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Comparative Colonisation Ability of Human Faecal Microbiome Transplantation Strategies in Murine Models

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ABSTRACT

The gut microbiome plays a crucial role in maintaining intestinal homeostasis and influencing immune-mediated diseases. Human faecal microbiota transplantation (FMT) is often employed in murine models to investigate the role of human microbes in disease regulation, but methods for effective colonisation require refinement. This study aimed to assess the colonisation efficiency of human microbiota in a murine model using FMT with human faeces, focusing particularly on the impact of gut microbiota depletion via polyethylene glycol (PEG) and comparing oral-gastric gavage with enema administration routes. Our findings revealed that PEG-induced depletion enhanced human microbiome colonisation in mice. Oral-gastric gavage prolonged colonisation, while enema administration facilitated quicker resolution of dysbiosis, both inducing selective human microbial colonisation in a time-dependent manner. Notably, genera such as *Bacteroides*, *Blautia*, *Medicaternibacter* and *Bifidobacteria* were successfully colonised, whereas *Roseburia*, *Anaerostipes*, *Anaerobutyricum* and *Faecalibacterium* failed to establish in the murine gut post-FMT. These findings highlight the challenges of replicating human gut microbiota in murine models and underscore the importance of selecting appropriate FMT methods based on desired outcomes. This study provides valuable insights into the colonisation dynamics of human microbiota in mice, contributing to the development of more effective FMT strategies for disease treatment.

1 | Introduction

Approximately 10–100 trillion microorganisms harbour an extensive reservoir of genetic information encoded by the human gastrointestinal tract (Sender et al. 2016). Intestinal epithelial cells, immune cells and gut microbiota constitute an ecosystem that contributes to the maintenance of intestinal tissue homeostasis. Accumulating data suggest that the human microbiota is closely associated with the development and progression of immune-mediated diseases (Zheng et al. 2020). In line with this, normalisation of disrupted microbiota by various ways such as dietary intervention improves the pathological phenotype of inflammatory diseases (Zhang et al. 2023). The administration of live microbes, such as gut microbiota, is considered a promising

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therapeutic approach. Faecal Microbiota Transplantation (FMT) is used to treat inflammatory diseases that may be induced by microbiota disruption. Eiseman et al. (1958) were first to propose FMT, and they transplanted the functional flora from the faeces of healthy individuals into the gastrointestinal tract of patients to re-establish a new intestinal microbiota for the treatment of intestinal and extraintestinal diseases. For decades, FMT has been considered a special type of organ transplantation and has been successfully used to eradicate pathogenic toxin-producing bacteria from patients with *Clostridium difficile* infection and for the treatment of various diseases, such as IBD (Shi et al. 2016; Khoruts 2017; Rees et al. 2022; Yadegar et al. 2023). Among microbiome-based techniques, FMT is recently recognised as the most feasible technique to treat gut inflammatory disease patients in the clinical area (Park and Im 2020).

Using human faeces for FMT or administering human-derived strains in mice provides researchers with valuable opportunities to understand the immune-regulatory functions of human gut microbes in disease progression (De Palma et al. 2017; Britton et al. 2019; Sharon et al. 2019). These methods are crucial for predicting the efficacy and potential side effects of microbiota normalisation strategies before they can be safely applied to human patients. Germ-free (GF) mice are free of bacteria, viruses, fungi, protozoa and other putrefactive and parasitic organisms. Due to their uniqueness in lacking any microbiota, GF mice have a big advantage for FMT study. Using a humanised, germ-free mouse model of FMT, the functional role of human gut microbiota in the regulation of intestinal inflammatory diseases was determined (De Palma et al. 2017; Britton et al. 2019; Sharon et al. 2019). However, GF mice are costly, difficult to manage and have critical immunological deficiencies including incomplete immune development, which may result in immune phenotypes that differ from those observed in human diseases (Hansen et al. 2012). In contrast, specific pathogen-free (SPF) mice are more affordable and readily available, making them the preferred choice for many researchers. However, the presence of an existing intestinal microbiota in SPF mice makes colonisation with new microbiota more challenging (Caballero-Flores et al. 2023; Lee et al. 2024). Additionally, antibiotics-induced microbial depletion requires caution depending on disease context when assessing FMT efficacy. For example, different antibiotics can lead to distinct lung inflammation phenotypes (Normansell et al. 2018; Park et al. 2020). Moreover, antibiotics can affect gut immune function, requiring time for recovery to restore normal function and eliminate residual antibiotics before FMT to ensure successful microbiota transplantation (Francino 2016; Palleja et al. 2018). Several important questions remain unresolved, including the efficiency of human microbiota colonisation in the murine gut, the most effective transplantation methods and the specific strains most likely to successfully colonise the murine gut.

