Introduction

This document guides researchers in performing viral or bacterial infectious disease studies with Emulate Organ-on-a-Chip technology. Although Emulate does not currently provide validated protocols for this type of research, more than 12 peer-reviewed papers have been published using Emulate Organ-Chips to study infectious disease, demonstrating the utility of the technology for this research area.

This document includes links to culture protocols and guidelines for various organ models, considerations for BSL3 studies, important factors for designing studies, and reference tables containing key details for peer-reviewed publications.

Organ-Chip Models for Infectious Disease Research

A variety of Organ-Chip models can be used to study infectious diseases. Below is a non-exhaustive table of commonly used models for infectious disease studies and their associated instructions for cell culture.

Organ Model	Protocol / Guideline
Alveolus Lung-Chip	*Culture guideline under development*
Airway Lung-Chip	*Culture protocol under development*
Colon Intestine-Chip	Colon Intestine-Chip Culture Protocol
Duodenum Intestine-Chip	Duodenum Intestine-Chip Culture Protocol
Liver-Chip	Liver-Chip Quad-Culture Protocol
Proximal Tubule Kidney-Chip	Proximal Tubule Kidney-Chip Co-Culture Protocol
Additional Organ Models	Basic Research Kit Protocol

BSL Considerations

The Emulate Human Emulation System® has been designed with biosafety level 2 (BSL2) environments in mind; however, several users have adopted the platform for use in biosafety level 3 (BSL3) environments. While Emulate has not tested the suitability of these systems for BSL3 laboratories, this document has been prepared to help these environments determine their suitability and evaluate which additional safety measures may be needed.

Modeling Viral and Bacterial Infection

Using Emulate Organ-Chips, researchers have studied a variety of viruses and bacteria, including SARS-CoV-2, human rhinovirus, influenza, tuberculosis, and E. Coli.

Infectious agents can be administered in the epithelial channel to model direct exposure to the epithelium, or in the endothelial channel to model systemic exposure. Drug candidates can be administered in either channel to model direct (e.g., oral) or intravenous exposure (see Figure 1).

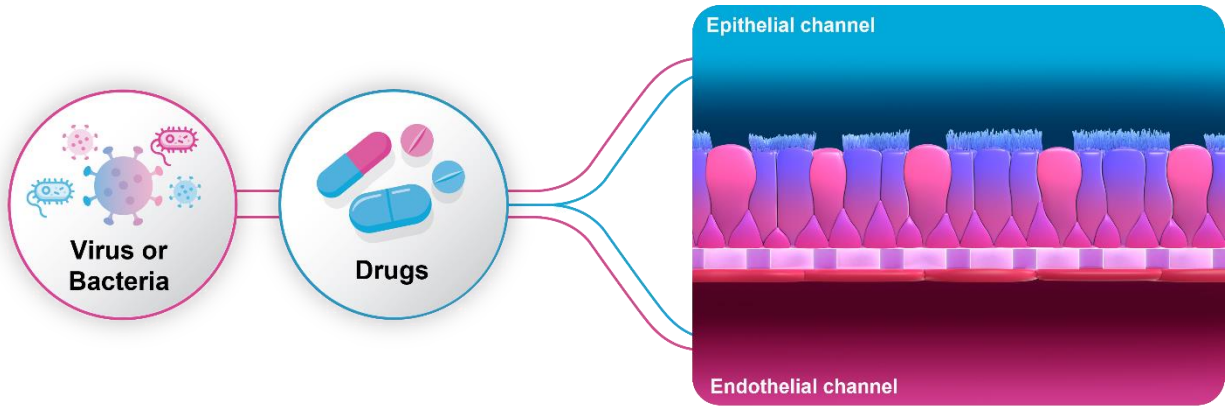

Figure 1: Route of Administration

When designing an infectious disease study using Organ-Chips, researchers should consider optimization of the infectious agent and drug dosing regime, such as dosing channel, concentration, day(s) of administration, and dosing order (e.g., dosing drugs before, during, or after dosing the infectious agent).

To assist researchers in designing their infectious disease studies, the below summary tables outline the study parameters used in peer-reviewed infectious disease publications using Emulate Organ-Chips. For any additional questions contact your Emulate Scientific Liaison or email the support team at support@emulatebio.com.

