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HIGHLIGHT

PEGylated bacteria restore intestinal mucosal barrier

KEY WORDS

PEGylation;
Probiotic;
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Colonic mucosal barrier;
Colitis;
Diabetes

The mucosal barrier of the gastrointestinal tract (GIT) is crucial for the overall health of the body. Its disruption permits the infiltration of intestinal microorganisms into the underlying mucosa, resulting in infections and inflammatory conditions, such as inflammatory bowel disease (IBD), metabolic disorders, and autoimmune diseases[1,2]. Therefore, strategies to protect the mucosal barrier from exogenous stimuli or to facilitate its recovery have become increasingly important. Currently, immunomodulators, corticosteroids, and biological agents have been widely employed to treat IBD by alleviating inflammation and enhancing mucosal healing. Unfortunately, they can also produce serious systemic toxicity[3,4]; therefore, there is an urgent need for exploiting innovative strategies to fortify the intestinal mucosal barrier against the invasion of exogenous damaging factors.

Published in *Nature Biomedical Engineering*, the work of Chen et al.[5] demonstrates that a surface coating of poly(ethylene glycol) (PEG) could enhance the penetration of commensal bacteria through the mucus layer and reinforce the intestinal mucosal barrier. In their study, a multidimensional approach was used to address the limitations in repairing damage to the intestinal mucosal barrier. Mucin glycoproteins often rapidly clear oral drugs, hindering their penetration through the mucus layer to reach mucosal tissues, and leading to poor therapeutic outcomes. Reducing the interaction between drug molecules and mucins is critical for improving their bioavailability, therapeutic effectiveness, and treatment outcomes.

In the first set of experiments, the surface of the selected bacteria (*Escherichia coli* Nissel 1917) was coated with polydopamine and then functionalized with PEG (average molecular weight of 1, 2, 5, or 10 kDa) to enhance bacterial motility and mucus penetration (Fig. 1A). It was observed that bacteria with surfaces modified with 2 kDa PEG exhibited the strongest mucus penetrating capacity among all PEGylated bacteria (Fig. 1B). Furthermore, the Transwell assay was used to quantify the mucus-penetrating activities of PEGylated bacteria, and the results revealed that the numbers of PEGylated bacteria in the basolateral chamber increased 25- and 26-fold, compared to uncoated bacteria after incubation for 1 and 2 h, respectively.

Encouraged by the enhanced *in vitro* permeability of PEGylated bacteria in the mucus layer, the authors performed *in vivo* experiments to evaluate their mucus-penetrating capability in mice. Tests with oral administration of PEGylated and non-PEGylated bacteria revealed that the colon segment contained the largest numbers of PEGylated bacteria, suggesting that surface modification with 2 kDa PEG could promote bacterial colonization in the mucus layer and prolong their retention in the GIT (Fig. 1C and D). Commensal microbiota plays crucial roles in resisting the growth and colonization of intestinal pathogens[6]. To benchmark whether PEGylated bacteria could prevent the invasion of an external pathogen, mice were gavaged with *Salmonella typhimurium* (STm) after oral administration of PEGylated bacteria for seven days. The experimental results demonstrated that following exposure to the PEGylated bacteria, the counts of *Staphylococcus aureus* and STm were about 17- and 11-fold lower than those of uncoated bacteria, respectively. This suggests that PEGylated bacteria could effectively prevent the invasion of external pathogens (Fig. 1E).

The gut microbiota plays an important role in maintaining intestinal homeostasis, and accumulating evidence suggests that alterations in the gut microbial community can lead to IBD, obesity, insulin resistance, and other diseases[7]. To evaluate the efficacy of oral PEGylated bacteria in maintaining a healthy

