

Video Article

A Novel Method for the Culture and Polarized Stimulation of Human Intestinal Mucosa Explants

Katerina Tsilingiri¹, Angelica Sonzogni², Flavio Caprioli³, Maria Rescigno¹

¹Department of Experimental Oncology, European Institute of Oncology

²Department of Pathology and Laboratory Medicine, European Institute of Oncology

³U.O. Gastroenterologia 2, IRCCS Ca' Granda, Ospedale Policlinico di Milano

Correspondence to: Maria Rescigno at maria.rescigno@fom-ieo-campus.it

URL: <https://www.jove.com/video/4368>

DOI: [doi:10.3791/4368](https://doi.org/10.3791/4368)

Keywords: Microbiology, Issue 75, Cellular Biology, Medicine, Molecular Biology, Biomedical Engineering, Anatomy, Physiology, Bacteria, Tissue Engineering, Tissue culture, intestinal mucosa, polarized stimulation, probiotics, explants, Lactobacilli, microbiota, cell culture

Date Published: 5/1/2013

Citation: Tsilingiri, K., Sonzogni, A., Caprioli, F., Rescigno, M. A Novel Method for the Culture and Polarized Stimulation of Human Intestinal Mucosa Explants. *J. Vis. Exp.* (75), e4368, doi:10.3791/4368 (2013).

Abstract

Few models currently exist to realistically simulate the complex human intestine's micro-environment, where a variety of interactions take place. Proper homeostasis directly depends on these interactions, as they shape an entire immunological response inducing tolerance against food antigens while at the same time mounting effective immune responses against pathogenic microbes accidentally ingested with food.

Intestinal homeostasis is preserved also through various complex interactions between the microbiota (including food-associated beneficial bacterial strains) and the host, that regulate the attachment/degradation of mucus, the production of antimicrobial peptides by the epithelial barrier, and the "education" of epithelial cells' that controls the tolerogenic or immunogenic phenotype of unique, gut-resident lymphoid cells' populations. These interactions have been so far very difficult to reproduce with *in vitro* assays using either cultured cell lines or peripheral blood mononuclear cells. In addition, mouse models differ substantially in components of the intestinal mucosa (mucus layer organization, commensal bacteria community) with respect to the human gut. Thus, studies of a variety of treatments to be brought in the clinics for important stress-related or pathological conditions such as irritable bowel syndrome, inflammatory bowel disease or colorectal cancer have been difficult to carry out.

To address these issues, we developed a novel system that enables us to stimulate explants of human intestinal mucosa that retain their *in situ* conditioning by the host microbiota and immune response, in a polarized fashion. Polarized apical stimulation is of great importance for the outcome of the elicited immune response. It has been repeatedly shown that the same stimuli can produce completely different responses when they bypass the apical face of the intestinal epithelium, stimulating epithelial cells basolaterally or coming into direct contact with lamina propria components, switching the phenotype from tolerogenic to immunogenic and causing unnecessary and excessive inflammation in the area.

We achieved polarized stimulation by gluing a cave cylinder which delimited the area of stimulation on the apical face of the mucosa as will be described in the protocol. We used this model to examine, among others, differential effects of three different *Lactobacilli* strains. We show that this model system is very powerful to assess the immunomodulatory properties of probiotics in healthy and disease conditions.

Video Link

The video component of this article can be found at <https://www.jove.com/video/4368/>

Protocol

1. Obtaining and Preparing the Tissue

1. Tissue (healthy or IBD mucosa) is obtained during surgery. Once the specimen is excised transfer to the lab as soon as possible, keeping it in $\text{Ca}^{++}/\text{Mg}^{++}$ -free HBSS buffer supplemented with Pen/Strep at 4 °C or on ice.

*The size of the specimen depends on the availability of the tissue: the part of the tissue obtained, is strictly what is not needed for diagnosis and can vary greatly between healthy and IBD subjects. Healthy tissue is excised from patients undergoing surgery for colon cancer.

2. Wash the tissue gently and clean it of all mesenteric material. Separate the mucosa layer from the submucosa using sterile scissors and forceps while keeping the tissue in HBSS buffer in a Petri dish (not necessarily immersed). Touch the mucosal surface with the forceps as little as possible.
3. Cut mucosa in stripes at least 1 cm wide and as long as possible. Use two sterile scalpels, much as though you were cutting your food.
4. Wash clean mucosa gently in HBSS and extend it on a Petri dish with the apical side facing upwards.

2. Mounting the Tissue

1. Prepare fresh medium (tisDMEM). Use DMEM supplemented with Glutamine (2 mM) and NaPyr (1 mM), and add 15% FBS-Na (Fetal Bovine Serum - North American), 1% ITS-X and 200 ng/ml EGF at the time of use.
2. Fill a 10 cm Petri dish with the rest of the FBS-Na (use about 30 ml so that metal grids will be completely submerged) and submerge the metal grids. Keep the plate open and support the plastic cylinders on its tap.

