HuMiX: A MICROFLUIDICS-BASED IN VITRO CO-CULTURE DEVICE FOR INVESTIGATING HOST-MICROBE MOLECULAR INTERACTIONS

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ABSTRACT

Lack of *in vitro* co-culture devices hinder investigations aiming to uncover the role of human-associated microbial community imbalances in causation of numerous medical conditions. We describe the development and validation of a microfluidics-based co-culture device (HuMiX) allowing co-cultivation of human and microbial cells. The modular device architecture provides access to individual co-cultured contingents following targeted perturbations which facilitate high resolution systemic investigations into the hypothesized role of the complex host-microbial molecular interactions in the pathogenesis of idiopathic medical conditions.

KEYWORDS: Microbiome, Gut on chip, Host-Microbe Interaction, Co-culture, Organs on chip

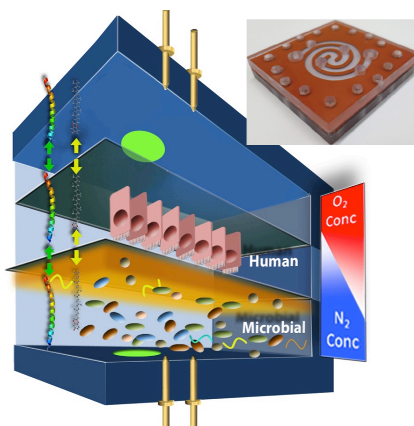

Figure 1. The model and image (inset) of HuMiX device design & integrated sensors.

INTRODUCTION

Human microbiome plays a central role in human health and disease [1]. Recently, imbalances (dysbiosis) in the microbial communities inhabiting the human body has been associated to many diseases [1], yet causal links are difficult to ascertain because of a distinct lack of in vitro human-microbial co-culture systems where emergent hypotheses can be tested [2]. We have developed a modular microfluidics-based co-culture device (HuMiX) that allows the partitioned yet proximal cultivation of human cells and microbial communities while at the same time allowing molecular interactions between both contingents across semi-permeable membranes. As a proof of concept, we demonstrate the establishment of a human gut model on the HuMiX platform. Utilizing laminar flow profile of the HuMiX device, we recreate gut homeostasis in the individual chambers. To harbor anaerobic bacteria residing in the human gut, we create anaerobic niches in the microbial chamber by perfusion of anoxic medium. Integrated of oxygen sensors as well as ports allowing evaluation of transepithelial electrical resistance (TEER) via commercial Epithelial Voltohmmeter and standard chopstick electrodes (STX01, WPI Inc) allow monitoring of the physicochemical parameters of the co-cultured contingents. Finally, modular architecture permits systemic omic investigations questioning the role of the host-microbial molecular interactions in relation to disease pathogenesis.

RESULTS AND DISCUSSION

The HuMiX device consists of three distinct chambers: perfusion chamber, human cell chamber and microbial chamber [Fig. 1]. We inoculated the human chamber with human epithelial cells (caco-2) and cultured them via diffusion of DMEM medium from perfusion chamber [3]. The human cells grow on collagen-coated membrane into a monolayer and undergo an enterocytic differentiation into an epithelial barrier, which is monitored by measuring the transepithelial electrical resistance (TEER) [Fig. 2]. The microbial membrane is coated with mucin to ensure adhesion of microbial communities. To ensue co-culture, the microbial isolates or communities are introduced into the microbial chamber [Fig. 2]

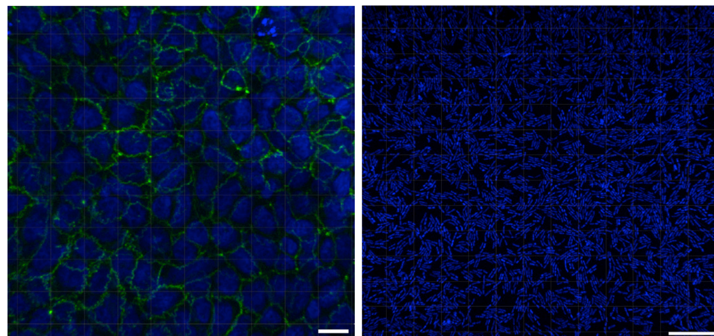

Figure 2. (Left) Epithelial barrier formation of caco-2 cells showing tight junctions (Right) Biofilm of co-cultured Lactobacillus Rhamnosus LGG. Scale bars: (10 µm)

The microbial chamber is perfused with bacterial culture medium. In order to harbor anaerobic bacteria, the microbial culture medium is bubbled with N_2 gas bringing O_2 concentration below 1% [Fig. 4]. Integrated oxygen sensors (optodes) allow continuous monitoring of oxygen concentration.

