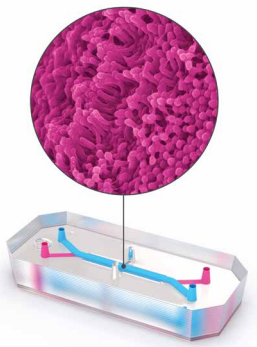
Cytokine-Mediated Inflammation in the Colon Intestine-Chip



Abstract

Inflammatory bowel disease (IBD) covers disorders causing chronic inflammation in the gastrointestinal tract with long term effects on patients. Costs of disease management and treatment are high while therapeutic relief remains underwhelming, and new models to study intestinal barrier function are needed to improve clinical translation of novel drugs. Organs-on-Chips technology has paved the way for the development of the Colon Intestine-Chip, emulating *in vivo* physiological responses. Here we present data demonstrating that the Colon Intestine-Chip platform is closer to *in vivo* relevant responses consistent with barrier disruption when inducing inflammation through the administration of interferon gamma (IFN-γ), which then can be treated with anti-inflammatory therapeutics—such as tofacitinib, a janus kinase (JAK) inhibitor—to reduce the inflammatory response. We also demonstrate concentration-dependent increases in epithelial permeability in the presence of the cytokine interleukin 22 (IL-22). Taken together we propose the Colon Intestine-Chip as a preclinical model for investigation of barrier disruption to facilitate improved clinical translation.

Key Highlights

- The Colon Intestine-Chip is a more physiological platform for investigating inflammatory diseases affecting the intestinal barrier
- The presence of endothelium leads to enhanced barrier function, morphology, polarity, and gene expression
- Researchers can study mechanisms of cytokine-mediated inflammation in a time-, concentration-, and donor-dependent manner
- Efficacy studies of anti-inflammatory drug candidates can be performed on-chip

Introduction

Compromised barrier integrity is increasingly recognized as part of the pathogenesis of a number of chronic conditions such as inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), disorders that involve non-infectious chronic inflammation within the gastrointestinal tract. Incidence of IBD has increased globally from 3.7 million in 1990 to 6.8 million people in 2017[1] and in the U.S., annual healthcare costs for IBD patients ($23,000) are almost three times the cost for non-IBD patients ($7,000).[2] The loss of intestinal barrier integrity, also known as leaky gut, is thought to initiate the pathogenesis of IBD, causing uncontrollable inflammatory signal cascades.[5] Disruption of the intestinal barrier is not only observed in IBD but also in gastrointestinal infections and a variety of autoimmune and metabolic diseases.[5,6]

During stress or inflammation, proinflammatory cytokines are produced in excess including IFN-γ, tumor necrosis factor alpha (TNF-α), and interleukin 13 (IL-13), which increase epithelial permeability. To date, understanding of barrier regulation remains limited, leaving effective therapeutic targeting for the

compromised intestinal barrier a clinical need.[7]

A range of models of the intestinal barrier are utilized to evaluation organ function, disease pathophysiology, and drug interaction. Murine models of intestinal inflammation are the most commonly used animal models in IBD research, with several genetic, cell-transfer based, or chemically induced colitis models having been developed and applied to better understand the disease pathophysiology. However, species differences—such as differences in gut microbiota and immune-microbiota cross-talk—limit translation to human biology.[8,9]

Of the *in vitro* models, Caco-2 cells in Transwell™ inserts are often considered the gold standard intestinal model due to their ability to develop intracellular tight junctions, creating a physical and biochemical barrier.[10–12] However, there are several limitations using Caco-2 cells due to their carcinogenic origin, lack of expression of the full set of gene pathways needed for target validation and mechanism of action studies, and reduced sensitivity to barrier disruption, requiring a significantly higher, non-clinically-relevant concentration of pro-inflammatory cytokines to elicit barrier damage.[13]

