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Review

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Probiotics and prebiotics: new treatment strategies for oral potentially malignant disorders and gastrointestinal precancerous lesions

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Oral potentially malignant disorders (OPMDs) and gastrointestinal precancerous lesions (GPLs) are major public health concerns because of their potential to progress to cancer. Probiotics, prebiotics, and engineered probiotics can positively influence the prevention and management of OPMDs and GPLs. This review aims to comprehensively review the application status of probiotics, prebiotics and engineered probiotics in OPMDs and GPLs, explore their potential mechanisms of action, and anticipate their future clinical use.

The digestive tract, which consists of the oral cavity and upper/lower gastrointestinal (GI) tract, is a high-incidence area of precancerous lesions in the body[1]. Conditions such as oral leukoplakia (OLK), proliferative verrucous leukoplakia (PVL), oral lichen planus (OLP), oral submucous fibrosis (OSF), and oral lichenoid lesion (OLL) constitute the majority of oral potentially malignant disorders (OPMDs). These OPMDs are early-stage lesions within the oral cavity that carry a heightened risk of evolving into oral cancer[2]. Gastrointestinal precancerous lesions (GPLs), including Barrett's esophagus, chronic atrophic gastritis (CAG), gastric intestinal metaplasia (GIM), aberrant crypt foci (ACF), and colorectal adenomas are a series of pathological conditions associated with a significant risk of malignant transformation[3]. These lesions usually progress slowly and often require prolonged clinical observation and follow-up.

The gut and oral cavity harbor the body's first and second largest populations of microbiota, respectively. It is widely recognized that bacterial dysbiosis, resulting from an imbalance between probiotics and harmful pathogens, is strongly associated with numerous disorders, including OPMDs and GPLs. Recent advancements have underscored the potential of probiotics, prebiotics and engineered probiotics in regulating the microbiota and boosting mucosal immunity. It has been determined that several probiotic strains could reduce lesional size in patients with OLP, while some prebiotics could alleviate limited mouth opening in patients with OSF. Moreover, the administration of several probiotic strains contributed to a significantly higher *Helicobacter pylori* (*H. pylori*) eradication rate in CAG patients. Furthermore, several probiotic strains have been confirmed to prevent the progression of Barrett's esophagus to esophageal adenocarcinoma and suppress the formation or progression of colorectal adenomas. These developments present promising strategies for the prevention and treatment of such conditions.

This review explores the existing evidence regarding the use of probiotics, prebiotics and engineered probiotics for treating OPMDs and GPLs. It further delves into their possible mechanisms of action and assesses their potential for clinical integration. The ultimate goal is to offer valuable insights and direction for future research and therapeutic approaches, providing a foundation for future clinical applications.

OPMDs and GPLs

Risk factors of OPMDs and GPLs

OPMDs are a group of conditions within the oral cavity that carry an increased risk of developing oral cancer. Common OPMDs include OLK, PVL, OLP, OLL, and OSF[4]. GPLs are abnormal changes in the GI tract that may precede the development of cancers, such as Barrett's esophagus, CAG, GIM, ACF, and colorectal adenomas[5]. Complex interactions of genetic, environmental, and lifestyle factors influence the development of OPMDs and GPLs. Key risk factors for OPMDs include the use of tobacco, alcohol consumption, betel quid chewing, and infection with human papillomavirus (HPV). Chronic irritation and inflammation also play a significant role[4]. An array of factors, such as chronic gastroesophageal reflux disease (GERD), *H. pylori* infection, dietary habits, obesity, and genetic predisposition, are crucial contributors to GPLs[5] (Fig. 1).

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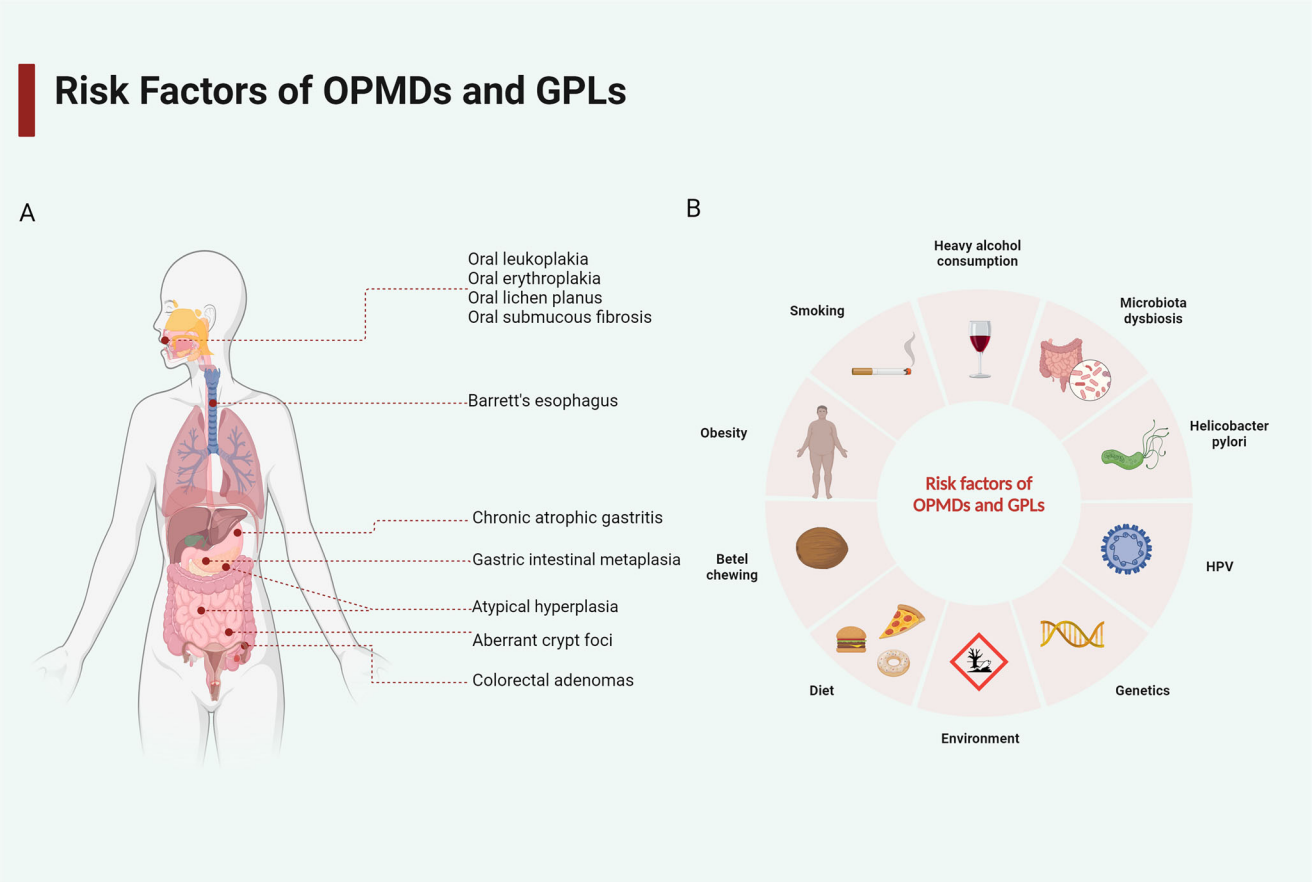

Fig. 1 | A Common OPMDs and GPLs. **B** Risk factors for OPMDs and GPLs include smoking, obesity, HPV, diet, and environment. (Created with BioRender.com).

Current treatment strategies for OPMDs and GPLs

The current management of OPMDs focuses on reducing risk factors, monitoring malignant transformation, and early interventions such as chemoprevention. For OLK, current chemopreventive compounds include retinoids, carotenoids, epidermal growth factor receptor inhibitors/antagonists, cyclooxygenase-2 inhibitors, p53 modulators, and bleomycin, among others[6]. The most common interventions for OLK remain the use of various techniques for excision or ablation, including cold knife excision, CO_2/Nd:YAG/KTP lasers, or a combination of excision and laser therapy[7]. However, patients who undergo multiple excisions and experience recurrence may develop tissue fibrosis and scar formation[8]. Photodynamic therapy (PDT), as a minimally invasive approach, has demonstrated high selectivity and repeatability in treating OLK. It has a low incidence of complications and can yield favorable functional and aesthetic outcomes with minimal scarring[9]. Cryotherapy and PDT combined with cryotherapy are also effective treatment options for OLK[10,11].

