CED/NOF-IADR
2024 Oral Health
Research Congress
12 — 14 Sept 2024
Geneva, Switzerland

0336

"Is Dead Really Dead?" Membrane-Potential and Intracellular-pH Affect Viability Staining

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Objectives The uptake of propidium iodide (PI) in bacterial cells during viability staining correlates with both membrane potential (Dψ) and intracellular pH (pH_i) distribution. A boosted Dψ or pH_i decrease enables increased accumulation of PI and can yield falsely positive results for viable bacterial cells.
Methods In the present study, the uptake of PI across intact cell membranes for facultative and obligate anaerobic oral microorganisms was examined. The aerobic *Micrococcus lylae* was used as a reference to enable comparison. The tested bacterial cells were screened during anoxic (aerobic, facultative anaerobic; 1 h anoxia, 37°C), oxic (all anaerobic; 1 h aeration in CO_2, 37°C) and oxic-anoxic (all aerobic and anaerobic; 1 h aeration in CO_2 followed by 1 h anoxia or conversely, 37°C) transitions. Untreated cells in exponential growth phase served as negative control. To visualize changes in membrane potential the carbocyanine dye $DiOC_2$ (3; 3,30-Diethyloxacarbocyanine Iodide) was applied, whereas BCECF-AM (2',7'-Bis-(2-Carboxyethyl)-5-(and-6)-carboxyfluorescein) acetoxymethyl ester) was used as intracellular ratiometric pH indicator. The Live/Dead® BacLight™ Kit (Syto 9/PI) combined with confocal laser scanning microscopy (CLSM) was applied to monitor bacterial viability. Finally, image quantification and statistical analysis (Kruskal-Wallis test) were conducted.
Results Short-term changes in oxygen supply induced bidirectional changes in Dψ and pH_i. The anaerobic preincubation of facultative anaerobic microorganisms resulted in high PI uptake and thus, the presence of falsely marked "dead" cells in the untreated controls. In *Streptococcus mutans* an aeration-related decrease in Dψ and increase in pH_i led to a lower PI uptake in the streptococcal cells compared to the untreated control.
Conclusions Overall, Dψ and pH_i seem to contribute actively to ATP regeneration and thus, PI uptake in aerobic and anaerobic oral bacterial cells. New alternatives to PI should aim at reducing falsely positive cells marked as dead during viability staining.