BJS Academy

Exploring microbial engineering for enhanced mucosal healing in inflammatory bowel disease

Kamacay Cira, MD; Philipp-Alexander Neumann, MD

Department of Surgery, Klinikum rechts der Isar, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany

Article review

*Praveschotinunt, P., Duraj-Thatte, A.M., Gelfat, I. et al. Engineered E. coli Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut. Nat Commun **10**, 5580 (2019). https://doi.org/10.1038/s41467-019-13336-6*

Inflammatory bowel diseases (IBD) are characterized by factors such as distorted expression of inflammatory cytokines, dysfunction in the epithelial and mucosal barriers, and dysbiosis of the intestinal flora.[1] The disease itself not only induces morbidity but also complicates treatments, including surgical interventions. The associated immunosuppression, inflammation, and dysbiosis may contribute to abnormal wound healing, further complicating the postoperative course.[2] In Crohn's disease (CD), rates of anastomotic leakage (AL) can reach up to 23%,[3-6] underscoring the challenges in managing these patients.

Restoring mucosal continuity to the gut lining is imperative for re-establishing barrier function, forming the basis for a pro-healing environment. Mucosal epithelial restitution, occurring rapidly within hours of injury, plays a crucial role not only in IBD but also in anastomotic healing following intestinal resection.

In this review, we explore an approach to engineered microbial therapies for promoting mucosal healing, presenting insights that may have implications for perioperative care and providing a novel perspective for addressing the complexities of compromised intestinal wound healing in patients with IBD.

The study introduces a novel approach called Probiotic-Associated Therapeutic Curli Hybrids (PATCH) forming an *Escherichia coli (E.coli)* biofilm matrix, potentially useful in treating IBD. Unlike conventional soluble therapeutic proteins, PATCH involves an engineered beneficial bacterium, *E.coli Nissle 1917* (EcN). In the PATCH system, a csg (curli) operon was deleted from the bacterial chromosomal DNA (PBP8 strain), and a plasmid was engineered to encode a synthetic curli operon capable of producing a chimeric CsgA protein. The CsgA protein, after extracellular secretion, self-assembles to build therapeutic curli hybrid fibers. The bioactive component linked to the CsgA protein is the trecoil factor (TTF) 3 which belongs to a family of human cytokines, primarily secreted by mucus producing cells in the gastrointestinal tract to promote epithelial restitution. The TTF's also exhibit beneficial effects such as tumour suppression or apoptosis blockade. The researchers used EcN as the microbial chassis for PATCH, taking advantage of EcN's safety record in humans and its compatibility with genetic engineering techniques. However, the use of EcN alone in previous studies for IBD treatment showed limited efficacy, prompting the authors to engineer it for enhanced therapeutic potential.

In vitro assessments confirmed the safety of PATCH, as the CsgA-TFF3 fusion did not exhibit increased invasiveness or compromise epithelial integrity in polarized Caco-2 cell models, indicating their non-pathogenic nature. The study confirmed *in vivo* production of engineered curli fibers, as detected by ELISA and immunohistochemistry, with CsgA-TFF3 showing mucin-binding activity, suggesting mucus integration of the synthetic curli fibers.

To assess the therapeutic potential, the study employed a mouse model of colitis induced by dextran sodium sulfate (DSS). The researchers administered the engineered EcN strains expressing curli-bound TFF3 rectally before, during, and after induction of colitis. This delivery method aimed to co-localize the therapeutic bacteria with the affected tissues. The results indicated that the engineered bacteria, particularly those producing CsgA-TFF3 curli fibers, ameliorated disease symptoms (reduced disease activity index and weight loss),

reduced inflammation, and enhanced the healing process compared to controls, only receiving DSS and phosphate buffered saline solution. In comparison to the control group, mice within the PBP8 CsgA-TFF3 group exhibited better crypt structure, lower inflammatory cell infiltration, less tissue edema, and more intact epithelium on histology.

The researchers investigated the potential mechanisms underlying the therapeutic effects. Gene expression and protein production profiles in colonic tissue homogenates suggested that the PATCH system with CsgA-TFF3 led to a reduction in pro-inflammatory cytokines (IL-6, IL-17A, TNF-α) associated with Th17 cell differentiation. Furthermore, the engineered bacteria contributed to the upregulation of tight junction protein-1 mRNA, suggesting a potential role in enhancing epithelial barrier function. Furthermore, the CsgA-TFF3 group showed significantly lower metalloproteinase 9 expression, and prostaglandin-endoperoxidase synthase 2 compared to the DSS group.

As the authors stated as a limitation, the plasmids have shown to be dependent on concomitant antibiotic application, which might further worsen the already thriving dysbiosis in IBD. Therefore, a second-generation PATCH should be developed with higher plasmid stability independent of antibiotic use to be more suitable in clinical practice.

In summary, the researchers have shown in an animal model that the application of PATCH was able to prevent the disruption of the intestinal barrier during colitis, which represents a hallmark of IBD, by reinforcing the barrier function through a *E.coli* biofilm matrix and thereby promoting epithelial restitution and wound repair. The latter might also be a promising approach for supporting anastomotic healing in inflammatory conditions by creating a pro-healing environment.

References

1. J. Torres, S. Mehandru, J.-F. Colombel, and L. Peyrin-Biroulet, "Crohn's disease," The Lancet, vol. 389, no. 10080, pp. 1741-1755, 2017, doi: 10.1016/S0140-6736(16)31711-1.

2. J. T. Schweer et al., "Crohn's Disease as a Possible Risk Factor for Failed Healing in Ileocolic Anastomoses," (in eng), J Clin Med, vol. 12, no. 8, Apr 11 2023, doi: 10.3390/jcm12082805.

3. F. H. M. Chaim et al., "Aspects Towards the Anastomotic Healing in Crohn's Disease: Clinical Approach and Current Gaps in Research," (in English), Frontiers in Surgery, Mini Review vol. 9, 2022-June-24 2022, doi: 10.3389/fsurg.2022.882625.

4. W. F. Johnston et al., "What Is the Risk of Anastomotic Leak After Repeat Intestinal Resection in Patients With Crohn's Disease?," Diseases of the Colon & Rectum, vol. 60, no. 12, pp. 1299-1306, 2017, doi: 10.1097/dcr.0000000000000946.

5. R. S. Shah et al., "Hypoalbuminaemia, Not Biologic Exposure, Is Associated with Postoperative Complications in Crohn's Disease Patients Undergoing Ileocolic Resection," Journal of Crohn's and Colitis, vol. 15, no. 7, pp. 1142-1151, 2021, doi: 10.1093/ecco-jcc/jjaa268.

6. A. Tzivanakis, J. C. Singh, R. J. Guy, S. P. L. Travis, N. J. Mortensen, and B. D. George, "Influence of Risk Factors on the Safety of Ileocolic Anastomosis in Crohn's Disease Surgery," Diseases of the Colon & Rectum, vol. 55, no. 5, pp. 558-562, 2012, doi: 10.1097/DCR.0b013e318247c433.