofiber carriers	[35]
Bacillus subtilis	Emerging	Produces surfactins (anti-biofilm), degrades IL-1β	Biofilm disruption, thermostability	Peri-implantitis, deep periodontal pockets	Alginate-gelatin dual-network microspheres	[93]
Escherichia coli Nissle 1917	Emerging	Polarizes macrophages (M1→M2), scavenges ROS	Immune compatibility, ROS sensitivity	Severe periodontitis with oxidative stress	ROS-responsive alginate hydrogels	[94]
Faecalibacterium prausnitzii	Emerging	Produces anti-inflammatory butyrate, restores mucosal homeostasis	Butyrate yield, oxygen sensitivity	Periodontitis-linked systemic inflammation	Colon-targeted guar gum/SA microcapsules	[50]
Akkermansia muciniphila	Emerging	Enhances mucus layer integrity, regulates TLR4 signaling	Mucin degradation, immune tolerance	Metabolic syndrome-associated periodontitis	Light-triggered HA-methacrylate hydrogels	[66]

efficacy in curbing Th17 polarization. This metabolic-epigenetic crosstalk mirrors host-modulation therapies (e.g., SIRT1 activators like resveratrol) and fasting-mimicking diets (FMDs), where ketogenesis epigenetically primes anti-inflammatory pathways [23,31,32,51].

As a result, the application of probiotics in anti-inflammatory and immune modulation offers not only novel avenues for disease prevention and treatment but also critical insights into the intricate interactions between microbiota and host physiology.

4. Study of microenvironment-sensitive hydrogels

Hydrogels with three-dimensional networks of polymeric chain structures cross-linked via chemical or physical cross-linking methods [52] are widely used during different pharmaceutical studies because of their biodegradability and biocompatibility. Microenvironment-sensitive hydrogels possess a range of properties that not only safeguard drugs from physiological degradation but also enable prolonged action at the administration site and controlled drug release. By investigating the composition, types, and applications of these hydrogels, their potential in periodontitis treatment can be harnessed, thereby offering a more comprehensive therapeutic strategy.

4.1. Materials and its characteristics of microenvironment-sensitive hydrogels

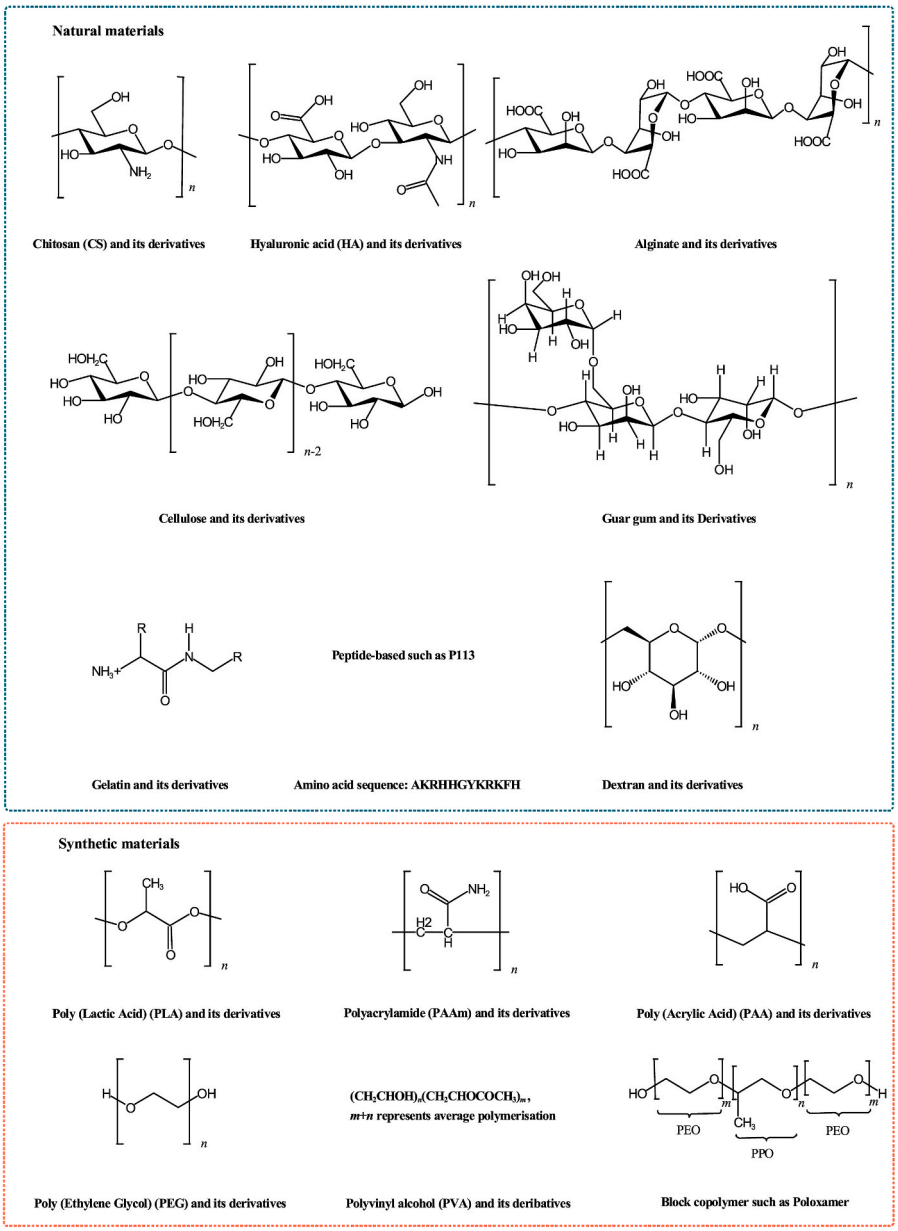
Commonly used materials are corresponding assembled as miscellaneous functional hydrogels by natural and synthetic biomaterials summarized in Fig. 3, and their basic structural formula are drawn. Significantly, these materials can not only be designed according to their raw types but also can be combined in situ cross-linking by chemistry and physical methods or the structure of some materials can be altered as needed to improve the strength of hydrogels [53,54]. Different microenvironment-sensitive hydrogel materials have different characteristics focusing on solubility, cross-linking sites and degree of cross-linking, cross-linking conditions, microenvironment-sensitive properties and stability, etc.

4.1.1. Natural materials

Chitosan (CS), Hyaluronic acid (HA) and Alginate are most commonly used for food, drug and cosmetic industries due to their natural biocompatibility and biodegradability.

4.1.1.1. Hyaluronic acid. HA is one of the most popular material as excellent fillers and lubricants for medical and aesthetic industry. Unmodified HA is easy to be degraded in clinical application with short half-time, needing frequent administration, therefore modifying hyaluronic acid is an important way to change its properties which also should take clinical translation into consideration [55]. HA is rich in physical and chemical properties, which has been manufactured by many companies using different cross-linking technologies to obtain different viscoelastic properties of hydrogels according to clinical necessity [56], but should not be modified so much degree as to resulting in biological immiscibility [57]. HA is a natural linear polysaccharide polymer with wide range molecular weight having different stickiness and viscoelasticity, which has biocompatibility, biodegradability, nontoxicity, and nonimmunogenicity properties [58]. The solubility of HA is dependent on pH value. It can be insoluble under acidic conditions and soluble under neutral to alkaline conditions [59]. According to the rigid or viscous requirements for hyaluronic acid in different dosing modes, we can modified HA by different cross-linking methods through its functional groups: hydroxyl group (-OH), carboxyl group (-COOH), and N-acetyl group (-$NHCOCH_3$) [55]. Hydrogels based on HA have demonstrated significant clinical efficacy in periodontitis management. A randomized controlled trial (RCT) revealed that local application of 0.8 % HA gel in peri-implantitis reduced probing pocket depth (PPD) by 1.8 mm and decreased IL-1β levels in gingival crevicular fluid by 42 % at 90 days post-treatment [60]. High-molecular-weight HA (>1 MDa) exerts anti-inflammatory effects by suppressing TLR4/NF-κB signaling, while its antioxidant properties counteract ROS-induced tissue damage [61]. These findings validate HA hydrogels as a clinically viable strategy for modulating periodontal inflammation.

