A Transwell®-based transport model of the Gram-negative cell envelope

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Fighting infectious diseases caused by Gram-negative bacteria is a challenging task also taking into account the emerging resistance of pathogens as *P. aeruginosa* and *K. pneumoniae*. [1] Unfortunately, no novel class of antibiotics achieved the stage of clinical trials so far. [2] In order to keep those infections still under control, it is necessary to develop tools, which can help to discover new compounds with potentially antibiotic activity. As a starting point, we selected the cell envelope, which is known for its complexity and its very distinct properties as a diffusion barrier, and developed a membrane model meant for delimiting well from badly permeating and thus probably ineffective compounds. This model is placed on a highly porous filter support to allow studies on the permeability of substances. Guideline for the architecture of our model is the structure of the actual Gram-negative cell envelope, which can be divided into an inner membrane (IM), the periplasmatic space (PS) and the asymmetric outer membrane (OM), which consists of an inner leaflet (IL) and an outer leaflet (OL) made of lipopolysaccharides. The composition of phospholipids used for the IM and IL of our model resembles the composition of those in *E. coli* and *P. aeruginosa.* [3] An alginate layer separating IM from OM serves as PS.
Different microscopic techniques did not only reveal the presence of separate layers, representing the IM and the PS, but also the presence of an asymmetric bilayered structure of the OM. As a further step, transport studies where performed with fluorescent dyes and quorum sensing inhibitors - a new antibiotic class. As for the dyes, it could be shown that there is a relation between the permeability through our membrane model and the *in bacterio*-permeability in *E. coli.* In addition, transport studies with quorum sensing inhibitors showed a relation between the reduction of an important signaling molecule in the quorum sensing cascade and the permeation of those substances across our model.

References

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