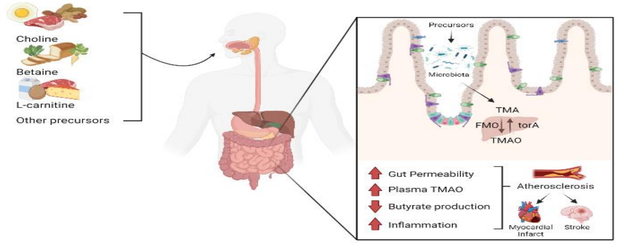
Functional Characterization of a High-Throughput *in vitro* Model to Predict Faecal Microbiota Transplantation (FMT) Donor Success

Catherine M. Andary [1], Shaeley Gibbons [1,4], Wongsakorn Kiattiburut [1,4], Kait F. Al [1,4], Jeremy P. Burton [1,2,4], and Michael S. Silverman [1,3,4]

[1] Department of Microbiology and Immunology, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada
[2] Department of Surgery, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada
[3] Division of Infectious Disease, St. Joseph's Health Care, London, ON, Canada
[4] Lawson Health Research Institute, London, ON, Canada

INTRODUCTION

- FMT has emerged in recent years as a potential therapy for a variety of microbiome-associated diseases, such as atherosclerosis.
- *In vitro* models are lacking to study mechanisms of treatment success and donor-recipient compatibility.
- Trimethylamine (TMA) is an atherosclerosis-linked metabolite generated by the gut microbiota from dietary precursors, which is then oxidized to trimethylamine N-oxide (TMAO) by the liver.
- FMT may alter or restore the gut microbiome of recipients to reduce plasma TMAO levels.
- A 96 well plate-based culturing model (MiPro)[1] was adopted and modified to identify the response of FMT donor and atherosclerosis patient microbiomes to TMA metabolites.
- The modified model was used to investigate TMA production and its influence on barrier integrity in a sample population of atherosclerosis patients with high plasma TMAO (n=2) and donors (n=4).



Objective: To characterize the effects of microbiome samples from individuals with divergent disease phenotypes on intestinal barrier integrity to identify characteristics implicated in FMT donor success.

METHODS

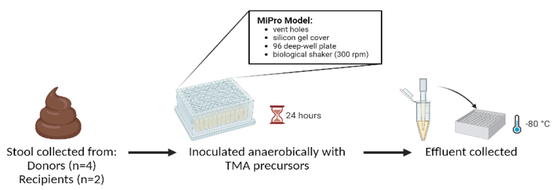


Fig. 1. Method of effluent collection from the modified MiPro model

Differences in TMA production and its effects on intestinal barrier integrity were evaluated by:

1. TEER of Caco-2:HT29-MTX-E12 co-culture
2. Confocal imaging of tight junctions (ZO-1)
3. qPCR for abundance of TMA production genes

Gene	Precursor to Production	Reference
CntA	Carnitine	Rath et al., 2017
CutC, Cut_Kp, Cut_Dd	Choline	Rath et al., 2017 & Via et al., 2020
GrdH	Betaine	Rath et al., 2017
16S rRNA	Control	341F/534R