Human intestinal organoids (HIOs) are a more recent modality for investigating intestinal physiology and pathology and offer several advantages over 2D cell lines, such as a 3D cytoarchitecture which contains spatial organization that captures cell-cell interactions, cellular diversity, and the retention of some physiological function.[14,15] A significant experimental challenge of HIOs is that they have reversed polarity, with the lumen in the middle of the organoid and inaccessible.[16] Additional limitations of organoids include the lack of the endothelial component (critical for assessing barrier disruption and *in vivo*-like gene expression), lack of *in vivo*-like morphology, and lack of organ-relevant mechanical forces. Without these components, the mini intestines do not fully express gut-relevant gene pathways necessary for target validation, mechanism of action, and drug targeting studies.[17,18] However by using organoids as a cell source in a more physiological environment, many of these limitations could be overcome.

Translational failure and limited efficacy of IBD therapeutics are testaments to the limited quality of intestinal preclinical models. Failure to respond to therapy occurs in 40-55% of patients, and 65-80% fail to enter full remission. Effective and safe drugs with durable responses are an important unmet clinical need.[19]

Goal

To improve potential for drug translation to the clinic by using the Colon Intestine-Chip to generate a model of cytokine-mediated barrier disruption, a key aspect implicated in leaky gut pathologies.

Materials

Below is a list of materials needed for creating the Colon Intestine-Chip in research use. For a detailed description, additional resources, and full protocol, please see emulatebio.com/protocols.

Hardware & Consumables	Cell Sources	Cytokines
• Chip-S1® Stretchable Chip • Zoë® Culture Module • Orb™ Hub Module	• Emulate Colon Intestine Bio-Kit containing qualified biopsy-derived human colonic organoids and primary microvascular colonic endothelial cells • Bio-kit Commercial availability coming in second half 2021	• IFNγ • IL-22

Results

The Colon Intestine-Chip combines organoids & Organs-on-Chips technology to overcome many of the limitations of organoid suspension culture. As seen in **Figure 1**, the model features two parallel channels creating a cell-cell interface between the apical (luminal) channel and basal (vascular) channel. For full characterization, please see the Colon Intestine-Chip Characterization Technical Note. Once the Colon Intestine-Chip was established, principal component analysis (PCA) analysis was performed comparing epithelial cells on-chip, in organoids, and in tissue. Gene expression in the Colon Intestine-Chip more closely resembled *in vivo* expression, with significantly enriched terms for cell epithelial differentiation, metabolism, and ion transportation (**Figure 2A**). Colonic organoids cultured on-chip acquired an *in vivo*-relevant cytoarchitecture containing highly polarized cells with a mature brush border with densely packed microvilli, and mature epithelial phenotypes, in addition to *in vivo*-like ratios of key cell subtypes (**Figure 2B**). Comparing the co-culture Colon Intestine-Chip with an epithelial-only version the importance of the endothelial co-culture becomes evident. The co-culture Intestine-Chip produced well-defined tight junctions (**Figure 3A**) and exhibited enhanced epithelial barrier integrity demonstrated by increased apparent permeability (P_{app}) to 3kDa dextran (**Figure 3B**).

Pro-inflammatory cytokines—such as IFNγ—can disrupt intestinal barrier function.[20,21] Prolonged exposure to elevated IFNγ in the mucosa can disrupt tight junctions and activate apoptotic mechanisms, increasing barrier permeability. IFNγ is commonly used experimentally to investigate barrier performance and give insights into function of healthy and diseased states such as IBD.[20–23]

To stimulate *in vivo*-like action of IFNγ, different human-relevant concentrations of IFNγ were administered to the basolateral side of the epithelial monolayer in the Colon Intestine-Chip via flow through the bottom