Building on this background, our study aimed to develop more effective strategies for enhancing colonisation during human faeces FMT in SPF murine models. We specifically investigated whether gut microbiota depletion prior to human faeces FMT influences colonisation. Furthermore, we compared the colonisation ability of two administration routes—oral-gastric gavage and enema—following gut microbiota depletion using polyethylene glycol (PEG) and assessed whether repeated FMT administration would provide additional benefits. Additionally, our study sought to identify which genera from human faeces are most likely to successfully colonise in the mouse FMT model. These findings offer valuable insights for researchers aiming to optimise human faeces FMT in disease models, potentially enhancing the translational relevance of gut microbiome studies.

2 | Results and Discussion

2.1 | The Colonisation Efficiency of Oral FMT Without Prior Gut Microbiota Clearance

First, to assess the efficiency of human faecal microbiota transfer to recipient animals, we compared the faecal microbiome profiles between humans (donor) and mice (recipient). The NC group (negative control, non-treated animals) exhibited a higher Chao1 index compared to the human faeces, although the Shannon index was similar (Figure S1A). In the human faeces used in this study, the families Bacteroidaceae, Tannerellaceae and Veillonellaceae were significantly more abundant than in the NC group. At the genus level, *Phocaeicola, Bacteroides*, *Blautia*, *Parabacteroides*, *Veillonella*, *Fusicatenibacter*, *Mediterraneibacter* and *Faecalibacterium* were notably more prevalent. Conversely, the NC group showed higher distributions of Muribaculaceae, Lactobacillaceae, Akkermansiaceae, Prevotellaceae and Rikenellaceae at the family level, and *Duncaniella*, *Ligilactobacillus*, *Akkermansia*, *Muribaculum*, *Kineothrix*, *Sangeribacter*, *Prevotella* and *Alistipes* at the genus level (Figure S1B–E). Prevotellaceae is a bacterial family commonly found in humans that often competes with Bacteroidaceae (Gorvitovskaia et al. 2016). Thus, the human faeces used in this study exhibited a Bacteroidaceae-dominant profile and contained a substantial presence of commensal clostridia such as *Faecalibacterium* and *Blautia*, which are commonly observed in human faeces (Gu et al. 2023).

When the human faeces were transplanted via FMT to recipient mice without gut microbiome depletion by PEG pretreatment, the recipient's microbiome remained comparable to that of the NC. To improve reproducibility, our experimental design and reporting were aligned with the Guidelines for Reporting on Animal Faecal Transplantation (GRAFT) framework, recently proposed by Secombe et al. (2021). We administered oral-gastric gavage with human faeces to animals that had not been treated with PEG (NPO) (Figure 1A). Faecal samples were analysed on Days 1, 3 and 5 post-FMT to compare colonisation efficiency. No significant differences were observed in alpha diversity and bacterial load (Figure 1B,C). When comparing beta diversity, the distance between the NPO group and the human faeces and PEG-treated mice was similar to that of the NC group, with no significant differences in composition compared to the NC group (Figure 1D–G). This suggests that the provision of human faeces without gut microbiota depletion results in only minimal changes in the gut microbiome.

2.2 | Significant Changes of Faecal Microbiome in Mice After PEG Treatment

In recipient animals, the resident gut microbiota act as a barrier against colonisation by the donor faecal microbiota. Antibiotic treatment is commonly used in FMT research to deplete gut

