Biomimetic tools enabling *in vitro* studies of host-pathogen interactions under physiologically relevant conditions

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Introduction: Tissue physiology defines the outcome of host-pathogen interactions. In pyelonephritis, uropathogenic *Escherichia coli* (UPEC) infects the renal epithelium despite the shear stress from the renal filtrate. It is therefore a balancing act between adhesion to renal cells and discharge by the renal filtrate. Apart from UPEC's adhesion, the renal filtrate also affects the local concentration of both bacterial and host secreted factors. These factors will fluctuate rather than statically accumulate, acting both locally and distally due to transfer by the filtrate flow. Current *in vitro* tools study these host-pathogen interactions under static conditions, disregarding the importance of the physiological parameters during infection.

Methods: To simulate the dynamic environment of the renal proximal tubules, we established a biomimetic tool that simulates the dynamic environment of the human renal proximal tubule. Renal cells were cultured in the channels of a microfluidic device and subjected to the flow of cell culture medium at a flow rate equivalent to the primary filtrate flow rate in the S1 part of the proximal tubules. The device was maintained at 37°C throughout the experiment, inside a portable incubator on a microscope stage. Renal cells in the biomimetic device were infected under flow with UPEC CFT073 and mutants for adhesion organelles and toxins. The host response to infection was monitored live by injecting fluorescent probes and applying time-lapse fluorescent microscopy. To study the host response on the gene and protein level, PCR arrays and a cytometric bead assay were also performed.

Results: Under exposure to physiological flow rate, the majority of bacteria were prevented from binding on the renal cells. The few adherent bacteria, however, started to rapidly multiply, and established an infection within a few hours. The P fimbriae tip adhesin, PapG, was important for initial adhesion to the renal epithelium, whereas the Type 1 fimbriae tip adhesin, FimH, enabled inter-bacterial binding and microcolony formation. As α-hemolysin levels secreted from UPEC increased from sublytic to hemolytic, renal cells started undergoing morphological changes leading to downregulation of inflammation, apoptosis and cell detachment. In the absence of α-hemolysin, however, renal cells remained viable and developed an inflammatory response.

Conclusions: By using biomimetic models to study pyelonephritis in a biomimetic model, we can study UPEC's virulence and fitness factors in physiologically relevant conditions *in vitro*. By introducing the parameter of the flow, fimbriae are tested for their ability not only to adhere but also maintain this adhesion over time, against the shear stress of the urine. Secreted factors from UPEC and the host are not merely accumulating in a well but constantly fluctuate, simulating the dynamic host-pathogen interactions observed *in vitro*. By combining microfluidics, cellular microbiology and organ physiology, biomimetic models provide an advanced *in vitro* tool to study host-pathogen interactions under physiologically relevant conditions.