4.1.1.2. Chitosan. CS is also widely used in pharmaceutical industries produced from deacetylation of chitin. Pure chitosan may have the disadvantages of low rigidity and low viscosity, but it can be modified to improve its pH-sensitivity, solubility and targetability through acylation, carboxylation, alkylation, quaternization [62], antioxidant and strength [63]. CS is insoluble in water and most organic solvents but can improve solubility through chemical modification such as hydroxyl group (-OH) and amino group (-NH_2), that is not influence the original properties of chitosan but on the contrary improve the chemical and physical properties [62]. It is temperature sensitive and can be designed to form a gel at body temperature [64,65] and metabolized by enzymes such as lysozyme of gut microorganisms [66]. The adhesion strength of microenvironment-sensitive hydrogels was quantified via tensile testing on ex vivo porcine buccal mucosa, revealing values of 5–6 N/cm^2 for



*m, n represents degree of polymerization; PEO: Polyoxyethylene; PPO: Polyoxypropylene

Fig. 3. Commonly used materials for microenvironment-sensitive hydrogels
*m, n represents degree of polymerization; PEO: Polyoxyethylene; PPO: Polyoxypropylene

xanthan gum (XTGM)-based formulations, significantly higher than Carbopol-based hydrogels (4.2 N/cm^2 [67]. In vivo retention time was validated using fluorescence imaging in murine periodontitis models, demonstrating ≥6-h mucosal adhesion under simulated masticatory forces [68]. These metrics correlate with clinical outcomes, as evidenced by reduced gingival bleeding index (GBI) and probing depth (PD) in a phase II trial of chitosan hydrogels co-loaded with probiotics [69].

4.1.1.3. Alginate. Alginate is a kind of smart material which is sensitive to ions and can self-assemble into microspheres under the action of ions. It is insoluble in water and organic solvents, but its monovalent salts and esters are soluble in water and the water solution is stable and viscous. It has the advantages of stomach acid resistance, non-toxicity, good biocompatibility and low cost [70]. But in simulated intestinal fluid (SIF), the structure of microspheres was looser and drug release was accelerated [71]. However, It can be modified by chemistry method and prepared into hydrogels, microspheres, fibers and sponges [72], which is a good choice to encapsulate drugs or probiotics. Moreover, there are manual and automatic [73] techniques for preparing hydrogel microspheres, which lays the foundation for the automated production of hydrogel microspheres.

Other natural materials also have been selected for drug delivering.

4.1.1.4. Dextran. Dextran is an extracellular polysaccharide produced from bacteria. It is essentially composed of α-1,6 linked D-glucopyranose residues with α-1,2, α-1,3, or α-1,4 linked side chains. It has many hydroxyl group (-OH) that can be dissolved in water easily and cross-linked through ester bonds which can be degraded by abundant human body

esterases [74].

4.1.1.5. Cellulose. Cellulose is consisted of D-glucose linked through glycosidic bonds with a lot of hydroxyl groups. It was modified through adding glycerol and glycol to improve its strength because of its high brittleness, low flexibility/stretchability, wfracture strength, poor mechanical elasticity, and limited optical clarity properties and can be dissolved and formed gel with the help of molten salt hydrates [75].

4.1.1.6. Gelatin. Gelatin is a protein-based macromolecule with week mechanical strength and poor water barrier which is dissolved by heat and agitation [76]. So cross-linking agents such as tannic acid and its oxidized form with biocompatible and nontoxic properties were used to enhance the strength of gelatin. Gelatin methacryloyl (GelMA) is made by chemical modification of gelatin with glycidyl methacrylate. As the raw material of microenvironment sensitive in-situ hydrogel, GelMA is widely used in various treatments [77].

4.1.1.7. Guar gum. Guar gum can be miscrible with water due to having a great many hydroxyl groups (-OH). It can be transformed from solution to gel by adding cross-linking agents such as sodium periodate (NaIO4) and borax through oxidizing hydroxyl groups into aldehyde groups or forming ester bond [78].

4.1.1.8. Peptides. Peptides are water soluble and can self-assemble to form gel through chemical (covalent bond) and physical (hydrogen bonding, hydrophobic forces, electrostatic attractions, orvan der Waals forces) cross-linking methods. For example, polyethylene glycol was used to covalently link with peptides to improve the stiff of hydrogels [79].

4.1.2. Synthetic materials

Except of natural materials of microenvironment-sensitive hydrogels, synthetic materials maybe more achievable, cheaper, stable and flexible designing. Commonly used poly-compounds such as Poly (Lactic Acid) (PLA), Polyacrylamide (PAAm), Poly (Acrylic Acid), Poly (Ethylene Glycol) (PEG), Polyvinyl Alcohol (PVA), Poluphosphazene (PPZ), Poly(organophosphazene), block copolymer (such as poloxamer 407/P407, poloxamer 188/P188) and their derivatives are the polymers of monomers of lactic acid, acrylamide, acrylic acid, ethylene glycol, vinyl alcohol, organophosphazene, and triblocking of polyoxyethylene and polyoxypropylene.

4.1.2.1. PLA. PLA is a biocompatible, biodegradable, and non-toxic polymer, however, its low melt strength, slow crystallisation, poor processability, high brittleness, low toughness, and low service temperature have limited its applications [80]. The formation of new polymers, such as PLGA (Poly(lactic-co-glycolic acid) [81] and PEG-PLGA [82], by combining with other structural fragments, have enabled the development of polymers with different performances, including thermosensitive properties.

4.1.2.2. PAAm. PAAm is also biocompatible and biodegradable, and can be designed with different structures to form a number of polymers, such as N-isopropyl acrylamide (NIPAm) and acrylamide (AAm) as copolymer monomers allows the preparation of a bis pH- and thermosensitive copolymer hydrogel (HG) system (PNIPAm-co-PAAm HG), which is both thermo-sensitive and pH-sensitive [83]. The hydrogel formed by PAA is a rigid and brittle, tightly connectable three-dimensional network structure. Sodium alginate (SA) is a soft and ductile polymer, PAA and SA were combined by cross-linking agent to form a dual network hydrogel network structure with PAA in the first layer and SA in the second layer, which was prepared as a pH- and temperature-sensitive hydrogel by Ghasem Rezanejade Bardajee and co-workers [84].

4.1.2.3. PEG. PEG is a synthetic polymer that exhibits hydrophilic, biocompatible, and low immunogenic properties [85]. Its structure contains hydroxyl groups that can be linked to a range of functional groups, enabling the alteration of its hydrogel rigidity. PEG is a widely utilized compound in the fields of pharmaceuticals and biology [86].

4.1.2.4. PVA. PVA is a non-toxic, biocompatible, thermally stable, highly hydrophilic polymer with excellent mechanical properties. It is easily processed and can be used to optimize the rigidity of hydrogels formed from natural polymeric materials such as CS [87].

4.1.2.5. Poloxamer. Poloxamer possesses biocompatibility and good hydrophilicity [88]. According to the varying number of its block structure, a variety of products have been produced. Among them, P407 and P188 are more widely utilized in either single or combined applications, and they are the relative highly commonly used temperature-sensitive hydrogel materials. P407 and P188 are dissolved with water under cold and room temperature, respectively.

These commonly used hydrogel materials possess distinct advantages and limitations. The limitations can be mitigated through physical or chemical modification methods. Many researchers, while modifying a single material, also consider combining multiple materials to leverage their respective strengths and optimize their weaknesses. Materials such as HA, CS, alginate, PLGA, PEG, and others have been utilized clinically. These hydrogels have demonstrated remarkable efficacy in basic research. Numerous studies have leveraged the benefits of hydrogels, highlighting their potential. Therefore, in the development of hydrogel products, a critical factor for promoting their clinical application maybe is to establish a robust production process that ensures consistent product quality and efficacy.