Due to the high risk of disease recurrence and cancer development in PVL patients, current management and treatment strategies primarily focus on close monitoring and early intervention[12,13]. For the PVL patients with multifocal lesions, mapping incisional biopsies are typically performed to establish baseline histopathology. For the PVL patients without carcinoma, management options include continuous biopsies for close monitoring, cold knife excision, and CO_2/Nd:YAG laser excision/ablation, though a previous study reported a high post-surgery recurrence rate, with approximately two-thirds of patients experiencing relapse[14]. It has been shown that PDT has some efficacy in treating PVL, particularly in large, thick, or highly keratinized lesions, although the evidence remains limited. A case report also described the successful treatment of refractory PVL using local 5-aminolevulinic acid-mediated PDT combined with diode laser drilling pretreatment, with no signs of recurrence within a 10-month follow-up[15,16]. Considering the high cancer occurrence risk in PVL patients, it has been shown that immunotherapy (such as PD-1 inhibitors: nivolumab) has therapeutic potential in clinical trials, especially for patients with high PD-L1 expression[17].

Pharmacological treatment remains the primary strategy for managing OSF. Hyaluronidase, collagenase, and elastase are commonly used to degrade the fibrotic soft tissue matrix, although these enzymes lose their activity completely over a short period[18]. Other medications include anti-inflammatory/immunomodulatory agents (such as corticosteroids, interferon-γ, colchicine, immunoglobulins, and placental extracts). Local steroid injections, particularly triamcinolone, can also be administered to relieve mouth opening restrictions and oral burning sensations[19]. Some vasodilators, such as buflomedil hydrochloride, naftidrofuryl oxalate, and azathioprine, are used to treat OSF by relaxing peripheral blood vessels, restoring blood flow to ischemic tissues, and reducing local tissue hardness[20]. Additionally, natural compounds with antioxidant effects, such as β-carotene, tanshinone, and lycopene, can be used to reduce or eliminate reactive oxygen species generated by areca nut metabolism, inhibiting fibroblast activation and collagen aggregation[19,21,22]. However, current studies have not provided sufficient evidence to confirm their long term effects. Surgical treatments include the use of local/regional flaps and microvascular flaps to excise fibrotic tissues and release fibrotic bands, thereby alleviating trismus[7]. Some in vitro studies have indicated that new therapies, such as sodium hyaluronate/bioactive glass composite hydrogels (BG/HA), mesenchymal stem cells and adipose stem cell-derived extracellular vesicles, have efficacy to treat OSF but further clinical validation are still required[23–25].

Topical high-potency corticosteroids (such as triamcinolone and betamethasone) are the first-line treatment for symptomatic flare-ups of OLP, while broader second-line/third-line treatments include topical calcineurin inhibitors (tacrolimus, pimecrolimus, and cyclosporine), topical or systemicretinoids (vitamin A analogs), systemic corticosteroids (prednisone, prednisolone), and systemic immunomodulators (methotrexate,

Table 1 | Oral microbial genera reported to be associated with OPMDs

OPMDs Type	Sample	Microbial Profile Variations	References
OLK	Saliva	↑: *Haemophilus, Bacillus, Salmonella, unclassified Enterobacteriaceae, Prevotella, Megasphaera* ↓: *Streptococcus, Abiotrophia, Gemella*	59,60
	Buccal mucosa	↑ : *Rothia, Alloprevotella, Neisseria, Leptotrichia, Fusobacterium, Campylobacter* ↓ : *Streptococcus, Gemella*	182
PVL	Biopsy	↑: *Prevotella, Actinomyces, Gemella, Campylobacter, Veillonella, Granulicatella* ↓: *Leptotrichia, Porphyromonas, Capnocytophaga, Haemophilus, Neisseria*	64
OLP	Saliva	↑ : *Solobacterium, Porphyromonas, Rothia, Phyllobacterium, Megaspaera, Prevotella, Fusobacterium, Alloprevotella* ↓: *Haemophilus, Corynebacterium, Streptococcus, Micrococcus, Campylobacter, Abiotrophia, Aggregatibacter, Bacteroides, Neisseria*	183–186
	Buccal mucosa	↑: *Leptotrichia, Acinetobacter, Actinomyces, Veillonella, Fusobacterium, Granulicatella* ↓ : *Streptococcus, Escherichia, Neisseria*	187–190

OLK oral leukoplakia, *PVL* proliferative verrucous leukoplakia, *OLP* oral lichen planus; (↑) Increased or (↓) reduced in patients compared to healthy subjects.

azathioprine, thalidomide)[26]. Some biologics and targeted inhibitors, such as TNF-α inhibitors, interleukin inhibitors, Apremilast (oral phosphodiesterase type 4 inhibitor), and JAK inhibitors (tofacitinib, baricitinib, ruxolitinib), have also been reported for use in the clinical treatment of refractory OLP[27–30].

Early detection and intervention of GPLs are of great importance in reducing the incidence of gastrointestinal cancers.

Barrett's esophagus, as a precursor to esophageal cancer, remains unclear in terms of the efficacy of chemopreventive agents, although some medications, such as proton pump inhibitors, have been widely recommended to reduce gastric acid reflux and decrease the risk of cancer progression[31]. For the patients with low-grade dysplasia or high-grade dysplasia, endoscopic eradication therapy is considered more effective than monitoring to reduce the risk of cancer development. Endoscopic treatments include endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). EMR is generally used for the initial resection of smaller lesions, while ESD is more suitable for larger lesions, allowing for precise resection of the target area[32]. Radiofrequency ablation (RFA) is widely used in the treatment of non-nodular Barrett's esophagus, particularly for ablating the lesions with high-grade dysplasia, showing significant efficacy, although its side effects, such as post-ablation strictures and high surgical costs, are drawbacks[33]. Cryoablation, another effective treatment method with liquid nitrogen or carbon dioxide, can be used to treat Barrett's esophagus, providing durable effects in certain cases[34]. Other available ablation methods include argon plasma coagulation and multipolar electrocoagulation[35].

Early intervention (such as *H. pylori* eradication and dietary adjustments) and regular monitoring are crucial to delay the progression of CAG and GIM and reduce the risk of cancer development[36,37]. Green tea extract, sulforaphane-rich broccoli sprouts, buckwheat, and oat β-glucan diets can prevent and reverse early-stage CAG, but their effectiveness is limited in advanced stages[38–40]. In pharmacological interventions, H2 receptor antagonists (e.g., ranitidine, nizatidine) and proton pump inhibitors (e.g., omeprazole, rabeprazole) are used to regulate gastric acid secretion[41]. However, long-term use of proton pump inhibitors may increase the risk of gastric cancer, kidney disease, and other complications[42–44]. *H. pylori* infection is the most common cause of the development of CAG and GIM. *H. pylori* eradication is crucial to prevent CAG and GIM progression, with common treatments including triple therapy (a proton pump inhibitor plus two antibiotics) and quadruple therapy (a proton pump inhibitor, bismuth, and two antibiotics)[45–47]. Gastric mucosal protectants such as sucralfate, bismuth potassium citrate, and teprenone can enhance mucus secretion and maintain the integrity of the gastric mucosa[48]. For ACF, the existing strategies mainly include chemoprevention (such as the use of drugs like aspirin and celecoxib), dietary modification (increasing fiber intake and reducing the consumption of red meat and fat), as well as preventing the occurrence and development of colorectal cancer through endoscopic monitoring and the resection of high-risk lesions[49,50].

Chemopreventive agents such as metformin, aspirin, the combination of difluoromethylornithine and sulindac, as well as natural calcium sources like dairy products and foods, have been shown to reduce the incidence and recurrence risk of precursor colorectal adenomas[51,52]. For smaller adenomas, EMR or ESD are commonly used for excision, as these methods are minimally invasive and allow for quick recovery. For larger adenomas or those with suspected malignancy, surgical resection of the intestinal segment may be necessary[53,54].

