Host-microbe interplay analysis using “apical anaerobic chamber”, a device that enables co-culture of anaerobic gut microbes and host epithelial cells

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Report: The apical anaerobic chamber developed in this study was found to be effective in keeping the dissolved oxygen concentrations of the apical side and the basal side of the Caco-2 monolayer at < 0.3% and > 60%, respectively, for at least 5 days. The transepithelial electrical resistance value of the cells was comparable to that of the cells maintained in the CO_2 incubator. Fluorescence immunostaining of claudin-2 revealed clear tight-junction formation of the cells. When obligate anaerobes belonging to the *Bacteroides* and *Bifidobacterium* genera were added to the apical medium of the Caco-2 cells in the apical anaerobic chamber, the growth of these bacteria was stimulated by 100- to 10,000-folds. The transepithelial electrical resistance both prior to and post co-culturing was comparable. These results indicate that the device serves as a useful tool for the analysis of symbiosis between intestinal epithelial cells and gut microbes.

Research objective

Gut microbes have significant impact on host health and disease. Animal experiments have greatly contributed to the understanding of the mechanism underlying the crosstalk between them; however, our community needs to develop alternate methodologies to address the increasing attention to animal welfare. In addition, recent studies have pointed out the difference in the microbiota composition between mice and humans. I recently commenced the research to develop a specific device (apical anaerobic chamber) that enables co-culturing obligate anaerobic gut microbes with human intestinal cell lines, by keeping the apical side of the epithelial cells anaerobic while keeping the basal side of the cells aerobic. Here I describe the usefulness of the apical anaerobic chamber in analyzing the molecular basis of symbiosis between gut microbes and humans.

Methods

The apical anaerobic chamber was designed and constructed by WakenBtech (Fig. 1). The Caco-2 cell line was grown in DMEM containing 10% FBS in a CO_2 incubator to form a monolayer in the culture insert. The insert with the layered cells was placed in the chamber

and transferred to the anaerobic station. The dissolved oxygen concentration was monitored using Micro Fiber Optic Oxygen Transmitter (PreSens). The nucleus was stained with DAPI, while claudin-2 was stained with the anti-claudin-2 monoclonal antibody, which was visualized via fluorescence. The bacterial strains were obtained from the RIKEN Bioresource Center. The GAM medium was routinely used for bacterial growth, while DMEM (FBS) was used for co-culture with Caco-2 cells.

Results

When the Caco-2 cells were incubated in the apical anaerobic chamber, the dissolved oxygen concentrations of the apical side and the basal side were kept < 0.3% and > 65%, respectively, for at least 5 days. When the culture insert was directly placed in the anaerobic station, the dissolved oxygen concentrations of both the sides dropped to 0% within a day. As shown in Fig. 2, the transepithelial electrical resistance values were quite similar between the cells incubated in the apical anaerobic chamber and the cells maintained in the CO_2 incubator. The values became significantly low when the culture insert was directly placed in the anaerobic station. Lactate dehydrogenase activity in the supernatant significantly elevated when the cells were incubated anaerobically. The clear tight-junction was visible in the cells incubated in the CO_2 incubator as well as in the apical anaerobic chamber, as revealed via fluorescence staining of claudin-2. On the other hand, when the cells were exposed to anaerobic conditions, the tight-junction collapsed (Fig. 3).

When *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides caccae*, *Parabacteroides merdae*, *Dorea longicatena*, *Lactococcues lactis*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* were added to the apical side of the Caco-2 cells incubated in the apical anaerobic chamber, the growth of these bacteria enhanced by 100- to 10,000-folds. The transepithelial electrical resistance values both prior to and post co-cultivation were comparable.

Conclusion

These results revealed the usefulness of the device in co-culturing the anaerobic gut microbes with human cell lines, enabling us to decipher the mechanism underlying the symbiosis between them. The device also serves as a tool to analyze the drug and food metabolism in intestines, on which gut microbiota should have significant influence.

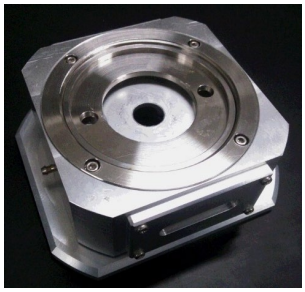

Fig. 1. Apical anaerobic chamber（11 cm x 11 cm x 5 cm）
The device is transferred into the anaerobic station once the transwell is placed into the center hole of the chamber.

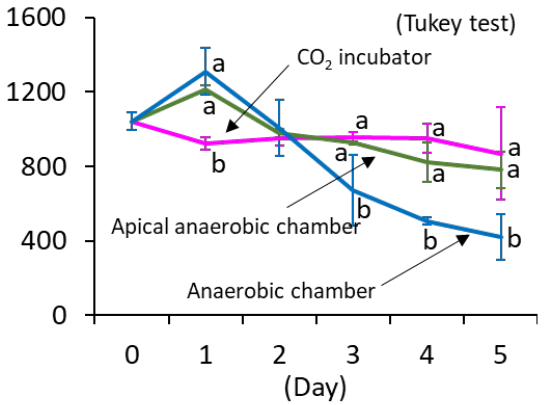

Fig. 2. Transepithelial electrical resistance
Caco-2 monolayers were incubated under the respective conditions. Transepithelial electrical resistance was monitored each day.

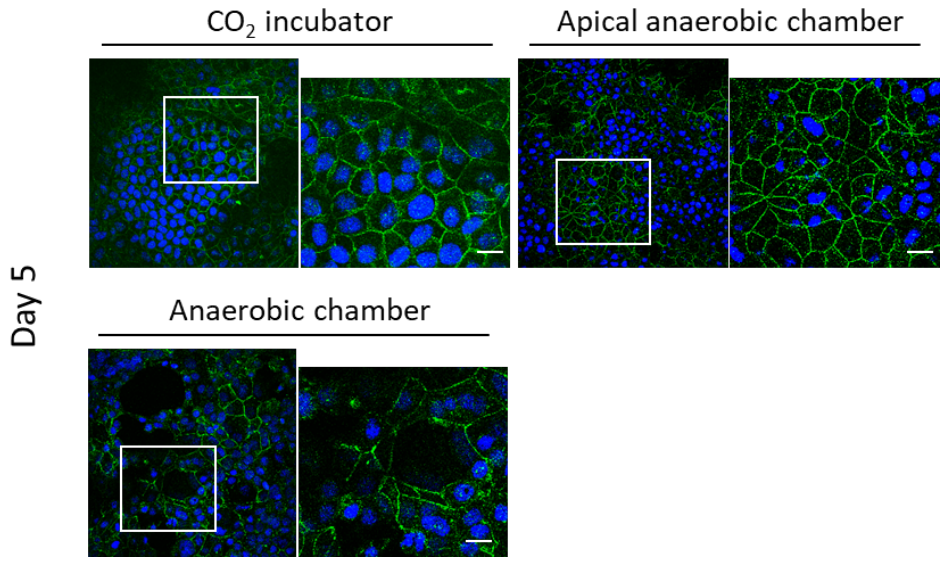

Fig. 3. Tight junction formation, evaluated via fluorescent immunostaining
Caco-2 cells at day 5 were used for staining. Nucleus (blue), claudin-2 (green). Scale bar: 20 μm.