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Optimising Bacterial Co-Culture with Vaginal Epithelial Cells

Rebecca S. Posner, Sogol Salamipour, Victoria Horrocks, Sarah J. Chapple, A. James Mason, Rachel M. Tribe

Department of Women & Children's Health, Institute of Pharmaceutical Science, Department of Vascular Biology & Inflammation, King's College London, London UK

Introduction

The cervicovaginal environment (microbiome, innate immune system, and metabolome) is an important contributor to a healthy pregnancy. Risk of premature birth (PTB) is associated with changes in resident microbial community, altered immune responses and inflammation. However, the mechanistic pathways leading to PTB have yet to be fully established. It is hypothesised that the microbiota modifies both vaginal epithelial cell (VEC) barrier integrity and host innate immune response, and that the resulting inflammation increases risk of cervical shortening ± ascending infection.

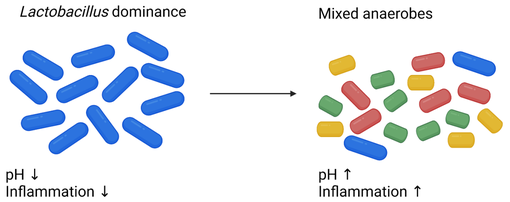

Figure 1: Hypothesis: Changes in vaginal microbiota lead to changes in physiological responses. A reduction in *Lactobacilli* and an increase in a heterogenous mix of anaerobic bacteria increases pH and raises inflammation and risk of adverse health outcomes such as PTB.

Objective

To further develop our 3D VEC model to enable co-culture and imaging together with bacteria associated with a diverse microbiota

Methods

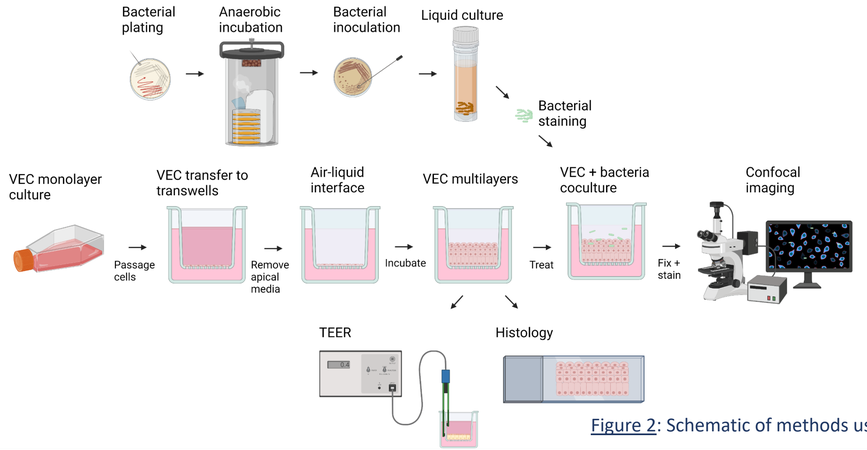

Figure 2: Schematic of methods used.

Summary

We have validated optimum and physiologically relevant growth conditions for VECs and bacteria prior to co-culture. VEC multilayer growth was evident, and there was an increase in barrier resistance, and thus barrier integrity from day 2 to day 10 of VEC growth. Co-culture of VECs with bacteria can be imaged using fluorescent stains, to determine bacterial infiltration into multilayers.

This provides the groundwork for assessing the impact of bacterial metabolites and bacterial co-culture on VEC structural integrity and host response. The aim of this study was to use our robust and physiologically relevant VEC model with bacterial cell interactions to test whether this provides insights into spontaneous PTB prediction.

References

Flaviani *et al.*, JCI Insight 2021. https://doi.org/10.1172/jci.insight.149257

Horrocks *et al.*, mSphere 2022. https://doi.org/10.1128/msphere.00166-22

Results

VEC model displays multilayers

Figure 3: Haematoxylin and Eosin staining of VECs shows multilayer formation of 3D culture after 10 days (40x magnification), *n*=6.

Barrier integrity increases in VEC layers over 10 days

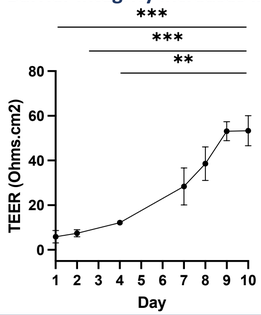

Figure 4: Daily trans-epithelial electrical resistance (TEER) measurements over ten days (ordinary one-way ANOVA followed by Tukey's multiple comparisons test **(p<0.01) ***(p<0.001), n=3).

Fluorescent staining of VECs with bacterial cells

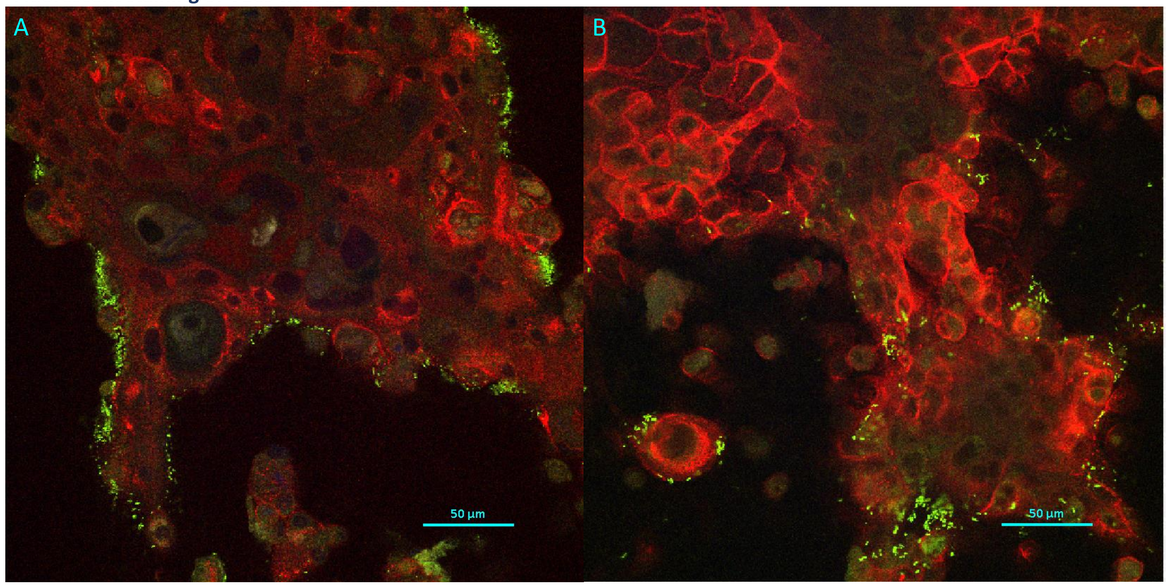

Figure 5: Fluorescent imaging of VECs (red) co-cultured for 24 h with *G. vaginalis* (A, green) and *P. bivia* (B, green), 40x magnification, n=3.