Current treatment strategies for OPMDs and GPLs primarily focus on surveillance, prevention, and surgical intervention. However, novel and more effective approaches are urgently needed to reduce and resolve post-treatment recurrence, medicine resistance in refractory cases, and carcinogenesis in chronic lesions. With the deepening understanding of the oral and Gl microbiome and microbiological technology development, an increasing number of studies are exploring the potential of probiotics, prebiotics, and engineered probiotics in the management of these diseases. These emerging therapies offer new perspectives and promising avenue for the prevention and treatment of OPMDs and GPLs.

Microbiota in OPMDs and GPLs

Oral microbiota and OPMDs (Table 1)

Increasing evidence emphasizes the link between oral microbiota and systemic diseases, as well as overall health[55–57]. However, the study of oral microbiota in OPMDs lags far behind that of periodontal disease or caries. The predominant genera showed both similarities and differences across different OPMDs. For example, compared with that in healthy controls, the relative abundance of *Firmicutes* is lower, whereas the relative abundance of *Bacteroidetes* is greater in patients with OLP and OLK[58]. A study of OLK revealed that *Streptococcus* abundance was low and *Prevotella* abundance was high in both saliva and mucosal tissues[59,60]. Among OLKs, PVL is a rare, progressive, persistent, and irreversible form[61]. In patients with PVL, there is a significant decrease in oral microbial diversity, along with an increase in the presence of pathogenic bacteria such as *Streptococcus*, *Actinomyces*, *Fusobacterium*, *Haemophilus*, *Treponema*, *and Campylobacter*[62]. In OLP patients, the abundance of common bacteria such as *Prevotella*, *Rothia, Fusobacterium*, and *Tannerella* is significantly increased[63]. OLLs significantly differed from the other OPMDs, with high abundances of *Cupriavidus* and *Roseomonas*[58].

At the species level, *S. pneumoniae* and *R. mucilaginosa* were the dominant species associated with OLPs. In OLK, *Meiothermus silvanus* exhibited the highest proportion. *Cupriavidus metallidurans* dominated in OLL[58].

The proliferation of these bacteria may be related to the chronic inflammatory response of OPMDs, further aggravating mucosal damage and lesions[64].

In addition to bacterial involvement, the role of fungi in OPMDs is noteworthy. *Candida albicans* is the primary *Candida* species associated with OLP and plays a role in the malignant transformation of OLP[65]. The

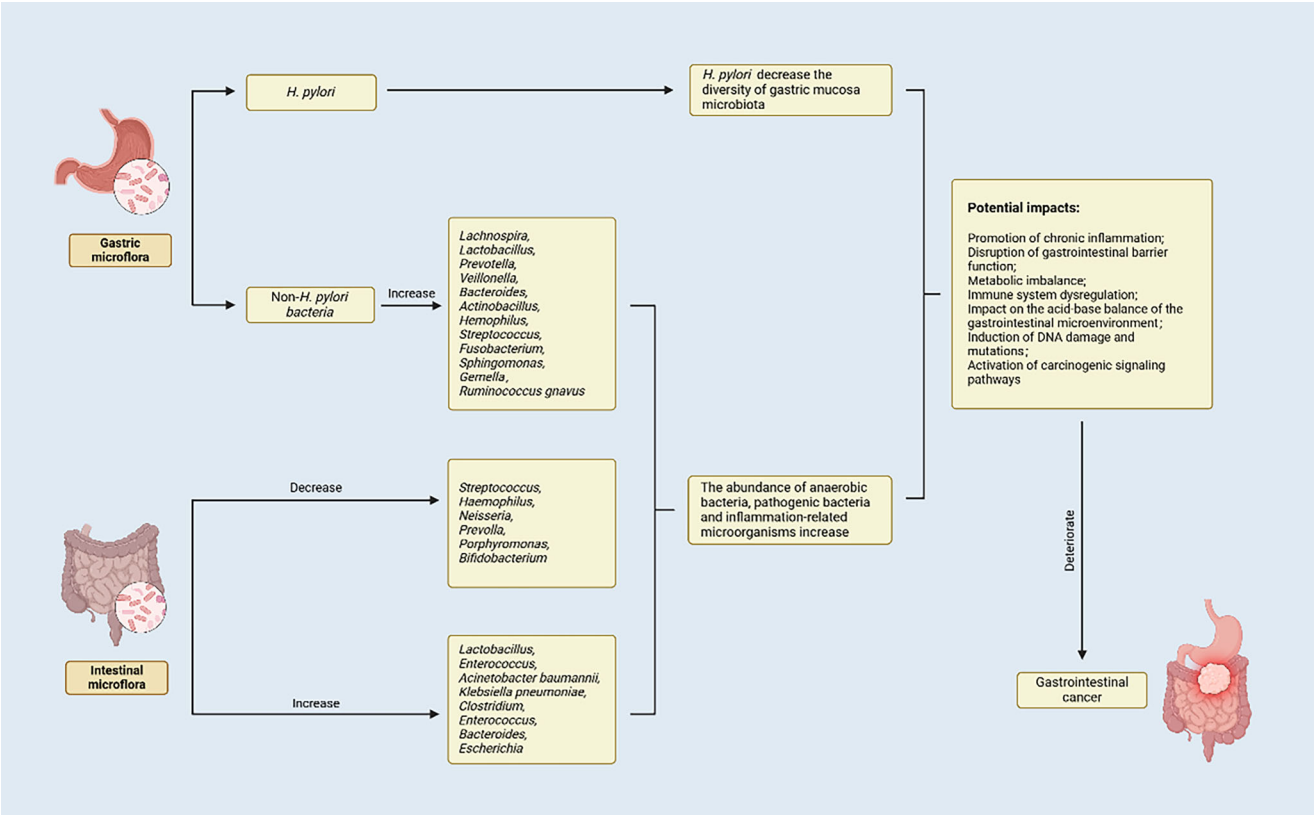

Fig. 2 | Relationship between GI microflora and GPLs. (Created with BioRender.com).

presence of *C. albicans* is significantly greater in patients with erosive OLP than in those with nonerosive OLP or healthy controls. Candida not only directly damages the oral mucosa but also triggers inflammation through its metabolites, increasing the risk of oral mucosal cancer[66].

GI microbiota and GPLs (Fig. 2)

The gastric microbiota, particularly *H. pylori*, plays a crucial role in the development of gastric cancer (GC) and GPLs[67]. *H. pylori*, a gram-negative bacterium found in the gastric mucosa, has been extensively researched for its involvement in gastritis, gastric ulcers, and progression to GC. The studies have shown that *H. pylori* significantly affects the stomach microbiota, leading to a notable reduction in bacterial diversity, along with notable enrichment of *Epsilonbacteraeota* and depletion of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* at the phylum level[68]. Effectively eradicating *H. pylori* can restore the stomach microbiome and positively influence the overall gut microbiome[69,70].

The diversity and interactions among gastric mucosal bacteria significantly decrease the incidence of gastric intraepithelial neoplasia. In these biopsies, genera such as *Veillonella*, *Gemella*, *Actinobacillus*, *Streptococcus*, and *Haemophilus* presented a relatively high degree of centrality and strong co-occurrence. Additionally, *Acinetobacter* shows frequent co-occurrence with various genera in intraepithelial neoplasia[68]. Specific genera are notably enriched at different stages: *Prevotella* and *Sphingomonas* are predominant in gastric atrophy; *Caulobacter*, *Dorea*, and *Bacteroides* are enriched in GIM; *and Bradyrhizobium*, *Sphingomonas*, *Curvibacter*, and *Acinetobacter* are more prevalent in *H. pylori*-negative intraepithelial neoplasia[68].

Researchers have investigated the gastric microbiota in individuals with nonatrophic gastritis, GIM, and GC[71]. The study results demonstrated that the abundances of *Neisseria*, *Porphyromonas*, and *Streptococcus* species decreased as the disease progressed, whereas those of *Lachnospira*, *Lactobacillus*, *Prevotella*, and *Veillonella* species increased. A subsequent study highlighted significant changes in the diversity and composition of the intestinal flora as lesions progress from nonatrophic gastritis to precancerous conditions and GC. Specifically, beneficial bacteria such as *Streptococcus*, *Haemophilus*, and *Neisseria* decreased, whereas *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterococcus* showed an increasing trend[58,72]. These findings emphasize the crucial role of the intestinal microbiota in the development of GI diseases and provide new perspectives for future research.