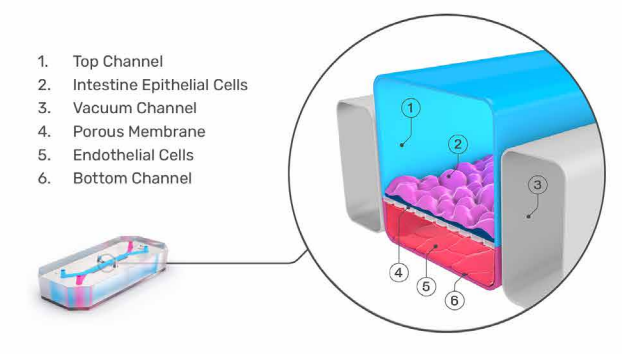
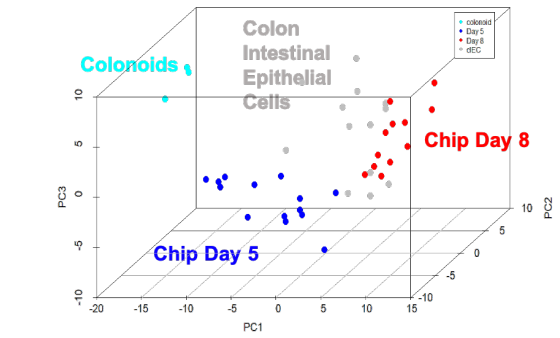


Figure 1. Colon Intestine-Chip Schematic



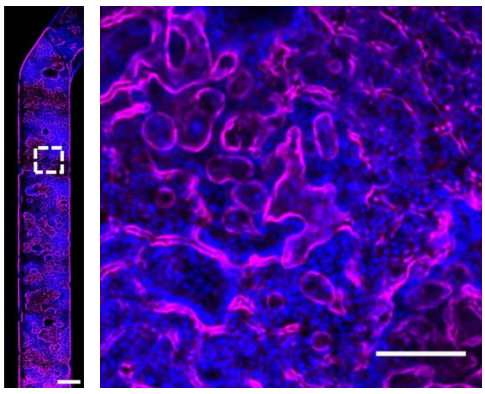


Figure 2. Characterization of epithelial cells on chip. *A. Principal component analysis (PCA) analysis comparing epithelial cells on chip and on tissue for 8 days.* ***B.*** *Confocal Image of epithelial cells on chip.*

channel. After 48 hours, epithelial cells degenerated, as observed by morphology (**Figure 4A**), and epithelial P_{app} increased (**Figure 4B**). There was a gradual deformation of epithelial tight junctions demonstrated by the relocation of ZO-1 and restructuring of the cytoskeletal protein F-actin (**Figure 5**).

The epithelial layer showed a gradual decrease in confluency as IFNγ concentrations increased 48 hours post-treatment (**Figure 6A**). Activation of apoptosis was observed, as indicated by a significant increase of cleaved caspase 3 (**Figure 6B**). Lastly, a concentration- and time-dependent increase in the secretion of proinflammatory molecules was seen, delimitating the propagation of the inflammatory response triggered by the injured barrier (**Figure 7**). Taken together, these data establish the Colon-Intestine Chip as a model of cytokine-mediated barrier disruption.

Next, we performed a study to evaluate the ability to test drug candidate efficacy for the treatment of barrier disruption. Tofacitinib is an FDA approved small molecule that protects against inflammation-induced damage and is used for treatment of several autoimmune disorders, including moderate to severe UC. Administration of tofacitinib in patients demonstrated inhibition of multiple pro-inflammatory cytokines and resulted in both protection and rescue of IFNγ-mediated loss of tight junctions and barrier integrity damage.[24,25]

Both tofacitinib and IFNγ were co-administered to the Colon Intestine-Chip, resulting in a delayed increase of IFNγ-driven epithelial P_{app}. When IFNγ was removed (after 36 hr) and tofacitinib was maintained, the loss of the epithelial barrier integrity was negated (**Figure 8**). Tofacitinib, co-administered with IFNγ, was able to prevent the loss of the epithelial tight junction integrity (**Figure 9**), as indicated by staining against ZO-1 and F-actin. Additionally, tofacitinib treatment of the Colon Intestine-Chip reduced the IFNγ-triggered secretion of the proinflammatory molecules ICAM-1, VCAM-1 and IL-6, which are associated with the acute phase of the intestinal inflammatory response (**Figure 10**). Taken

