27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: OS065 Biofilm: novel research tools and properties

Category: 9c. Preclinical biofilm studies

23 April 2017, 11:30 - 11:40
OS0324

Biofilm formation of Staphylococcus aureus under CuCl2 or CuSO4 influence: impedance analyses as tool for early biofilm development detection

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Background: The preferred form of growth of bacteria in nature is biofilm formation. Biofilms often lead to significant problems in clinical settings as it is difficult to remove them from surfaces (e.g. catheters) and they show increased antimicrobial resistances due to physical protection of the cells and due to the lower exchange rate of the cells with their environment.

Material/methods: *Staphylococcus aureus* (DSM799 and Newman) biofilms were grown in increasing concentrations of $CuCl_2$ or $CuSO_4$ for 48h in order to establish a copper level at which the biofilm formation of the bacteria was inhibited. Electrical impedance sensing (EIS) was performed using an ECIS Model Z Theta (Ibidi, Germany). *S. aureus* biofilms were grown in ECIS culture ware 8 well arrays with 10 electrodes per well optimized for adhesion studies and other cellular assays. Starting value impedance was measured using 150 µL of LB media and 150µL of the copper solution in the desired concentration. This step was done for all eight cells and the solution was carefully removed with a pipette after the measurement. Afterwards again 150µL of the desired copper concentration were added to the empty cells and inoculated with 150µL of the diluted ONC (OD600 of 0.5). The impedance plates were sealed using breathable membranes. Impedance was measured directly afterwards and after 24 and 48 hours of incubation at 37°C and 90 rpm, respectively. Additionally typical laboratory measurements for biofilms (polysaccharide and protein content, life/dead cell flow cytometry) were performed. For these additional measurements the biofilms where grown in 24 well plate assays.

Results: When uninfluenced, biofilms develop on the electrodes of the system, causing the impedance values to drop because the biofilm precludes electrode polarization. When cell growth and biofilm formation become inhibited this effect does not take place. Polysaccharide, protein and live-dead measurements support these findings. *S. aureus* Newman is more sensible to copper ions in the medium than *S. aureus* DSM799, and it is also more sensitive to $CuCl_2$ than to $CuSO_4$. Inhibition of biofilm formation was reached at different concentrations up to 6mM copper sulfate and copper chloride; however, concentrations up to 6mM did not cause cell dead.

Conclusions: Impedance analyses proved to be an interesting tool for a fast and easy assessment of antimicrobial substances. Future studies are planned to evaluate the feasibility of the presented method for routine antimicrobial assessment. Other species and different antimicrobial substances will be used.