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## Development of a Scalable Coculture System for Gut Anaerobes and Human Colon Epithelium

Nobuo Sasaki<sup>1,2,3</sup>, Kentaro Miyamoto<sup>1,4</sup>, Kendle M. Maslowski<sup>5</sup>, Hiroshi Ohno<sup>6</sup>, Takanori Kanai<sup>1,3</sup>, and Toshiro Sato<sup>1,2</sup>

<sup>1</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Otemachi, Tokyo, Japan

<sup>2</sup>Department of Organoid Medicine, Keio University School of Medicine, Otemachi, Tokyo, Japan

<sup>3</sup>AMED-CREST, Otemachi, Tokyo, Japan

<sup>4</sup>Mizarisan Pharmaceutical Co., Ltd, Tokyo, Japan

<sup>5</sup>Institute of Immunology and Immunotherapy and Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK

<sup>6</sup>Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan

### Abstract:

The intestinal epithelium resides at the interface of the gut microbiota and plays a pivotal role in shaping the gut ecosystem. Owing to a lack of tractable coculture systems, the cell biological understanding of host-microbe interactions remains elusive. Organoid technology allows propagation of colonic epithelium under normoxia; however, the conflicting oxygen demands between epithelium and gut anaerobes makes their coculture difficult. We established a simple 2-chamber culture system for human colonic epithelium, termed as Intestinal Hemi-Anaerobic Coculture System (iHACS), consisting of a hypoxic apical chamber and a normoxic basal chamber. The medium in the apical chamber was equilibrated with anaerobic gas and subsequently sealed by inserting a plug made of butyl rubber (AsONE international, Santa Clara, CA). The oxygen concentration of the apical chamber was measured by a fiberoptic oxygen meter (PreSens, Regensburg, Germany). For bacterial coculture, we inoculated anaerobic bacteria ( $5 \times 10^4$  cell/mL in *Bifidobacterium adolescentis*, *Bacteroides fragilis*, *Clostridium butyricum*, and  $5.0 \times 10^5$  cell/mL in *Akkermansia muciniphila*) in the apical chamber medium.

Keywords: intestinal epithelium, gut microbiota, oxygen demand, coculture, normoxia, host-microbe interactions