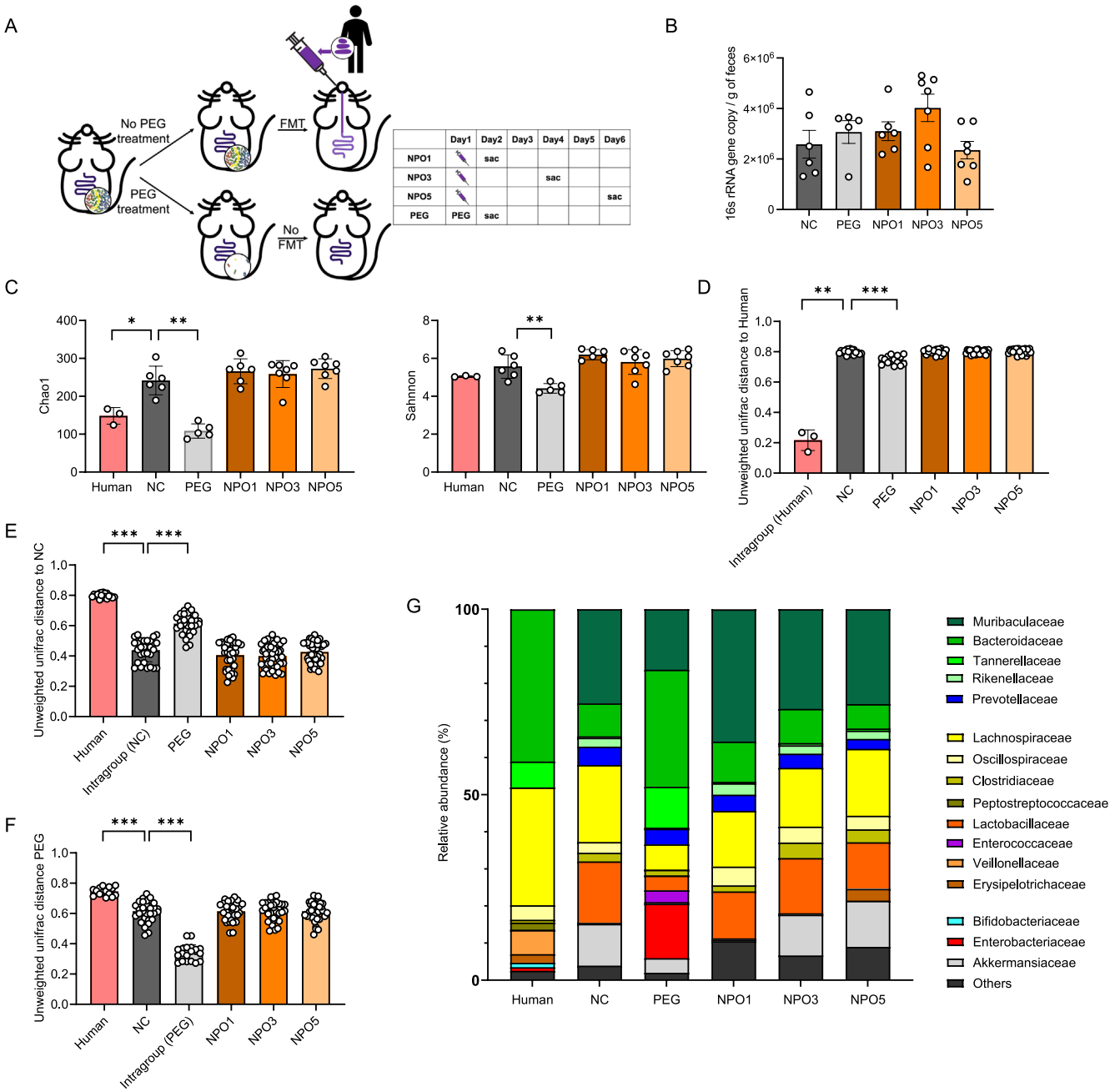

FIGURE 1 | Oral gavage of human faecal microbiota transfer without PEG pretreatment did not alter faecal microbiome of recipients. (A) Experimental scheme. (B) Bacterial load. (C) Chao1 and Shannon index for alpha diversity. (D–F) Unweighted unifrac distance according to FMT groups. (D) Distance to Human faeces. (E) Distance to NC. (F) Distance to PEG Group. (G) Relative composition of taxa in family level (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, Mann–Whitney U test). Human, the faeces from the human donor; NC, negative control (non-treated animals); NPO, oral-gastric gavage without PEG pretreatment group; ns, not significant; PEG, polyethylene glycol treated group.

microbiota in recipient animals, as it provides an easy method to overcome colonisation resistance (Kennedy et al. 2018; Tan et al. 2022; Fishbein et al. 2023). However, this approach has significant limitations, including unintended effects on symbiotic bacteria in other regions like the airways and skin, as well as impacts on the host immune system, which may alter physiological and immunological phenotypes, making the model less reflective of natural conditions (Sauer et al. 2021; Venditto et al. 2021).