Crosstalk between oral and GI organisms via the oral-gut axis

The oral-gut axis refers to the complex network of interactions between the microbiomes of the oral cavity and the GI tract, as well as their associated immune and metabolic systems. This axis connects the mouth and gut through neural, endocrine, and immune pathways, creating a dynamic regulatory system. Recent studies have highlighted the bidirectional influence between the oral and gut microbiota, with specific oral pathogens potentially translocating from the oral cavity to the gut, where they may alter the gut microbial composition and promote inflammation or other pathological responses. For example, typical oral species such as *Streptococcus mutans, Porphyromonas gingivalis*, and *Fusobacterium nucleatum* can be found along the GI tract[73]. A number of periodontal pathogens including *Fusobacterium, Veillonella, Porphyromonas*, and *Campylobacter* have been shown to be associated with the onset of inflammatory bowel disease (IBD)[74–76]. In cases of IBD, bacteria from the oral cavity, including *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, may translocate to the gut, where they can disrupt tissues outside of the oral environment, partly through the production of proteases such as gingipain[77]. Additionally, oral bacteria can intensify gut inflammation by triggering T helper 1 (Th1) and T helper 17 (Th17) cells, which are reactive to oral pathobionts[73]. Conversely, IBD has also been associated with an increased risk of periodontitis. Experiments on SAMP1/YitFc mice (a spontaneous model of Crohn's disease) revealed that these mice naturally developed periodontitis[78,79]. In addition, the gut microbiota itself can be involved in periodontal tissue bone metabolism by producing short-chain fatty acids (SCFAs) and other metabolites that circulate throughout the body[80].

The oral-gut axis exemplifies a multifaceted system of microbial and immunological interactions that impact both local and systemic health. This complex crosstalk suggests that the maintenance of a balanced microbial community in both the oral cavity and the gut is critical for overall health.

Probiotics, prebiotics and engineered probiotics

Probiotics

The term "probiotic" is derived from the Greek word "pro-bios," meaning "for life". In 2001, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly defined probiotics as "live microorganisms, which, when administered in adequate amounts, exert a beneficial effect on the host's health." However, the International Scientific Association for Probiotics and Prebiotics (ISAPP) revised this definition in 2014, describing probiotics as "living microorganisms that, when administered in sufficient amounts, confer health benefits on the host. "[81]. According to the requirement of ISAPP, probiotic should be used only on products that deliver live microorganisms with a suitable viable count of well-defined strains with a reasonable expectation of delivering benefits for the wellbeing of the host. The following bacterial species, when delivered in food at a level of 1×10^9 colony forming units (CFU) per serving, as probiotics with nonstrain-specific claims might be made: *Bifidobacterium adolescentis* (*B. adolescentis*), *B. animalis*, *B. bifidum*, *B. breve, and B. longum*, and *Lactobacillus acidophilus* (*L. acidophilus*), *Lacticaseibacillus casei* (*L. casei*), *Limosilactobacillus fermentum* (*L. fermentum*), *Lactobacillus gasseri* (*L. gasseri*), *Lactobacillus Johnsonii* (*L. Johnsonii*), *Lacticaseibacillus paracasei* (*L. paracasei*), *Lactiplantibacillus plantarum* (*L. plantarum*), *Lacticaseibacillus rhamnosus* (*L. rhamnosus*), *and Ligilactobacillus salivarius* (*L. salivarius*)[81,82]. In addition, the framework for probiotics should include some well-defined beneficial symbiotic microorganisms, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, along with other butyrate-producing bacteria such as *Roseburia* spp. and *Eubacterium hallii*, provided that appropriate safety assessments are conducted[81]. Probiotic-containing products typically include foods, meal replacements, dietary supplements, over-the-counter (OTC) drugs, and prescription medications. The required dosage of probiotics varies depending on the strain and product, with many OTC products offering doses ranging from 1 to 10×10^9CFU/dose. The dosage should be based on human studies that demonstrate health benefits. These probiotics play a crucial role in enhancing intestinal barrier function, modulating the gut microbiota balance, and reducing inflammatory responses in the GI tract [83,84]. Additionally, probiotics have been utilized for various other purposes, such as antioxidation, cancer treatment, autoimmune disorder treatment, allergy prevention, and the management of neurodegenerative diseases[85].

Prebiotics

In 2016, ISAPP updated the definition of prebiotics to: "a substrate that is selectively utilized by host microorganisms giving a health benefit." Unlike probiotics, which are live microorganisms, prebiotics are non-living substrates that serve as nutrients for beneficial microorganisms carried by the host[86]. They are naturally found in foods such as garlic, onion, bananas, and whole grains and can also be added to foods or supplements to increase their health benefits. Common prebiotics include fructooligosaccharides (FOS), inulin, lactulose, galactooligosaccharides (GOS), and β-glucans[87].

Prebiotics provide prominent benefits by stimulating the growth of beneficial bacteria such *as Bifidobacterium*, *Lacticaseibacillus, Roseburia, Eubacterium* or *Faecalibacterium* spp[84,86]. By modulating the gut microbiota, prebiotics can inhibit the growth of pathogens and stimulate the immune system, thereby improving gastrointestinal health. In addition, they help lower blood lipid levels and improve insulin resistance, while also enhancing brain function, energy levels, and cognitive abilities through the regulation of metabolites. Furthermore, prebiotics are believed to support bone health by promoting the bioavailability of minerals[83,86,88].

The definition and functions of prebiotics are continually evolving. Certain fermentable soluble fibers are considered potential prebiotics, while the ability of other types of dietary fiber to act as prebiotics depends on whether the host microbiota can effectively utilize these components to promote health[89]. Plant polyphenols are another class of compounds that meet the criteria for prebiotics. They cannot be absorbed in the small intestine but they can be transformed by the gut microbiota in the colon into beneficial metabolites that contribute to health[90]. However, evidence for these emerging prebiotics is limited, and further research is needed to assess their health benefits and confirm their prebiotic status. Human milk oligosaccharides (HMOs) play a critical role in the development of the newborn's gut microbiota, metabolism, and immune system, with long-term implications for health[91]. HMOs promote the growth of specific beneficial bacteria, such as *B. longum* subsp. *infantis*, which has evolved to specifically degrade HMOs[92]. Additionally, HMOs modulate immune responses and protect the newborn from infections[93]. However, further research is needed to determine if HMOs meet the prebiotic definition by selectively promoting beneficial microbiota in the host.

Engineered probiotics

The development of engineered probiotics represents a cutting-edge advancement in microbiome research and therapeutic applications[94,95]. These entities are genetically modified probiotics engineered to possess superior or novel functionalities that are absent in their natural counterparts[94]. Gene editing technologies enable the modification of existing probiotics to create new strains with desired characteristics. These techniques allow for direct validation of changes in the genetic material, proteins, and functions of these engineered microorganisms[96]. The development of gene editing tools has evolved from homologous recombination to first-generation zinc finger nucleases (ZFNs), second-generation transcription activator-like effector nucleases (TALENs), and the more recent clustered regularly interspaced short palindromic repeats (CRISPR)-associated system, which has revolutionized life sciences[97]. ZFNs and TALENs are artificial nucleases that use zinc finger DNA domains or TAL effector DNA domains to edit or cut specific target DNA[98]. However, these tools face challenges such as low specificity and time-consuming construction, which have hindered their widespread use[96]. In contrast, CRISPR and associated Cas genes, part of a bacterial immune system that provides resistance to bacteriophages, offer higher editing efficiency and more straightforward, flexible applications[99,100]. The Type II CRISPR-Cas9 system consists of the Cas9 protein, crRNA, and tracrRNA, with Cas9 being the only DNA-catalytic protein among the many Cas proteins of *Thermus thermophilus*[101]. The crRNA and tracrRNA can be combined into small guide RNA (sgRNA), which directs Cas9 for site-specific DNA cleavage, gene knockout, and insertion[102]. This technology, including CRISPR activation and interference, supports the development of next-generation probiotics, enabling functional screening and genetic modification with significant therapeutic potential[97]. Other common synthetic biology methods for engineered probiotics include expression systems, genetic circuits, and more[96].