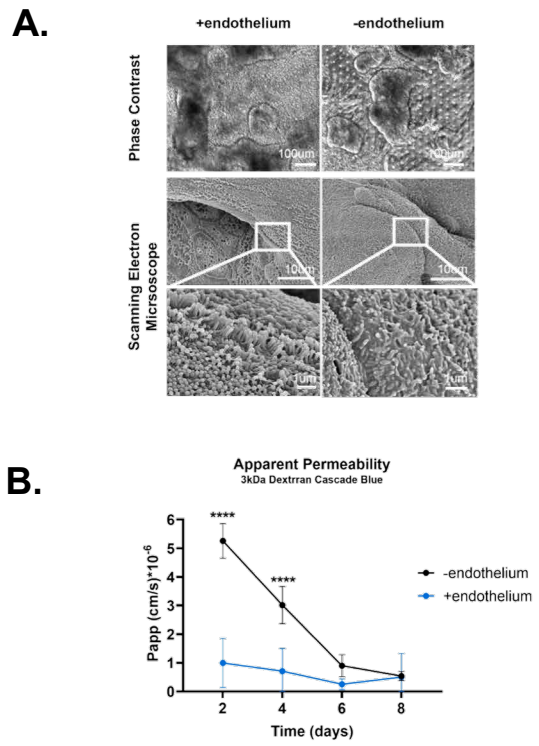


Figure 3. Tight junction characterization on chip with or without endothelium. A. *Scanning electron microscope (SEM) images of epithelial morphology.* ***B.*** *Apparent permeability (Papp) with 3kDa dextran over 8 days. ****p<0.0001*

together, these data demonstrated that the Colon Intestine-Chip can emulate *in vivo*-like responses when administered a drug to treat inflammation-induced barrier damage.

IL-22 is a cytokine that is part of the IL-10 family which can either protect against tissue damage or contribute to disease progression. Different colitis mouse models utilizing IL-22 application exhibited both pro-inflammatory and anti-inflammatory properties in the homeostasis of the gut. Therefore, a better understanding of the role IL-22 in barrier function could provide insight into the mechanism of action of disease progression in IBD.[26,27]

Basolateral stimulation of the Colon Intestine-Chip with IL-22 demonstrated loss of epithelial barrier integrity as well as degeneration of the epithelial cell morphology (**Figure 11A**). Epithelial P_{app} was increased (**Figure 11B**). When the IL-22 soluble receptor IL-22 binding protein (IL22-BP) was present, loss of epithelial integrity and increase in permeability were negated, indicating the specificity of the response. Similar to IFN γ, the administration of IL-22 activated epithelial apoptosis as early as 24 hours post-simulation, evidenced by increased cleaved caspase 3 (**Figure 11C**). These data indicate that IL-22 targets colonic epithelial cells and disrupts barrier function.

Conclusion

The human Colon Intestine-Chip supported the formation of an intestinal barrier from biopsy-derived organoids with cytoarchitecture and a transcriptome profile that emulates human *in vivo* function. Barrier function was confirmed by stimulation with IFNγ, a prototype cytokine in barrier disruption studies, in a concentration, time, and donor dependent manner. Results demonstrated increased P_{app}, degeneration of epithelial cell morphology and apical tight junction complexes, apoptosis activation, and secretion of pro-inflammatory cytokines and chemokines. Application of a clinically approved drug, tofacitinib, with IFNγ demonstrated that the Colon Intestine-Chip can be induced to exhibit