In contrast, PEG is a nondigestible, nonabsorbable agent that induces diarrhoea via osmotic effects, thereby clearing intraluminal contents, including gut microbiota. Widely used in humans for bowel preparation, PEG generally has fewer systemic effects than antibiotics (Juluri et al. 2011). By providing a shorter, more controlled window for microbiota removal, PEG-based depletion allows researchers to examine its direct impact on gut microbiota without confounding immune or systemic influences, while also avoiding potential interference from residual antibiotics post-FMT. Indeed, PEG treatment has also been shown to deplete mouse gut microbes in FMT applications. A previous study reported that bowel cleaning with PEG effectively depleted gut bacterial numbers in conventional mice

(Wrzosek et al. 2018). The use of PEG for bowel cleansing in humans suggests that its application is a more rational choice for translational relevance.

Thus, we treated mice with PEG to remove the resident gut microbiota from the recipient animals. Faecal contents were collected 1 day post-PEG treatment, and the composition of the faecal microbiome was analysed. PEG treatment substantially decreased gut microbial alpha diversity, as evidenced by lower Chao1 and Shannon index scores in the faecal microbiome of PEG-treated animals compared to NC with similar bacterial load (Figure S2A,B). Beta diversity also markedly differed between the NC and PEG groups (Figure S2C). A decrease in commensal bacteria was observed following PEG treatment, with a particularly pronounced reduction in Bacillota, including families such as Lactobacteriaceae, Oscillospiraceae and Lachnospiraceae. Muribaculaceae, which had the highest abundance within Bacteroidota, also showed a marked decrease in abundance. However, the removal of Bacteroidaceae and Tannerellaceae from the commensal bacteria was relatively inefficient. Notably, PEG treatment led to the expansion of potential pathobionts. Similar to the phenomena observed in gut microbiome depletion using antibiotics (Grazul et al. 2016; Hagan et al. 2019), the relative compositions of Enterobacteriaceae (*Escherichia*) and Enterococcaceae (*Enterococcus*), which tend to increase during dysbiosis, were substantially elevated following PEG treatment (Figure S2D–G). Strains within Enterobacteriaceae, including *Escherichia*, are known to increase not only due to antibiotic use but also in conditions such as inflammatory bowel disease and severe systemic inflammation. Thus, the relative increase in pathobionts appears to be a consistent outcome of intestinal bacterial depletion, even after PEG treatment. Collectively, one-time PEG treatment substantially decreased gut microbial diversity and induced significant alterations in the gut microbiome of mice, including the expansion of potential pathogens.

While PEG-based depletion effectively targets gastrointestinal physiology, it can still disrupt gut function. Even mild osmotic shifts can lead to lasting changes in microbiota composition and host immune responses (Tropini et al. 2018). Alterations in gut pH, motility or pathobionts may increase permeability or trigger local inflammation, potentially resulting in systemic effects. Thus, despite having a lower systemic impact compared to antibiotics, PEG has clear limitations in studies focused on gut function and gastrointestinal physiology. Depending on the study design—particularly those centered on gut functional parameters—researchers should adopt depletion strategies that minimise unintended effects on gut function.

2.3 | The Efficiency of FMT Administration via Two Delivery Routes After PEG Depletion

While oral gastric gavage is the most commonly used method for FMT due to its simplicity, it carries risks such as inducing stress in the animals, and it does not guarantee that the faecal microbiota will survive and reach the cecum and large intestine. Although less common, rectal administration by enema may provide more effective delivery to the colon with fewer enzymatic reactions. However, it carries risks such as rectal leakage and infection, and limited data are available comparing the colonisation ability of these two methods.

So, we conducted experiments to assess the efficiency of FMT via two different administration routes (Figure 2A). We administered oral–gastric gavage (PO) or intra-rectal injection (PR) to PEG-treated animals with human FMT the following day. The faecal microbiome of PEG-treated animals was examined at 1 (PO1, PR1), 3 (PO3, PR3) and 5 (PO5, PR5) days post-FMT and compared with other groups, including the human (donor human faecal microbiome), NC and PEG groups. Faecal microbiome analysis at 1, 3 and 5 days post-FMT showed that PEG-induced gut depletion improved human faeces colonisation compared to the non-depletion group, but the microbiota gradually reverted to the original mouse pattern over time. Specifically, in comparison with the NC group, the Chao1 and Shannon indices of the FMT-treated recipients reached comparable levels of 5 and 3, respectively (Figure 2B). Bacterial loads were not significantly increased by FMT, possibly due to factors such as microbial composition, immune regulation and environmental limitations in the gut (Figure 2C). When comparing the beta diversity distance from the human sample, both PO and PR groups showed a substantial decrease in similarity by Day 5. Analysing the efficiency of the FMT methods between PO and PR, at 5 days post-FMT, PR5 had a greater distance from the human faecal microbiome than PO5 (Figure 2D,E). PR5 maintained a shorter distance from the faecal microbiome of NC than PO5 (Figure 2F). These findings suggest that oral administration may support the prolonged colonisation of human faeces and allow for longer residence of the targeted bacterial strains in the gut. However, at 1 day post-FMT, PR1 exhibited greater divergence from conventional mice than PO1 did (Figure 2F and Figure S3A). Compared to the PEG-treated control group, there was a more pronounced difference, particularly in the rapid reduction of pathobionts such as *Enterococcus* and *Escherichia* (Figure 2G and Figure S3B). In summary, while oral administration may prolong colonisation, enema administration may have quicker effects. Therefore, the choice between oral and enema administration for FMT should be based on the desired experimental outcomes.