Engineered probiotics can target specific pathogens and their associated toxins, and mediate the directly targeted delivery of vaccines, drugs, and immunomodulators to host cells. These applications hold great potential in the treatment of various diseases, including diabetes, AIDS, oral mucositis, bacterial infections, inflammatory bowel diseases, cancers, and metabolic disorders.

Mechanisms of probiotic and prebiotic effects (Fig. 3)

Modulating microbiota balance

The modulation of the oral and gastrointestinal microbiota by probiotics occurs through multiple mechanisms, including competitive exclusion of harmful pathogens, forming biofilmbarrier, fermentation to produce SCFAs, and the production of antimicrobial compounds such as bacteriocins to inhibit pathogenic bacteria[103–105]. For example, strains such as *B. animalis* DN-173 010, *L. rhamnosus* GG (LGG), *Limosilactobacillus reuteri* (*L. reuteri*) ATCC55730, and *L. casei* have shown the ability to alter the colonization of caries-causing bacteria, thereby preventing tooth decay[106]. *Streptococcus salivarius* (*S. salivarius*) K12 is used in the treatment of oral candidiasis by reducing biofilm formation and inhibiting the dimorphic

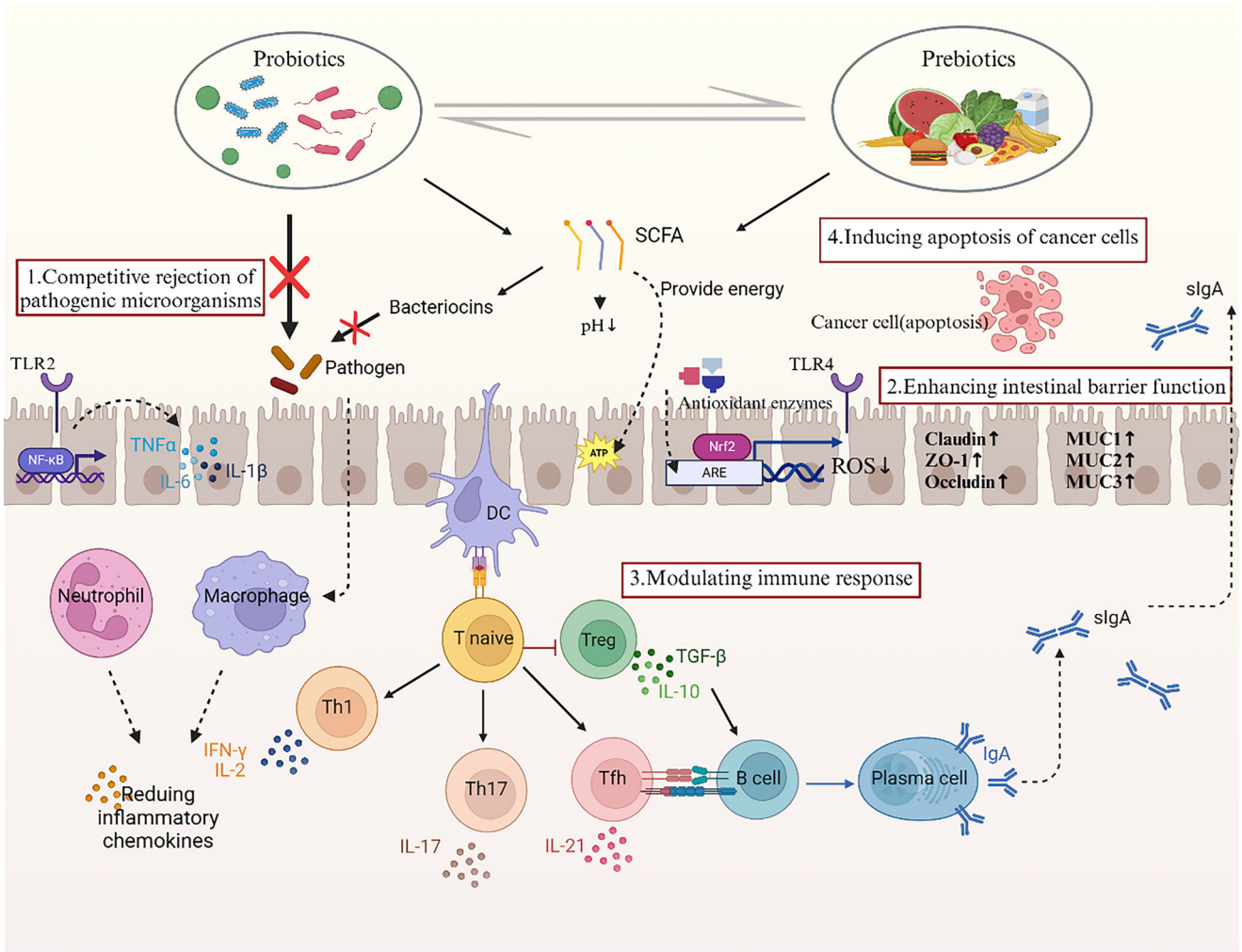

Fig. 3 | Mechanisms of probiotic and prebiotic effects. The mechanisms by which probiotics and prebiotics are applied to precancerous lesions include repelling competing pathogenic microorganisms, enhancing intestinal barrier function, modulating the immune response and inducing apoptosis of cancer cells. (Created with BioRender.com).

aggregation of *C. albicans*[107]. *L. acidophilus* CGMCC0460.2 and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (*L. bulgaricus*) NQ2508 can inhibit the adhesion of *H. pylori* by producing acetic acid and other antimicrobial substances[108]. Two strains of *L. reuteri* ATCC PTA 6475 and ATCC 53608, can reduce infections caused by enteropathogenic *Escherichia coli* (EPEC)[109]. Strains of *L. reuteri* ATCC PTA 6475 and ATCC PTA 5289 prevent the overgrowth of pathogens and symbiotic bacteria by forming biofilms and producing antimicrobial compounds such as reuterin, helping to maintain the health of the gut and oral mucosa[110].

Prebiotics help probiotics grow and establish themselves in the gut, enhancing their ability to combat pathogenic bacteria. An animal study investigated the effects of GOS on the prevention and alleviation of *E. coli* O157 invasion and colonization, and the results demonstrated that GOS effectively promoted the growth and activity of beneficial bacteria such as *Akkermansia*, *Ruminococcaceae*, and *Bacteroides*, and facilitated the production of SCFAs[111]. Additionally, compared with a placebo, GOS increased the abundance of *Lacticaseibacillus* and *Lactococcus* in constipated rats, thereby alleviating constipation and positively affecting colon health[112].

Enhancing barrier function

The barrier function of the gut shields the digestive tract from toxins, pathogens, and other forms of damage. Probiotics enhance intestinal barrier function by increasing the expression of tight junction proteins (claudin-1, ZO-1, and occludin), improving transepithelial resistance, and increasing mucus production (MUC2, MUC3, and MUC1)[113]. Researchers have shown that some probiotics, such as LGG and *L. reuteri* ZJ617, can reduce oxidative stress and inflammation, thereby increasing the expression of tight junction proteins and restoring intestinal barrier function[114,115]. *L. acidophilus* induces a rapid and strain-specific enhancement of intestinal epithelial tight junction barrier function through TLR complexes, particularly TLR-2/TLR-1 and TLR-2/TLR-6, thereby helping protect against intestinal inflammation[116]. Probiotics can also induce mucin expression and promote goblet cell secretion of mucus. Studies have shown that treating mucin-secreting colon epithelial cells with the supernatant of a probiotic-rich yogurt mixture can enhance the expression of mucin, particularly MUC2, which is a key component of the protective mucus layer[117]. Prebiotics also demonstrate beneficial effects on the intestinal mucosal barrier function. In a human study, the consumption of pasta enriched with 11% inulin was demonstrated to significantly increase the level of glucagon-like peptide-2 while reducing the serum zonulin levels, thereby contributing to the maintenance of mucosal barrier integrity[118]. Additionally, a previous study indicated that in obese adults, daily intake of GOS reduced sucralose excretion, suggesting that GOS may help enhance barrier function[119].

