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Synthetic Engineering of Probiotic Biofilms for Targeted Immune Enhancement and Intestinal Health- A Review

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Abstract

Probiotic therapeutics represent a rapidly advancing field, with particular interest directed toward the engineering of probiotic biofilms for immune modulation and gastrointestinal (GI) health. In contrast to conventional planktonic probiotic cells, biofilm-forming strains exhibit enhanced resilience, prolonged mucosal colonization, and improved immunological interactions facilitated by their extracellular polymeric substances (EPS). These attributes confer superior therapeutic consistency and stability, thereby addressing the limitations of traditional probiotic formulations that are often characterized by transient survival and limited clinical efficacy. This review critically examines the emerging landscape of synthetic engineering of probiotic biofilms, with emphasis on their design strategies, functionalization approaches, and biomedical applications. Advances in the genetic modification of probiotics have enabled precise delivery of therapeutic molecules at mucosal sites, while encapsulation systems have been developed to shield biofilm-based therapeutics from gastrointestinal stressors. Furthermore, inactivated biofilm matrices are gaining recognition as innovative modalities for regulating host immunity, fortifying epithelial barriers, and mitigating enteric infections, offering safer alternatives to live microbial therapies. The translation of these advances into clinical practice, however, is not without challenges. Issues relating to biosafety, regulatory compliance, and ethical considerations remain critical barriers to widespread adoption. Additionally, the personalization of probiotic biofilm therapies must account for inter-individual variability in microbiome composition and host responses. Large-scale manufacturing, standardization, and quality assurance also pose significant obstacles to clinical deployment. Despite these limitations, emerging progress in precision microbiome engineering underscores the potential of synthetic probiotic biofilms as next-generation biotherapeutics, with far-reaching implications for gastrointestinal health, systemic immune regulation, and human well-being.

Keywords: Synthetic biology, Engineered probiotics, Biofilm formation, Immune modulation, Gut microbiota, Nanotechnology

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Introduction

The gastrointestinal (GI) tract harbors a dense and dynamic population of microbes, collectively known as the gut microbiota, which is essential to host metabolism, immune regulation, and mucosal defense. Perturbations in microbial balance (dysbiosis) have been linked to a spectrum of conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), colorectal cancer, and systemic autoimmune disorders (Duan *et al*., 2020). Probiotics, which are live microbial supplements that promote health, have emerged as promising adjuncts in controlling dysbiosis and restoring mucosal homeostasis. However, conventional probiotics, mostly in the form of free-living planktonic cells, exhibit limitations such as low gastric survivability, transient gut residency, and inconsistent clinical outcomes (FAO/WHO, 2021). To address these issues, attention has shifted to biofilm-forming probiotics. Biofilms are organized microbial communities embedded within a self-produced matrix of EPS, which facilitates protection against environmental stressors and enhances adherence to intestinal epithelium. The biofilm mode of existence allows sustained therapeutic interactions with the host immune system, improved persistence in the gut, and synchronized release of beneficial metabolites (Ramezani *et al*., 2021). Synthetic engineering of probiotic biofilms incorporates principles from genetic engineering, materials science, and systems biology to manipulate probiotic strains and design microenvironments conducive to biofilm formation (Xu *et al*., 2021). Engineered biofilms can be tailored to deliver specific therapeutic payloads, activate host immune pathways, and engage in dynamic crosstalk with the resident microbiota. This review provides a comprehensive overview of the immunological functions of probiotic biofilms,

synthetic biology approaches to probiotic enhancement, biofilm stabilization technologies, and their therapeutic roles in disease management (Aldhairyan *et al*., 2022; Alhazmi *et al*., 2022; Almohmmadi *et al*., 2022; Almuhanna *et al*., 2022; Alqurashi *et al*., 2022; Alsayed *et al*., 2022). Furthermore, we examine challenges in biosafety, regulatory pathways, personalization strategies, and industrial scalability, proposing future directions in clinical translation and microbiome-targeted therapies.

