(1) Submission ID#1527434

Development of a human cervicovaginal biomimetic model to examine gonococcal infection and immune response

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Background
The limitations of animal models of Neisseria gonorrhoeae (Gc) reproductive tract infection and human experimental urethral infection prevent a full understanding of Gc-host interactions that could aid in the establishment of new therapeutics. In particular, the mechanisms that allow Gc to thrive despite a robust immune response remain poorly understood.

Aim/Methods
We developed a biomimetic human cervicovaginal microfluidic chip to model the lower female reproductive tract in the context of Gc infection. This chip consists of a fluidic “cassette” and a removable “insert” with two fibronectin-coated channels cut in medical-grade silicone, separated by a porous polycarbonate membrane. A2EN human endocervical or VK2 human vaginal cells were seeded onto the membrane in the channel of the insert. Once epithelial cells were adhered, the insert was inverted, and endothelial cells were seeded into the second channel on the opposite side of the membrane. Epithelial barrier integrity was examined by fluorescence microscopy and by exclusion of FITC-dextran. For infection, GFP-labeled piliated, Opa+ Gc strain FA1090 was added to the epithelial chamber. To assess polymorphonuclear cell (PMN) transmigration through the epithelial and endothelial layers, PMNs were isolated from the venous blood of healthy human subjects, and 1e6 PMNs were added to the endothelial chamber. PMN transmigration into the epithelial compartment was measured by myeloperoxidase (MPO) activity and immunofluorescence, and PMN activation was measured by flow cytometry.

Results
A2EN cells established monolayers that exhibited barrier function by excluding FITC-dextran from the endothelial channel. VK2 cells also grew in the epithelial channel but did not establish barrier function, as expected for this squamous cell type. Gc adhered to and grew upon both the A2EN and VK2 cells. PMNs added to the endothelial cell channel transmigrated through the endothelial and epithelial layers in response to Gc in the epithelial channel. The transmigrated PMNs released MPO and displayed an activated phenotype.

Conclusions
Microphysiological devices consisting of epithelia, endothelia, PMN, and Gc recapitulate numerous aspects of Gc host-pathogen interactions. Further advancements of these in vitro systems, including adding microbiota, laminar flow in the cassette, and extracellular matrix, along with finer measures of host responses, are ongoing.