Human urothelial microtissue model for *in vitro* studies of urinary tract infection and its treatment

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Urinary tract infections (UTIs) are a major cause of morbidity, hospitalization and prescription of antibiotics worldwide. The primary source of UTIs are uropathogenic *Escherichia coli* (UPEC), responsible for approximately 80% of the cases. High recurrence rates of UTIs show that even the gold-standard treatments are suboptimal, and UPEC are associated with a high prevalence of antimicrobial resistance (AMR). There is a need for urothelial models that comprehensively recapitulate the human ultrastructure, physiology and immunity of the bladder to investigate UTI. The recently developed 3D urine-tolerant human urothelial model (3D-UHU) (Flores et al., 2023, Sci. Adv. 9:eadi9834) allows studying UTI in a relevant bladder-mimicking environment. In this project, the 3D-UHU model is augmented to facilitate a refined analysis of UTI dynamics and treatment outcome.

Here, the 3D-UHU model was inverted to allow for live cell imaging. Human epithelial bladder (HBLAK) cells were grown on the bottom side of Transwell® inserts and stratified and differentiated, generating microtissues with ~7 cell layers that embody the three main urothelial subtypes (basal, intermediate and umbrella), on the bottom side of the Transwell® membrane. Inoculation of the inverted version of the 3D-UHU model with UPECs enabled to follow the dynamics of infection by live cell imaging at high spatiotemporal resolution. Currently, immune cells are added to the model to create an immunocompetent model of UTI, facilitating studies of the immune response to bacterial infection.

Overall, a relevant human urothelial *in vitro* model was established, which will enable the investigation of antibiotic treatment in the context of UTIs. In the future, the infected tissue will be treated with antibiotics to investigate treatment failure and infection recurrence. In addition, this model could be used to test novel antibiotics in a human-relevant context.