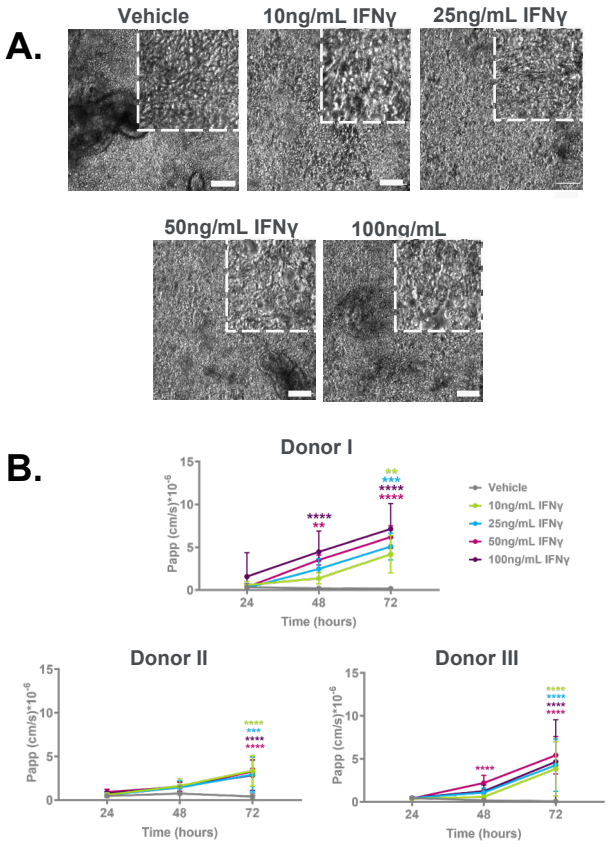


Figure 4. Characterization of epithelial cell with administration of INFγ. A. *Epithelial morphology after exposure to multiple concentrations.* ***B.*** *Apparent permeability (Papp) results with 3kDa dextran after 72 hours of varying IFNγ doses in 3 donors. Scale bar, 100 mm. **p<0.01; ***p<0.001; ****p<0.0001*

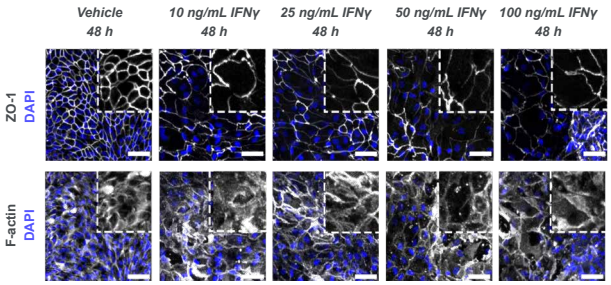


Figure 5. Confocal images of epithelial tight junction structure after the administration of multiple dose concentrations of IFNγ after 48 hours. Scale bar, 50 mm.

cytokine-mediated barrier disruption suggesting great potential to utilize the model to evaluate the efficacy of a drug candidate to treat/prevent that disruption. The IL-22-driven barrier disruption, characterized by increased P_{app} and activation of apoptosis, is abrogated after the administration of the soluble IL-22 receptor, IL-22BP. Taken together, these results support the clinically relevant behavior and physiology of the Colon Intestine-Chip in both healthy and pathological conditions. This would be a novel and efficient preclinical model for testing drug candidates in development for colonic barrier disruption in diseases like IBD, identifying druggable targets, mechanism of action studies, and assessing efficacy of drug candidates to prevent/treat cytokine-driven barrier disruption. Other applications include developing on-chip IBD disease models and investigating mechanisms implicated in barrier damage/disruption specifically in GI disorders including IBD or other diseases like cancer or type 2 diabetes, and research into the gut microbiome.