*The metal grids are made of iron. The mesh is square-shaped with a side of 0.5 mm.

3. Take 3 μ l of surgical glue with a 10 or 20 μ l pipette. Apply it carefully to one of the borders of a plastic cylinder while holding it firm with the forceps.
4. Gently place the cylinder on the apical side of the mucosa, as close to one of the borders of the strip as possible.
5. To give the glue time to firmly attach the cylinder on the tissue, prepare the centre-well organ culture plate: Dispense 850 μ l-1 ml of medium in the centre well and support the metal grid on the edges of the plate's central well.
6. Cut away excess tissue from the borders of the cylinder using two sterile scalpels as before.
7. Gently lift the cylinder with the attached tissue and place the basolateral side on the metal grid in the centre of the plate.

3. Stimulation

1. Use the stimuli of your choice in your preferred concentration.
2. Apply appropriate volume in the centre of the plastic cylinder without disturbing the cylinder or the mucosal surface with the pipette tip. Make sure your chosen volume uniformly covers the surface inside the cylinder. Indicated volumes:
Large plastic cylinder: 200 μ l
Medium plastic cylinder: 100 μ l
Small plastic cylinder: 50 μ l
3. Incubate for up to 3 hr in a conventional incubator.

*If you wish to incubate for longer time points, make sure you use a much smaller volume of stimuli (10-20 μ l) and directly place the tissue in an atmosphere of 100% O₂ in the pressure of 1 Atm (see 3.5).

4. Take the medium with the stimuli off the apical surface of the tissue and DO NOT REPLACE with fresh medium, because the tissue does not get enough O₂ if a thick layer of medium on the apical face prevents gas exchange. **Cover the plate.**
5. Place the tissue in an atmosphere of 100% O₂ in the pressure of 1 Atm.

4. Harvesting

1. After 24 hr of total culture time ($\pm$ 2.5 hr) carefully remove the cylinder from the tissue with the help of a scalpel gently scraping glue off and store it depending on the desired analysis (fix for immunohistochemistry and/or immunofluorescence assays, or snap freeze in liquid N₂ for RNA purification and gene expression profiling or protein purification and Western blot assays).
2. Harvest the basolateral medium for cytokine secretion profiling. Centrifuge at 8,000 rpm for 7 min to pellet debris, transfer the supernatant in an Eppendorf tube (alternatively aliquot) and immediately store at -20 °C.

*if you want to keep supernatants for longer, store them at -80 °C.

Representative Results

We succeeded in maintaining healthy and IBD human intestinal mucosa in culture for at least 24 hr preserving the wellbeing of the tissue. In accordance with previous observations¹, this was only possible if the majority of the culture time (at least 85% of total) took place in O₂, as the tissue did not survive for 24 hr in conventional incubators (**Figure 2**). We have also shown that different responses of the explants can be observed on this model depending on the polarity of the stimulation (apical vs basolateral) and the immunogenicity of the stimuli^{2,3}.

Using this protocol, we show that different probiotic strains can elicit different responses on the tissue's part. *Lactobacillus plantarum* NCIMB8826 proved to be surprisingly immunogenic on healthy tissue. Contrary to the two other *Lactobacilli* strains, not only did it induce migration of lymphoid cells towards the uppermost surface of the mucosa, but it also significantly upregulated a chemokine relevant to this migration, CCL4, as well as IL-1b, a cytokine traditionally related to pro-inflammatory activity. To our surprise, even though we had previously observed a preventive action of *L. paracasei* in a mouse model of colitis⁴, this did not translate to a curative effect when live bacteria of this strain were applied on inflamed IBD tissue. Rather, all three strains proved detrimental when used on IBD mucosa in the acute phase of the disease².

Further, we showed that the metabolic product of a probiotic, called postbiotic, is capable of shielding healthy epithelium against a rather invasive *Salmonella* strain (FB62), while at the same time significantly ameliorates inflammation when applied on IBD mucosa².