Some studies have reported that the cecal and faecal microbiomes are different in humans and other species (Marteau et al. 2001; Stanley et al. 2015). Thus, we further examined the cecal microbiome in the FMT models at 3 days post-FMT. Faecal and cecal microbiomes were similar across the NC, PO3, PR3 and NPO3 conditions (Figure S4A). The cecum and faeces from the same individual showed substantially higher similarity than the faeces from different individuals within the same group (Figure S4B). Additionally, unweighted UniFrac distance analysis of the cecal microbiome showed a similar pattern of changes compared to the faecal microbiome. Similar to faecal microbiome changes induced by FMT, the cecal microbiome showed an increased unweighted UniFrac distance to the human faecal microbiome on Day 5 post-FMT (PO5, PR5) (Figure S4C,D). Intragastric gavage FMT without PEG treatment showed a similar distance from humans at 1, 3 and 5 days post-FMT (Figure S4E). Collectively, the cecal microbiome was similar to the faecal microbiome in our model.

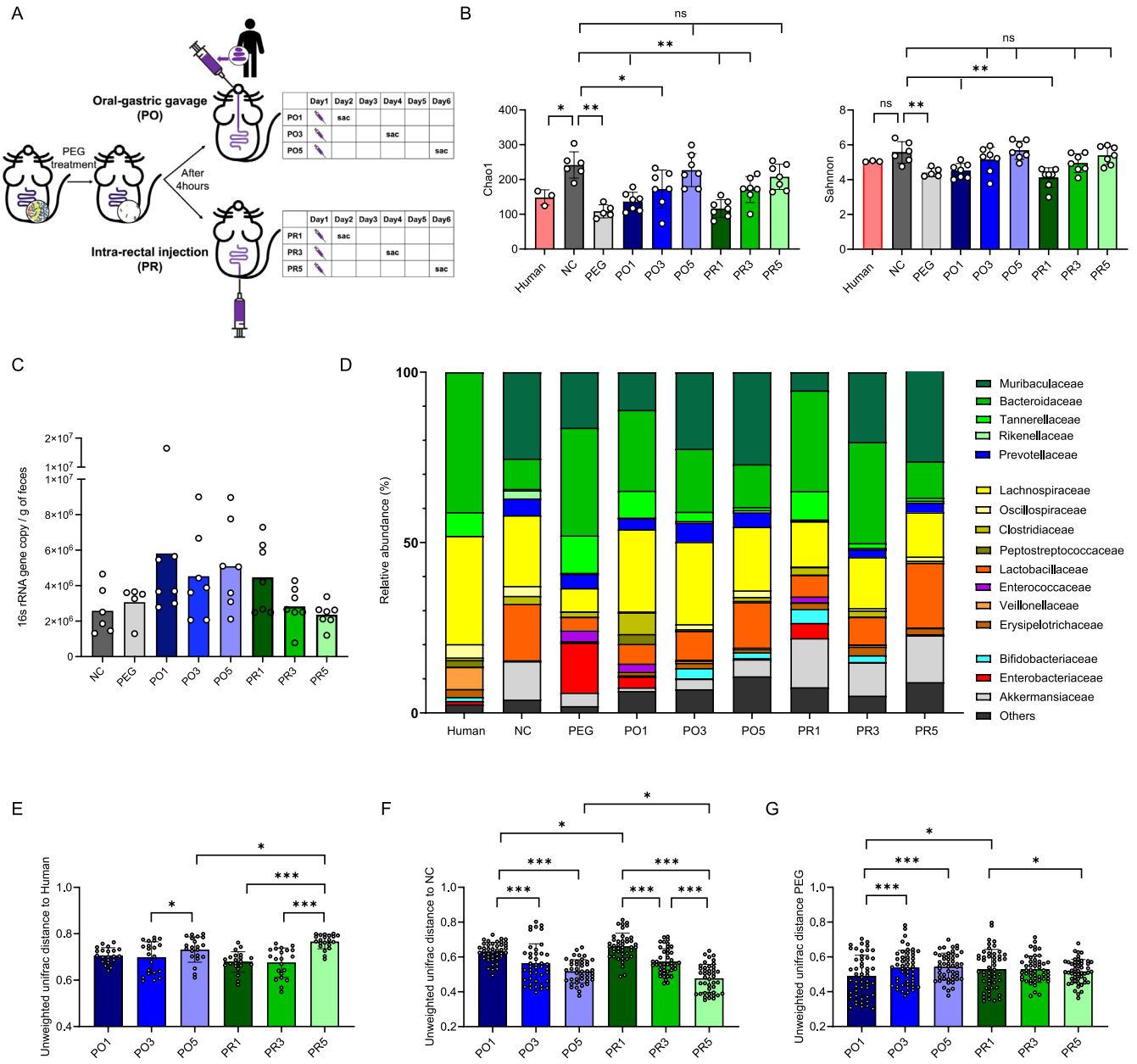

FIGURE 2 | Each oral gavage and intrarectal injection of human faecal microbiota transfer induce differential changes of faecal microbiome in PEG-treated mice. (A) We adopted oral-gastric gavage (PO) and intra-rectal injection (PR) for PEG treated animals. (B) Chao1 and Shannon index for alpha diversity. (C) Bacterial load. (D) Relative composition of taxa in family level. (E–G) Unweighted unifrac distance according to