Immunological Functions of Probiotic Biofilms

EPS-Mediated Immunomodulation

The EPS matrix of probiotic biofilms acts as a multifunctional interface, facilitating direct interaction with the host immune system. Composed of polysaccharides, glycoproteins, lipids, and extracellular DNA, EPS molecules are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and C-type lectins. These interactions initiate signaling cascades that regulate the production of cytokines, chemokines, and antimicrobial peptides (Nowak *et al*., 2021; Yuan *et al*., 2021). EPS produced from Lactobacillus plantarum stimulates TLR2 and increases the release of anti-inflammatory cytokines such as IL-10 and TGF-β. EPS from Bifidobacterium breve affects both pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory pathways, depending on the illness environment (He *et al*., 2023). Advances in synthetic carbohydrate engineering and genome editing allow for the customization of EPS structures to enhance immune recognition. For example, altering EPS glycosylation patterns can improve TLR engagement and modulate dendritic cell maturation, thereby directing T-cell polarization toward regulatory or effector phenotypes (Zhou *et al*., 2023).

Biofilm-Derived Antigen Presentation

The prolonged adherence of biofilms to the intestinal mucosa enhances opportunities for antigen uptake by immune cells residing in gut-associated lymphoid tissues (GALT), including Peyer's patches and mesenteric lymph nodes. Probiotic biofilms release antigens and microbe-associated molecular patterns (MAMPs) in a sustained fashion, allowing dendritic cells to process and present these molecules to T cells, thereby initiating adaptive immune responses (Chen *et al*., 2023). Biofilm-enhanced antigen presentation facilitates the induction of regulatory T cells (Tregs) and IgA-producing plasma cells, which are pivotal in suppressing intestinal inflammation and reinforcing barrier integrity. Such mechanisms are particularly beneficial in the management of chronic inflammatory diseases and allergic responses (Zhou *et al*., 2023).

Modulation of Macrophage Plasticity

Macrophages residing in the intestinal lamina propria are critical components of the gut immune system, where they perform dual and dynamic roles in both immune defense and tissue repair. Their functional versatility is driven by their capacity to undergo phenotypic polarization in response to environmental cues and microbial signals. Upon stimulation with pro-inflammatory cytokines or microbial products such as lipopolysaccharides, these macrophages differentiate into classically activated M1 phenotypes, which produce pro-inflammatory mediators like TNF-α, IL-6, and nitric oxide to combat pathogens. Conversely, in the presence of anti-inflammatory signals such as IL-4 or IL-13, they polarize into alternatively activated M2 phenotypes, which secrete factors like IL-10 and transforming growth factor-beta (TGF-β) to promote resolution of inflammation, tissue remodeling, and wound healing.

Recent studies have shown that extracellular polymeric substance (EPS) components derived from probiotic biofilms can actively influence this polarization process, skewing macrophages toward the M2 phenotype and thereby exerting anti-inflammatory and tissue-protective effects. EPS molecules act as immunomodulatory agents that interact with pattern recognition receptors (PRRs) on innate immune cells, shaping downstream signaling pathways involved in macrophage activation and function. Zhao *et al*. (2021) provided compelling evidence for this phenomenon, demonstrating that EPS derived from *Lactobacillus plantarum* biofilms significantly promoted M2 macrophage polarization in murine models of colitis. Their study reported elevated levels of anti-inflammatory cytokines IL-4 and IL-10, accompanied by marked reductions in epithelial tissue damage and inflammatory infiltrates in the colon. These findings underscore the therapeutic potential of biofilm-mediated immune modulation, suggesting that engineered or natural probiotic biofilms may serve as targeted tools for managing inflammatory bowel diseases and promoting mucosal healing through controlled immune cell reprogramming (Zhao *et al*., 2021).

