



## Introduction

Dental implants are one of the most frequently used treatment options in the replacement of missing teeth. The oral microflora and biofilm-related peri-implant infections seem to be defining factors for the success or the failure of a dental implant. Therapeutic strategies proposed for managing peri-implantitis appear to be largely based on clinical empirical values but not on particular scientific findings. Therefore, the aim of this human *in vivo* pilot study was to evaluate the efficacy of six antimicrobial agents on the surface decontamination of an oral biofilm attached to titanium implants.

## Methods

Machined pure titanium specimens (9 mm in diameter and 2 mm in thickness) were fixed to individual removable acrylic upper jaw splints for *in vivo* biofilm formation. The splints were worn by two female and two male volunteers overnight for 12 h. The plaque-covered specimens were then treated with different antimicrobial agents for 1 min (cf. Fig. 1 & Tab. 1). Afterwards the total bacterial load and the viability of adhering bacteria were quantified by live (green) or dead (red) cell labeling (LIVE/DEAD BaLight; Molecular Probes, Eugene, USA) in combination with fluorescence microscopy (Axiovert 200M fluorescence microscope; Carl Zeiss GmbH, Göttingen, Germany).

Continuous data were summarized with medians and interquartile ranges (25/75 percentiles) and analyzed using non-parametric Friedman and pairwise Wilcoxon's test ( $\alpha = 0.05$ ).



Figure 1. Individual removable acrylic upper jaw splint *in situ*, used for positioning the titanium specimens.

Specimen	Antimicrobial agent	Manufacturer
1 (control)	Phosphate-buffered saline (PBS)	Sigma-Aldrich, St Louis, USA
2	Sodium hypochlorite 1% (NaOCl)	Pharmacy, Regensburg University Medical Center, Germany
3 & 4	Hydrogen peroxide 3% (H <sub>2</sub> O <sub>2</sub> )	Herbeta, Berlin, Germany
5 & 6	Chlorhexidine gluconate 0.2% (CHX)	GlaxoSmithKline, Bühl, Germany
7 & 8	Plax (triclosan 0.3%)	Colgate Palmolive, Hamburg, Germany
9 & 10	Listerine coolmint (alcoholic based)	Pfizer, Karlsruhe, Germany
11 & 12	Citric acid 40%, pH 1	Pharmacy, Regensburg University Medical Center, Germany

Table 1. Antimicrobial agents used for treating the titanium specimens covered with human *in vivo* biofilm; application time: 1 minute.

## Results

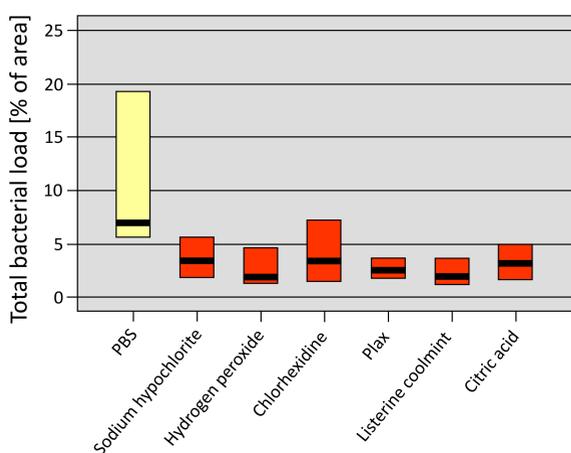


Figure 2. Areas [%] covered by bacteria after 12 hours of *in vivo* biofilm formation and one minute disinfection time for different peri-implantitis disinfection procedures on titanium implant surfaces (medians and 25/75 percentiles; combined data of all four patients).

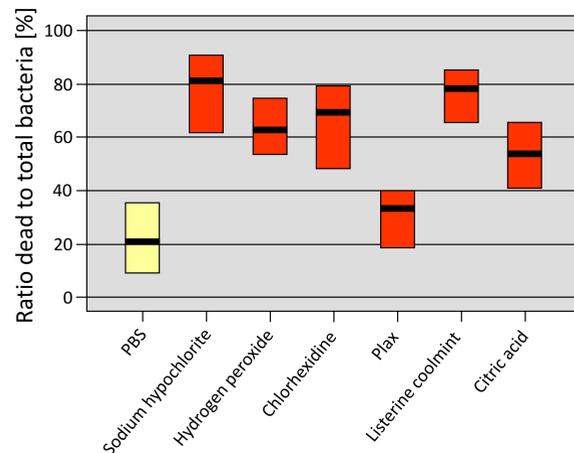


Figure 3. Percentage of dead to total bacteria after 12 hours of *in vivo* biofilm formation and one minute disinfection time for different peri-implantitis disinfection procedures on titanium implant surfaces (medians and 25/75 percentiles; combined data of all four patients).

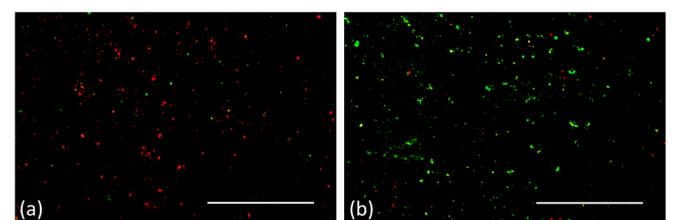


Figure 4. Fluorescence micrographs (live/dead staining) of titanium surface after 12 hours of *in vivo* biofilm formation and one minute disinfection in (a) chlorhexidine and (b) Plax (scale bar = 50 µm).

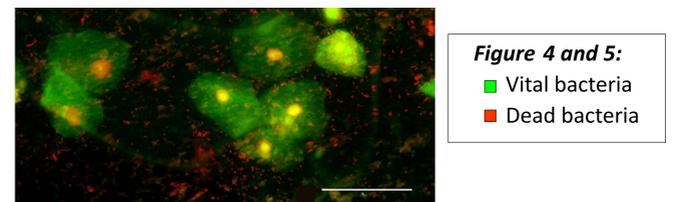


Figure 5. Fluorescence micrographs after disinfection in Listerine. Adherent oral mucosal epithelial cells on dead (red) bacteria (scale bar = 50 µm).

Figure 4 and 5:  
■ Vital bacteria  
■ Dead bacteria

## Conclusions

- ▶ All tested peri-implantitis disinfection procedures were shown to significantly reduce the total number of attached bacteria on titanium surfaces after one minute of immersion.
- ▶ Except for Plax, all tested solutions were capable of inactivating attached bacterial cells compared with the PBS control.
- ▶ These observations may support the regular additive use of antibacterial agents in peri-implantitis therapy.