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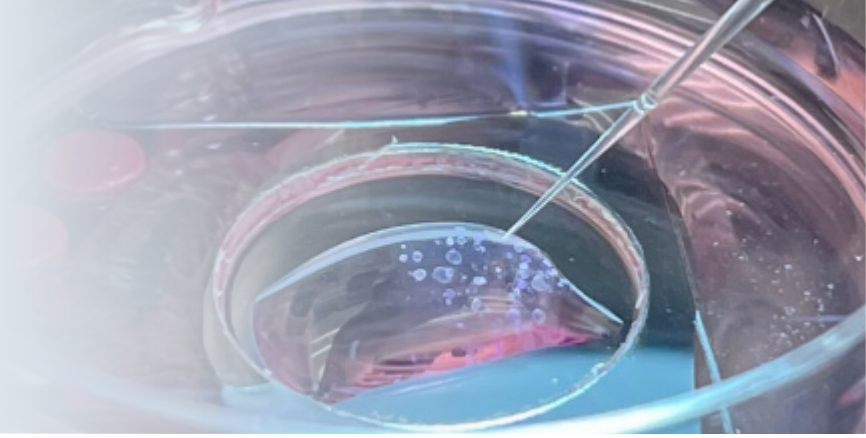

Profiling pH in Gastrointestinal Human Organoids

pH Microelectrode reveals distinct environment inside 3D organoid

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

Lyon et al.

The application note is written by:

Petersen et al., Unisense

Introduction

Stomach ulcers are a widespread condition often triggered by infection with *Helicobacter pylori*, a pathogen capable of penetrating the gastric mucus layer. While this mucus is known to serve as a first line of defense, our understanding of its regulation and its coupling with other gastroprotective mechanisms (such as acid secretion) remains limited. Traditional research has relied heavily on animal models, immortalized cell lines, or mucin protein solutions, which fall short in replicating human physiology.

Human organoids - three-dimensional, multicellular structures derived from patient tissue - have emerged as a promising alternative, and they offer more physiologically relevant features. However, it remains a challenge to replicate the stomach's extreme pH environment - particularly the steep gradient from the near-neutral epithelium to the highly acidic gastric lumen (pH 1-3).

Lyon and colleagues addressed this by leveraging tissue-derived organoid models to explore their suitability for modeling the dynamic gastric luminal environment. Conventional pH measurement methods, such as pH-sensitive dyes, suffer from limited spatial resolution and low accuracy. To overcome these limitations, the researchers aimed to perform high-resolution, full-range pH profiling within organoids.

"The Unisense microelectrodes and profiling system gave me the unique and exciting opportunity to interact with organoids - my favorite model system - with my own two hands.
Automation is great, but the heterogeneity of organoid culture sometimes demands a more hands-on approach."

Dr. Katrina Lyon, Dept Microbiology and Cell Biology, Montana State University

Laboratory Setup

To establish the organoids, Lyon et al. obtained tissue samples from patients undergoing upper endoscopy and isolated the gastric glands. The glands were embedded in an extracellular matrix to support organoid formation in 3D culture. After several passages, the organoid cultures were established in glass-bottom dishes containing culture media. These were positioned on a stereomicroscope stage with a surrounding Unisense MicroProfiling System (Figure 1).

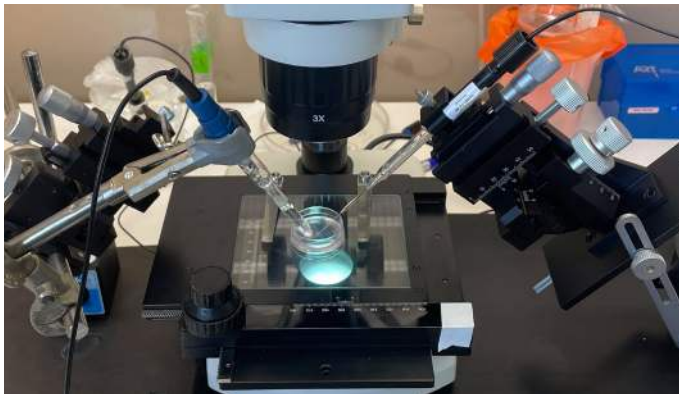

Figure 1: Microscope stage with the pH microelectrode in a Unisense Micromanipulator to the right and the reference electrode to the left.

