Immature Intestinal Tissue-derived Enteroids - A Novel Model to Study GBS Pathogenesis

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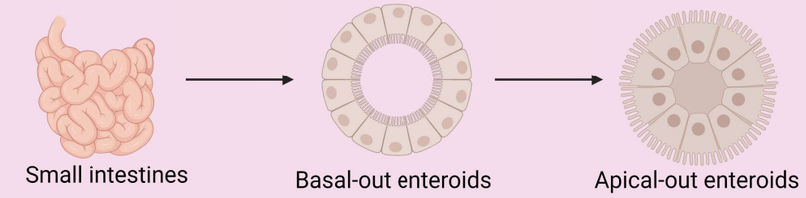
BACKGROUND

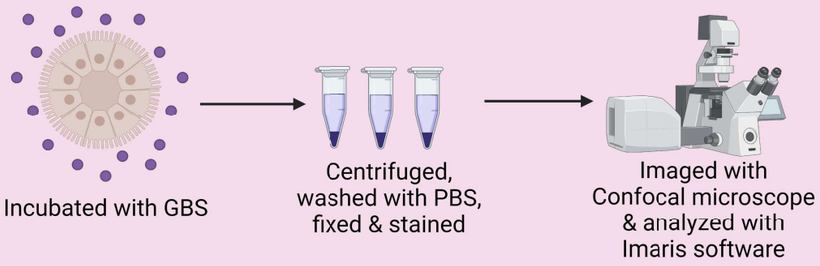
- Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis globally.
- GBS colonization of the gastrointestinal tract is a critical precursor to late-onset disease in exposed neonates.
- Studies of GBS-host interactions have primarily relied upon animal models with limited human relevance or *in vitro cell* culture using adult human intestinal cell lines that do not recapitulate the unique vulnerabilities of neonatal epithelial barrier.
- Previous studies using human fetal tissue-derived enteroids (HFTE) to model interactions with enteric pathogens have yielded valuable insights but have yet to be employed in studies of GBS.

Aim: Establish a model of GBS-exposed apical out HFTE to study GBS-host interactions.

METHODS



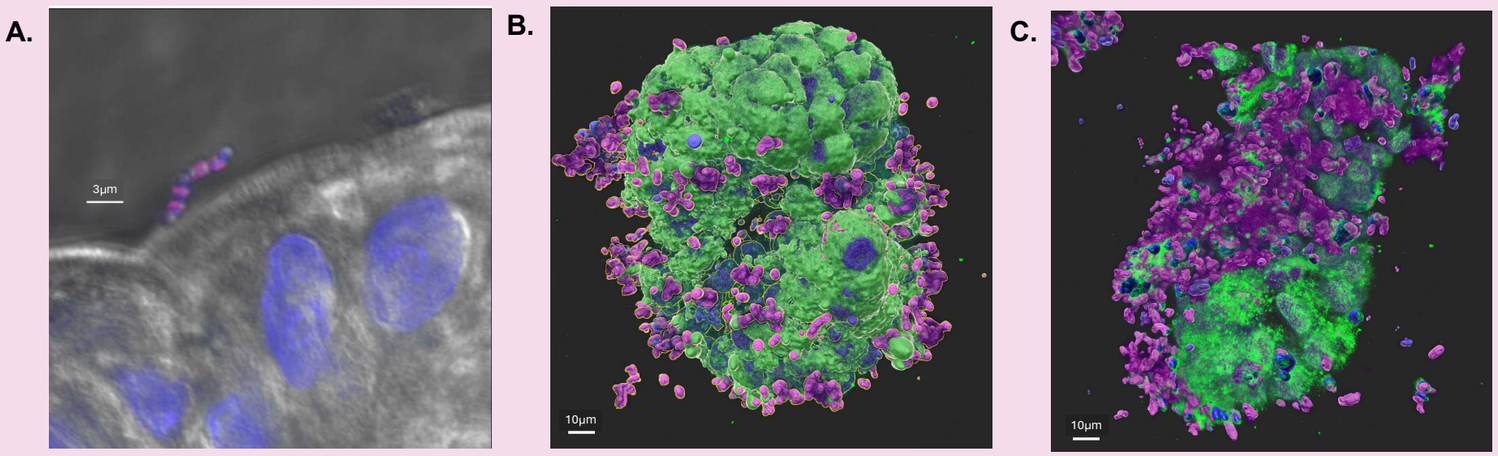
- The enteroids were cultivated from human fetal intestinal tissues obtained from the Birth Defects Research Laboratory at the University of Washington.
- HFTE were generated with Intesticult media and Matrigel to produce basal-out HFTE.
- The polarity of epithelial cells was reversed using EDTA to separate cells from the Matrigel to generate apical-out HFTE.



- HFTE were exposed to GBS (COH-1, sequence type-17) grown to optical density (OD) 600nm of 0.4 or 0.8 for 2 hr at 37°C.
- Samples were fixed and stained with fluorescently-labeled antibodies directed against villin (enterocyte brush border) and GBS for visualization using confocal microscopy. Images were analyzed using the Imaris software.

RESULTS

GBS attaches to apical surfaces of HFTE and is capable of translocating through the intestinal epithelial barrier.



Laser confocal scanning microscopy (LCSM) of GBS exposed enteroids. A) GBS chain adhering to epithelial cell surface. **B-C)** GBS adhesion and invasion of HFTE spheroid using 3D rendering of confocal images. **D)** GBS invasion into apical-out spheroid. **E)** 3D rendered of image D.

[blue = nuclei, DAPI; green = villin, anti-villin IgG Alexa Fluor™ 488; magenta = GBS, anti-GBS IgG Alexa Fluor™ 568]

CONCLUSIONS & DISCUSSION

- Our data demonstrate that HFTE function as a useful model to study GBS pathogenesis in the intestinal epithelium.
- Antibody-based labeling of specific epithelial cell sub-types (Paneth cells, goblet cells, enteroendocrine cell) and tight junction proteins may reveal site-specific preferences for GBS adhesion and invasion.
- This model can serve as a platform to test novel preventative strategies targeting late-onset GBS disease.

ACKNOWLEGDEMENTS

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Graphics generated using BioRender.