FMT groups. (E) Distance to Human faeces. (F) Distance to NC. (G) Distance to PEG Group (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, Mann–Whitney U test). Human, the faeces from the human donor; NC, negative control (non-treated animals); PEG, polyethylene glycol treated group; PO, oral-gastric gavage group; PR, intra-rectal injection group.

2.4 | Additional FMT via Oral–Gastric Gavage Does Not Improve the Colonisation Ability of the Initial FMT

To determine whether additional FMT increased the efficacy of initial FMT, we treated PEG-animals with oral–gastric gavage two more times (PO3times) and the faecal microbiome was examined on Day 3 after the initial FMT treatment (Figure S5A). The faecal microbiome of the PO3times group had a similar unweighted UniFrac distance to that of the human faecal microbiome compared to that of the PO3 group (Figure S5B) The unweighted UniFrac distances to NC and PEG controls were similar between PO3times and PO3 (Figure S5C,D). The relative abundance of the human-dominant bacterial taxa was similar (Figure S5E). Collectively, the additional FMT by oral gavage did not cause changes in human microbiota colonisation. To assess whether the additional administration of human microbiota to PEG-treated animals improved the colonisation ability of FMT, we performed two more oral gavage treatments on consecutive days (PO3times) and compared the faecal microbiome with PO3. We did not find any marked changes in the faecal microbiome, suggesting that sequential

