emulate

Protocol for Emulate Organ-Chips:

Cytokine-Mediated Barrier Disruption

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EP213 v1.0

Goals:	Key Steps:	Other Required Materials:
• Prepare treatment solution • Treat cells in Emulate Organ-Chips with IL-22	• Prepare stock solution • Prepare working solution • Treat chips with cytokine	• IL-22 (R&D, Cat. # 782-IL-010) • IL-22BP(R&D, Cat. # 8498-BP-025) • IFNγ (Peprotech, Cat. # 300-02) • Tofacitinib (Sigma, Cat. # PZ0017) • DMSO (Sigma, Cat. # D2650) • BSA (Sigma, Cat. # A1595) • DPBS (Sigma, Cat. # D8537) • Medium

Introduction

The Colon Intestine-Chip provides a human organoid-based platform to study cell-cell interactions and mechanisms that elicit collapse of the intestinal epithelial homeostasis. We employed the cytokines IFN**γ**, a prototype cytokine on intestinal epithelial barrier disruption studies, or IL-22. IL-22 acts as a barrier-insulting cytokine when applied to healthy tissue in our Colon Intestine-Chip, in line with recent findings[1,2,3]. For barrier disruption of customer-originated cell material, this protocol may need to be optimized. Please reach out to Emulate Field Science Support for additional guidance.

Method

Before starting this process, ensure that all treatment solutions prepared in Part I and II of this protocol are warmed up to 37°C. Label all chips and Pods with their corresponding treatment conditions prior to adding the compound.

Part I - Barrier function mediated by IL-22

1. Prepare the stock solution, diluting the cytokine in the appropriate solvent (e.g., DMSO, water, DPBS), as per manufacturer's instructions. Ensure that all materials and reagents required for the preparation of the stock solution are sterile and ready.
2. Calculate the volume of solvent to prepare a stock solution of the test article based on mass / molecular weight / molarity of the compound and the desired concentration / molarity of the stock solution. Preparation of 1000X target concentration of stock solution is typically recommended as this allows you to maintain solvent at 0.1%.
3. Resuspend IL-22 in DPBS at stock concentration of 10 µg/mL (606.06 nM).
4. Resuspend the IL-22 BP in 0.1% BSA in DBPS at concentration of 250 µg/mL (9,615.38 nM).
5. Ensure all chip carriers are labeled and identify the different conditions clearly.

Part II — Barrier Function mediated by IFNγ

1. Reconstitute the IFNγ in sterile cell culture grade water. We recommend to resuspend the 20 µg vial in 200 µL of prepare the stock solution at a final concentration of 10 µg/mL.
2. Once reconstituted, aliquot and store the stock solution in -80°C. Do not keep the aliquots for longer than one week.
3. Reconstitute 5 mg of Tofacitinib in 495.55 µL of DMSO to prepare a stock solution at a concentration of 20 mM.
4. Aliquot the reconstituted Tofacitinib in 20 µL portions. This amount is sufficient to dose 12 chips.

Part III – Dosing the Cells in the Colon Intestine-Chip

1. Carefully remove trays with Pods from the Zoë™ Culture Module and transfer to the biosafety cabinet. We recommend removing one tray at a time to minimize stress experienced by cells because of fluctuations in temperature. Ensure that the biosafety cabinet light is turned off if you are working with a light sensitive compound.

   Note: If you have multiple collection time points in your experiment, it helps to organize your conditions so that you have one time point per tray. This will allow you to pause flow only on the tray from which you need to collect, while letting other trays flow uninterrupted, resulting in a more accurate assessment of elapsed time.
2. Fully aspirate both inlet and outlet reservoirs of each Pod, while avoiding direct contact with the Pod reservoir vias.
3. Add the calculated volume of warm, freshly-prepared treatment media to the appropriate channel.
4. Once all Pods have been refreshed, ensure that all trays are returned to the appropriate Zoë. Flush chips at 600 µL / hour for 5 minutes to introduce dosing solution in the chip channels.
5. After flushing Pods and chips, carefully remove trays from Zoë one at a time. Once again, transfer to the biosafety cabinet, and aspirate from the flow-through medium in the outlet reservoirs.
6. Return all trays to Zoë, reset the Zoë settings to the correct experimental conditions (e.g., flow rate, stretch) and note the time as the experimental start time (t=0).
7. Each outlet reservoir can be sampled independently at each timepoint following Protocol EP124 Effluent Sampling.
8. Replenish Pods with freshly prepared treatment medium daily, regardless of the collection timepoints, until the end of your treatment period or experiment.

Test Article	Concentration	Starting Day	Treatment Duration	Flow rate
IL-22	1 nM	Day 5	1-72 hours	30 µL/h
IL-22BP	30 nM	Day 5	1-72 hours	30 µL/h
IFNγ	10 ng/mL	Day 4	48 hours	30 µL/h
Tofacitinib	20 uM	Day 4	72 hours	30 µL/h

Suggested Readouts:

- RNA Isolation and Purification Protocol
- cDNA Preparation Protocol
- Quantitative Polymerase Chain Reaction (qPCR) Protocol
- Protein Samples Isolation Protocol
- Total Protein Quantification Protocol
- Assessment of Cleaved Caspase 3 Protocol
- Assessment of Phosphorylated STAT3 Protocol
- Quantification of Cytokines and Acute Inflammatory Phase Proteins Secretion Protocol
- Immunofluorescence Staining

References

1. Wang, Y., Mumm, J. B., Herbst, R., Kolbeck, R. & Wang, Y. IL-22 Increases Permeability of Intestinal Epithelial Tight Junctions by Enhancing Claudin-2 Expression. *J. Immunol.* (2017). doi:10.4049/jimmunol.1700152
2. Powell, N. *et al.* Interleukin-22 orchestrates a pathological endoplasmic reticulum stress response transcriptional programme in colonic epithelial cells. *Gut* (2019). doi:10.1136/gutjnl-2019-318483
3. Tsai, P. Y. *et al.* IL-22 Upregulates Epithelial Claudin-2 to Drive Diarrhea and Enteric Pathogen Clearance. *Cell Host Microbe* (2017). doi:10.1016/j.chom.2017.05.009