Modulating immune response

Probiotics interact with immune cells such as dendritic cells and macrophages through pattern recognition receptors (such as TLRs, NLRs) to induce the secretion of anti-inflammatory cytokines (such as IL-10, TGF-β), promote the polarization of macrophages to the anti-inflammatory M2 phenotype, and reduce the release of pro-inflammatory cytokines (such as

TNF-α, IL-6)[120]. This regulation helps maintain the balance of T cell subgroups. For example, *L. rhamnosus* RHT3201, LGG, *L. acidophilus* L92, *L. plantarum* LM1004, etc. can inhibit the overactivation of Th2 cells, thereby alleviating allergic reactions, while *B. adolescentis* ATCC15703 suppresses chronic colitis by regulating Treg/Th2 response[121,122]. At the molecular mechanism level, probiotics regulate immunity by intervening in key inflammatory signaling pathways. For example, strains like *L. acidophilus* can reduce the production of pro-inflammatory cytokines (such as TNF-α, IL-1β) via a TLR-2 and PI3K-dependent inhibition of NF-κB activation[123]. The surface protein called SpaCBA in LGG affects the levels of TNF-α, IL-10, IL-6, and IL-12 through a TLR2-dependent mechanism, stimulating immune responses[124]. Some probiotics produce various antioxidant enzymes, such as NADH oxidase, glutathione reductase, catalase, glutathione peroxidase, and feruloyl esterase[125–127]. By activating the antioxidant pathway Nrf2, they enhance the activity of enzymes like superoxide dismutase (SOD), reduce the expression of cyclooxygenase-2 (COX-2), and alleviate oxidative stress damage to the immune system[128]. Probiotics can also promote the activity of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), increasing the production of kynurenine. Kynurenine and its derivatives (such as kynurenic acid and quinolinic acid) play important roles in regulating the immune system, inflammatory responses, and neuroprotection[129]. These metabolites, together with extracellular polysaccharides (EPS) and other components, not only enhance the phagocytic capacity of macrophages and the antigen-presenting function of dendritic cells (DCs), but also extend local immune modulation effects to the entire body through the "gut-lung axis" and "gut-brain axis"[130].

Inhibition of carcinogenic agents

Probiotics deal with carcinogens in the gut through two mechanisms: attachment and inactivation. For example, the two lactic acid bacteria strains *B. longum* and *L. acidophilus* can effectively bind with carcinogenic compounds such as benzo[a]pyrene (B(a)P) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2)[131]. The studies have shown that probiotics and prebiotics are able to activate apoptosis signaling pathways, such as mitochondria-dependent pathways and death receptor-dependent pathways, thereby initiating a series of biochemical reactions that lead to the apoptosis of cancer cells[132]. An in vitro study on KB, an oral cancer cell line, demonstrated that *L. plantarum* probiotics reduce the mRNA expression of MAPK (a key pathway in cancer progression) and increase the mRNA expression of PTEN (a tumor-suppressor pathway) within 24 h post-treatment[133]. Oral administration of the *L. salivarius* REN probiotic or its postbiotics effectively protects DNA from oxidative damage induced by carcinogens, downregulates COX-2/PCNA expression, and induces apoptosis, thereby inhibiting 4NQO-induced oral carcinogenesis[134]. Additionally, researchers have demonstrated that probiotics can induce apoptosis of gastric cancer cells (KATO3) by disrupting the NF-κB and mTOR-mediated signaling pathways[135].

In addition to their direct effects on carcinogens, probiotics and prebiotics can also reduce cancer risk by regulating gut microbial metabolism. They act to suppress the function of harmful enzymes such as β-glucuronidase and nitroreductase within the GI tract. This suppression diminishes the process by which inactive precarcinogens are transformed into their active, carcinogenic forms[136,137]. For example, a study indicated that *Bifidobacterium* species have the ability to decrease β-glucuronidase activity in the human gut. LGG has been shown to lower the activities of β-glucuronidase and nitroreductase[138]. Furthermore, *B. adolescentis* was found to decrease the activities of intestinal β-glucosidase, tryptophanase, and urease[139].

Application of probiotics and prebiotics in OPMDs and GPLs

The application of probiotics and prebiotics in OPMDs and GPLs has attracted increasing attention. This review categorizes and examines the findings of several studies, highlighting their role in various precancerous conditions (Tables 2 and 3).

OLP

Probiotics can regulate immune responses in a strain-specific manner and interact with many pathways involved in the pathogenesis of OLP, including inhibiting T cell activation, infiltration, and proliferation, preventing keratinocyte apoptosis and NF-κB signaling, modulating the production of inflammatory cytokines and microRNAs, and suppressing MMP-9 expression and mast cell degranulation[140]. A clinical study observed the effectiveness of *S. salivarius* K12 lozenge (1 tablet twice daily, which contained no less than 1×10^9 CFU/tablet of *S. salivarius* K12 for 4 weeks) in the treatment of OLP. After 4 weeks of treatment, significant reductions were noted in the size of the lesions, including the extent of the white striations and the areas of atrophy/congestion and ulceration. The Visual Analogue Scale (VAS) scores for pain and burning sensation also significantly decreased, indicating that probiotic treatment can alleviate pain and improve symptoms in OLP patients. Moreover, the relative abundance of *S. salivarius* significantly increased following treatment[141]. Another randomized controlled trial investigated the effects of probiotics on recurrent oral candidiasis in OLP patients. Participants took lozenges containing *L. reuteri* DSM 17938 and ATCC PTA 5289 for 16 weeks, and clinical parameters such as VAS scores, *Candida* counts, plaque indices, and gingival indices were collected. Compared with the placebo group, the probiotic treatment group did not experience a reduction in *Candida* counts over time but showed a decrease in the gingival index, OLP severity score and lower VAS scores compared to those of the placebo group[142]. In a 1-year randomized clinical trial, 22 OLP patients received conventional treatment (antifungal drugs or corticosteroids) combined with probiotics (two strains of *L. reuteri* DSM 17938 and ATCC PTA 5289) or a placebo. The results showed no significant impact of the probiotic supplement on the oral microbiome in the conventional OLP treatment, despite recruitment issues and the premature termination of the study[143]. *Marlina* et al. preliminarily evaluated the clinical effects of VSL#3 (a product with a group of multiple bacterial strains and species [containing *L. acidophilus* BA05, *L. Bulgaricus* BD08, *L. paracasei* BP07, *L. plantarum* BP06, *B. longum* BL03, *B. infantis* BI04, *B. breve* BB02, and *Streptococcus thermophilus* BT01]) in patients with symptomatic OLP. Although the results revealed some biological and clinical effects, they were not statistically convincing, indicating that further research is needed to validate these findings[144].

OSF

Currently, there is no direct evidence suggesting that probiotics or prebiotics can be directly applied in the treatment of OSF. However, polyphenolic compounds, as a class of plant-based chemicals with prebiotic effects, have been used in the treatment of OSF due to their antioxidant and anti-inflammatory properties[145]. Common polyphenolic compounds include flavonoids, lycopene, epigallocatechin gallate (EGCG), resveratrol (RSV), curcumin, and others[146]. A clinical study on the efficacy of lycopene showed that 21 OSF patients treated with 16 mg/d lycopene for 2 months exhibited significant improvement in mouth opening[147]. In OSF rats induced by betel nut extract administration through the buccal mucosa, EGCG hydrogel significantly reduces the expression of TGF-β1 and type I collagen, with significant improvements in SOD and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) clearance rate[145]. Curcumin, a polyphenol found in turmeric rhizomes, is widely used as a dietary supplement and food additive. Curcumin is recognized for its anti-inflammatory, antioxidant, and anticancer properties, and can interfere with the TGF-β and iNOS pathways, thus alleviating cell fibrosis[148]. A randomized clinical trial involving 15 OSF patients assessed the therapeutic effects of curcumin. The results showed that curcumin effectively alleviated the burning sensation and improved mouth opening in OSF patients, with no side effects reported[149].