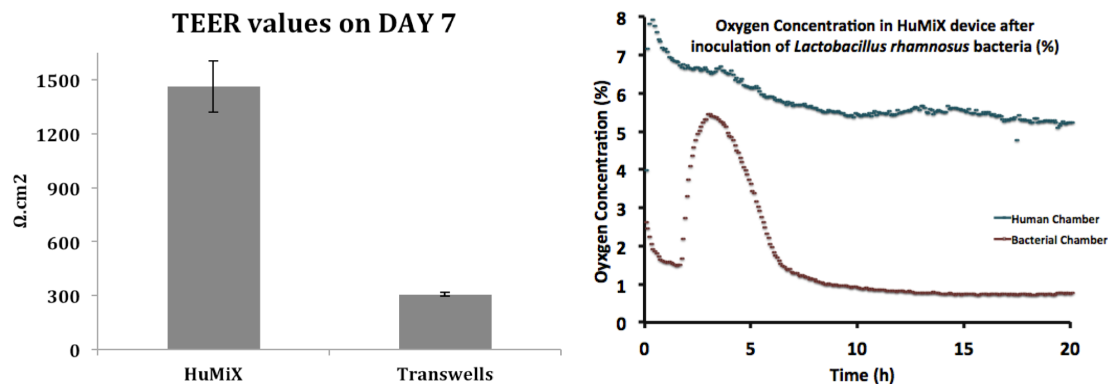

Figure 3. (Left) Comparison of caco-2 cell growth and epithelial barrier formation on HuMiX as well as control (Transwell inserts) via TEER measurements on DAY 7. Establishment of anaerobic niches in HuMiX device.

The co-cultures are monitored via metabolomics on spent medium. Furthermore, the modular device architecture allows independent probing of the both cell contingents using the latest high-resolution omic analyses. Hence, post co-cultures, the human and microbial contingents are processed with our comprehensive bio-molecular extraction protocol [4] for isolating high quality metabolites, DNA, RNA and proteins fractions from individual samples. Molecular fractions are analyzed via dedicated high throughput multi-omic analyses to understand the effects of co-culture on human cellular metabolism and gene expression. Differences in intra and extracellular metabolite profiles before and after addition of the bacteria highlight salient features of the host-microbial interaction [Fig. 4].

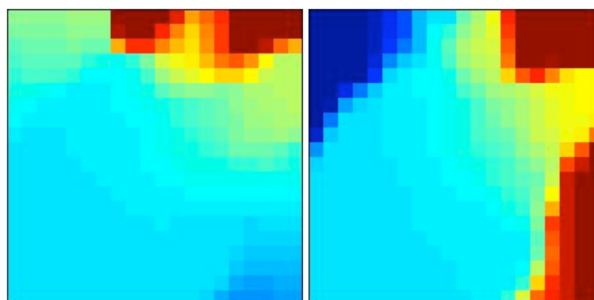

Figure 4. Metabolomic analysis of spent medium (Left) Before inoculation of LGG (Right) After co-culture.

RNA fractions are analyzed via microarray analysis to study changes in the gene expression of the human epithelial cells [Data not included]. Further, the spent medium is analyzed for cytokine profile to evaluate immunomodulatory effects of the co-culture on the epithelial cells [Fig. 5].

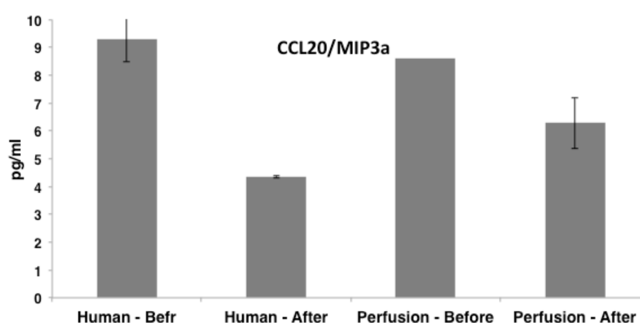

Figure 5. Cytokine profile (CCL20/MIP3a) in different chambers (perfusion chamber and human cell culture chamber) before and after co-culture with LGG. As LGG is a probiotic strain, it leads to anti-inflammatory conditions for the caco-2 cells evident by the decrease in the expression of CCL20 chemokine.

CONCLUSION

The HuMiX device is the state-of-the-art platform for studying the human microbiome and to expand our understanding of fundamental molecular processes related to syntrophy and antagonism between human cells and microbial communities. The flexible HuMiX device can be customized to mimic various host-microbiome interaction sites across the human body to understand the possible mechanisms underlying such co-habitation and its hypothesized role in the pathogenesis of numerous idiopathic syndromes.

ACKNOWLEDGEMENTS

Financial support from Fonds National de la Recherche, Luxembourg (CORE/BM/11/ 1186762). Technical assistance from M. Niegowska and M. Barrett.

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