4.2. Types of microenvironment-sensitive hydrogels

Microenvironment-sensitive hydrogels can be classified into a lot of types such as pH-sensitive, ROS-sensitive, thermosensitive, NO-sensitive, enzyme-sensitive, ultrasound-sensitive, magnetic-sensitive, light-sensitive and multifactor-sensitive smart hydrogels. The typical types and materials of different smart hydrogels and the conditions for the transformation of the phase that drives the gel to occur are described in Table 2. For example, Yuting Zhang et al. 2023 presented a pH- and temperature-sensitive hydrogel that made from bamboo parenchymal cellulose and in situ modified with β-cyclodextrin through DMAc (Dimethylacetamide)/LiCl (Lithium chloride) dissolution system in wounding healing filed. Besides, except of obvirously microenvironment-sensitive hydrogel, an in situ forming, bio-orthogonally cross-linked, nanocluster (NC)-reinforced collagen and hyaluronic acid hydrogel (NCColHA hydrogel) with enhanced structural integrity and both pro-regenerative and anti-inflammatory effects was invented by Nae-Won Kang et al. 2024 [89]. Basma Ibrahiem et al. 2024 innovatively investigated a thermosensitive in situ forming hydrogel loaded with superparamagnetic iron oxide nanoparticles (SPIONs) for intra-muscular (IM) administration to improve joint targeting and localization by applying an external magnet to the joint [90].

4.3. Application of microenvironment-sensitive hydrogels in inflammatory diseases

In situ microenvironment-sensitive hydrogels play an important role in drug delivery for different diseases. The process of gel formation is that drug assembled into hydrogels administered as a liquid and transformed into a soft gel through touching microenvironment, which have the ability to form various sizes of hydrogels according to the shape of the diseased tissue cavity. Conversely, hydrogels are also degraded by the microenvironment in the body. The characteristics of these hydrogels are enhancing local bioavailability, reducing dose concentration,

Table 2
Examples of main types of microenvironment-sensitive hydrogel and their materials.

Types	Natural materials	Synthetic materials	Phase transition condition	References
Forming gel when mixed	Hyaluronic acid	Alkyne	Mixed	[89]
pH-sensitive hydrogels	Hyaluronic acid; Humic acids	/	Acidity and alkalinity	[59]
ROS-sensitive hydrogels	/	PVA	ROS-responsive borate ester bonds broken when ROS level increased	[95]
NO-sensitive hydrogels	/	Polyacrylamide	Nitrogen-oxygen bond broken by NO-cleavable cross-linker	[96]
Enzyme-sensitive hydrogels	/	Poly(lactic-co-glycolic acid) (PLGA)	RA-related enzymes	[97]
Thermosensitive hydrogels	/	Poloxamer 407	37 °C	[98]
Ultrasound-sensitive hydrogels	/	Polyacrylamide	Local fluidic vortices	[99]
Magnetic-sensitive hydrogels	Cationic guar gum	β-cyclodextrin	Magnetic field	[100]
Light-sensitive hydrogels	Hyaluronic acid	Cyclic o-nitrobenzyl-sulfide phototriggers	Photo triggered S-nitrosylation coupling reaction	[101]
Ion-sensitive	Peptide V1-Cal	/	Exogenous saline such as PBS, cell culture mediumor tear fluid	[102]
pH- and temperature-sensitive	Bamboo raw materials	β-cyclodextrin	Releasing faster when temperature and pH decreased from 32 to 40 °C and pH1.5~7.4	[103]
Temperature- and Magnetic-sensitive	/	Superparamagnetic iron oxide nanoparticles assembled into Synperonic™ PE/F 127 (PE/F127) hydrogel	External magnet and temperature was 37 °C	[90]

decreasing administration frequency, ensuring uniform dispersion and accurate control of the material's properties and reducing side effects [104–107]. Given these advantages in situ hydrogels have drawn attention to treating a lot of diseases especially focusing on anti-inflammatory. The examples of microenvironment-sensitive hydrogels loading anti-inflammatory drugs for treating typical inflammatory diseases are summarized in Table 3. As will be readily seen that hydrogels could be designed with flexibility as needed because of their different properties of inflammatory microenvironment at the disease site with the commonly purpose of increasing adhesion and prolonging duration time to improve bioavailability. Throughout all studies, it is amazing and excellent that these smart hydrogels can load small molecule (such as ciprofloxacin), biomolecule (such as antigen), inorganic metal nanoparticles (such as $Cu_{2-x}Se$ nanoparticles), probiotics (such as *Lactobacillus rhamnosus*), and cells (such as stem cells [64]), etc. Therefore, these smart hydrogels can be used as a sole delivery vehicle or as a bearer of other delivery vectors to target therapeutic agents well to the site of inflammation.

Table 3
Examples application of microenvironment-sensitive hydrogels in some disease.

Disease	Type of hydrogel	Loading drugs	References
Dry eye disease	TRPV1-targeted ion-responsive	KFQ12	[102]
	Gel formation when schiff-base bonds formed through reaction of aldehyde groups of F127 and pending amine groups of proteins	$Cu_{2-x}Se$ nanoparticles	[108]
Otitis media	Temperature-sensitive	Ciprofloxacin	[109]
	Temperature-sensitive	Anti-bacterial components	[110]
Periodontitis	ROS-responsive	Doxycycline/ Metformin	[111]
	pH-responsive	Macrophage membrane loaded with metronidazole	[112]
Hepatitis B	pH-sensitive	Hepatitis B antigen	[113]
	Gel formation when mixed	Ovalbumin and hepatitis B surface antigen	[114]
Osteoarthritis	Light-sensitive	Rapamycin	[115]
	Temperature-sensitive	Piezoelectric short nanofibers of poly-L-lactic acid	[116]
Wound healing	Temperature-sensitive	Nicotinamide mononucleotide	[117]
	ROS-sensitive	Tea polyphenol silver nanoparticles	[118]
	ROS-sensitive	Platinum	[119]
	ROS-sensitive	Platinum-deposited epigallocatechin-3-gallate	[120]
	pH- and Temperature -sensitive	Berberine	[103]
Wound infections	pH- and ROS-sensitive	*Lactobacillus rhamnosus*	[121]

5. Study of microenvironment-sensitive hydrogel loaded probiotics

Probiotics are often prepared as probiotic agents because of their capacity for regulation of dysbiosis in human body, can treat some disease such as cancer and inflammatory diseases. Key probiotic strains have been introduced in Table 1. The structures in question exhibit a variety of forms, which can be broadly classified into two main categories: rod-shaped (with long and short rods) and spherical. However, oxygen, temperature, gastric acid, bile salts, and digestive enzymes could influence the activity of these probiotics in different degree. Therefore, pharmaceutical technologies should be designed to protect probiotics.

5.1. Microenvironmental hydrogel encapsulated probiotics

A variety of microenvironment-sensitive hydrogels and their wide range of applications in inflammatory therapies have been summarized previously, which can encapsulate small molecules or macromolecules, organic or inorganic substances, chemical or biological drugs and active probiotics, etc. Moreover, these hydrogels can be prepared in forms capable of topical, oral, injectable and implantable application, etc, and the dosage can be arbitrarily adjusted as required, which is one of the reasons for its wide application. Based on technologies for loading probiotics, the structure and composition of the various types of hydrogel formulations are analyzed and summarized.

5.1.1. In situ microenvironment-sensitive hydrogel loaded probiotics

In situ microenvironment-sensitive hydrogels can be designed

flexibility according to the pH, ROS, temperature and enzymes, etc of body microenvironment, and also can cross-link with the help of external means such as light, magnetism, ultrasound, and temperature, etc. These factors may trigger the properties of in situ gels to undergo a sol-gel transition. Probiotics existing in a three-dimensional network structure can remain viable and be protected from damage by acid, base, enzyme, and high temperature. Coincidentally, these factors can also form hydrogels or destroy them. It is a robust physical barrier to protect probiotics from environmental hazards and could be slowly released as required. It can be classified into single-responsive, dual-responsive and multi-responsive hydrogels according to responsive factors.

Single-responsive hydrogels are where the formation of a hydrogel is due to a single factor. Probiotics are encapsulated directly by one-step and forming gel through microenvironment factors such as endogenous factors (pH, ROS, temperature, ion, and enzyme) and exogenous factors (light, ultrasound, and magnetic). For example, Huizhen Zheng et al. 2017 developed a pH-responsive hydrogel to load *Lactobacillus rhamnosus* ATCC 53103 that were assembled with ethylenediaminetetraacetic, calcium, and alginate, and it would gel when the pH was below 4.0 (Fig. 4. A). P407 is one of the commonly used temperature-sensitive hydrogel material with "sol-gel" transformation property for delivering drugs. Its gelling time and viscosity are proportional to concentration and temperature. Aoxing Chen et al. 2024 encapsulated *Lactococcus lactis* by P407 solution at 25 °C, gelation at 37 °C (Fig. 4. B). HA modified with methacrylic anhydride and cross-linked with thiolated thioketal for gelation with 37 °C, then the hydrogel slowly degraded with the increase of ROS at the inflammatory site because of its ROS-responsive property, and the probiotics were slowly released, which was studied by Lijie Huang et al. 2022 (Fig. 4. C). Alginate is a ion-sensitive hydrogel. Xue Dou et al. 2023 introduced a sturdy calcium alginate hydrogel cross-linked with Ca^{2+} to deliver *Lactobacillus rhamnosus* and a marine prebiotic fucoidan (Fig. 4. D). *Lactobacillus paracasei* was added in hyaluronic acid methacrylate (obtained by reacting HA with methacrylic anhydride) and this material cross-linked extracellular polysaccharides via hydrogen bonding and triggered forming hydrogel with 405 nm blue light (Fig. 4. E).

