PROJECT

Supervisor:	Nicola Clementi
Title:	Advanced In Vitro Models of Mucosal Barriers for Microbiota-Pathogen Interaction Studies
Curriculum:	Experimental and Clinical Medicine
Link to the personal page of the University or relevant hospital site website:	https://www.unisr.it/docenti/c/clementi-nicola

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Mucosal barriers (respiratory, vaginal, intestinal) represent the primary interface between host and microbial communities, where commensal flora maintains homeostasis while defending against pathogens. While static culture models have provided insights into host-pathogen interactions, they fail to recapitulate the complex microenvironment including fluid flow, mechanical forces, oxygen gradients, and microbiota-epithelium crosstalk. Current knowledge gaps include: (i) how commensal flora composition influences susceptibility to viral and bacterial coinfections across different mucosal sites; (ii) dynamic interactions between resident microbiota, invading pathogens, and epithelial barriers under physiologically relevant conditions; (iii) translation of patient-derived microbiota profiles into predictive infection models. Conventional transwell systems lack physiological flow dynamics, while advanced microfluidic platforms remain underutilized for multi-kingdom infection studies.

Rationale and hypothesis

Physiologically relevant *in vitro* models incorporating flow dynamics, controlled oxygen tension, and patient-derived microbiota are essential to understand infection susceptibility and coinfection mechanisms. We hypothesize that: (1) microbiota composition critically modulates epithelial barrier function and pathogen colonization resistance in a site-specific manner; (2) viral infections disrupt microbiota-epithelium homeostasis, predisposing to secondary bacterial infections; (3) microfluidic organ-on-chip systems will reveal pathogen-microbiota dynamics invisible in static cultures, enabling patient-stratified infection risk prediction.

Objectives and specific aims

Year 1: Establish and validate three mucosal barrier models (lung: air-liquid interface with bronchial epithelial cells; vaginal: lactobacillus-epithelium co-culture; intestinal: enterocyte-mucus layer system) in transwell and microfluidic platforms (IVTech/commercially available chips). Compare barrier integrity, cytokine profiles, and microbial adhesion between static and flow conditions.

Year 2: Characterize microbiota-pathogen interactions by introducing patient-derived microbial communities followed by viral (influenza, SARS-CoV-2, HSV, HPV, rotavirus) and bacterial (*S. pneumoniae, E. coli, S. aureus*) mono- and coinfections. Quantify pathogen load, microbiota shifts (16S/ITS sequencing, culturomics), barrier disruption, and immune responses across models.

Year 3: Integrate patient cohort data by modeling individual microbiota profiles from infected patients. Correlate in vitro infection outcomes with clinical parameters to identify microbiota signatures predictive of infection severity and coinfection risk.

Expected outcomes

This project will deliver: (1) validated, flow-based mucosal barrier platforms for microbiology-virology research; (2) mechanistic insights into microbiota-mediated protection and coinfection susceptibility across mucosal sites; (3) patient-derived microbiota biomarkers for infection risk stratification; (4) high-impact publications and translational data supporting microbiota-targeted therapeutics. The candidate will gain expertise in advanced cell culture, microfluidics, multi-omics, and clinical translation.

Skills that the student should acquire (max. 600 characters including spaces):

Technical: Advanced epithelial cell culture (primary cells, air-liquid interface, 3D models); microfluidic/organ-on-chip operation and optimization; aseptic handling of viral and bacterial pathogens (BSL-2); anaerobic culturomics; microbiota profiling (16S/ITS rRNA sequencing, metagenomics); molecular biology (qPCR, RNA extraction); immunoassays (ELISA, multiplex cytokine analysis); microscopy (confocal, live-cell imaging). Analytical: Bioinformatics (microbiome data analysis, statistical modeling); experimental design and troubleshooting. Professional: Scientific writing, presentation skills, interdisciplinary collaboration, laboratory management.

References (max. 15)

Park SE, et al. Organoid-on-a-chip. Science 2019;364:960-965.

Beaurivage C, et al. Development of a human primary gut-on-a-chip to model inflammatory processes. Sci Rep 2020;10:21475.

Blaskewicz CD, et al. In vitro model of the lactobacillus-dominated vaginal microbiome. mBio 2021;12:e02329-21.

Thacker VV, et al. A lung-on-chip model for infectious disease research. Nat Protoc 2021;16:3401-3432.

Sencio V, et al. Gut dysbiosis during influenza contributes to pulmonary pneumococcal superinfection. Cell Rep 2020;32:107934.

Wypych TP, et al. The influence of the microbiome on respiratory health. Nat Immunol 2019;20:1279-1290.

Gozzi N, et al. Microfluidic models of the human respiratory system for virus studies. Adv Biol 2022;6:2101633.

Kasendra M, et al. Duodenum Intestine-Chip for preclinical drug assessment in a human relevant model. eLife 2020;9:e50135.

Nikolić MZ, et al. Human lung development: recent progress and new challenges. Development 2021;148:dev194654.

Banerjee A, et al. Lung-on-a-chip models of SARS-CoV-2 infection. Trends Pharmacol Sci 2022;43:482-495.

van der Velden VHJ, et al. The vaginal microbiome in health and disease. Trends Microbiol 2023;31:78-91.

Yeoh YK, et al. Gut microbiota composition reflects disease severity in COVID-19 patients. Gut 2021;70:698-706.

Shilts MH, et al. Severe COVID-19 is associated with an altered upper respiratory tract microbiome. Front Cell Infect Microbiol 2022