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## AIM OF THE STUDY

The aim of the study was to assess the impact of laser irradiation during antimicrobial photodynamic therapy in an artificial 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 40 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) within a layer of 10  $\mu\text{m}$  at intervals of 1  $\mu\text{m}$  from the pellicle layer. Phenothiazine chloride (Helbo, Grieskirchen, Austria) was used as a photosensitizer and added to all 40 test chambers. 20 chambers were irradiated with a diode laser (wavelength: 660 nm, output power: 100 mW, Helbo) (fig. 1) for 2 min each. An interval of 5 min was used as residence time for the photosensitizer in the remaining 20 chambers.

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: Diode laser (660 nm, 100 mW, class 2M) of the aPDT system, the respective photosensitizer (black lined insert) and the security switch to activate the laser device (red lined insert)

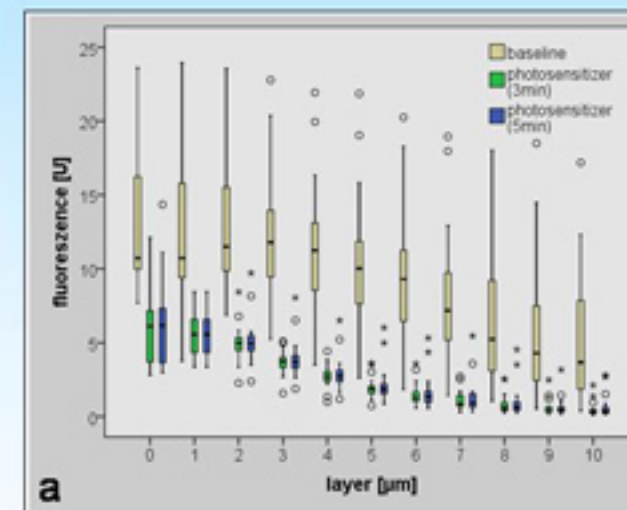


Fig. 2: Fluorescence at baseline, after adding the photosensitizer and after a residence time at intervals of 1  $\mu\text{m}$  from the pellicle layer (a) and overall values (b) ( $n=20$ )

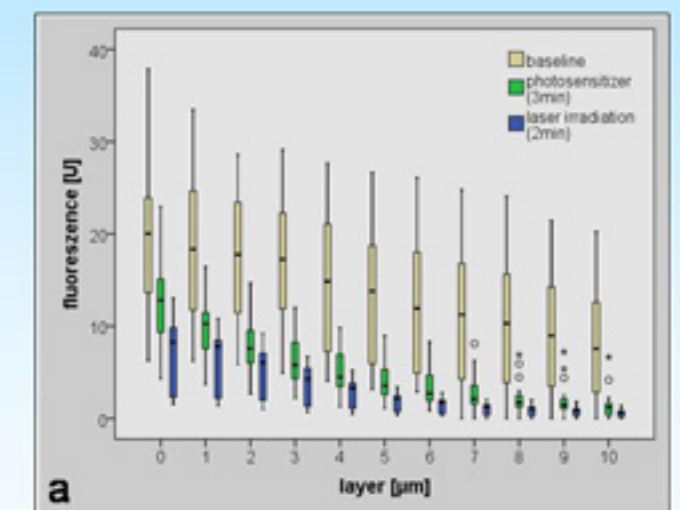
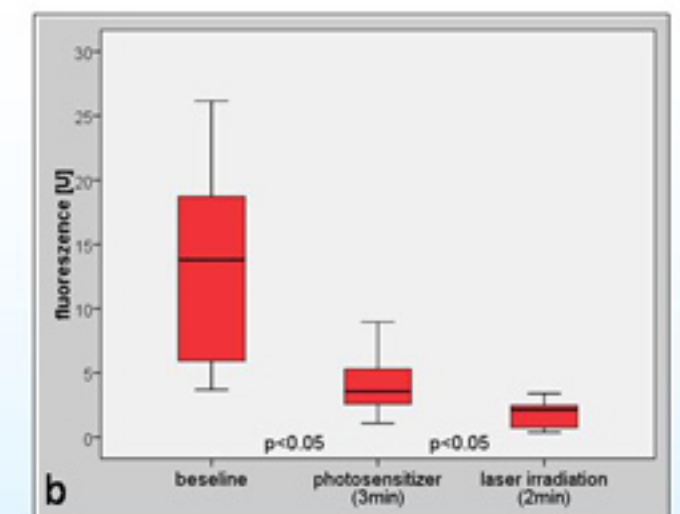
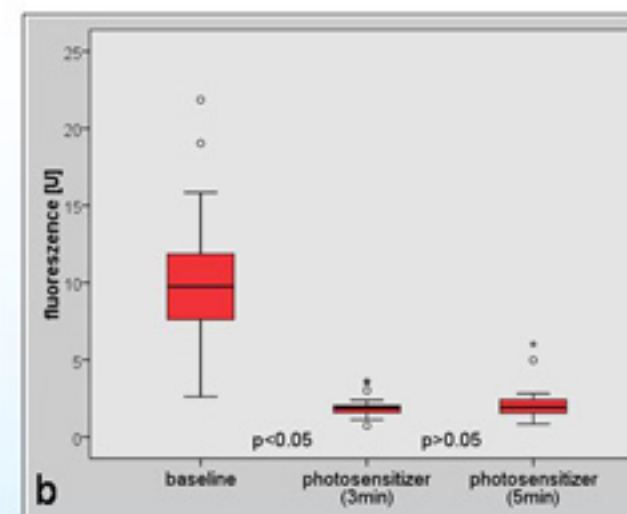