Dual-responsive hydrogel is a more prevalent form of hydrogel, which exploits the property that the hydrogel is sensitive to two response factors, such as pH/ROS and pH/temperature, etc. The strength of the hydrogel can be enhanced to fulfill the purpose of protecting and slowly releasing drugs according to different environment. Yu Miao et al. 2024 obtained a pH/ROS dual-responsive hydrogel. It modified the natural polysaccharide SA through forming a boric acid ester bond. This modification introduced a cross-linker synthesized by a boric acid molecule from succinic acid and 4 -(bromomethyl)phenylboronic acid. The hydrogel is injectable, exhibits self-healing properties and undergoes swelling, and displays pH and ROS responsiveness (Fig. 4. F).

Designing multiple responsive hydrogels for probiotic encapsulation is also a good strategy, but there are fewer studies of this type, although many researchers have designed encapsulation of other drugs that can be used as a reference for probiotic encapsulation. For example, Fanjia Dai and co-workers designed a pH/ROS/glucose/photothermal multi-responsive hydrogel to load insulin-like growth factor 1 C domain (IGF-1C) and deferoxamine-loaded polydopamine nanoparticles by the materials of phenylboronic acid-modified carboxymethyl CS and oxide dextran (Fig. 4. G). Qi Zhou and co-workers produced a temperature/pH/salt/redox/glucose/light multi-responsive hydrogel through mixing phenylboronic acid-modified gelatin (GA-DBA), catechol-modified carboxymethyl chitosan (CCS-PCA), 3,5-dinitrosalicylic acid (DNSA), and Eu^{3+} ions (Fig. 4. H).

5.1.2. Hydrogel microspheres

Alginate, as the commonly utilized material, can form single-network or dual-network in combination with other microenvironment-sensitive materials such as pectin. However, it is typically present in the form of its sodium or calcium salt, and can be chemically cross-linked with other reagents to yield materials with enhanced properties. Furthermore, it can also be enveloped through alternative preparation methods, ensuring better protection for probiotics. It can be employed both for forming gel 3D network structures and as a surgical procedure for gel microspheres, demonstrating a wide range of application capabilities. In recent years, microencapsulation and nanoparticles can encapsulate probiotics with good encapsulation rates. Alginate, CS and cellulose are commonly used for encapsulating probiotics using extrusion, emulsion, spray drying, freeze drying, impinging aerosol, emulsification, electrospinning, electrospraying and electrohydrodynamics [122,129–133].

Hydrogel microspheres with a single-layer network structure are formed by using a hydrogel material, undergoing structural modification and cross-linking to create microspherical hydrogels that protect probiotics or other drugs. Based on the characteristics of the materials, their degradation ability in different in vivo environments can be designed according to actual needs. For example, pectin through methyl esterification cross-linked with Ca^{2+} to form a single-layer microsphere can encapsulate *Lactiplantibacillus plantarum* and have different release rates depending on various in vivo pH values [134]. In a study by Timothy W. Yeung et al. 2016, the probiotics were thoroughly mixed with the alginate solution and subsequently introduced into the calcium chloride solution. In the presence of Ca^{2+}, calcium alginate self-assembled, forming hydrogel microspheres that encapsulated the probiotics (Fig. 5. I).

In order to enhance the strength and viscosity of hydrogels and to avoid probiotic damage by the in vivo environment, multifactorial hydrogels are often designed. Dual-network hydrogel, as the name implies, provides the probiotic with two layers of protection to enhance the protection of probiotics. For instance, Wen-Can Huang et al. 2024 designed a double-layer hydrogel. The inner layer was carboxymethyl cellulose supramolecular, the outer layer was a dialdehyde alginate cross-linked carboxymethyl chitosan. This double-layer hydrogel can protect probiotics from gastric environment (Fig. 5. II). Shuxin Wang et al. 2024 developed a thiolated oxidized guar gum (first network, disulfide-linked)/SA (second network, cross-linked with Ca^{2+}) dual cross-linked microspheres with intestinal-targeted and mucoadhe sive properties for targeted delivery of *Lactobacillus rhamnosus* GG (LGG), and LGG was existed in the interpenetrating dual-network microspheres for good protection [143]. Alginate and gelatin formed dual-network structure intersecting hydrogel through electrostatic reaction and hydrogen bonding and encapsulated *L. plantarum* by self-assembled microspheres with Ca^{2+}, which was designed by Fangfang Ni et al. 2023 (Fig. 5. III). Haritha Asokan-Sheeja et al. 2024 enhanced the robustness of peptide-based bydrogel through adding a second hydrogel network via PEG [79].

The combination of hydrogels with other dosage forms is also an excellent approach for protecting probiotics. For example, LGG was encapsulated in water-oil-water double emulsion with liquid compatibility, small droplet size, ease of handling and efficient production and the emulsion decorated in sodium alginate (SA)-carboxymethyl chitosan (CMCS) hydrogel shells which were cross-linked by hydrogen bonding. The multi-layer formulation hydrogel was designed by Yi Li et al. 2024 (Fig. 5. IV). Another novel multi-layer formulation hydrogel was invented by Xiaoqing Ding et al. 2022. Ding used carboxymethyl konjac glucomannan with positively charged CS particles self-assembled through intermolecular electrostatic interaction to form nanogel to stabilize water-oil-water double emulsion which encapsulated *Lactobacillus reuteri*, and then wrapped in alginate hydrogel. Except of combination of hydrogel and other dosage forms, hydrogel can also construct multi-layer hydrogel for probiotics delivering (Fig. 5. V). Alginate solution usually formed hydrogel under adding Ca^{2+}, so the first step was to encapsulate probiotics with alginate cross-linking by Ca^{2+}, and then isolated through the oil phase to obtain hydrogel-(oil-hydrogel-)$_n$ multi-layer hydrogel, which was a good favorable biological carrier to deliver probiotics. The porous internal structure and sturdy shell contribute to

