**BBSRC MIBTP Studentship Project**

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| **Project Title** | How does spatial organisation impact host-microbiome interactions in human airways? |
| **Project Summary** | |
| The spatial organisation of microbial communities can be influenced by chemical gradients (e.g. metabolite concentrations), physical features (e.g. spatial scale, airflow), and biological factors (e.g. immune processes, microbial species growth rates). For example, microbial colonies in lower human airways differ from those found in upper airways due to the latter’s exposure to external environment and activity of immune cells in lower airways1. These factors influence microbial function and mediate interactions with host cells, with implications for human health1.  How microbial spatial organisation influences community dynamics and human health remains poorly understood in the context of human airways. Lack of suitable model systems that can capture features of higher vs lower airway microbiome, especially the spatial scale, poses a significant challenge. This gap can be addressed by co-culturing human airway and microbial cells on extracellular matrix micropatterns of defined shape and size. These micropatterns can be created using deep ultraviolet (UV) lithography. This entails exposing UV light on polymers (e.g. Lipidure) via a photomask whose opaque areas act as a stencil of the desired pattern. The exposed regions are chemically processed (e.g. via carbodiimide and succinimide chemistry) to ensure covalent binding with extracellular matrix. The photomask can be designed to yield desired pattern shapes (circle, ellipse) and sizes (30µm–3mm). Human pluripotent stem cell micropatterns revealed insights into the gene regulatory networks mediating human gastrulation2 that were previously inaccessible.  This proposal aims to develop an in vitro human airway microbiome model and use it to understand how spatial organisation impacts microbial-host cell interactions. The student will test the hypothesis that release of microbial metabolites with inflammatory potential depends on the size of micropatterns of co-cultured airway cells and microbial communities. Project objectives are:  Objective 1: Develop a protocol to co-culture airway cells and microbial populations. The student will create high-throughput microtitre plates with micropatterns, as reported elsewhere2. Next, they will optimise the protocol to co-culture relevant airway (e.g. epithelial cells and fibroblasts) and microbial cells (e.g. Proteobacteria). This entails identifying the optimal cell density ratios, media supplements, extracellular matrix, and culture duration.  Objective 2: Test the impact of spatial organisation on host-microbiome interactions. The student will create circular and ellipsoid micropatterns of lengths 50 µm, 500 µm, and 1000 µm. Following cell seeding, the colonies will be analysed for morphology and architecture (via confocal imaging), release of inflammatory cytokines (via ELISA, qPCR), and bacterial metabolites (via mass spectrometry). The student will also co-culture multiple microbial populations to study how size impacts interactions of microbes with each other and host cells.  Objective 3: Develop an automated data-analytic pipeline. First, the student will create an image analysis pipeline in CellProfiler to identify regions of interest (e.g. nuclei, bacterial communities) based on intensities of relevant stains. Second, they will write a Python code to pool the collected multimodal data and conduct statistical tests (e.g. PCA) to generate integrative insights into how size shapes host-microbe and microbe- microbe interactions.  This project will yield a novel high-throughput system to study microbiome interactions with human airway cells at multiple spatial scales. This will yield insights into how size and spatial organisation shape these interactions. The student will work in an interdisciplinary environment and gain transferable skills that will broaden their career prospects.  Techniques that will be undertaken during the project:  The student will (be offered training to) directly work with the following techniques: lithography, human cell co-culture, microbial culture, confocal imaging, ELISA, RT-qPCR, mass spectrometry, and principal component analysis (PCA). The diversity of these techniques will enable the student to develop a strong foundation to lead future interdisciplinary projects and pick up transferable skills that will help them succeed in any working context.  BBSRC Strategic Research Priority: Understanding the Rules of Life – Immunology, Biology | |
| **References** | |
| 1. W Barcik et al. Immunity, 2020, doi: 10.1016/j.immuni.2020.01.007  2. H Kaul et al. Stem Cell Reports, 2022, In Press (pre-print doi: 10.1101/2020.10.06.327650) | |