Simultaneous optical and electronic monitoring of epithelial monolayer formation and disruption

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Introduction: Understanding the dynamics of multicellular interactions during infection is a vital part of finding new antimicrobial agents and ways to treat infections. To generate data under dynamic conditions closely mimicking the cellular microenvironment, improved *in vitro* platforms are needed allowing for data generation without constant manipulation of the specimen. Here, we present a method that allows dynamic monitoring of epithelial monolayer formation and disruption using highly sensitive electronic detection, optionally combined with simultaneous light microscopy imaging.

Method: Phase angle spectroscopy was performed on a device containing two transparent conducting polymer electrodes serving as cell culture substrate at he same time. An AC potential with 5 mV rms was applied at frequencies ranging from 0.1 Hz – 100 kHz and the phase delay between the sourced potential and the response current was measured as phase angle ϕ at each frequency step. A peak in ϕ amplitude between 10^3 and 10^5 Hz was found to be an indicator of cell density on the active cell culture substrate. Amplitudes of relevant ϕ peaks were monitored over time to track formation and toxin or pathogen induced disruption of a Madin-Darby canine kidney (MDCK) epithelial monolayer.

Results: Continuous data on the formation of an MDCK monolayer were obtained and the electronic measurement was correlated with cell counts and fluorescence microscopy. Kinetic data on toxin-induced disruption of the epithelial barrier were obtained using ionomycine and a half-life time of epithelial monolayer integrity of 2.76 ± 0.62 min could be established and was corroborated by simultaneous optical observation. Kinetic data on pathogen induced epithelial monolayer disruption were collected using uropathogenic *Escherichia coli* (UPEC) as a model for extracellular, toxin producing bacteria. Using a α-hemolysin (hlyA) knockout mutant lead to considerably slower progression of infection, but eventually progresses to the same final outcome as obtained with the wild-type strain. Interestingly, accelerated loss of epithelial barrier integrity was observed with a secreted autotransporter toxin (sat) knockout mutant. As a model for invasive bacteria, *Salmonella enterica* serovar *typhimurium* was used. Our data show that infection with a knockout mutant of the invasion protein regulator hilA causes initial epithelial tightening or accumulation of bacteria, but eventually the infection progresses as observed with the wild-type strain. This suggests the existence of a hilA independent pathway to Salmonella invasion gene transcription. Infection with a knockout mutant of the secretion system protein prgH leads to successful infection to some degree, but does not cause complete disruption of the epithelial monolayer.

Conclusions:
Collectively, our data demonstrate that this novel tool provides a highly sensitive, simple platform allowing us to study subtle changes in the epithelium undergoing bacteria infection. The sensitivity of our method and the possibility to record high-resolution temporal data open a new window for the study of host-pathogen interactions.