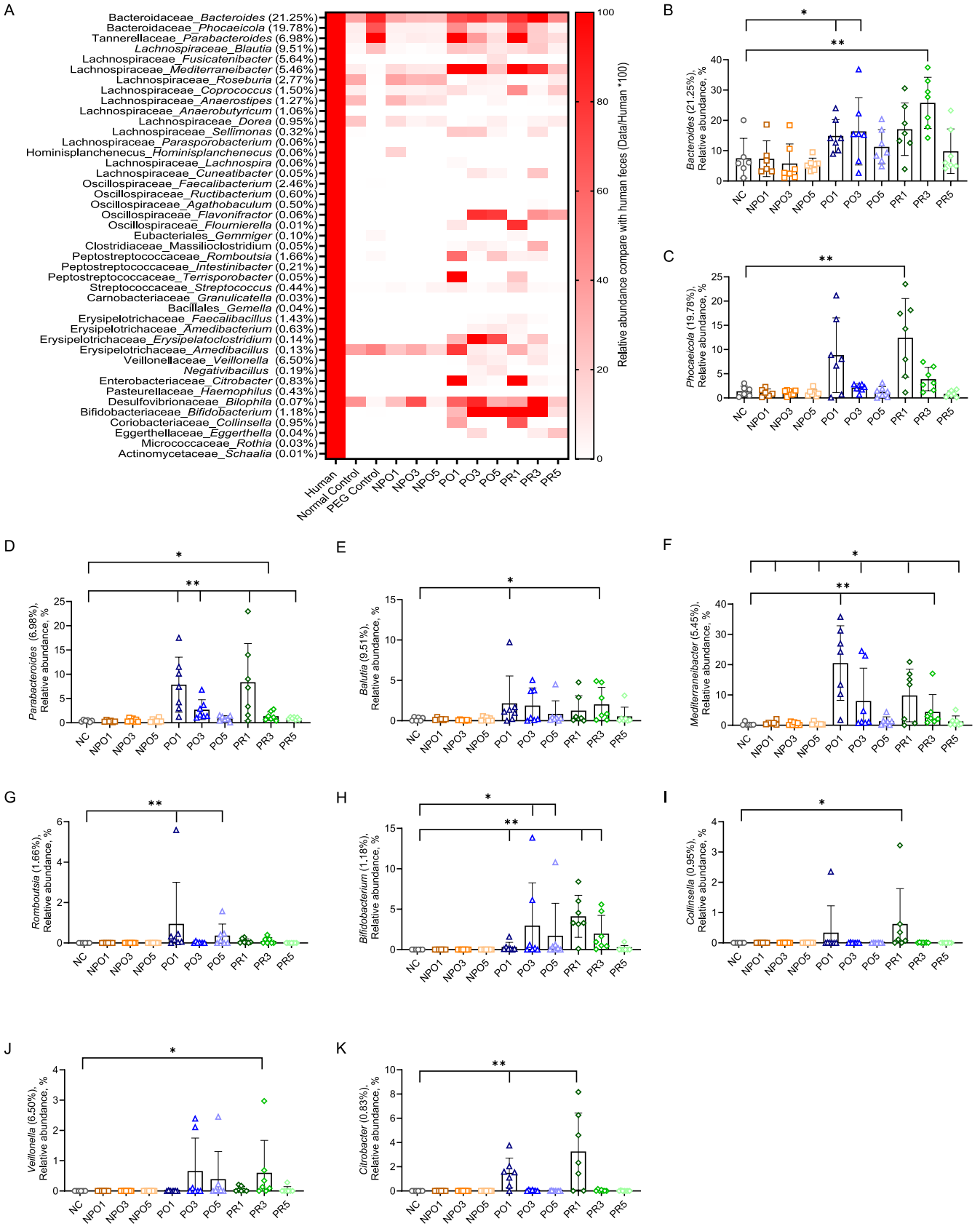

FIGURE 3 | Legend on next page.

FIGURE 3 | Comparison of colonisation based on FMT strategies for human faeces-dominant genera. (A) Visualisation of post-FMT changes in significantly increased taxa within the human group compared to NC at the genus level via heatmap. The values in brackets indicate the relative abundance in human faeces. (B–K) Genus taxa with a significant increase in composition compared to NC after FMT strategies. (B) Bacteroides. (C) Phocaeicola. (D) Parabacteroides. (E) Blautia. (F) Mediterraneibacter. (G) Romboutsia. (H) Bifidobacterium. (I) Collinsella. (J) Veillonella. (K) Citrobacter (*$p < 0.05$ and **$p < 0.01$, Mann–Whitney U test). Human, the faeces from the human donor; NC, negative control (non-treated animals); NPO, oral-gastric gavage without PEG pretreatment group; PEG, polyethylene glycol treated group; PO, oral-gastric gavage group; PR, intra-rectal injection group.

FMT did not improve the efficacy of the initial treatment. Wrzosek and colleagues compared four different strategies based on the frequency of FMT over 4 weeks: (1) twice a week, (2) once a week, (3) two FMTs and (4) one FMT. They detected human microbiota after 4 weeks even if only one FMT was performed, but there was a shift in the microbiota over time (Wrzosek et al. 2018).