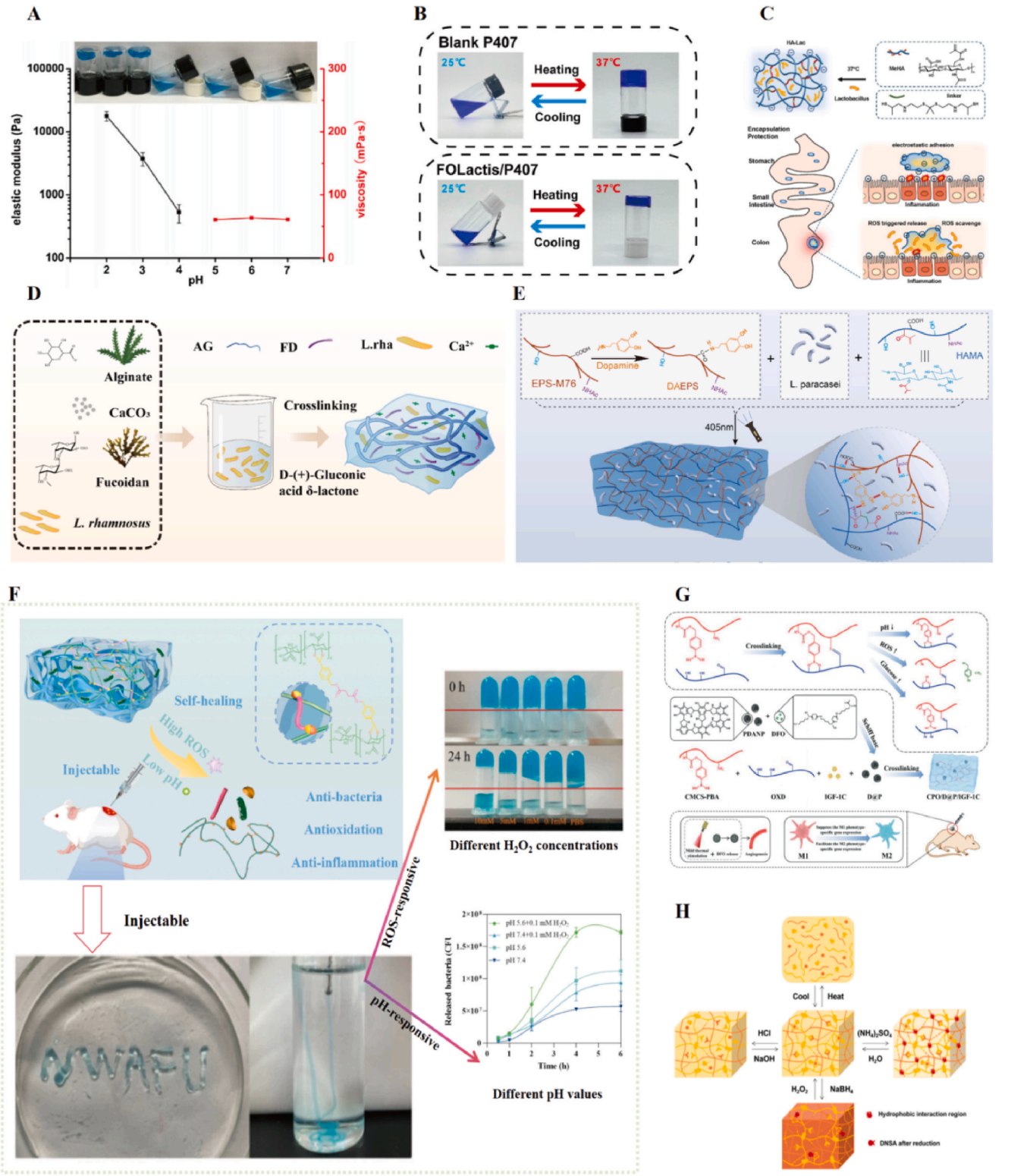


Fig. 4. Diagram of in situ hydrogels. A) Hydrogel formation at pH below 4.0, while a solution at pH 5.0 to pH 7.0. The elastic modulus (left) and viscosity (right) of the hydrogel are also shown. Reproduced with permission [122]. Copyright 2017, Elsevier. B) Hydrogel formation at 37 °C, while being a solution after cooling. Reproduced with permission [123]. Copyright 2024, Elsevier. C) Hydrogel formation at 37 °C, and probiotics were released from the hydrogel in the presence of ROS. Reproduced with permission [124]. Copyright 2022, Elsevier. D) Hydrogel formation at crosslinking with Ca^{2+}. Reproduced with permission [125]. Copyright 2023, Elsevier. E) Hydrogel formation with 405 nm blue light. Reproduced with permission [126]. Copyright 2024, Elsevier. F) Dual-responsive hydrogel with pH and ROS responsive properties. Reproduced with permission [121]. Copyright 2024, Elsevier. G) Multi-responsive hydrogel with pH, ROS, glucose and photothermal responsive properties. Reproduced with permission [127]. Copyright 2024, John Wiley and Sons. H) Multi-responsive hydrogel with temperature, acid/alkali, different ions responsive properties. Reproduced with permission [128]. Copyright 2020, American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

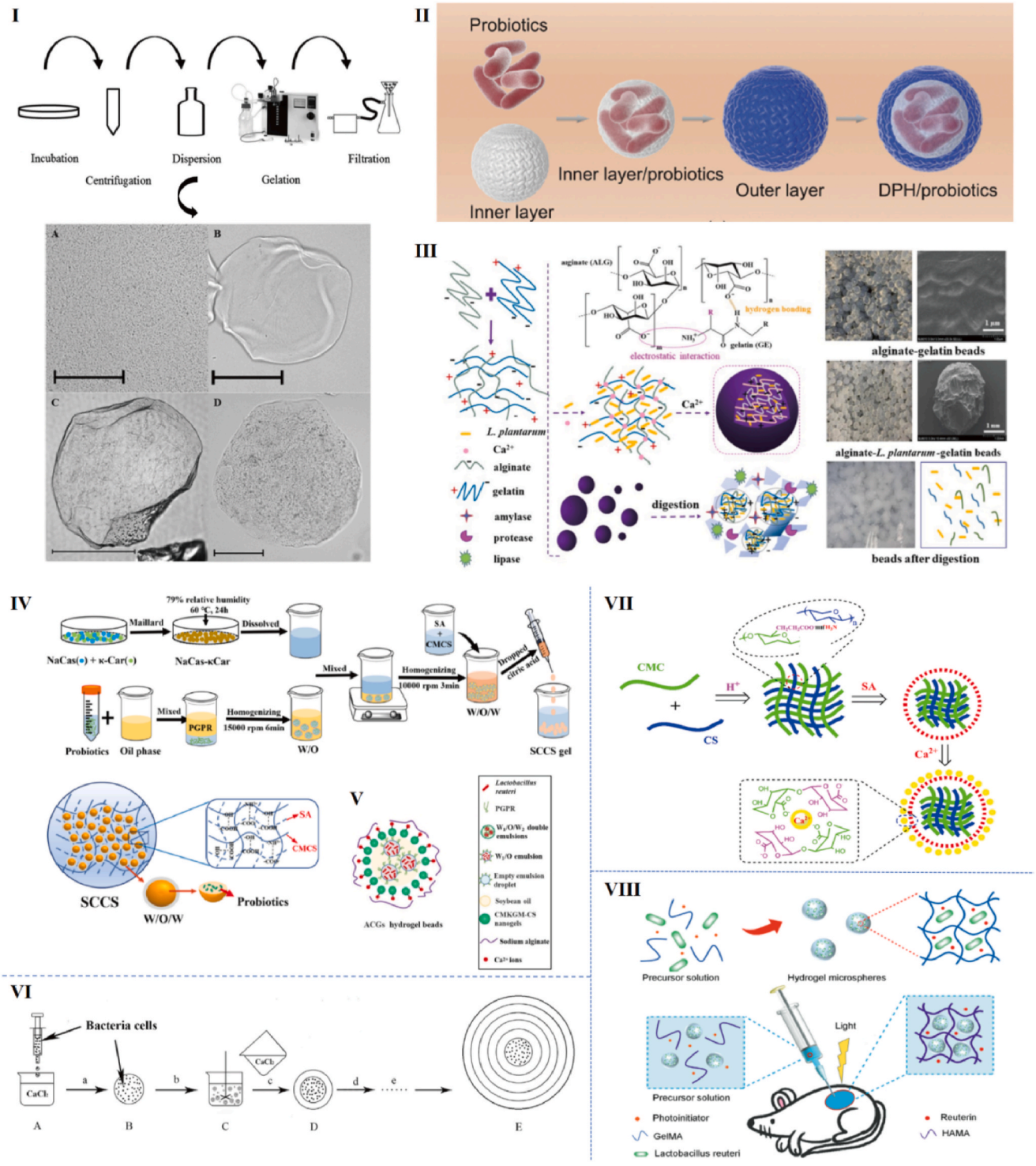


Fig. 5. Diagram of examples of hydrogel microspheres. I) Single-network hydrogel microspheres formation when alginate contacts Ca^{2+}. Reproduced with permission [135]. Copyright 2016, Royal Society of Chemistry. II) Dual-network hydrogel microspheres composed of carboxymethyl cellulose supramolecular inner layer and a dialdehyde alginate cross-linked carboxymethyl chitosan outer layer. Reproduced with permission [136]. Copyright 2023, Elsevier. III) Dual-network hydrogel microspheres assembled by alginate and gelatin forming structure of a cross-linked hydrogel network. Reproduced with permission [137]. Copyright 2023, Elsevier. IV) Multi-layered formulation consisted of W/O/W emulsions encapsulated probiotics and dispersed in sodium alginate-carboxymethyl chitosan hydrogel shells. Reproduced with permission [138]. Copyright 2024, Elsevier. V) Multi-layered formulation consisted of W/O/W emulsions encapsulated probiotics and protected by CMKGM-CS nanogel cross-linked with sodium alginate hydrogel network. Reproduced with permission [139]. Copyright 2022, Elsevier. VI) Multi-layered formulation formed by layer-by-layer cross-linking of alginate with Ca^{2+}. Reproduced with permission [140]. Copyright 2017, Elsevier. VII) Multi-layered formulation, probiotics first dispersed in a dual-network hydrogel and both encapsulated in calcium alginate hydrogel microspheres. Reproduced with permission [141]. Copyright 2021, Elsevier. VIII) Multi-layered formulation, probiotics were encapsulated by hydrogel microspheres and dispersed in in-situ light-responsive hydrogel. Reproduced with permission [142]. Copyright 2021, John Wiley and Sons.