Synthetic Biology Approaches to Probiotic Biofilm Engineering

Genetic Circuitry and Strain Optimization

Advances in genome editing technologies, notably the introduction and refining of CRISPR-Cas systems, have transformed the area of synthetic biology, allowing for precise and efficient genetic alterations in probiotics. These tools allow researchers to reprogram the genetic circuitry of probiotics to perform highly specialized therapeutic functions tailored to host needs. Through targeted gene insertion, deletion, or regulation, engineered probiotic strains can be designed to express a variety of biologically active molecules, including immunomodulatory cytokines such as interleukin-10 (IL-10), short-chain fatty acids (SCFAs) like butyrate, antimicrobial peptides that suppress pathogenic bacteria, and adhesion molecules that enhance colonization and persistence at mucosal surfaces (Li *et al*., 2020; Feng *et al*., 2021). These modifications enable probiotics to exert localized, on-demand therapeutic effects, particularly valuable in managing chronic inflammatory conditions such as inflammatory bowel disease (IBD).

A striking example is the engineering of Escherichia coli Nissle 1917, a well-known probiotic strain, to express trefoil factors (TFFs) and anti-inflammatory cytokines, which aid in epithelial repair and barrier restoration in IBD models. Trefoil factors are tiny peptides that play important roles in mucosal healing; their production by a probiotic vehicle allows for tailored administration to regions of intestinal damage (Praveschotinunt *et al*., 2021). In preclinical experiments, this strain was shown to be

effective at decreasing inflammation and stimulating epithelial regeneration, demonstrating the translational potential of such synthetic constructions.

Furthermore, recent advances in multiplex CRISPR editing have expanded the medicinal potential of modified probiotics. This method allows for the simultaneous integration or regulation of several genes in a single editing cycle, resulting in multifunctional strains capable of executing complex, coordinated activities. These sophisticated strains may be trained to detect host-derived cues, such as pH changes, inflammatory cytokines, or metabolites, and respond dynamically by activating certain therapeutic pathways. Such responsiveness and flexibility pave the way for next-generation living biotherapeutics with adaptive and programmable functions for precision medicine applications in gastrointestinal and systemic illnesses.

Functional Amyloids and Curli Fiber Display

Curli fibers are extracellular amyloid proteins produced by certain members of the *Enterobacteriaceae* family, including *Escherichia coli* and *Salmonella* species. These highly ordered protein structures are key components of the bacterial extracellular matrix, contributing significantly to the mechanical strength, cohesion, and resilience of biofilms. By forming tightly interwoven fibrous networks, curli fibers provide a stable scaffold that enhances bacterial adhesion to abiotic surfaces, host tissues, and other microbes, thereby facilitating colonization and persistence in diverse environments.

Recent innovations in synthetic biology have enabled the functionalization of curli fibers, transforming them into versatile platforms for therapeutic applications. Through precise genetic engineering of the curli subunit protein CsgA, it is possible to append peptide sequences or protein domains that retain their biological activity when displayed on the extracellular fiber surface. This allows curli fibers to be modified to carry a wide range of bioactive molecules, including immunomodulatory cytokines (e.g., IL-10), growth factors (e.g., EGF, VEGF), antibodies, or enzymes, enabling the direct interface between engineered probiotics and host tissues at a molecular level.

Yan *et al*. (2021) demonstrated that probiotic strains engineered to express functionalized curli fibers exhibited enhanced adhesion to the intestinal mucosa, thereby improving their residence time and therapeutic potential in vivo. Importantly, these engineered fibers served as a delivery vehicle for therapeutic proteins, allowing localized and sustained release of functional biomolecules directly at the site of inflammation or tissue damage. Further supporting these findings, Kwon *et al*. (2022) reported that curli-functionalized probiotics were able to efficiently deliver bioengineered payloads to target tissues, enhancing therapeutic outcomes in models of mucosal disease. These studies underscore the transformative potential of engineered curli fibers in the development of next-generation probiotic-based therapeutics for precision medicine.

Quorum Sensing and Regulatory Feedback

Synthetic quorum sensing modules have emerged as potent synthetic biology techniques for controlling gene expression at the population level in designed probiotics. Bacteria employ quorum sensing, a naturally occurring cell-cell communication mechanism, to coordinate group activities such as biofilm formation, virulence factor synthesis, and bioluminescence dependent on population density. This connection is facilitated by the release and detection of tiny signaling molecules known as autoinducers. In synthetic systems, similar methods have been repurposed and developed to provide precise and programmable control over genetic circuits in probiotic populations.

