Novel approaches to mimic bacterial respiratory infection *in vitro* and *ex vivo*

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Background: Anti-infective drug development routinely involves standard in vitro assays to determine the antibacterial efficacy (minimum inhibitory concentration, MIC) and in vivo rodent pneumoniae models. However, these assays do not consider that most persistent respiratory infections in patients involve biofilm formation associated with higher resistance towards antimicrobial treatment. Our aim is to develop more predictive models for preclinical efficacy testing with a focus for inhalative drug development.

Methods: To assess the efficacy of e.g. inhalative antimicrobial drug candidates a test battery of *in vitro* and *ex vivo* models for bacterial infection using *Pseudomonas aeruginosa* (PA) was developed, including i) *in vitro* PA biofilm exposure to antibiotic aerosol, ii) *in vitro* PA infected human airway epithelial cells (AEC) and iii) *ex vivo* PA infection of vital human lung tissue (precision-cut lung slices, PCLS). Endpoints are bacterial load, biofilm visualization via Syto9/PI staining, host tissue viability using LIVE/DEAD staining and analysis via confocal microscopy, as well as evaluating the host immune response.

Results: The *in vitro/ex vivo* test battery was successfully set up using PA as relevant pathogen and tobramycin as clinical standard treatment. PA biofilms exhibiting strongly reduced tobramycin susceptibility compared to MIC were efficiently treated by exposure to tobramycin aerosol. Using AEC and PCLS, PA infection was mimicked in *vitro/ex vivo* and the virulence of PA versus human cells efficiently reduced after tobramycin treatment resulting in sustained host tissue viability.

Conclusion: Bacterial pneumonia can be mimicked in our *in vitro/ex vivo* test battery and applied to investigate efficacy of pharmacological intervention. Furthermore, initial data on safety/biocompatibility by addressing the effect on human host cells as the relevant species can be generated. For testing of inhalative drugs and formulations the exposure of infected AEC and PCLS is possible using the Fraunhofer patented P.R.I.T. ExpoCube® system.