A Unisense pH microelectrode with a 25 µm tip was mounted in a micromanipulator, while a reference electrode was placed in the medium. The microelectrode was customized with a beveled tip to minimize damage to the organoids. Under continuous visual guidance through the stereomicroscope, the pH microelectrode was carefully advanced into the organoid lumen, to ensure precise insertion. pH measurements were taken both in the culture medium and across spatial gradients from the extracellular matrix to the organoid interior.

Results and conclusion

The internal pH of the organoids was significantly lower than that of the surrounding matrix and culture medium (Figure 2). This indicates that the organoids can maintain distinct internal environments, underscoring their physiological relevance. Spatial profiling further showed a marked pH drop from the interface between the extracellular matrix and the epithelium toward the center of the organoid lumen (Figure 3).

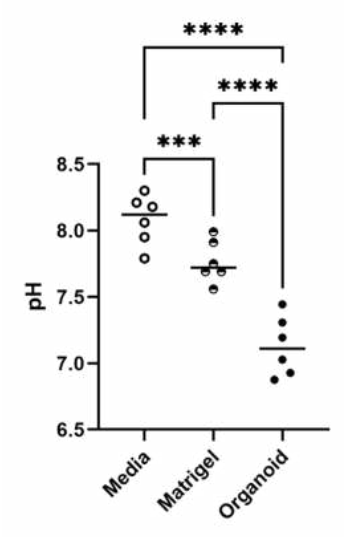

Figure 2: The pH was measured by the pH microelectrode in the media, the extracellular matrix that the organoids were embedded in (matrigel) and in the organoids.

In their experimental protocol, Lyon et al. described the use of pH microelectrodes to obtain pH measurements with high spatiotemporal resolution. The authors validated the use of these microelectrodes in a gastrointestinal organoid model and concluded that they represent a valuable complement to conventional pH measurement methods. Finally, the approach described by Lyon et al. may be adapted for the measurement of other analytes in organoid systems.

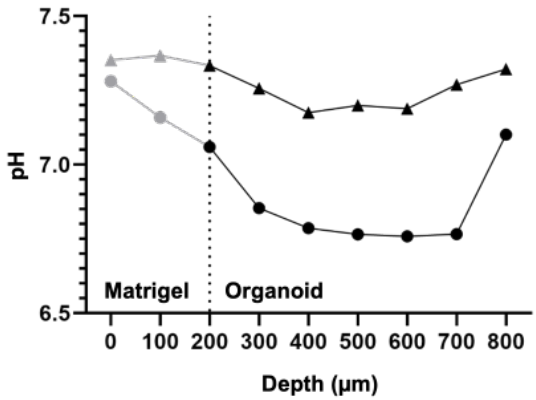

Figure 3: pH profiling through the extracellular matrix (matrigel) and into the organoid.

You can read more in the article by Lyon et al. "Profiling Luminal pH in Three-Dimensional Gastrointestinal Organoids Using Microelectrodes", Journal of Visualized Experiments (JoVE) 209 (2024): e66900.

Suggested products

- pH-25 microelectrode
- Reference electrode
- MicroProfiling System
- Zeiss stereomicroscope
- Single or Multi Channel UniAmp
- SensorTrace Profiling

Related publications

Fofanova, Tatiana Y., et al. "A novel system to culture human intestinal organoids under physiological oxygen content to study microbial-host interaction." Plos one 19.7 (2024): e0300666.

Voss, Logan J., and D. Alistair Steyn-Ross. "Tissue oxygen partial pressure as a viability metric for ex vivo brain tissue slices." Journal of Neuroscience Methods 396 (2023): 109932.

Voss, Ninna CS, et al. "Targeting the acidic tumor microenvironment: unexpected pro-neoplastic effects of oral NaHCO3 therapy in murine breast tissue." Cancers 12.4 (2020): 891.

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.

Murphy, Kaitlin C., et al. "Measurement of oxygen tension within mesenchymal stem cell spheroids." Journal of The Royal Society Interface 14.127 (2017): 20160851.

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