Assessing the Physical and Inflammatory Response of Intestinal Organoids for Necrotizing Enterocolitis using a NEC-in-a-Dish

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Eve Melbouci[1], Stephen Mackay[1], Natalia S Akopyants[1], Misty Good[1]

[1] Division of Neonatal-Perinatal Medicine, Department of Pediatrics, University of North Carolina at Chapel Hill, USA, 27599

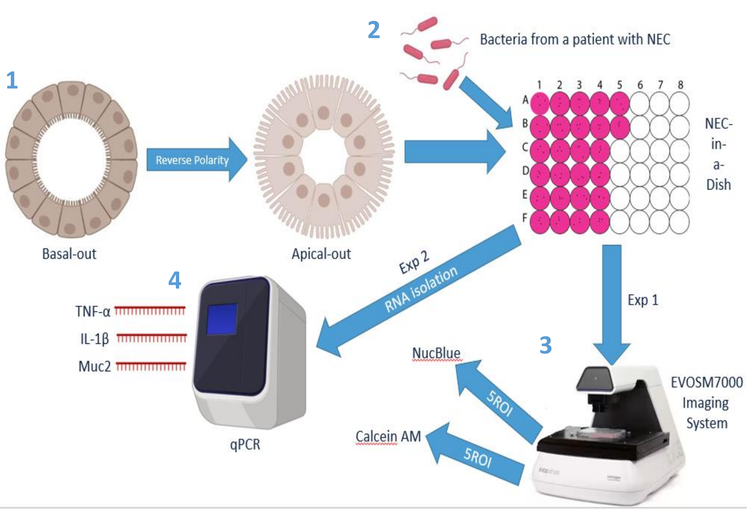
Introduction

- Necrotizing enterocolitis (NEC) is a devastating disease that impacts the intestinal tract, and most commonly occurs in preterm infants.
- Infants with an immature gut barrier and disymbiosis in the gut microbiome are at risk for NEC, which can lead to epithelial injury, sepsis from bacterial translocation, and intestinal necrosis.
- **We predict that the extent of epithelial damage and associated inflammatory response is increased in enteroids exposed to gut bacteria isolated from a patient with NEC, compared to the control.**

Methods

"NEC-in-a-Dish" experiments were performed by co-culturing apical-out intestinal organoids (enteroids) with bacteria isolated from a patient with NEC.

1. Basolateral-out enteroids are reverse polarized to apical-out.
2. Apical-out organoids are co-cultured with human gut bacteria.
3. The inflammatory response is observed by image analysis over time (Fig. 1).
4. Transcription of pro-inflammatory cytokines is determined by qPCR (Fig. 2)



Results

Figure 1. Intestinal organoids (enteroids) infected with NEC gut bacteria show increased cell damage.