Table 1: Viral Infection Studies Using Emulate Organ-Chips

Tissue	Infection	Drugs	Cells	Title	Year	Authors	Infectious Agent Concentration	Channel Dosed	Day of Dosing	Infectious Agent Exposure Time	Endothelium Seeding Day	Note
Lung: Alveolus	Influenza	Azeliragon (RAGE inhibitor), GSK2193874 (TRPV4 inhibitor)	• Primary alveolar epithelium & endothelium • PBMCs	Mechanical control of innate immune responses against viral infection revealed in a human lung alveolus chip (Link)	2022	Wyss	MOI = 0.01	Top	Day 15 after seeding	2 h static, then removed top channel media to re-establish ALI	Seeded same day as epithelium	
Lung: Alveolus	SARS-CoV-2	H-151 (STING inhibitor)	• Primary alveolar epithelium & endothelium • Macrophages	The cGAS–STING pathway drives type I IFN immunopathology in COVID-19 (Link)	2022	EPFL	400–600 PFU in a volume of 30 µl	Top	3-5 days	1h static, then removed top channel media to re-establish ALI	Seeded same day as epithelium	
Lung: Airway/ Alveolus	SARS-CoV-2	Short duplex RNAs	• Primary alveolar/ airway epithelium & endothelium	Self-assembling short immunostimulatory duplex RNAs with broad-spectrum antiviral activity (Link)	2022	Wyss	MOI = 0.01	Top	Day 15 after seeding	6 h static then ALI then samples were collected 72h later	Seeded same day as epithelium	
Lung: Airway	Influenza A Virus	Nafamostat	• Primary alveolar epithelium & endothelium	Clinically Relevant Influenza Virus Evolution Reconstituted in a Human Lung Airway-on-a-Chip (Link)	2021	Wyss	MOI = 0.01, 0.1, 1 and 2	Top	3-4 Weeks	2 h static, then removed top channel media to re-establish ALI	Seeded same day as epithelium	
Intestine	Pseudo SARS-CoV-2		• Primary duodenal organoids • Primary colonic endothelium • PBMCs	Enteric Coronavirus Infection and Treatment Modeled With an Immunocompetent Human Intestine-On-A-Chip (Link)	2021	Wyss	MOI 0.01 in 50 µl NL63 or OC43	Top	Dosed after seeding endothelium	6 h static then added fresh virus, incubate overnight (16h) static then on flow for 48h	Seeded between day 16-24 (2 days after villi formation)	
Lung: Alveolus	SARS-CoV-2	Tocilizumab	• Primary alveolar epithelium & endothelium • Macrophages	Rapid endotheliitis and vascular damage characterize SARS-CoV-2 infection in a human lung-on-chip model (Link)	2021	EPFL						
Lung: Airway	Pseudo SARS-CoV-2		• Primary alveolar epithelium & endothelium • Neutrophils	A human-airway-on-a-chip for the rapid identification of candidate antiviral therapeutics and prophylactics (Link)	2021	Wyss, etc.	MOI = 0.1	Top	3-4 Weeks	2 h static, then removed top channel media to re-establish ALI	Seeded same day as epithelium	
Lung: Airway	HRV	MK-7123 (CXCR2 antagonist)	• Primary airway epithelium • HUVECs • Neutrophils	A Microengineered Airway Lung Chip Models Key Features of Viral-induced Exacerbation of Asthma (Link)	2020	Emulate, Merck	MOI = 1	Top	3 weeks ALI	3 h static @ 33 degrees	Seeded after 3 weeks of ALI	Prototype chip design: 3 µm pores
Intestine	Coxsackie B1		• Caco-2	Human Gut-On-A-Chip Supports Polarized Infection of Coxsackie B1 Virus In Vitro (Link)	2017	Wyss, FDA	5 µl of coxsackievirus B1 of stock	Top	Day 6 after cell seeding	2 h static, wash, then flow for 6,24, and 48h (sampling)		Prototype chip design: 10 µm pores, 200 µm channel height

Table 2: Bacterial Infection Studies Using Emulate Organ-Chips

Tissue	Infection	Cells	Title	Year	Authors	Infectious Agent Concentration	Channel Dosed	Day of Dosing	Infectious Agent Exposure Time	Endothelium Seeding Day
Vagina	Lactobacillus Crispatus, Gardnerella, Prevotella, Atopobium	• Primary vaginal epithelium and cervical fibroblasts	Vaginal microbiome-host interactions modeled in a human vagina-on-a-chip (Link)	2022	Wyss	CFU: 10^5	Top	2 weeks post cell seeding	Overnight static, then connected to flow	Fibroblast seeded same day as epithelium
Intestine: Jejunum	E. Coli	• Primary jejunal organoids	Mechanical Stimuli Affect Escherichia coli Heat-Stable Enterotoxin-Cyclic GMP Signaling in a Human Enteroid Intestine-Chip Model (Link)	2020	Johns Hopkins, Emulate	ST (Virulence factor of E. Coli)	Top	Day 5 after seeding	6 h	
Lung: Alveolus	Myco-bacterial tuberculosis	• Mouse alveolar epithelium and endothelium	A lung-on-chip model of early Mycobacterium tuberculosis infection reveals an essential role for alveolar epithelial cells in controlling bacterial growth (Link)	2020	EPFL		Top	Day 7 of ALI	2-3 h, then ALI	Seeded same day as epithelium
Intestine: Colon	E. Coli	• Primary colonic organoids • Primary endothelium	Species-specific enhancement of enterohemorrhagic E. coli pathogenesis mediated by microbiome metabolites (Link)	2019	Wyss, ETH Zurich	1.7×10^5 EHEC	Top	• Day 8: chip conditioning • Day 9: dosing	3 h static, then 60ul/h for 24 h	Seeded 7 days after epithelium
Intestine: Caco-2	Shigella	• Caco-2	Bioengineered Human Organ-on-Chip Reveals Intestinal Microenvironment and Mechanical Forces Impacting Shigella Infection (Link)	2019	Pasteur Institute	MOI = 0.1 Shigella WT-GFP	Top	Day 6 after seeding	30, 60, 120 min post - inoculation. Second experiment performed at MOI = 1 with 2 h inoculation, washed of non-adherent bacteria, incubated overnight	