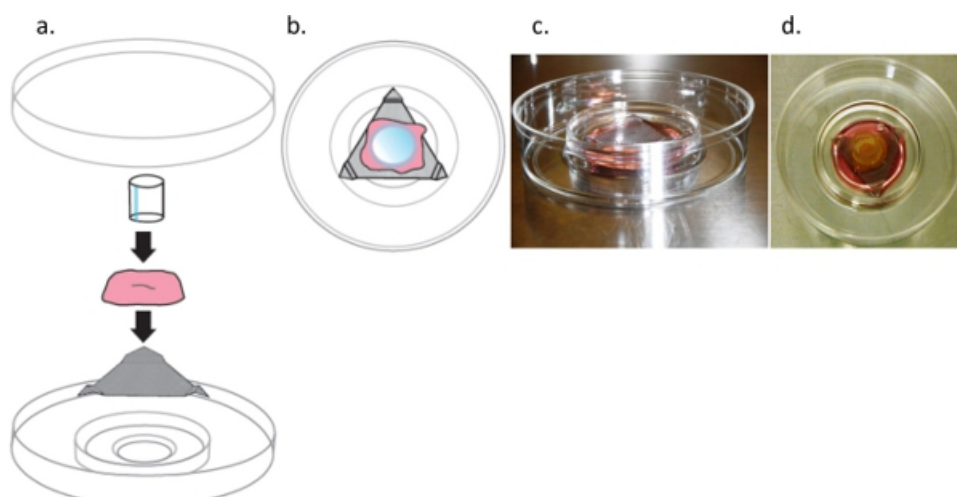


Figure 1. Schematic representation of the set-up and photos. **a.** schematic representation of the set up viewed from the side, **b.** schematic representation of the set up viewed from the top, **c.** photo of the metal grid used as support for the explant. When correctly placed, only the basolateral side gets in contact with the central medium chamber, **d.** photo of the entire ensemble. [Click here to view larger figure.](#)

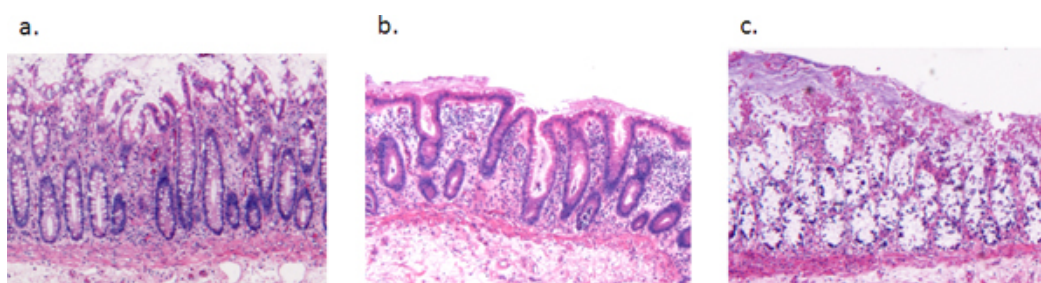


Figure 2. The tissue only survives culture in O_2 . **a.** uncultured tissue, fixed upon arrival from the operating room, **b.** tissue cultured for 24 hr in O_2 , **c.** tissue cultured for 24 hr in a conventional incubator. Original magnification: 5X.

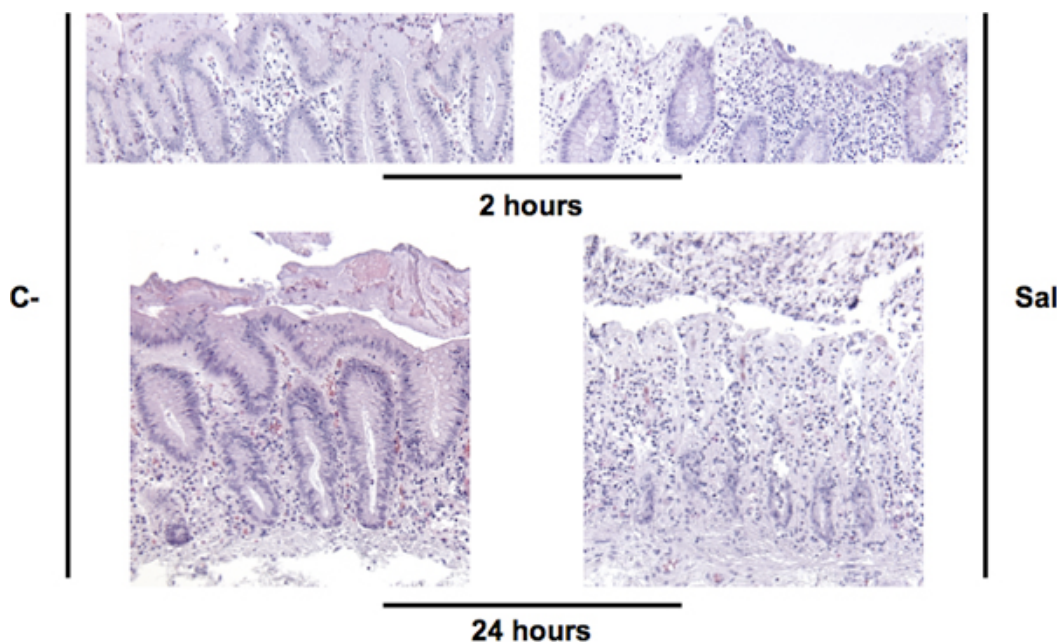


Figure 3. The effect of a pro-inflammatory stimulus on the explants. **C-:** Unstimulated tissue cultured for the indicated time points; **Sal:** Tissue challenged with *Salmonella* and cultured for the indicated time points. The destruction of the upper layer of the epithelium is already visible at 2 hr. The explant presents extensive degeneration after 24 hr. Original magnification: 10X.

