

An anaerobic gastrointestinal host-microbe flow model

Measuring oxygen levels inline using optodes

The application note is based on the research and article by:

Bang et al.

The application note is written by:

Petersen et al., Unisense

Introduction

Understanding interactions between the human gut epithelium and anaerobic bacteria remains a central challenge in microbiology and infection research. Traditional in vitro cell culture models lack the complexity of the gut environment, while animal models do not fully replicate human susceptibility to intestinal pathogens, such as *Clostridioides difficile*. This has left a need for robust and accessible models that can mimic both the physiological shear stress and the anaerobic environment of the colon.

Maintaining stable, anaerobic conditions mimicking the human gut is central to the success of such a model. Existing models have utilized materials that are highly oxygen permeable, which compromises the models' compatibility with microorganisms that require strict anaerobic conditions.

To address this challenge, Bang et al. developed a dual flow chamber model that supports long-term co-culture of intestinal epithelial cells with obligate anaerobic bacteria under controlled conditions.

Laboratory Setup

To ensure sufficient oxygen supply to the epithelial cells while maintaining strict anaerobic conditions for the bacteria, the team implemented a flow-based dual chamber design integrated with an anaerobization unit (Figure 1). In this setup, culture medium was supplied to both the apical and basal sides of the chamber, with the apical medium being continuously deoxygenated before entering the channel to maintain oxygen levels below 1%. Because the basal medium remained oxygenated, epithelial viability was not compromised.

Inline monitoring of oxygen levels was performed using a Unisense oxygen optode mounted in a PEEK flow-through cell. This enabled real-time verification of oxygen depletion efficiency and stability throughout the experiments, ensuring that the model accurately reproduced the low-oxygen environment of the human gut.

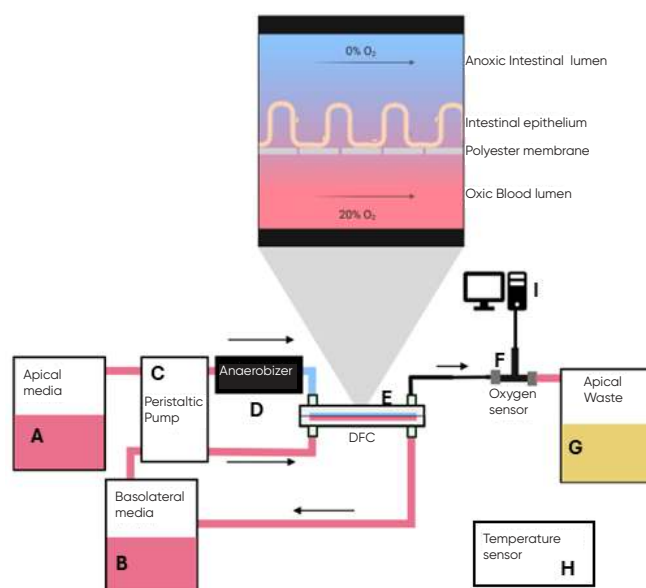
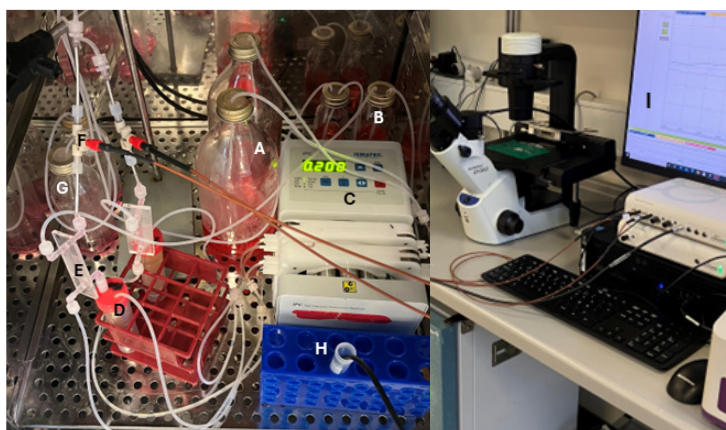


Figure 1: Laboratory setup of the Unisense OPTO-PEEK sensor connected to the dual flow cell model.