2.5 | Differential Colonisation Efficiency of Human Gut Microbes in FMT Model

Based on the previously mentioned FMT methods, we aimed to evaluate whether colonisation efficiency varied across different genus-level taxa. Interestingly, we found that while some taxa colonised the murine model relatively well, others did not colonise at all. Genus-level taxa that were dominant in human faeces were established in mice from Day 1 to Day 5 following FMT (Figure 3A). The group without PEG pre-treatment showed a similar taxonomic distribution to the NC group. In the group with PEG pre-treatment, some strains were relatively well established in mouse faeces depending on the taxon, while others were not detected in mouse faeces even after FMT. Genus taxa within Bacteroidota that are dominant in humans, such as *Bacteroides*, *Phocaeicola* and *Parabacteroides* (Figure 3B–D), were successfully colonised in the murine model but gradually decreased by Day 5. Genus taxa within Lachnospiraceae showed variation depending on the genus; *Blautia* and *Mediterraneibacter* (Figure 3E,F) exhibited significant colonisation. *Bifidobacterium* (Figure 3H) also demonstrated a significant increase in colonisation. Additionally, *Collinsella, Veillonella* and *Citrobacter* (Figure 3I–K) were observed to colonise on day 1 or day 3. On the other hand, several Lachnospiraceae taxa, including *Roseburia*, *Coprococcus*, *Anaerostipes* and *dorea*, showed little to no colonisation. Notably, *Anaerobutyricum* was not observed at all. Additionally, genus taxa within Oscillospiraceae, including *Faecalibacterium*, which are known butyrate producers, were also not observed at all (Figure S6). In the experiment, several key bacterial strains, including *Anaerostipes*, *Anaerobutyricum* and *Faecalibacterium*, which are major commensals in human faeces and often reduce various inflammatory responses (Vieira-Silva et al. 2019; Gu et al. 2023) and influence the response to immune checkpoint inhibitors (Gopalakrishnan et al. 2018; Hezaveh et al. 2022), did not colonise post-FMT, regardless of PO or PR administration. The lack of colonisation by these particular strains, which are important targets in gut microbiota research because of their therapeutic potential, is an important consideration when assessing the efficacy of such strains in mouse models. Our study highlights the challenges of using human microbiota-associated mice to replicate the human gut microbiota-associated phenotype.

2.6 | Limitation

Our study had several limitations. First, we relied on 16S rRNA sequencing to assess bacterial colonisation following FMT of human faeces. Although this approach offers insight into overall community structure, it lacks the resolution of whole metagenomic sequencing, which could reveal more about the precise origin of transplanted species (i.e., whether they truly derive from the human donor) and their functional roles in detail. Second, the ability of FMT to colonise may not be solely attributed to bacterial colonisation, but also to the supplemented metabolites, extracellular vesicles, various proteins and nucleic acids, all of which can influence gut mucosal immunity. In particular, metabolites play a crucial role in interactions with gut immunity. However, this study evaluated the ability of FMT to colonise exclusively on bacterial colonisation, meaning that the potential effects of metabolites and other factors on disease models were not considered. Third, we did not evaluate the effects of FMT in disease models. Since we only assessed the ability of FMT to colonise in normal mice without any specific conditions, it remains unclear whether the same phenomena would occur in individual disease models. Lastly, the post-FMT observation period in this study was relatively short, limited to up to 5 days after either a single or three consecutive FMTs. This was based on our finding that, by Day 5, the microbial composition in recipient mice had largely reverted to a murine-dominant profile, and most human-derived taxa exhibited a marked decline. While this time point was sufficient to address our primary objectives related to early-phase colonisation efficiency, the exclusion of longer-term analysis represents a limitation. Further studies will be necessary to explore the durability of engraftment and the impact of repeated FMT over extended periods. Despite these limitations, our study compared the changes in the gut microbiota due to FMT using various methods, evaluated the actual bacterial colonisation efficiency and identified differences in colonisation at the genus level. This provides valuable information for future experiments utilising the gut microbiota.

3 | Conclusion

In conclusion, we explored the colonisation ability of the human microbiome in an animal model using FMT with human faeces. While the colonisation of the human microbiome in mice has its limitations, we found that effective colonisation can be improved by depleting the recipient's gut microbiota. Gut microbiota depletion facilitated by PEG followed by oral administration prolonged human microbiome colonisation. Taxa such as *Bacteroides*, *Blautia*, *Medicaternibacter* and *Bifidobacteria* were well-colonised in recipient animals through FMT. Taxa such as *Roseburia, Anaerostipes, Anaerobutyricum* and *Faecalibacterium* did not colonise the murine faeces post-FMT.

Author Contributions

Bon-Hee Gu: conceptualization, investigation, writing – original draft, writing – review and editing, project administration, validation, visualization, methodology. **Ho Young Jung:** investigation. **Chae-Yun Rim:** investigation. **Tae-Yong Kim:** investigation. **Sang-Jin Lee:** investigation. **Doo Young Choi:** investigation. **Han-Ki Park:** project administration, funding acquisition, visualization, supervision, writing – original draft, writing – review and editing, methodology, formal analysis. **Myunghoo Kim:** writing – original draft, writing – review and editing, supervision, funding acquisition.

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Ethics Statement

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Pusan National University.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.