enhancing probiotic activity and facilitating controlled release (Fig. 5. VI). Mengmeng Wang et al. 2021 grouped carboxymethyl cellulose (CMC), CS, and sodium alginate (SA) to load *Bacillus subtilis*. CMC and CS were utilized for loading *Bacillus subtilis* by constructing a dual-network hydrogel, and then encapsulated by SA hydrogel beads cross-linked with Ca^{2+}. It exhibited pH-sensitive release in different pH environments (Fig. 5. VII). Another interesting microsphere was using calcium alginate forming hydrogel microsphere cross-linked by Ca^{2+} to deliver LGG, this was the robust microsphere shells, and the probiotics were coated by double-layer CS and tannic acid [144]. In situ microenvironment-sensitive hydrogels incorporate hydrogel microspheres that can both protect the hydrogel and undergo sol-gel transformation in situ with injectability. Zunzhen Ming and co-workers developed a light-responsive hydrogel carrying hydrogel microspheres and hydrogel microspheres encapsulated *Lactobacillus reuteri*. *Lactobacillus reuteri* was firstly encapsulated by the covalent cross-linking of methacrylated gelatin solution containing living bacteria through emulsion polymerization. And then mixed both microspheres containing probiotics and HA hydrogels modified with methacrylate. The HA hydrogels have light sensitivity (Fig. 5. VIII).

5.2. Microenvironment-sensitive hydrogel co-loaded probiotics and anti-inflammatory drugs

Probiotics, as a class of beneficial bacteria, have been shown to prevent and treat diseases through anti-inflammation and immunomodulation. By co-assembling probiotics and anti-inflammatory drugs into a hydrogel, synergistic treatment and enhanced anti-inflammatory effect can be achieved by targeting and slowly releasing to treat inflammation through the sensitivity of the hydrogel to the microenvironment. Importantly, the microenvironment-sensitive hydrogels and hydrogel microspheres all can be applied for delivering probiotics and anti-inflammatory drugs previously. Researchers have found a number of hydrogels loaded with probiotics or probiotics together with anti-inflammatory drugs through chemical or physical methods to treat diseases more efficient.

Jagtar Singh et al. 2024 invented a capsule loaded with ion-sensitive hydrogel beads which loaded with both probiotic and anti-inflammatory drug by taking steps: 1) Sodium alginate polymeric solution was prepared. 2) Mesalamine and *B. bifidum* cell suspension were co-encapsulated in polymeric solution respectively. 3) Using 22G syringe

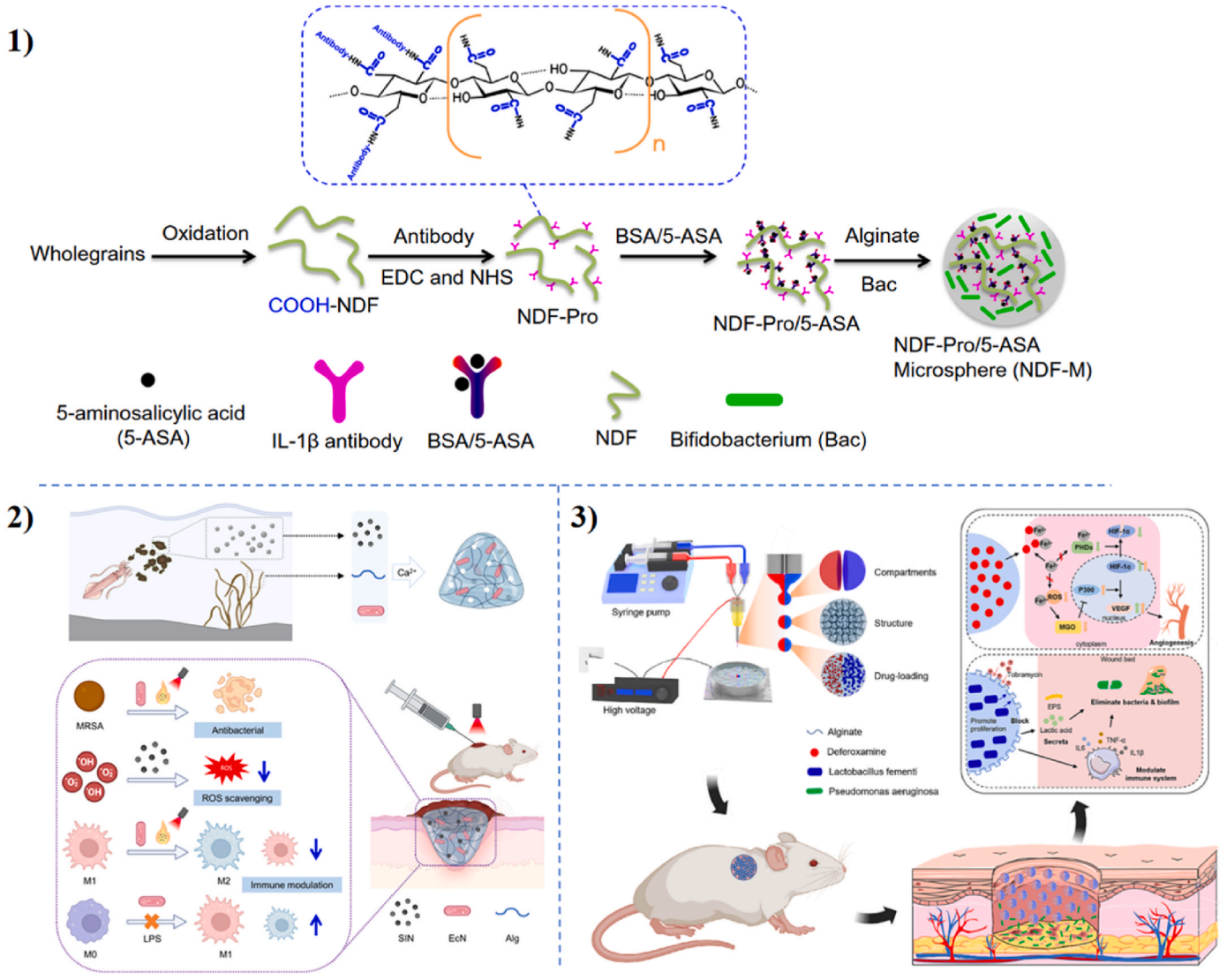


Fig. 6. Examples of co-encapsulated probiotics and anti-inflammatory drugs in a hydrogel to treat inflammatory diseases. 1) 5-ASA and Bac were co-encapsulated in Alginate hydrogel microsphere. Reproduced with permission [146]. Copyright 2023, Journal of Nanobiotechnology. 2) Squid ink nanoparticles and Escherichia coli nissle were co-dispersed in Ca^{2+}-crosslinked alginate hydrogel. Reproduced with permission [94]. Copyright 2024, Elsevier. 3) *Lactobacillus fermentum* and deferoxamine were encapsulated in different areas. Reproduced with permission [147]. Copyright 2024, Elsevier.

to drop 2) solution into crosslinking agents calcium chloride (2 %), chitosan (0.5 %), or dual-crosslinking solutions to form the hydrogel beads by external gelation. 4) Putting the hydrogel beads into capsule shells which can target colon successfully because of its pH-sensitive. In this study, the authors had to consider not only how to maintain the activity of *B. bifidum* but also target the hydrogel to the colon [145]. Lei Qiu et al. 2023 designed an alginate hydrogel microsphere encapsulating *bifidobacterium* and drug (5-aminosalicylic acid)-modified nanoscale dietary fibers with the help of an electrostatic droplet generator, which can protect both drugs from acidic and multi-enzymatic environments [Fig. 6.1).] Yutong Cui et al. 2024 innovated an interesting Ca^{2+} cross-linked alginate hydrogel co-loading probiotics *Escherichia coli* nissle1917 (EcN) and squid ink nanoparticles (SIN) with excellent biocompatibility, photothermal antibacterial activity, and reactive oxygen species (ROS) scavenging properties. Importantly, the SIN's photothermal ability was strengthened by *Escherichia coli* nissle to promote immune regulatory activities, shifting pro-inflammatory macrophages (M1) to anti-inflammatory macrophages (M2) [Fig. 6. 2).]