Barrett's esophagus

In one study, *B. longum* KACC 91563 and *L. acidophilus* NCFM were cocultured with two different Barrett's esophagus cell lines, and the expression of IL-18, TNF-α, P53, COX-2, and CDX1 was measured. The results revealed that these probiotic strains significantly inhibited the

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Table 2 | Application of probiotics/prebiotics for therapy of OPMDs

Disease	Probiotic/Prebiotic/Compounds with prebiotic effects (polyphenols) used	Dose	Object	Outcome	References
OLP	*S. salivarius* K12 lozenges	≥1 × 10^9 CFU/tablet of *S. salivarius* K12, 1 tablet, twice daily, 4 weeks	Human	A reduction in the size of the lesion, along with a decrease in pain and burning sensation	141
OLP	*L. reuteri* DSM 17938 and ATCC PTA 5289 lozenges	3 tablets daily, 16 weeks	Human	A decrease in gingival index with lower OLP severity score and VAS pain score	142
OLP	*L. reuteri* DSM 17938 and ATCC PTA 5289 tablets	3 tablets daily, 16 weeks	Human	No significant impact of the probiotic supplement on the oral microbiome in the conventional OLP treatment	143
OLP	VSL#3 (*L. acidophilus* BA05, *L. Bulgaricus* BD08, *L. paracasei* BP07, *L. plantarum* BP06, *B. longum* BL03, *B. infantis* BI04, *B. breve* BB02 and *S. thermophilus* BT01)	>4.5 × 10^9 CFU/packet (4.4 g), 4 packets daily, 30 days	Human	No statistically significant change in pain, disease activity, quality of life, serum/salivary CXCL10 or oral microbial composition	144
OSF	Lycopene	16 mg daily, 2 weeks	Human	Significant improvement in mouth opening	147
OSF	EGCG	3 times daily, 90 days	Mice	A significant decrease in the expression of TGF-β1 and type I collagen, along with significant improvements in SOD and DPPH clearance rate	145
OSF	Curcumin	2 g daily, 30 days	Human	Alleviation of burning sensation and improvement in mouth opening restrictions	149

OLP oral lichen planus, *OSF* oral submucous fibrosis, *EGCG* epigallocatechin-3-gallate, *DPPH* 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

Table 3 | Application of probiotics/prebiotics for therapy of GPLs

Disease	Probiotic/Prebiotic/Probiotic preparation	Dose	Object	Outcome	References
Barrett's esophagus	*B. longum* KACC 91563, *L. acidophilus* NCFM, *B. longum*	Barrett's esophageal cells co-cultured with *B. longum* and *L. acidophilus*	In vitro	Preventing the progression of Barrett's esophagus to esophageal adenocarcinoma	150
Barrett's esophagus	*L. acidophilus* 4356, *L. plantarum* 14917, *L. fermentum* 14931	Exposed to bile salts with a 1:1 ratio sodium cholate/sodium deoxycholate (CA/DCA)	In vitro	Accelerated repairing of bile-induced DNA damage. Reducing NF-κB-associated inflammation	151
H. pylori infection	*L. reuteri* DSM 17648	Combined triple therapy, one capsule (200 mg) daily, 30days	Human	A significantly higher *H. pylori* eradication rate	160
H. pylori infection	*L. rhamnosus* LRa05	Combined with bismuth quadruple therapy, one power (3 g, 1 × 10^{10} CFU) daily, 4 weeks	Human	A higher eradication rate of *H. pylori* and enhancing liver function	161
H. pylori infection	*Saccharomyces boulardii*	Combined triple therapy, 500 mg once, twice per day, 10 days or *S. boulardii* only, 14 days	Human	A significantly lower overall incidence of adverse reactions and diarrhea	162
ACF	XOS, FOS	Combine 1,2-dimethylhydrazine (DMH), +60 g XOS (FOS)/kg	Mice	A significant increase in SCFA levels	167
ACF	*L. delbrueckii* UFV-H2b20, *B. animalis* var. *lactis* Bb12	Oral administration in water, 3 × 10^8 CFU/ML, 14weeks	Mice	A significant reduction in the number of ACF	168
Colorectal tumors	*L. casei* strain Shirota (1.5 × 10^9 to 2.1 × 10^{10} viable cells/g)	1 g after each meal, 4 years	Human	A lower prevalence of metachronous AP with moderate or severe atypia	169
AP	Yogurt (*L. bulgaricus, S. thermophiles, L. acidophilus, Bifidobacterium*)	Never/rarely, monthly but less than weekly (1–3 times/month), weekly but less than daily (1–6 times/week), and daily (1 + /day)	Human	Weekly yogurt intakewas associated with decreased odds of AP among women	171
Colorectal adenomas	Fermented kimchi (*L. plantarum*)	10 weeks	Mice	Suppressing the formation or progression of colorectal adenomas	172

H. pylori Helicobacter pylor, ACF aberrant crypt foci, *XOS* xylooligosaccharide, *FOS* fructooligosaccharides, *SCFA* short-chain fatty acids, *AP* adenomatous polyps.

expression of these molecules, suggesting that the probiotics may have therapeutic potential for esophageal precancerous diseases[150].

In another study, researchers explored the bile tolerance and antioxidant properties of three lactic acid-producing bacterial strains (*L. acidophilus* 4356, *L. plantarum* 14917, and *L. fermentum* 14931) under simulated human reflux conditions. The investigation assessed DNA damage caused by bile exposure by measuring the ROS marker 8-oxo guanine and performing comet assays. The results revealed that all three bacterial strains exhibited substantial bile tolerance, which facilitated their colonization of the esophageal epithelium in GERD-like environments. Additionally, these lactic acid bacteria significantly promoted the repair of bile-induced DNA damage through the recruitment of pH2AX/RAD51 and a reduction in NF-κB-related inflammation[151].

CAG and GIM

The key to treating CAG and GIM is to eradicate *H. pylori*, as the majority of these patients have current or former evidence of *H. pylori* infection[152,153]. Probiotics, as an adjunct to *H. pylori* eradication therapy, not only improve the eradication rate but also reduce the side effects of conventional treatments[154].

Probiotics can produce antimicrobial substances such as SCFAs, lactic acid, and bacteriocins to inhibit or kill *H. pylori*[155]. Bacteriocins produced by *L. bulgaricus* strains (*L. bulgaricus* 14, *L. bulgaricus* 23, *L. bulgaricus* 35, *L. bulgaricus* 44, *L. bulgaricus* 47, *L. bulgaricus* 55, *L. bulgaricus* 60) exhibit strong anti-*H. pylori* activity against both antibiotic-sensitive and resistant strains[156]. Probiotics can also inhibit urease activity. *L. plantarum* ZJ316 blocks the expression of the *Ure* gene, thereby inhibiting urease synthesis[157]. Furthermore, probiotics reduce *H. pylori* colonization by competing with it for adhesion sites on epithelial cells or by inhibiting the expression of adhesion-related genes[158]. *B. animalis* CNCM I-745 can block the binding of *H. pylori* to host cells (mainly duodenal cells)[159]. *L. plantarum* ZJ316 effectively inhibits *H. pylori* adhesion to AGS cells, reducing adhesion by 70.14%[157].

A randomized, double-blind, placebo-controlled trial using *L. reuteri* DSM 17648 as an adjunct treatment showed that the probiotic group had a significantly higher *H. pylori* eradication rate compared with the placebo group, and also alleviated symptoms such as abdominal discomfort[160]. In another clinical trial, *H. pylori* positive patients received levofloxacin-based quadruple therapy with bismuth and *L. rhamnosus* LRa05 or placebo for two weeks. The results showed that the LRa05 group demonstrated a higher eradication rate of *H. pylori*, and LRa05 was able to modulate the inflammatory response and enhance liver function[161]. Another clinical trial showed that, compared with the standard triple therapy including proton pump inhibitors and bismuth quadruple therapy groups, the *Saccharomyces boulardii* powderquadruple therapy group had a significantly lower overall incidence of adverse reactions and diarrhea[162]. Supplementing the standard triple therapy with *S. boulardii* also improved the *H. pylori* eradication rate in children, while reducing the overall incidence of adverse events and gastrointestinal side effects[163].

Prebiotics can enhance the ability of probiotics to eliminate pathogens. A clinical study compared the effects of probiotics alone and probiotics (*Lactobacillus* and *bifidobacterium*) combined with prebiotics (Inulin + butyrate) in 120 patients with chronic gastric and duodenal diseases and *H. pylori* infection. The results showed that adding prebiotics to the treatment significantly increased the eradication rate of *H. pylori* and reduced side effects compared to probiotics alone (prebiotic group: 95%; probiotic group: 85.7%; and placebo group: 83.3%)[164].