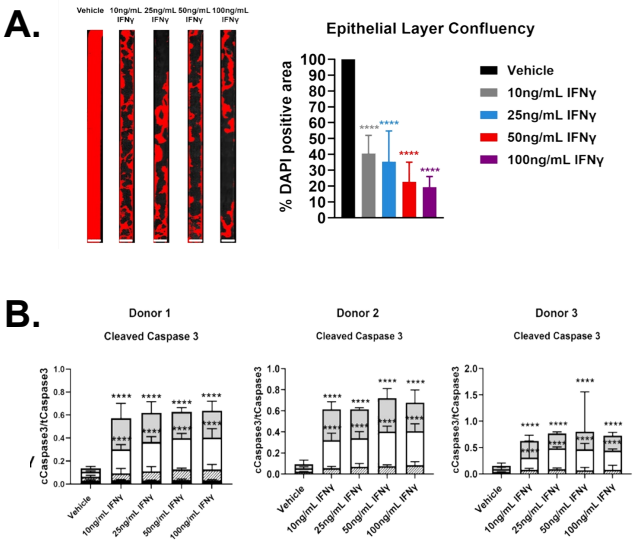


Figure 6. Epithelial confluency characterization post IFNγ treatment. A. *Representative of images epithelial cells and calculated confluency, 48 hours poststimulation with different concentrations of IFNγ.* ***B.*** *Caspase 3 cleavage with different IFNγ concentrations across 3 donors. Scale bar, 1000 mm. ****p<0.0001*

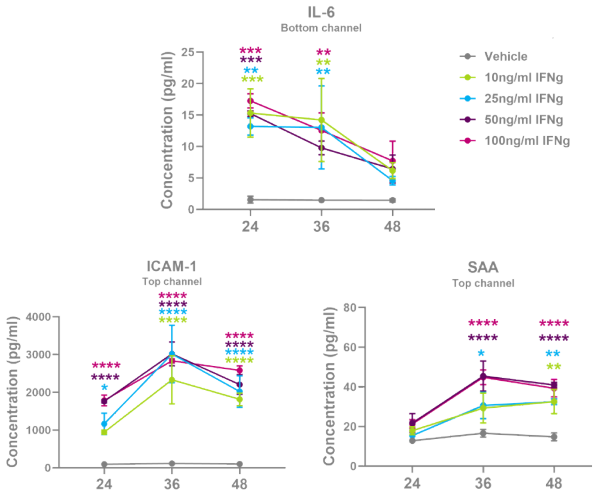


Figure 7. Concentration- and time-dependent secretion of IL-6, and ICAM-1 & SAA, in the bottom and top channel respectively

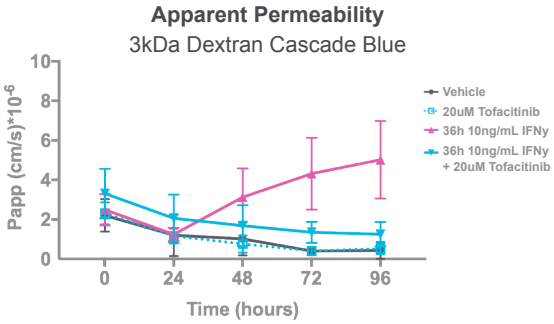


Figure 8. Epithelial P_{app} in the Colon Intestine-Chip during co-treatment with IFNγ and tofacitinib. ***p<0.001; ****p<0.0001

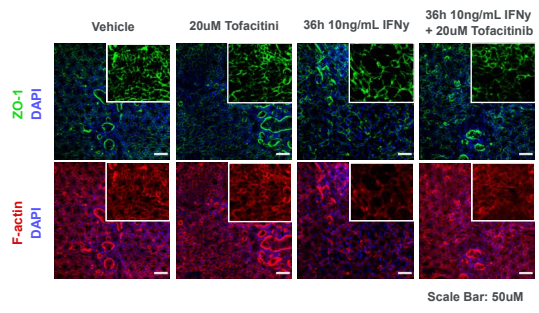


Figure 9. Confocal images of tight junctions after the co-administration of tofacitinib and IFNγ on day 7 of culture. Scale bar, 50 mm