What is very flexible is that, in addition to the even distribution of drugs in the hydrogel network, the hydrogel can also be partitioned and different drugs can be loaded in different areas, which also provides good protection of the drugs and probiotics from each other. For example, multiple-chambered microgels were designed by Chao Hua and co-workers using electrospray and microfluidics for accelerating wound healing. *Lactobacillus fermentum* and deferoxamine were encapsulated in different areas [Fig. 6. 3).]

5.3. Stability of encapsulated probiotics

Maintaining the activity of probiotics during the preparation process is crucial for ensuring their subsequent inclusion capacity, as well as accurately calculating the encapsulation efficiency. It is well established that probiotics exhibit sensitivity and instability under conditions of temperature fluctuation, strong acids, strong bases, strong oxidizing agents, etc. As shown in Table 4, commonly preparation techniques for hydrogels, such as emulsification, freeze-drying, microcapsule granulator, high-speed centrifugation or automation technologies have no obvious influence on stability of probiotics. The storagy stability demonstrated that the survival time of probiotics was shorter at high temperature and longer at low temperature. All of researchers wanted to desighed a stable delivery system to protect encapsulated probiotics from gastrointestinal environment, the results showed that the survival rate of probiotics can be significantly improved under the protection of the designed hydrogel delivery system. Due to the complexity of the gastrointestinal environment, which involves factors such as acidity, alkalinity, oxidation, and enzymatic activity, this provides valuable insights for the treatment of periodontitis or other disease.

In summary, in situ hydrogels and hydrogel microspheres encapsulating probiotics can be tailored to specific delivery sites and objectives. These delivery systems are developed to preserve the viability of probiotics, primarily by optimizing storage conditions and administration site environment stability.

5.4. Molecular mechanisms linking probiotic-hydrogel synergy to clinical outcomes

The therapeutic efficacy of probiotic-hydrogel combinations relies on their dual modulation of microbial dysbiosis and host immune responses: TLR4/MyD88/NF-κB pathway suppression: probiotics (e.g., *L. acidophilus*) reduce LPS levels in periodontal pockets, inhibiting TLR4 activation and downstream NF-κB nuclear translocation [150]. Hydrogels co-loaded with NF-κB inhibitors (e.g., BAY11-7082) further enhance this effect by blocking IκB degradation [151].

NLRP3 Inflammasome Inhibition: Probiotic metabolites (e.g., butyrate) directly suppress NLRP3 assembly and caspase-1 activation, while ROS-sensitive hydrogels release antioxidants (e.g., quercetin) to neutralize oxidative stress [152].

Microbiome-Immune Crosstalk: Hydrogels encapsulating Akkermansia muciniphila restore mucosal barrier integrity via TLR4/TLR2 balance regulation, reducing systemic inflammation in comorbid periodontitis and diabetes [153].

6. Study of characterization and mechanical properties of microenvironment-sensitive hydrogels

The investigation into the characterization and mechanical properties of hydrogels constitutes a critical aspect of assessing property and structural changes in hydrogels under physiological conditions. Due to the flexibility and variability of hydrogels, diverse design strategies can yield distinct hydrogel formulations. Consequently, evaluating their apparent and mechanical properties during in vivo testing becomes paramount. This paper summarizes the methodologies (as shown in

Table 4
Stability of hydrogel encapsulated probiotics using different technologies.

Encapsulation technologies	Possible destructive conditions	Stability of the final product	Encapsulation efficiency (%)	Survival rate (%) in gastrointestinal tract	References
Probiotics are simply mixed with a hydrogel solution	No	Stable at 4 °C 28d	91.02–94.63	84.40–90.30	[148]
Emulsification/internal gelation technique	Stirring strength	Stable under acidic conditions	90.00	94.60	[122]
Inverse emulsion method	Stirring strength	Not mentioned	88.67	Higher than unencapsulated	[143]
Emulsions technology, extrusion	Homogenization intensity, extrusion force and microsphere diameter	Stable at 60 °C 30min and 72 °C 15s	Not mentioned	90.69	[138]
Microcapsule granulator, freeze-drying	Squeezing power and automation intensity	Higher probiotic survival at 4 °C for 2 weeks	95.50	Higher than unencapsulated	[149]
Electrospinning	Automation intensity	Stable at 4 °C 3 months	89.06–91.30	Not mentioned	[131]
Coaxial wet electrospraying, freeze-drying	Automation intensity	Stable at −18 °C 5 months	80.06–93.77	86.46–92.06	[129]
Spray drying	High temperature (120 °C)	Not mentioned	Before modified by different degrees of N-succinylation is 40.84 %, after modified is 57.96 %	Higher than unencapsulated	[130]
Impinging aerosol, freeze-drying	Flow rate, spray angle, droplet size, spray pattern, and alginate and $CaCl_2$ concentrations	Not mentioned	48.61–97.00	Not mentioned	[132]
Electrohydrodynamics	High-voltage electric field, shear force, osmotic and oxidative stress	Not mentioned	92.98–96.50	Higher than unencapsulated	[133]

Table 5) for characterizing and assessing the mechanical properties of hydrogels, providing valuable methods in periodontitis.

7. Discussion of release mechanism of microenvironment-sensitive hydrogels

Regardless of the mechanical strength of the prepared hydrogel, its ultimate objective is to deliver and release the drug at the site of disease. As illustrated by the aforementioned examples, hydrogels possess a three-dimensional network structure where drugs or probiotics are encapsulated within the pores. For pH-sensitive hydrogels, the robustness of the network structure varies under different pH conditions, with physiological pH being a critical factor in controlling the release behavior. For temperature-sensitive hydrogels, temperature significantly influences gel strength. For instance, a specific concentration of P407 readily forms hydrogels at elevated temperatures, but below the gelling temperature, intermolecular interactions weaken, leading to changes in the gel state that are not linearly proportional to temperature fluctuations. For ROS-sensitive hydrogels, higher concentrations of ROS accelerate drug release, while lower ROS levels decelerate it. In the case of hydrogel microspheres, water absorption and expansion increase pore size, facilitating drug release. The microenvironment-sensitive hydrogel achieves spatiotemporal drug release through dual pH/ROS responsiveness. For example, carboxymethyl chitosan (CMCS)-based hydrogels swell rapidly in acidic periodontal pockets (pH 5.5–6.5), releasing metronidazole to suppress pathogenic biofilms [155]. Concurrently, disulfide bonds within the hydrogel network degrade under elevated ROS levels (50–100 μM H_2O_2), enabling sustained release of probiotics (e.g., *Lactobacillus reuteri*) to modulate TLR4/NF-κB signaling [156].

Importantly, beyond chemical bond degradation, the properties of hydrogel materials at varying concentrations and different mechanical strength such as gastrointestinal peristalsis, blinking, oral peristalsis also influence their in *vivo* behavior, however, every properties of hydrogels in physiological condition can be simulated by in *vitro* methods in Table 4. Hydrogels as an excellent drug carrier can be tailored to meet specific needs. For the co-delivery of anti-inflammatory drugs and probiotics using hydrogel carriers for periodontitis treatment, various types of hydrogels can be designed, such as in-situ hydrogel formation, sustained-release hydrogel microspheres, or a combination thereof, thereby enhancing the protection and controlled release of drugs or probiotics, thus strengthening the efficacy of periodontitis treatment. See the formation and release of microenvironment-sensitive hydrogels in Fig. 7.