Results and conclusion

Initial validation of the system confirmed that anaerobic conditions could be reliably maintained. Oxygen measurements from the dual flow chambers with mature epithelial cells demonstrated stable anaerobic levels between 0.1–1% over six days (Figure 2), confirming that the setup provided a consistent low-oxygen environment.

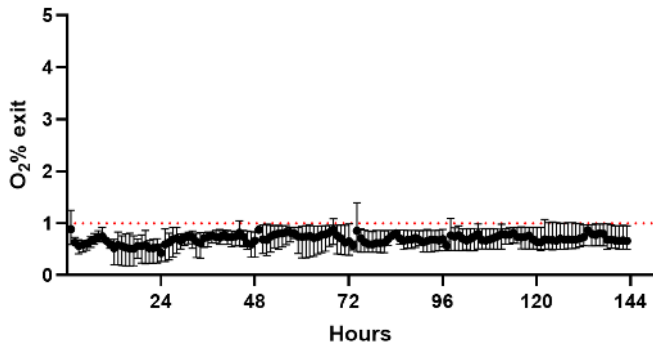


Figure 2: Effluent oxygen concentrations from flow cells with epithelial cells grown under anaerobic conditions for 6 days.

"Over the past few years, we have used the MicroOptodes from Unisense to validate the anaerobic conditions in our infection models.

We appreciate the user-friendliness of both the sensors and software from Unisense, and Unisense has been very helpful in customizing the sensors to meet our specific requirements"

PhD candidate Line Lundegård Bang,
Department of Clinical Research, Odense University Hospital

When the model was subsequently colonized with *C. difficile*, oxygen levels remained low despite bacterial growth and the barrier properties against oxygen diffusion were preserved, indicating an uncompromised epithelium even under infection conditions (Figure 3). Importantly, the model reflected a clinically relevant phenotype: persistence of *C. difficile* following vancomycin treatment, mirroring the antibiotic tolerance observed in patients.

Inline measurements with the Unisense oxygen optode were central to these insights, as they enabled continuous real-time monitoring of oxygen levels without disturbing the system.

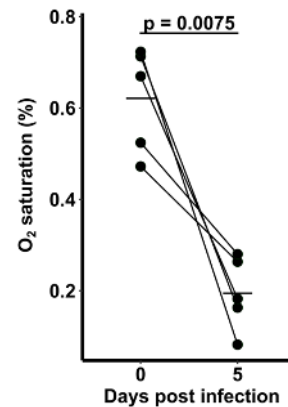


Figure 3: Effluent oxygen concentrations from flow cells with epithelial cells infected with *C. difficile*.

You can read more in the article by Bang et al. "An anaerobic in vitro flow model for studying interactions at the gastrointestinal host-microbe interface", *npj Biofilms and Microbiomes*, 2025, 11:160

Suggested products

- OPTO-PEEK
- Temp UniAmp
- Single or MultiChannel UniAmp
- uSense Log



Related publications

Tinevez, Jean-Yves, et al. "Shigella-mediated oxygen depletion is essential for intestinal mucosa colonization." *Nature microbiology* 4.11 (2019): 2001–2009.

Lyon, Katrina, Rama Bansil, and Diane Bimczok. "Profiling Luminal pH in Three-Dimensional Gastrointestinal Organoids Using Microelectrodes." *Journal of Visualized Experiments (JoVE)* 209 (2024): e66900.

Sebrell, Thomas A., et al. "A novel gastric spheroid co-culture model reveals chemokine-dependent recruitment of human dendritic cells to the gastric epithelium." *Cellular and molecular gastroenterology and hepatology* 8.1 (2019): 157–171.