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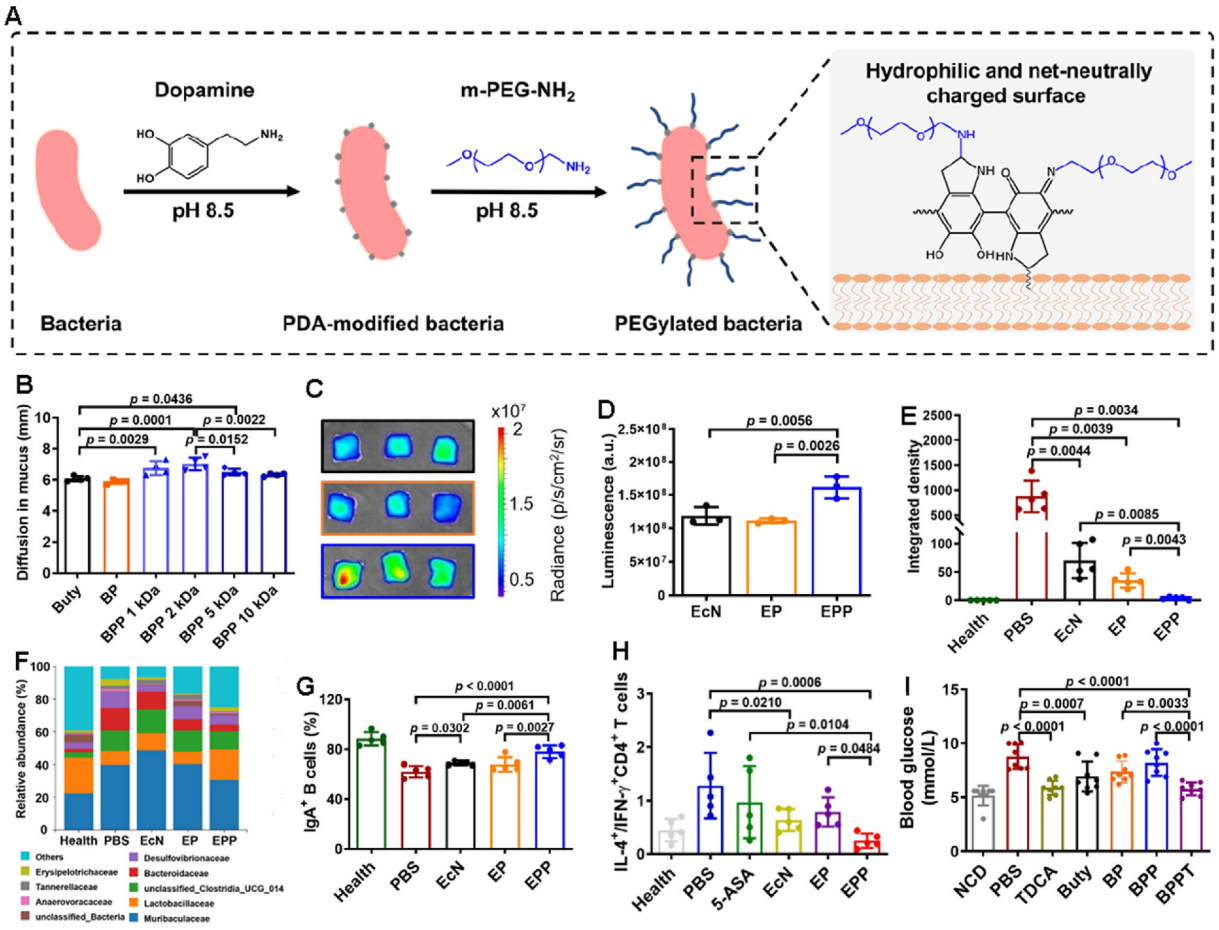

Figure 1 (A) Schematic illustration of PEGylated bacteria prepared *via in situ* self-polymerization of dopamine. (B) The penetration of *Clostridium butyricum* grafted with PEG of different molecular weights reached the depth of artificial mucus after 72 h of treatment. (C) IVIS images and (D) corresponding fluorescence intensities of porcine colon segments after incubation with different bacteria. (E) Cecum segments were collected to quantify the STm-integrated green channel density in immunofluorescence images of STm 3 days post-infection. (F) Abundances of the gut microbiota at family levels. (G) Percentage of IgA^+ B cells in the Peyer's Patches (PPs). (H) T cells, and ratio of IFN-γ^+CD4^+ to IL-4^+CD4^+ T cells in the mesenteric lymph nodes measured by flow cytometry. (I) Fasting blood glucose levels in mice after different treatments. Reprinted with the permission from Ref. 5. Copyright © 2024 Springer Nature Limited.

microbial balance and inhibiting the spread of opportunistic infections in the intestine, Chen and co-workers utilized dextran sulfate sodium (DSS)-induced IBD mice as a model for imbalanced gut microbiota. The study supported the hypothesis that PEGylated bacteria could successfully promote the growth of beneficial gut microbes and inhibit the proliferation of opportunistic pathogens (Fig. 1F). Intercellular tight junction proteins, including occludin and zonula occludens-1, are closely linked with epithelial barrier integrity, and are primary determinants of mucosal permeability. The researchers found that in mice with STm/DSS-induced IBD, oral administration of PEGylated bacteria could strengthen the gut mucosal barrier. This was achieved by increasing the proportion of IgA-secreting B cells, promoting the expression of tight junction proteins, and stimulating goblet cells to produce mucus (Fig. 1G).

Finally, the ability of oral PEGylated bacteria to prevent or alleviate inflammatory intestinal damage was evaluated. It was observed that oral PEGylated bacteria increased the percentage of $IL4^+CD4^+$ T cells, decreased the percentage of IFN-γ^+CD4^+ T cells, and inhibited leukocyte infiltration into colon tissues compared to control treatments (Fig. 1H). Chen et al. also evaluated the potential of PEGylated bacteria to alleviate insulin resistance. The results suggested that PEGylated bacteria possessed the ability to regulate glucose homeostasis, relieve adipose tissue hyperplasia, and reduce inflammatory responses (Fig. 1I).

This work demonstrates that PEGylation with 2 kDa PEG not only improves penetration, localization, and retention of the bacteria in the mucus layer, but also efficiently suppresses the invasion of pathogenic bacteria, restores beneficial gut microbiota composition, and promotes mucus secretion in the lower GIT. The enhancement of barrier integrity through the regulation of mucus secretion, tight junction expression, and improved bacterial penetration, localization, and retention in the mucus layer has shown promising results in alleviating the symptoms of colitis and diabetes. Oral administration of probiotics to reinforce the mucus barrier is becoming attractive. However, the regulation of mucosal barrier through therapeutic interventions is an emerging area of research, necessitating further investigations to elucidate the role

of mucosal barrier integrity in various diseases. Understanding the signaling pathways that regulate tight junctions and mucus secretion is crucial for restoring mucosal barrier function, which is essential for developing novel and effective medications.

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Author contributions

Menghang Zu: Writing − review & editing, Writing − original draft, Conceptualization. Xue Xia: Writing − review & editing, Writing − original draft. Bo Xiao: Writing − review & editing, Funding acquisition, Conceptualization.

Conflicts of interest

The authors have no conflicts to declare.

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