221

Developing a high-throughput screening assay for epithelial kinematics using organoid monolayers

Bart van der Hee[2], Marcela Fernandez-Gutierrez[2], Myrthe Gilbert[2], Michiel Kleerebezem[2], Nico Taverne[2], Anja Taverne-Thiele[2], Linda Loonen[2], Veerle Wolbers[2], Boudewijn Burgering[1], Walter Gerrits[2], Hauke Smidt[2], Jerry Wells[2]

[1] University Medical Center Utrecht, The Netherlands
[2] Wageningen University & Research, The Netherlands

Presenter: Bart van der Hee

Renewal and restitution are important protective properties of the intestinal epithelium, a physical barrier separating the inside of the body from luminal content. Metabolism of nutrients in the intestinal lumen produces numerous metabolites, some of which can be cytotoxic or influence turnover capacity of the epithelium. Static monotypic cell line monolayers are commonly used as models to study re-epithelialization, but they do not mimic the intricate crosstalk and interplay between specialized epithelial cell types that might impact on proliferation, migration, and differentiation. We developed a method using polarized monolayers of intestinal organoids to recapitulate intestinal cell type diversity, which were exposed to luminal metabolites. Inducing a scratch through the monolayer surface mimics wounding of the epithelium and allows measurement of cell migration and proliferation in the resulting gap. Differentiated organoids with crypt-like structures were enzymatically dissociated into single-cell suspensions. The cells were then seeded into 96-well plates, scratched using a replicator, and imaged on a high-content bioimager to monitor re-epithelialization. Combining organoid monolayer scratch assays with nuclear tracking, we could follow spatial-temporal kinematics of cells and the effects of different metabolites on this process. As an example, we exposed colon organoid monolayers to physiologically relevant concentrations of ammonia (NH3) or hydrogen sulfide precursor sodium sulfide (Na2S). NH3 slowed the re-epithelialization process significantly, although cells still exhibited directed migration towards the wounding-area. Na2S-treated cells showed highly localized cell displacement and velocity but slow re-epithelialization, whereas the control showed collective migration towards the scratched area. Individual cell tracking then provided a basis for testing multiple hypotheses on cellular responses to xenobiotics and ideas for further downstream analytical procedures, e.g. effects of Na2S on epithelial barrier functioning or autophagy pathways. We conclude that combining organoid monolayer scratch assays with computational methods provides an advanced model for epithelial migration and high-throughput metabolite assays.