

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 ₂ O ₂)	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

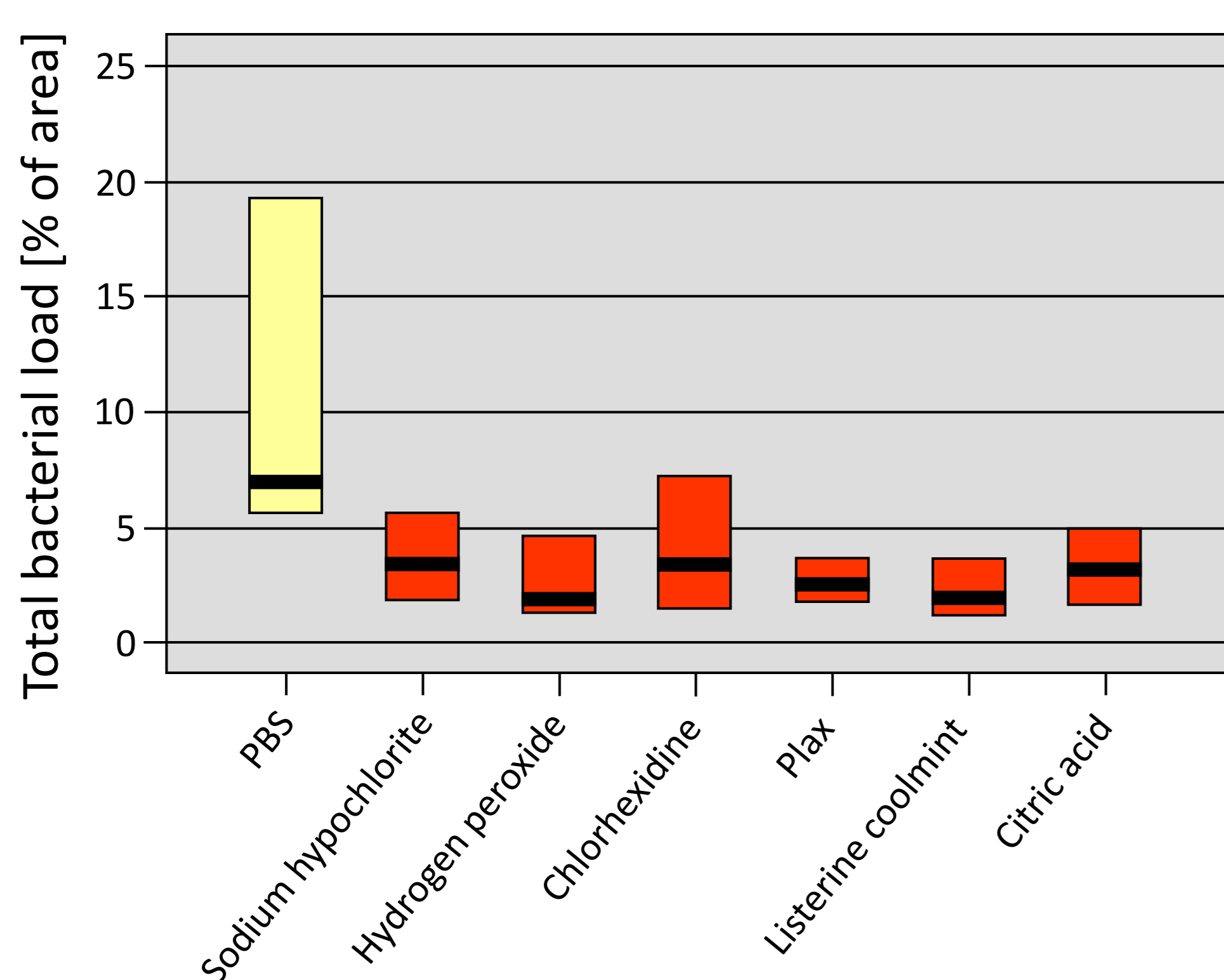


Figure 2. Areas [%] covered by bacteria after 12 hours of *in vivo* biofilm formation and one minute disinfection time for different peri-implantitis disinfection procedures on titanium implant surfaces (medians and 25/75 percentiles; combined data of all four patients).