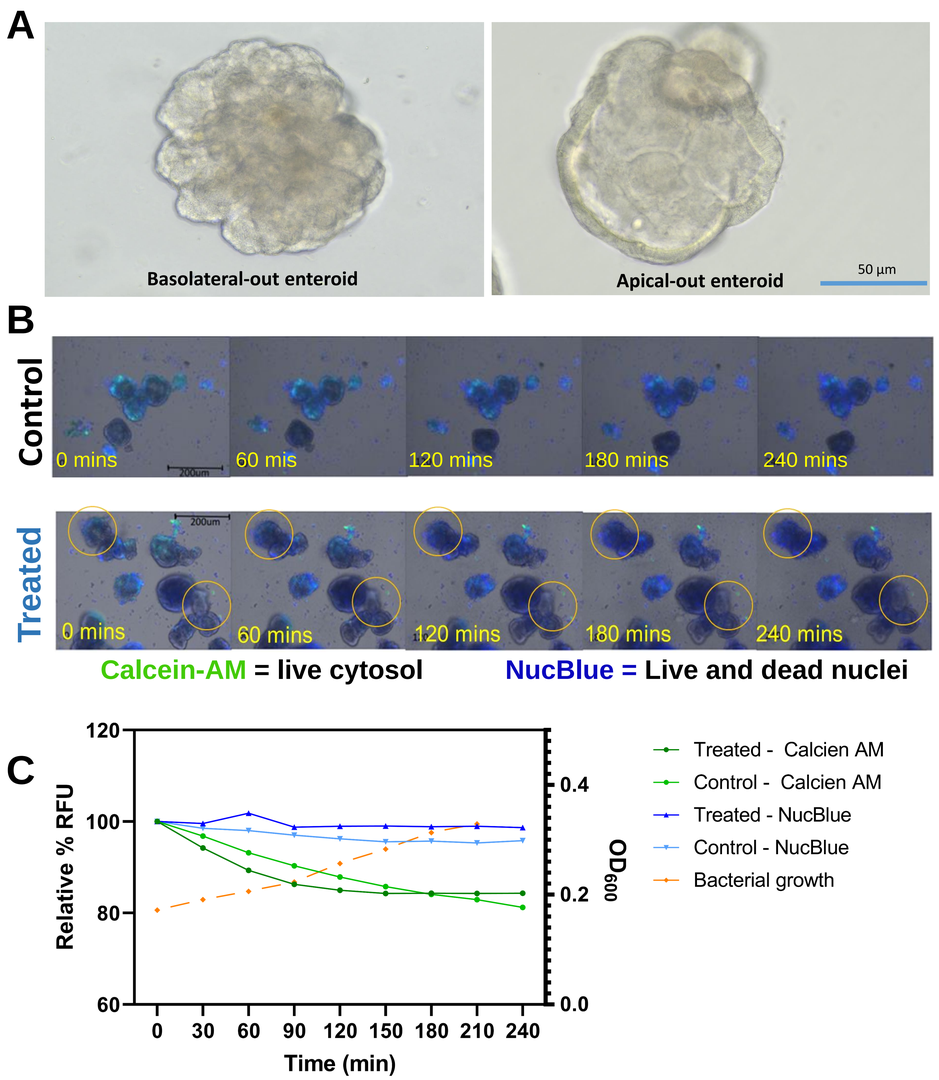


Fig. 1: Enteroids co-cultured with NEC gut bacteria with show increased cellular damage. **(A)** Images of an apical-out and basolateral-out enteroids. **(B)** Apical-out enteroids with and without bacteria. NucBlue (nuclear) and Calcein-AM (live cytosol) intensities taken at time intervals. Circles indicate areas of cell damage. All Images taken at 10X and merged using the DAPI, GFP, and Trans channels on the EvosM7000. ©. Quantitation of Relative % RFU (Calcein-AM = green lines, Nuclei = blue lines) for the infected and uninfected groups over time. % RFU normalized relative to uninfected controls. A Two way Anova analyzed across paired column values. *P*-values were calculated using the Tukey method. The relative bacterial growth rate over time (yellow line) was monitored at OD_{600}.

Figure 2. Enteroids infected with NEC bacteria show induction of proinflammatory cytokines.

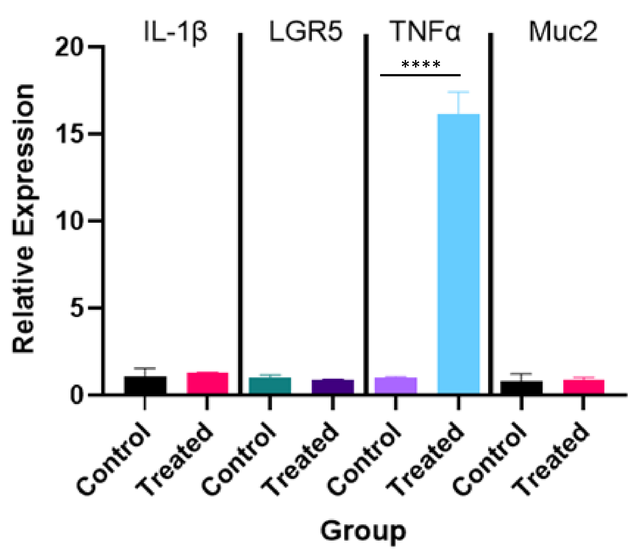


Fig. 2: Enteroids infected with NEC gut bacteria show induction of proinflammatory cytokine *TNF-α*. Preliminary qPCR data of treated and untreated enteroids showed induction of proinflammatory cytokine *TNF-α* but not *IL-1β*. Transcription of the stem cell marker *LGR5* and goblet cell marker *MUC2* did not change. Data was analyzed by unpaired two-tailed *t*-test, **** *<0.001.*

Conclusions

- Epithelial damage was observed during NEC bacteria inoculation compared to controls, suggesting the microbiome plays a role in inducing epithelial damage in patients with NEC**.**
- Increased expression of the proinflammatory cytokine *TNF-α* in response to bacteria indicates a role of the microbiome in the inflammatory response in NEC**.**
- These results act as preliminary data for establishing an enteroid inflammatory model for use in drug efficacy assays for NEC.