Fig. 3: Fluorescence at baseline, after adding the photosensitizer and after laser irradiation at intervals of 1  $\mu\text{m}$  from the pellicle layer (a) and overall values (b) ( $n=20$ )



## RESULTS

Comparing baseline fluorescence after adding the photosensitizer (baseline median: 1.9, min: 0.7, max: 3.6) with the values after the residence time of the photosensitizer (median: 1.9, min: 0.8, max: 6.0), no change in fluorescence could be observed (fig. 2) ( $p > 0.05$ ). The laser irradiated group (median: 3.6 [U], min: 1.1, max: 9.0) showed a decrease of fluorescence after irradiation (median: 2.1, min: 0.4, max: 3.4) (fig. 3, 4) ( $p < 0.05$ ).

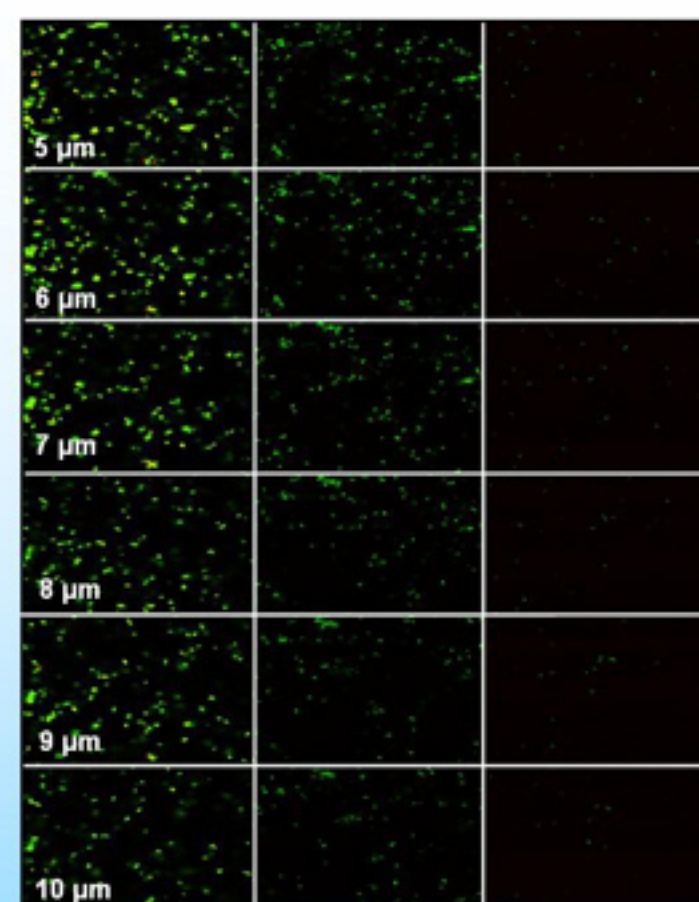
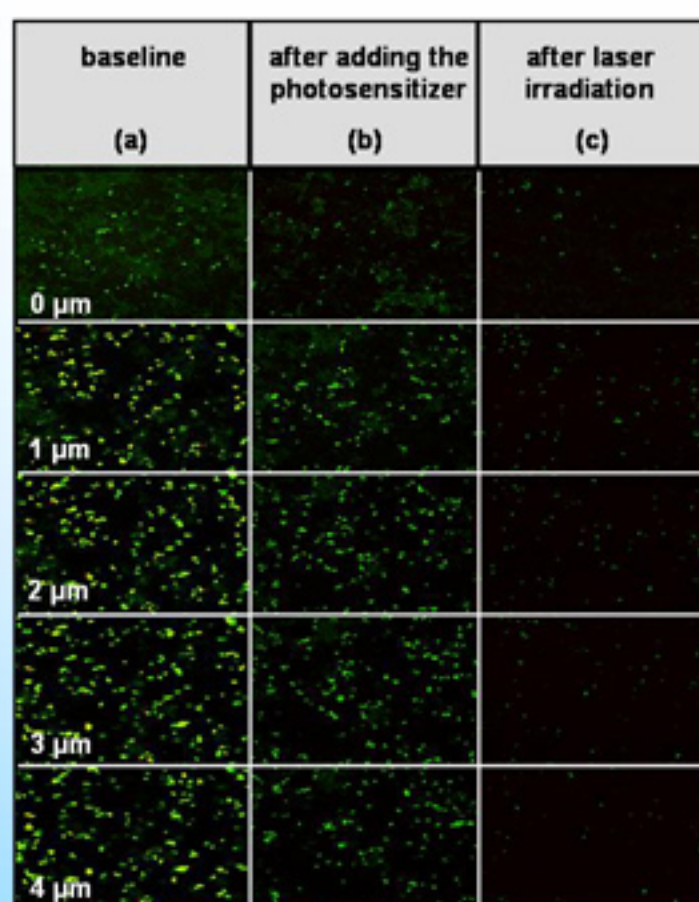


Fig. 4: Confocal laser scan microscopy of a representative sample at a distance of 0–10  $\mu\text{m}$  from the pellicle layer before (a) and after (b) adding the photosensitizer and after laser irradiation (c) (original magnification:  $\times 63$ ).

## CONCLUSION

The present study indicates that laser irradiation is an essential part to reduce bacteria by antimicrobial photodynamic therapy. The treatment protocol comprising photosensitization and laser irradiation should be followed carefully to obtain an antimicrobial effect on microorganisms.

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