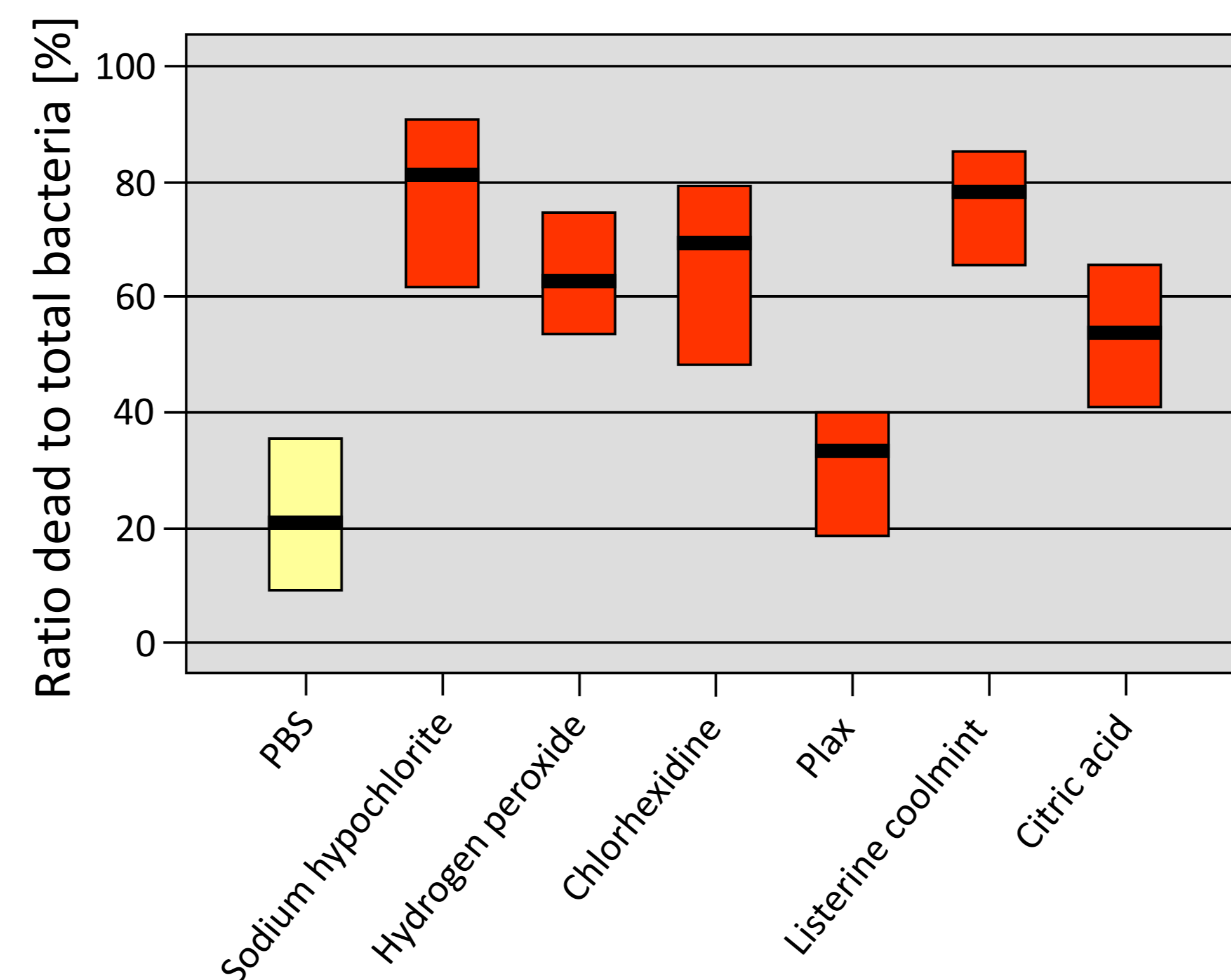


Figure 3. Percentage of dead to total bacteria after 12 hours of *in vivo* biofilm formation and one minute disinfection time for different peri-implantitis disinfection procedures on titanium implant surfaces (medians and 25/75 percentiles; combined data of all four patients).