One widely used approach involves autoinducer-2 (AI-2) or N-acyl homoserine lactone (AHL)-based quorum sensing systems, which enable synchronized expression of therapeutic genes once the bacterial population reaches a specific threshold density. These synthetic circuits allow for tunable, density-dependent control of critical functions, including the initiation or suppression of biofilm formation, the expression of bioactive compounds (such as anti-inflammatory cytokines or antimicrobial peptides), and even the controlled disassembly of biofilms after therapeutic action has been achieved. This ensures that therapeutic effects are exerted only under appropriate physiological conditions, minimizing off-target effects and improving biosafety.

Furthermore, synthetic quorum sensing modules can be engineered to respond to environmental cues such as pH changes, nutrient levels, oxygen tension, or markers of host inflammation, allowing the engineered probiotics to function as intelligent, responsive delivery systems. For instance, in an inflamed gut environment characterized by low pH and oxidative stress, quorum sensing signals can be integrated into logic-based genetic circuits that trigger targeted therapeutic responses. Tan *et al*. (2020) illustrated how such systems can be designed to precisely regulate probiotic behavior in situ, offering dynamic control over microbial functions in response to fluctuating environmental and host-derived signals. These advances represent a significant step toward the development of smart, self-regulating microbial therapies for a wide range of diseases.

Materials Science Innovations in Biofilm Stabilization

Nanoencapsulation and Controlled Release

Nanoencapsulation has emerged as a transformative strategy in enhancing the functional performance, stability, and targeted delivery of probiotic biofilms. One of the major challenges in oral probiotic therapy is the harsh gastrointestinal environment, particularly the acidic conditions of the stomach and the presence of digestive enzymes, which can significantly reduce probiotic viability before they reach the intestines. Nanoencapsulation addresses this limitation by forming a protective barrier around the probiotic cells or biofilm matrix, shielding them from premature degradation and facilitating controlled, site-specific release in the gastrointestinal tract (Alizadeh *et al*., 2022; Elshorbagy *et al*., 2022; Sabar *et al*., 2022; Akbari, 2023; Sari *et al*., 2023; Verevkina *et al*., 2024).

Several encapsulating materials, including alginate, chitosan, polydopamine, and metal-organic frameworks (MOFs), have been created and improved for this purpose. These materials are not only biocompatible and non-toxic but also offer tunable degradation rates and surface properties, allowing precise control over the release kinetics of encapsulated probiotics. For example, alginate and chitosan can form pH-responsive hydrogels that remain stable in acidic gastric fluid but dissolve or swell in the more neutral pH of the intestinal lumen, enabling targeted probiotic release at the site of action (Zhang *et al*., 2021). Polydopamine and MOFs further enhance encapsulation by providing mechanical stability, functionalization potential, and protection from oxidative stress.

In a recent breakthrough, Pan *et al*. (2022) introduced a single-cell nanoencapsulation platform that allows the encapsulation of individual probiotic cells within protective nanoshells. This high-resolution approach ensures uniform coating and maximizes survival through the gastric environment. Once in the gut, the encapsulating material dissolves in response to local factors, including pH, enzyme activity, and microbial signals, allowing live probiotics to be released precisely into the intestinal lumen. The end outcome is dramatically higher colonization efficiency, gut persistence, and therapeutic efficacy in regulating host immunity or restoring microbial balance.

Importantly, these encapsulation platforms are also being engineered for multifunctionality, enabling the co-delivery of prebiotics, immunostimulants, and bioactive compounds alongside the probiotic cells. This synergistic delivery strategy enhances probiotic growth and activity in situ while simultaneously stimulating host immune responses or modulating the gut environment to favor therapeutic outcomes. As such, nanoencapsulation not only improves the delivery efficiency and viability of probiotics but also opens new avenues for designing complex, targeted probiotic-based interventions for gastrointestinal and systemic diseases.