RESULTS

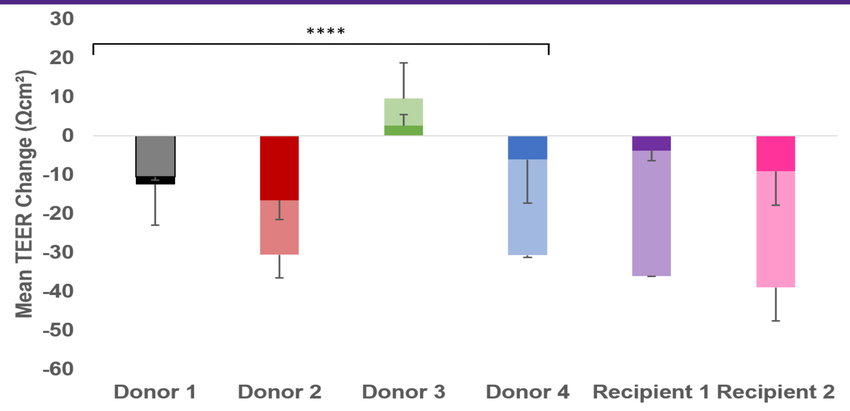


Fig. 2. Donors demonstrate significant differences in epithelial barrier permeability following treatment with filtered MiPro effluent. Caco-2:HT29-MTX-E12 cell monolayers were grown to confluency on a nitrocellulose transmembrane over 14 days. The mean change in transepithelial electrical resistance (TEER) (Ωcm²) was measured using an epithelial volt/ohm meter (EVOM) following 24 hours of filtered effluent treatment. Negative values indicate increased barrier permeability. Significant differences post-treatment (indicated by decreased opacity) were observed between donors (n=4) using a one-way ANOVA at ****p<0.0001. Error bars represent standard errors of the mean.

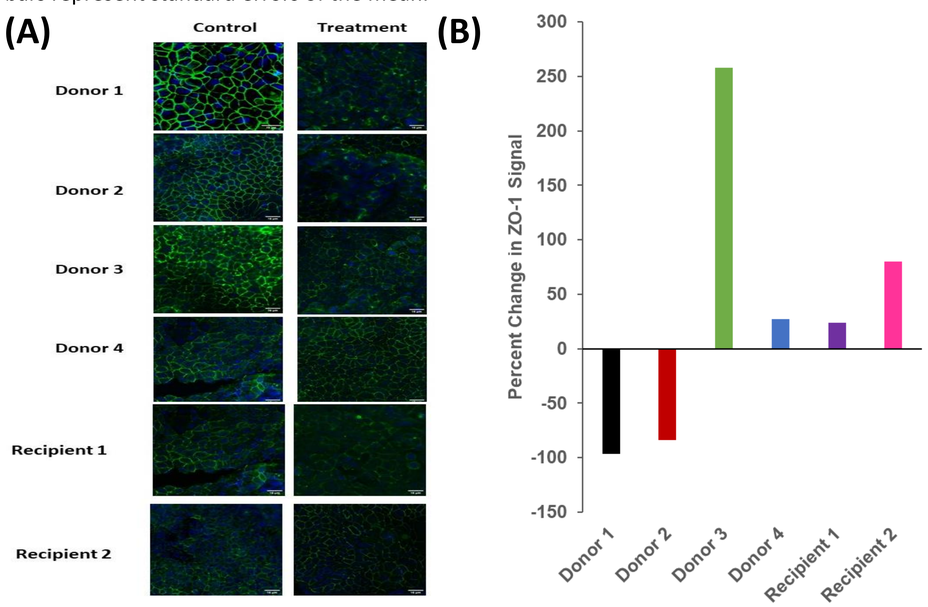


Fig. 3. (A) Confocal images and (B) quantified percent change of Zonula occludens-1 (ZO-1) signal are altered following effluent exposure in donors and recipients. Caco-2:HT29-MTX-E12 cell monolayers were grown to confluency on a nitrocellulose transmembrane over 14 days, treated with donor (n=4) or recipient (n=2) filtered effluent for 24 hours, and stained with antibody against ZO-1 before imaging. Expression of ZO-1, a tight junction peripheral membrane protein, is shown in green, while nuclei were stained with DAPI and shown in blue.

DISCUSSION

- Significant differences in monolayer barrier integrity measured by TEER were observed following treatment with filtered donor MiPro effluent.
- Donor 3 was especially resilient, with increases in cell barrier integrity observed post-treatment with filtered effluent.
- FMT recipients exhibited decreases in monolayer barrier integrity measured by TEER following treatment with filtered effluent.
- Barrier integrity measured by ZO-1 signal was notably disrupted amongst donors and recipients post-effluent treatment, with the largest changes observed in Donor 3.
- This study provides proof of concept of a novel high-throughput *in vitro* model to predict donor microbiota resilience upon exposure to toxic metabolites such as TMA.
- Future projects correcting for the small sample size of this study may allow for identification of specific FMT donor microbiota characteristics implicated in therapeutic success.

ACKNOWLEDGEMENTS

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