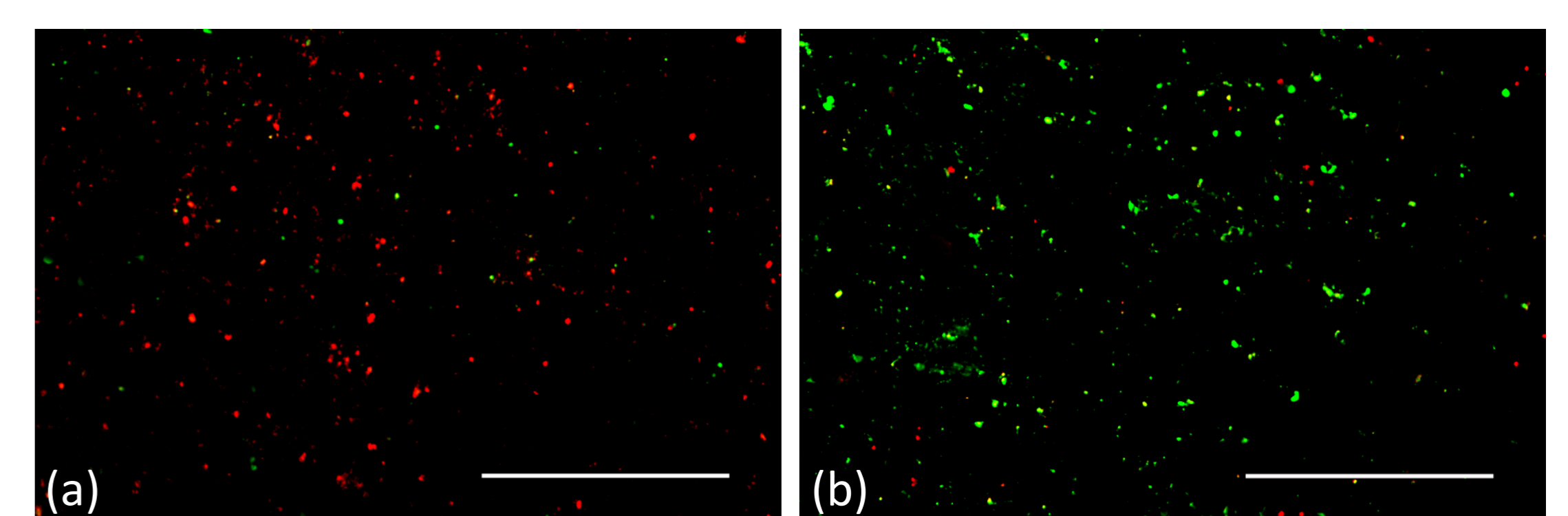


Figure 4. Fluorescence micrographs (live/dead staining) of titanium surface after 12 hours of *in vivo* biofilm formation and one minute disinfection in (a) chlorhexidine and (b) Plax (scale bar = 50 µm).

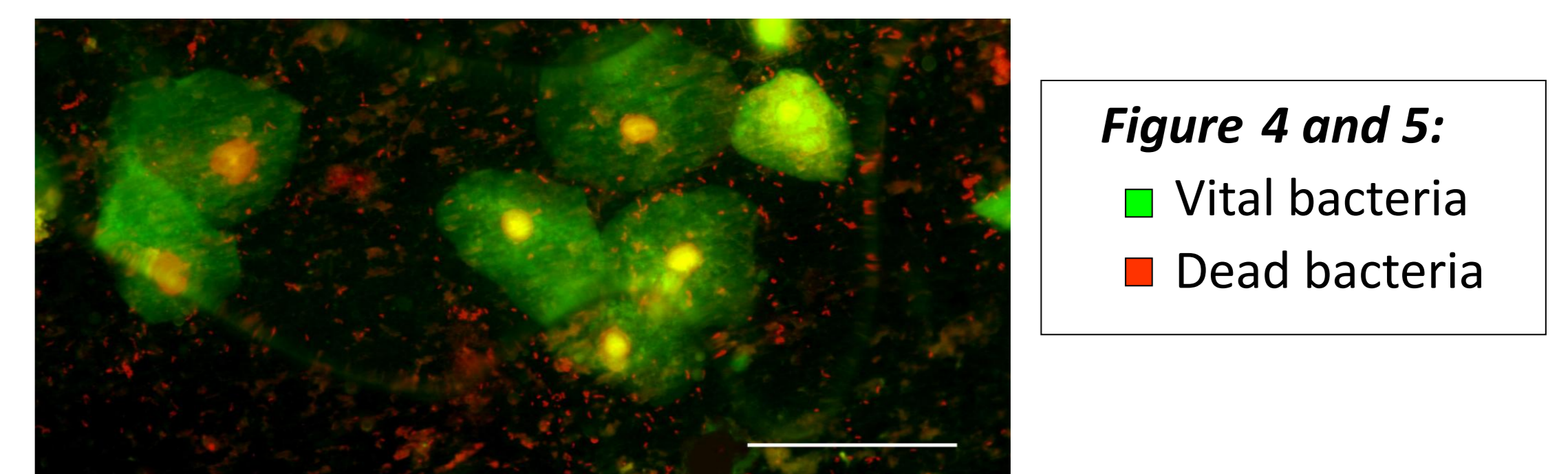


Figure 5. Fluorescence micrographs after disinfection in Listerine. Adherent oral mucosal epithelial cells on dead (red) bacteria (scale bar = 50 µm).

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.