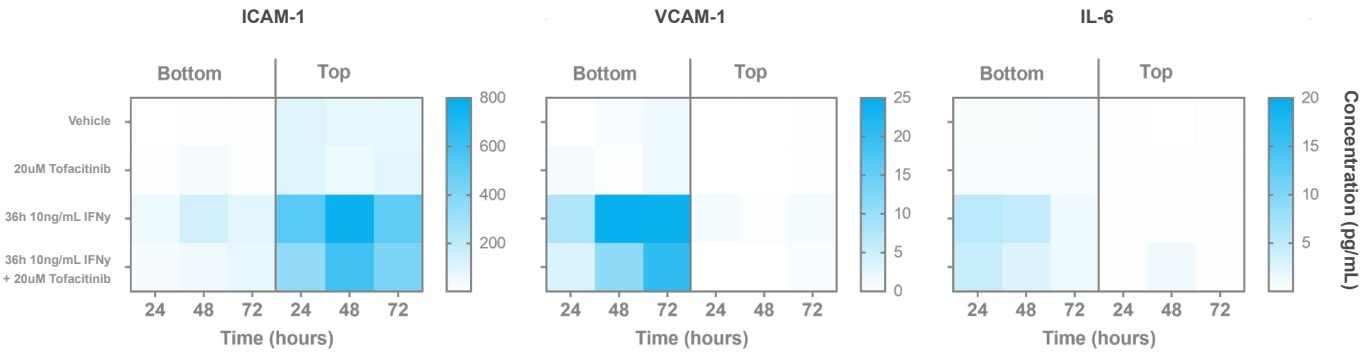


Figure 10. Inflammatory response after 36-hour stimulation with 10 ng/mL IFNγ and treatment with 20 μM tofacitinib.

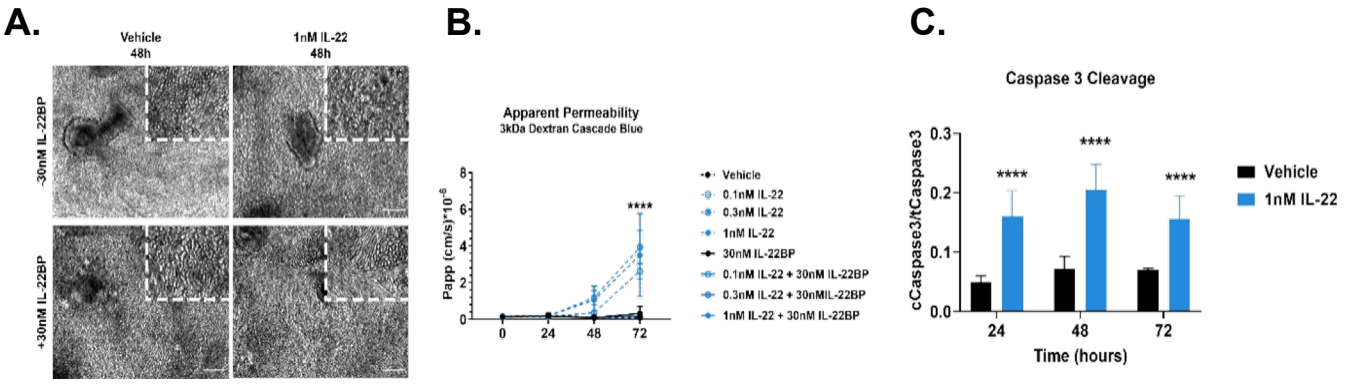


Figure 11. Characterization of epithelial barrier after administration of multiple concentrations of IL-22. ***A.*** *Images of epithelial morphology after 48 hours of vehicle or IL-22 treatment.* ***B.*** *Apparent permeability (Papp) of epithelial barrier after treatment with vehicle, IL-22 or ILL-ss and IL-22BP.* ***C.*** *Caspase 3 cleavage over 72 hours with vehicle or IL-22 treatment. Scale bar, 100 mm. ****p<0.0001*

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