Mucosal Targeting via Surface Functionalization

To improve the retention, colonization efficiency, and therapeutic efficacy of probiotic formulations within the gastrointestinal tract, probiotic cell surfaces can be functionally engineered by decorating them with targeting ligands. These ligands, such as lectins, monoclonal antibodies, or synthetic peptides, are intended to identify and selectively bind to particular biomolecular targets, such as mucin glycoproteins or epithelial cell surface receptors. This targeted interaction enhances the adhesion of probiotics to the intestinal mucosa, thereby prolonging their residence time and improving their ability to exert localized immunomodulatory or metabolic effects. A key advancement in this area involves the use of bioorthogonal chemistry techniques, particularly click reactions, which allow for the precise and stable conjugation of ligands to the probiotic surface under physiological conditions. These reactions are highly specific and do not interfere with the viability or functionality of the living probiotic cells, making them especially suitable for clinical and therapeutic applications (Song *et al*., 2023).

Inactivated Biofilm Scaffolds

UV- or chemically-inactivated probiotic biofilms offer a promising strategy for harnessing the beneficial properties of probiotics without the risks typically associated with administering live microbial agents, such as uncontrolled colonization, horizontal gene transfer, or infection in immunocompromised individuals. These inactivation methods preserve the structural integrity of the extracellular polymeric substances (EPS) matrix, which is crucial for maintaining the biofilm's physical stability, adhesive capacity, and immunomodulatory potential. As a result, the inactivated biofilms retain the ability to interact beneficially with host tissues and immune cells, eliciting protective or anti-inflammatory responses. Due to their safety and biofunctionality, these inert probiotic biofilms are being explored for a wide range of biomedical applications, including as biocompatible coatings for implants and medical devices, stabilizers in oral drug delivery systems, and biologically active layers in wound dressings that promote healing. In a recent study, Li *et al*. (2024) demonstrated that orthopedic implants coated with inactivated probiotic biofilms not only prevented post-surgical infections by resisting pathogenic colonization but also facilitated tissue regeneration, highlighting their dual functionality in clinical settings.

Therapeutic Applications of Engineered Probiotic Biofilms

Management of Inflammatory Bowel Disease

IBD is characterized by chronic intestinal inflammation and epithelial barrier disruption. Engineered probiotic biofilms expressing anti-inflammatory molecules and growth factors have been effective in restoring epithelial function and suppressing cytokine storms. B. breve biofilms have demonstrated reduced histopathological scores and improved weight gain in colitis models (Zhou *et al*., 2023).

Recovery from Antibiotic-Induced Dysbiosis

Following antibiotic therapy, gut microbiota is severely depleted, increasing the risk of opportunistic infections like Clostridium difficile. Probiotic biofilms are more resilient to antibiotics and can recolonize the gut more effectively than planktonic forms. MOF-encapsulated biofilms ensure prolonged viability and facilitate microbiota reconstitution (Gao *et al*., 2024).

Oral Mucosal Vaccination and Nutraceuticals

Oral delivery of biofilm-forming probiotics engineered to express antigens or immunostimulatory adjuvants has emerged as a promising strategy for inducing both mucosal and systemic immune responses. By leveraging the natural ability of probiotic biofilms to adhere to and persist at mucosal surfaces, these systems enable prolonged antigen presentation to immune cells within the gut-associated lymphoid tissue (GALT), thereby enhancing the magnitude and durability of the immune response. This approach offers significant advantages over conventional parenteral vaccines, as it bypasses the need for injections, reduces needle-associated risks such as pain, infection, and needle phobia, and eliminates the requirement for trained healthcare personnel to administer the vaccine.