Discussion

The need for physiologically relevant models on which treatments for human intestinal mucosa can be validly tested has long been emphasized. Many researchers have identified potential artifacts and defects in both cell⁵ and mouse models⁶ used so far for that purpose. Indeed, even though promising preclinical data is often obtained, these rarely translate to significant clinical benefit.

The method described in this work, presents a relatively simple, yet effective and novel adaptation to existing protocols^{7,8} for the culture and stimulation of human intestinal mucosa explants in a polarized fashion.

This model could provide an excellent complementary approach for the generation of valid pre-clinical data before taking a potential treatment of any kind to the clinics. Thus, unfortunate clinical outcomes⁹ could potentially be avoided and researchers could more safely promote treatments to the clinics for the handling of acute inflammatory conditions.

The major advantages of the model are the possibility to compare polarized versus non polarized stimulation and to follow the tissue's response over a 24-hour time frame. A very important parameter is that in every experiment, the different treatments are applied on explants coming from the same patient which allows taking into account variability between patients. The fact that the explants are of a relatively large size compared to biopsies also means that a variety of analyses can be performed on the same explant, confirming results in many different ways (ELISA assays, quantitative rtPCRs, microarray analyses, immunohistochemistry and/or immunofluorescence data). Further, other types of analyses could be optimized in the future, such as purification of cell populations of interest from stimulated explants and evaluation of their phenotype by cytofluorimetric analysis.

However, there are also important limitations to be taken into account. At its present form this model is not suitable for high throughput screening of a large number of treatments, as it relies on the availability of the tissue. Cell culture models are probably more suitable for that purpose as they are easier to handle and perform a large number of stimulations on¹⁰ whereas the model we describe would be more efficiently used as the end testing of candidate treatments before entering the clinics. Moreover, the time frame during which we have succeeded to preserve the viability of the tissue does not allow for experiments requiring long monitoring periods, such as RNA interference. Finally, the bacterial stimulation is carried out in a regular incubator and does not allow for preserving or testing the effect of anaerobic bacteria. It is our intention to work on these issues in the future.

Disclosures

No conflicts of interest declared.

Acknowledgements

We thank Erika Mileti for excellent technical support and Dr. Antonio Di Sabatino for providing the oxygen chambers.

Funding: This work was supported by grants from the 7th EU framework program (IBDase, ERC: Dendroworld) and Fondazione Cariplo to MR and support by a Marie Curie international training mobility network (Cross-Talk, grant agreement No: 21553-2) to KT.

References

- Schuller, S., Lucas, M., Kaper, J.B., Giron, J.A., & Phillips, A.D. The *ex vivo* response of human intestinal mucosa to enteropathogenic *Escherichia coli* infection. *Cell. Microbiol.* **11**, 521-530 (2009).
- Tsilingiri, K., *et al.* Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. *Gut.*, (2012).
- Tsilingiri, K. & Rescigno, M. Should probiotics be tested on *ex vivo* organ culture models? *Gut Microbes.*, Manuscript in preparation, (2012).
- Mileti, E., Matteoli, G., Iliev, I.D., & Rescigno, M. Comparison of the immunomodulatory properties of three probiotic strains of *Lactobacilli* using complex culture systems: prediction for *in vivo* efficacy. *PLoS One.* **4**, e7056 (2009).
- Cencic, A. & Langerholc, T. Functional cell models of the gut and their applications in food microbiology--a review. *Int. J. Food Microbiol.* **141 Suppl. 1**, S4-14 (2010).
- te Velde, A.A., Verstege, M.I., & Hommes, D.W. Critical appraisal of the current practice in murine TNBS-induced colitis. *Inflamm. Bowel Dis.* **12**, 995-999 (2006).
- Senior, P.V., Pritchett, C.J., Sunter, J.P., Appleton, D.R., & Watson, A.J. Crypt regeneration in adult human colonic mucosa during prolonged organ culture. *J. Anat.* **134**, 459-469 (1982).
- Browning, T.H. & Trier, J.S. Organ culture of mucosal biopsies of human small intestine. *J. Clin. Invest.* **48**, 1423-1432 (1969).
- Besselink, M.G., *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet.* **371**, 651-659 (2008).
- Lakhdari, O., *et al.* Identification of NF-kappaB modulation capabilities within human intestinal commensal bacteria. *J. Biomed. Biotechnol.* **2011**, 282356 (2011).