Colorectal adenoma

Aberrant crypt foci (ACF), glandular abnormalities detected during colonoscopy, serve as early indicators of colorectal adenomas and cancer. Early identification of the ACF plays a crucial role in detecting and studying the progression of colorectal cancer[165]. In recent years, increasing evidence has shown that various probiotics and prebiotics including Xylooligosaccharide (XOS), FOS, *L. delbrueckii* UFV-H2b20, LGG, and *B. animalis* var. *lactis* Bb12 can reduce the formation of ACF and polyps[166–168]. In a randomized trial evaluating the effects of probiotics on adenomatous polyps (AP), the patients with recent colorectal tumors (AP or early-stage cancer) were randomly assigned to one of four groups: dietary guidance, *L. casei* preparation, wheat bran, or *L. casei* preparation and wheat bran combined. After 4 years, individuals in the *L. casei* preparation group had a lower prevalence of metachronous AP with moderate or severe atypia[169]. A case-control study investigating the link between yogurt intake and the size of adenomatous polyps indicated that individuals who regularly consumed yogurt had a lower risk of developing large adenomas[170]. Another 24-year prospective follow-up study conducted in the United States investigated the impact of yogurt intake and probiotic use on colorectal polyps. The study showed that high yogurt intake (more than 2 cups per week) was associated with a reduced risk of AP in women[171]. Korean fermented kimchi, as one of the representative probiotic foods, provides beneficial microorganisms and exerts significant inhibitory effects in the APC/$^{Min+}$ polyp model and in colitis-associated cancer, and it has also been clinically proven to significantly suppress the formation or progression of colorectal adenomas[172].

Innovative applications of engineered probiotics

By adding, reducing, or altering their genetic material, probiotics such as *E. coli* Nissle (EcN) 1917, *B. longum* NCC2705, *L. reuteri* DSM17938, *L. casei* BL23, and *L. plantarum* WCFS1, etc. have been genetically engineered to create bacterial strains capable of treating specific diseases[94].

A genetically engineered strain, *Lactococcus lactis* ATCC334, can produce *Listeria* adhesion protein. This strain colonizes the mouse intestine and competitively reduces the colonization and systemic spread of *Listeria* on the mucosa, protecting the mice from fatal infections[173]. Some researchers have genetically modified *S. boulardii* to secrete a fusion protein (ABAB) that can neutralize four different *Clostridium difficile* toxins[174]. Preventive administration of these engineered bacteria significantly alleviates inflammation and tissue damage in the intestinal mucosa associated with *C. difficile* infection in mice, thereby reducing their mortality rate.

Additionally, probiotics can be engineered to detect pathogens by binding to novel metabolites or quorum sensing (QS) molecules produced by the pathogens. The researchers developed a genetically engineered *L. reuteri* DSM20016 capable of detecting real-time changes in autoinducing peptide-I (AIP-I) produced by *Staphylococcus aureus*[175].

Engineered probiotics can be used to sense and treat intestinal inflammation. The *Lactobacillus delbrueckii* subsp. *Lactis* (*L. Lactis*) NZ9000 has been engineered to produce REX-binding proteins, which are considered as IL-23 receptor (IL-23R) antagonists. These proteins inhibit the secretion of the pro-inflammatory cytokine IL-17A, thus suppressing the inflammatory cascade[176]. In a mouse model of colitis induced by dextran sulfate sodium, genetically engineered EcN 1917 expressed high levels of schistosome immunoregulatory protein (Sj16) in the gastrointestinal tract, significantly improving the colitis symptoms in the mice[177]. Probiotics are also designed to sense and even respond to treating inflammatory infections. A cellular biosensor could detect Crohn's disease by using a promoter that responds to nitric oxide (NO). This promoter regulates the expression of a reporter gene. The system guides EcN1917 to areas where NO concentration is elevated and induces it to produce therapeutic proteins, including granulocyte-macrophage colony-stimulating factor (GM-CSF)[178].

With the development of modern gene editing technology, more and more probiotics are designed to have anti-cancer effects. Dietary supplementation with microencapsulated *B. bifidum and L. gasseri*, either administered alone or in combination with quercetin, significantly reduced ACF and adenomas in Apc$^{Min/+}$ mice, inhibiting the canonical Wnt/β-catenin signaling pathway, thereby suppressing CRC development[179]. EcN 1917 was engineered to target the angiogenesis inhibitor TUM-5 and the tumor suppressor p53 to anaerobic tumor regions, and this treatment significantly inhibited the growth of transplanted tumors in mice[180]. The SYNB1891 strain (NCT04167137) made from non-pathogenic EcN 1917 can produce cyclic di-AMP to stimulate the interferon gene pathway, and then trigger innate immunity by activating antigen-presenting cells to

present tumor antigens[181]. In a Phase I clinical trial (NCT04167137), an engineered strain SYNB1891 constructed from EcN 1917 can stimulate the STING pathway, and then trigger anti-tumor immunity[181].

Conclusion

Traditional treatment methods for OPMDs and GPLs have shown some limited effectiveness, but novel and more effective approaches are urgently needed to reduce and resolve post-treatment recurrence, medicine resistance in refractory cases, and carcinogenesis in chronic lesions. With the deep understanding of the oral and Gl microbiome and microbiological technology development, increasing evidence indicated that probiotics, prebiotics, and engineered probiotics have the potential in the management of OPMDs and GPLs.

Probiotics and prebiotics not only help enhance barrier function and eliminate pathogenic microbes such as *H. pylori*, but also alleviate antibiotic resistance and reduce cancer risk, with better long-term effects and fewer side effects. As emerging therapies, applications of probiotics/prebiotics could offer new perspectives and promising avenue to improve the prevention and treatment of precancerous lesions, providing patients with more precise and personalized treatment options.

The oral cavity and GI tract are continuous segments of the digestive tract. Therefore, future probiotics and prebiotics products are expected to develop multi-target therapeutic strategies that can simultaneously regulate the oral and GI microbiomes. Moreover, personalized probiotic and prebiotic therapies in the future will be multi-layered and multidimensional, involving not only the application of single strains but also strain combinations, prebiotic pairing, dietary interventions, and comprehensive regulation of the microbiota environment.

Although engineered probiotics have not been applied in the clinical treatment of oral and GI precancerous lesions, their advantages, such as stability, specificity, preference, efficient, and relative safety, may make them a new therapeutic option in the future. However, the application of engineered probiotics still faces a series of challenges. For example, many microorganisms lack available genetic tools, limiting probiotic modification to a few strains. The existing basic mechanistic theories are insufficient, which may lead to improper modifications of probiotics. Additionally, the microbial stability, retention time, and colonization resistance of engineered probiotics need further improvement. Therefore, the safety of modified probiotics needs to be strictly validated during application, and the process of regulating engineered probiotics from clinical research to new drug production needs to be further standardized.

Data availability

No datasets were generated or analysed during the current study.

Abbreviations

ACF	aberrant crypt foci
AP	adenomatous polyps
COX-2	cyclooxygenase-2
EGCG	epigallocatechin gallate
FOS	fructooligosaccharides
GERD	gastroesophageal reflux disease
GI	gastrointestinal
GPLs	gastrointestinal precancerous lesions
HPV	human papillomavirus
NK	natural killer
NO	nitric oxide
OLK	oral leukoplakia
OLLs	oral lichenoid lesions
OLP	oral lichen planus
OPMDs	oral potentially malignant disorders
OSF	oral submucous fibrosis
PVL	proliferative verrucous leukoplakia
ROS	reactive oxygen species
SCFA	short-chain fatty acids
Th1	T helper 1
Th17 cells	T helper 17 cells
Tregs	regulatory T cells
VAS	visual analogue scale
XOS	xylooligosaccharide

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Author contributions

Z.H., J.Z., X.B., and S.L. contributed to the methodology, investigation, original draft writing, and visualization of the article. Y.L., T.L., and N.D. were responsible for data curation and conceptualization. W.W. and Y.W. contributed to the writing–review & editing and validation of the manuscript. X.W. provided supervision, writing–review & editing, and funding acquisition. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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