The impact of antimicrobial photodynamic therapy on *Streptococcus mutans* in an artificial biofilm model

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AIM OF THE STUDY

The aim of the study was to assess the impact of laser induced antimicrobial photodynamic therapy on the viability of *Streptococcus mutans* cells employing an aritificial biofilm model.

MATERIAL AND METHODS

Employing sterile chambered coverglasses (LAB-TEK Chambered coverglass w/cr #1, Neperville, USA), a salivary pellicle layer formation was induced in 19 chambers. *Streptococcus mutans* cells (Clarke 1924, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were inoculated in a sterile culture medium. Using a live/dead bacterial viability kit (LIVE/DEAD BacLight L7012, Invitrogen, Carlsbad, USA), bacteria with intact cell membranes stain fluorescent green. Test chambers containing each the pellicle layer and 0.5 ml of the bacterial culture were analyzed by confocal laser scan microscopy (LSM 710, Carl Zeiss MicroImaging, Jena, Germany) (fig. 1) within a layer of 10 µm at intervals of 1 µm from the pellicle layer. A photosensitizer was added to the test chambers and irradiated with a diode laser (wavelength: 660 nm, output power: 100 mW, Helbo, Grieskirchen, Austria) (fig. 2) for 2 min each. Additionally, two samples were not irradiated after adding the photosensitizer but analyzed by microscopy after 4 min of residence time to exclude a decrease of living bacteria caused by the photosensitizer agent itself.

For statistical analysis normal distribution of the values was assessed with the Shapiro–Wilk test. Since not all data were normally distributed, values were analysed with the nonparametric Wilcoxon two-sample paired signed rank test employing the SPSS®-software (SPSS Inc., Chicago, USA). Differences were considered as statistically significant at $p<0.05$.

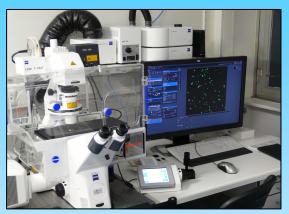

Fig. 1: confocal laser scan microscope used to evaluate fluorescence within a layer of 10 µm at intervals of 1 µm from the pellicle layer

Fig. 2: Diode laser (660 nm, 100 mW, class 2M) of the aPDT system and the respective photosensitizer (small picture)

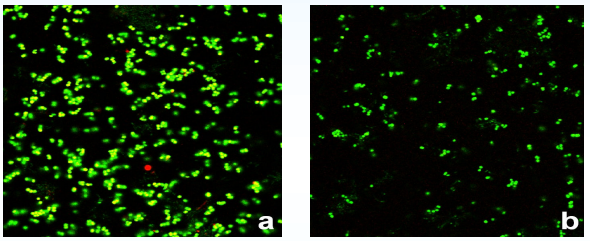

Fig. 3: Confocal laser scan microscopy of a representative sample at a distance of 3 µm from the pellicle layer before adding the photosensitizer (a) and after laser irradiation (b) (original magnification: x63).

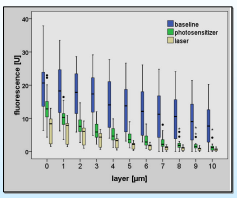

Fig. 4: Fluorescence at baseline, after adding the photosensitizer and after laser irradiation at intervals of 1µm from the pellicle layer

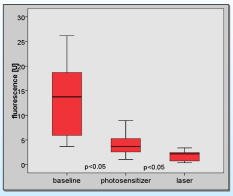

Fig. 5: Overall values for fluorescence at baseline, after adding the photosensitizer and after laser irradiation with statistically significant differences (p<0.05)

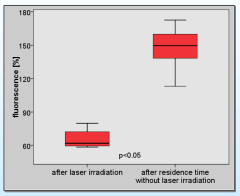

Fig. 6: Fluorescence in percentage of values after adding the photosensitizer after laser irradiation and after residence time without using the laser (p<0.05)

RESULTS

Comparing the baseline fluorescence (median: 13.8 [U], min: 3.7, max: 26.2) with the values after adding the photosensitizer (median: 3.7, min: 1.1, max: 9), a dilution caused decrease of fluorescence could be observed ($p<0.05$). After irradiation of the samples with a diode laser, an additional decrease of fluorescence became evident (median: 2.2, min: 0.4, max: 3.4) ($p<0.05$) (fig. 3, 4, 5). Comparing the samples with added photosensitizer but without laser irradiation at different times, no decrease of fluorescence could be measured ($p>0.05$) (fig. 6).

CONCLUSION

The present study indicates that antimicrobial photodynamic therapy can reduce live bacteria within a layer of 10 µm in an artificial biofilm model. Further studies have to evaluate the maximum biofilm thickness that still allows a toxic effect on microorganisms.

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