Importantly, oral probiotic-based vaccines are particularly well-suited for pediatric and geriatric populations, where compliance with injectable vaccines may be low due to discomfort or logistical barriers. Furthermore, biofilm-mediated antigen delivery enhances antigen stability in the harsh gastrointestinal environment, protecting it from degradation by gastric acid and digestive enzymes until it reaches immune inductive sites in the small intestine. Chen *et al*. (2023) highlighted the clinical potential of this approach, demonstrating that biofilm-forming probiotic vectors expressing target antigens elicited strong mucosal IgA responses alongside robust systemic IgG production, providing comprehensive immune protection. Such systems not only improve patient compliance but also open the door to thermostable, needle-free, and easily deployable vaccines for global immunization programs, especially in low-resource settings (Al-Johani *et al*., 2022; Chidambaranathan & Culathur, 2022; Mady *et al*., 2022; Mathew *et al*., 2022; Verma & Pandian, 2022; Wąsacz & Chomyszyn-Gajewska, 2022; Zahid & Khan, 2022).

Functional Foods and Personalized Nutrition

Engineered probiotic biofilms can be seamlessly integrated into functional food matrices, creating next-generation nutraceutical products that deliver targeted and sustained health benefits. Unlike free-living probiotic cells, biofilm-associated probiotics exhibit enhanced resistance to environmental stressors such as heat, oxygen exposure, and gastric acidity, thereby maintaining higher viability during food processing, storage, and passage through the gastrointestinal tract. When embedded within food carriers such as dairy products, plant-based beverages, cereals, or edible gels, these engineered biofilms can be designed to release bioactive compounds in a controlled manner, enabling site-specific delivery within the gut.

Functional food formulations can be tailored to provide specific health benefits, such as immune function modulation, regulation of metabolic pathways involved in glucose and lipid homeostasis, gut barrier integrity enhancement, and stress resilience promotion via gut-brain axis signaling. The use of engineered biofilms also enables the co-delivery of complementary bioactives such as prebiotics, antioxidants, and micronutrients, which increases their total physiological impact.

A particularly promising frontier in this field is the development of personalized probiotic biofilm-based functional foods guided by individual microbiome profiles (Alaghemandan *et al*., 2022; Haidar, 2022; Istyagina-Eliseeva *et al*., 2022; Oran *et al*., 2022; Polevoy *et al*., 2022; Shoghi & Kian, 2022; Garbarova & Vartiak, 2024). By using metagenomic sequencing and metabolomic profiling, researchers can identify deficiencies or imbalances in an individual's gut microbiota and design customized formulations that address these specific needs. Wu *et al*. (2024) reported ongoing work in this area, demonstrating that microbiome-informed engineered biofilm formulations could be optimized to enhance colonization efficiency and therapeutic activity, paving the way for precision nutrition products that align with personal health goals and genetic predispositions. This convergence of synthetic biology, microbiome science, and functional food technology represents a major step toward individualized dietary therapeutics.

Challenges and Future Prospects

Regulatory and Biosafety Considerations

The use of genetically modified organisms (GMOs) in human therapeutics necessitates stringent biosafety assessments. Synthetic kill switches, auxotrophy, and inducible suicide genes are being implemented to control proliferation and ensure containment (Kim *et al*., 2023). International harmonization of regulatory standards is critical for clinical translation.

Interindividual Variability and Microbiome Interactions

Host genetics, diet, and microbiota composition significantly influence the efficacy of probiotic biofilms. Personalized biofilm therapies, guided by microbiome sequencing and machine learning algorithms, offer a pathway to precision medicine (Wu *et al*., 2024).

Production Scalability and Shelf Stability

Manufacturing consistent, viable probiotic biofilms at scale remains a challenge. Innovations in continuous bioreactors, lyophilization techniques, and encapsulation processes are being explored to improve yield, cost-effectiveness, and product shelf life (Sharma *et al*., 2022).

Conclusion

Synthetic engineering of probiotic biofilms merges advances in microbiology, synthetic biology, and biomaterials to offer novel solutions for immune modulation and intestinal health. Engineered biofilms provide durable, responsive, and targeted interactions with the host, paving the way for personalized therapeutics. Overcoming current technical, regulatory, and personalization challenges will be vital to translating these innovations from bench to bedside. With continued research and development, synthetic probiotic biofilms are poised to become a cornerstone in the next generation of microbiome-centered healthcare.

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