8. Advantages and limitations of microenvironment-sensitive hydrogel in practical applications

The wide used of in situ microenvironment-sensitive hydrogel applications are mainly due to the following prospects: firstly, in situ hydrogels can be transformed from a less viscous liquid state to a semi-solid state or vice versa according to the microenvironmental sensitivity of the disease site, such as pH, ROS, temperature, etc., which can be injected or implanted into a cavity to control the release rate of the drug to achieve a slow-release effect and to protect drugs from damage in vivo environment. Secondly, these smart hydrogels can be structurally modified by physical and chemical cross-linking with other moieties to achieve gelation or to enhance the rigidity of the gel. Thirdly, natural and some synthetic materials are biocompatible, biodegradable, lower toxicity and higher water absorption, so it can be safely used in various pharmaceutical applications. What's more, these hydrogels are flexible to design as single-responsive, dual-responsive, and multi-responsive, and can be linked to targeting groups for precise drug delivery and achieved high bioavailability. It is noteworthy that hydrogels can also form microspheres to encapsulate the drug or probiotic. This process protects the drug from damage by the microenvironment, maintains the drug's activity, and prolongs the duration of its action at the site of

Table 5
Evaluation methods of characterization and mechanical properties.

Methods	Key parameters	Evaluation information	Examples
Differential Scanning Calorimetry (DSC); Thermogravimetric Analysis (TGA)	Temperature	Thermal stability	Yuanfei Ge et al., 2022 examined thermal stability of hydrogel [148].
^{1}H NMR spectra;^{13}C NMR spectra; FTIR spectra	1. Chemical shift values for hydrogen and carbon signals. 2. Resonance frequency of different functional groups	The breaking and formation of chemical bonds and the chemical environment around the elements	Fanjia Dai et al. 2024 demonstrated that phenylboronic acid has been successfully conjugated to carboxymethyl chitosan using ^{1}H NMR spectra and FTIR spectra [127].
Transmission Electron Microscope (TEM)	Resolution	Morphology, size and uniformity of particles	Fanjia Dai et al. 2024 demonstrated the particle was a hollow spherical structure [127].
Scanning electron microscopy (SEM)	Resolution	Surface morphology of hydrogel	Yi Li et al. 2024 observed spherical structure of the sample [138]. Nae-Won Kang et al. 2024 observed the surface of hydrogel [89].
Confocal laser scanning microscopy (CLSM)	Fluorescence	Particle morphology	Yi Li et al. 2024 revealed that the formation of W/O/W double emulsions [138].
Optical microscope	Magnification, resolution	Particle size	Yiik Siang Hii et al. 2021 examined the size of microbeads [132].
Rheological analysis	1. Temperature 2. Shear stress/rate 3. Vscosity 4. Time 5. Phase Angle 6. G′: viscosity modulus 7. G″: elasticity modulus	1. The relationship between viscosity and temperature. 2. The relationship between viscosity and shear stress/rate 3. Thixotropic. 4. Yield stress. 5. Frequency and amplitude scanning	Yuanfei Ge et al. 2022 examined G′ and G″ [148]. Cigdem Yucel Falco et al. 2017 examined the relationship between viscosity and shear rate [154].
Swelling behaviour	Swelling rate	Degree and rate of water absorption swelling of hydrogel	Cigdem Yucel Falco et al. 2017 examined hydrogel microsphere have different swelling rate under different pH conditions [154].

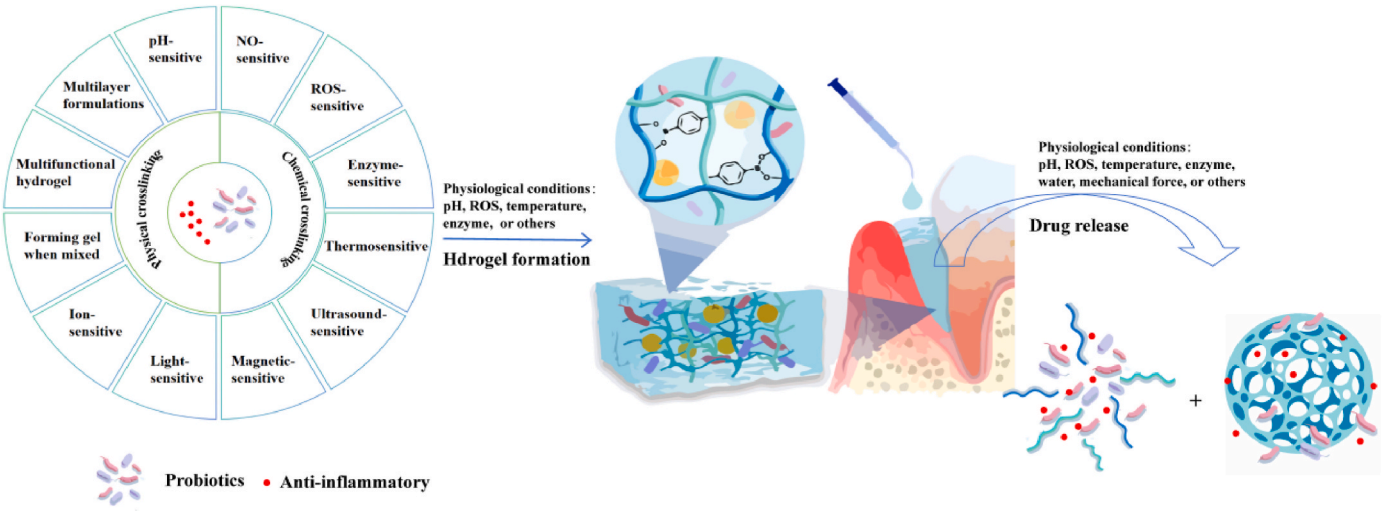


Fig. 7. Formation and release of microenvironment-sensitive hydrogels.

administration. Therefore, microenvironment-sensitive hydrogels that can improve bioavailability and increase medication safety may be a good choice for delivering drugs to treat multiple types of diseases.

Although microenvironment-sensitive hydrogels hold significant promise for protecting and releasing drugs and probiotics, several critical issues remain unexplored and unsolved. Firstly, limited research data on the long-term in vivo safety and biocompatibility of various hydrogels compromise patient drug safety assurances. Secondly, a comprehensive review of hydrogel studies reveals that the stability duration of coated drugs and probiotics is short, with stringent temperature control requirements. This may be attributed to the high water content in hydrogels, which can activate drug molecules and probiotics, promoting bacterial growth and complicating storage. Moreover, due to the strong adhesion properties of hydrogels, specialized equipment is required for production, leading to higher maintenance costs and increased production expenses. Additionally, establishing a robust quality control system for hydrogels remains a challenge. Despite these limitations, the flexible design of hydrogels offers promising application prospects, making it worthwhile to advance their development for clinical use.

It is evident from the introduction of our article that hydrogels have achieved significant advancements in basic research. To transition to clinical translational research, it is crucial to address the following considerations: firstly, we must identify preparation methods that ensure consistent quality and scalability for automated production; secondly, extensive biosafety evaluations must be conducted to demonstrate the safe application of hydrogels in patients.

9. Future directions

Looking ahead, we recommend conducting large-scale, multicenter clinical trials to validate the efficacy and safety of combining probiotics with anti-inflammatory therapies. Additionally, integrating advanced molecular biology and microbiome technologies will be crucial for exploring the intricate interactions between microbiota and the host, thereby enabling the development of more precise intervention strategies. Through these studies, we aim to provide more effective treatment options for patients with periodontitis and novel approaches for the prevention of cardiovascular diseases. One of promising methods is to leverage the synergistic effects of hydrogels, anti-inflammatory drugs, and probiotics by co-delivering inflammatory drugs and probiotics and utilizing the unique properties of hydrogels such as flexibility, slow degradation, protective capabilities, and sensitivity to the microenvironment, which is a novel therapeutic strategy for treating periodontitis and potentially cardiovascular diseases.

10. Conclusions

In conclusion, microenvironment-sensitive hydrogels exhibit excellent biocompatibility, robust adhesion, and biodegradability. They can be tailored into various types to meet specific clinical requirements and microenvironmental conditions. This adaptability effectively enhances drug bioavailability. Leveraging the superior water-retention and drug-loading capabilities of hydrogels, they serve as a promising strategy for the co-delivery of probiotics and anti-inflammatory drugs in periodontitis therapy.

CRediT authorship contribution statement

Yi Quan: Writing – review & editing, Writing – original draft. **Huihui Shao:** Writing – review & editing, Writing – original draft. **Nuoya Wang:** Writing – review & editing. **Zhonggao Gao:** Writing – review & editing. **Mingji Jin:** Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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