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ORIGINAL ARTICLE

Do edible oils reduce bacterial colonization of enamel in situ?

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Abstract
Objective Edible oils are an empiric approach for the prevention of oral diseases. The present in situ study investigated the effect of edible oils on initial bacterial colonization of enamel surfaces.
Methods and materials Initial biofilm formation was performed on enamel specimens mounted on maxillary splints and carried by eight subjects. After 1 min of pellicle formation, rinses with safflower oil, olive oil and linseed oil were performed for 10 min. Application of chlorhexidine for 1 min served as positive control. Afterwards, the slabs were carried for 8 h overnight. Samples carried for 8 h without any rinse served as negative controls. The amount of adherent bacteria was determined by DAPI staining (4′,6-diamidino-2-phenylindole) and live–dead staining (BacLight). Additionally, determination of colony forming units was performed after desorption of the bacteria. TEM evaluation was carried out after application of the rinses.
Results The number of adherent bacteria on control samples was $6.1 \pm 8.1 \times 10^5/cm^2$ after 8 h (DAPI). Fluorescence microscopic data from DAPI staining and live–dead staining as well as from the determination of CFU revealed no significant effects of rinsing with oils on the amount of adherent bacteria compared to the non-rinsed control samples. However, with chlorhexidine a significant reduction in the number of bacteria by more than 85 % was achieved (DAPI, chlorhexidine: $8.2 \pm 17.1 \times 10^4/cm^2$). The ratio of viable to dead bacteria was almost equal (1:1) irrespective of the rinse adopted as recorded with BacLight. TEM indicated accumulation of oil micelles at the pellicle's surface and modification of its ultrastructure.
Conclusion Rinses with edible oils have no significant impact on the initial pattern and amount of bacterial colonization on enamel over 8 h.
Clinical relevance Rinses with edible oils cannot be recommended for efficient reduction of oral biofilm formation.

Keywords Pellicle · Biofilm · In situ · Edible oil · Lipids · DAPI · BacLight

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Introduction

The worldwide prevalence of caries and periodontitis epitomizes that there is still a strong demand for the improvement of oral prophylaxis. Thereby, biofilm management is one key aspect [1]. Some chemotherapeutics such as chlorhexidine effectively reduce oral biofilm formation, but cannot be recommended for permanent application as they may

induce resistance of some bacterial strains as well as a general shift of the oral flora and irritations of the taste [2]. Furthermore, their application is sometimes problematical for patients suffering from mucositis due to xerostomia after radiation [3]. Therefore, mild but effective agents are demanded for adjuvant oral biofilm management and health care without irritating components and without negative effects on the ecology of the oral cavity. For this purpose, some teas and foodstuffs are of interest such as polyphenolic beverages or lipids [4]. Edible oils are one more or less empiric approach for the prevention of oral diseases. Especially the advantages for the prophylaxis of gingivitis and periodontitis have been described [5, 6]. Nevertheless, these effects and the so-called oil pulling are discussed controversially in the literature [7]. There are also some toothpastes based on lipids gained from edible oils such as olive oil or almond oil. These preparations have been shown to be quite effective [8, 9]. In addition, the efficacy of a two-phase oil:water mouthrinse for plaque management in vivo has been shown. However, this rinse also contained cetylpyridinium chloride [10]. Furthermore, the pure native oils are of interest due to the fact that they are easily accessible worldwide.

The first step of bacterial adhesion to the tooth surface is governed by the initial oral biofilm, the proteinaceous pellicle layer [11, 12]. Mechanisms like co-adhesion of bacteria as well as the interactions with the pellicle components contribute to this process considerably [11, 12]. Thus, bacterial adherence over the first 8 h is of interest as the starting point of oral biofilm formation. Lipids are assumed to provide hydrophobic properties to the pellicle layer thereby optimizing its protective efficacy [13]. This was discussed for bacterial adhesion as well as for acidic noxae [14–18]. Due to the fact that 23 % of the pellicle's dry mass is represented by lipids [13, 14], their accumulation at the tooth surface by rinsing with edible oils is conceivable.

Several edible oils are discussed in preventive medicine and preventive dentistry. Olive oil is well known as a key component of the cardio-protective Mediterranean diet [19, 20]. Linseed or flaxseed oil contains high amounts of alpha-linolenic acid, a fatty acid not present in the saliva or the pellicle [13, 14, 21, 22]. Safflower oil contains a high percentage of linolic acid and is rather tasteless. This might be an advantage if used as an oral rinse for patients suffering from xerostomia.

Nonetheless, the effect of different edible oils on the amount of adherent microorganisms in the in situ formed pellicle has not been quantified systematically until now. Even a promotion of bacterial metabolism and adhesion cannot be excluded.

Bioadhesion in vivo differs considerably from in vitro models [23]. This applies also for the interactions of the pellicle with foodstuffs and edible oils. Accordingly, in situ studies are recommendable as they closely mirror the situation in the oral cavity. Thereby, modern fluorescence microscopic methods i.e. DAPI staining allow quantification of adherent microorganisms [12, 24]. The aim of the present study was to investigate the impact of different edible oils on initial bacterial adhesion in situ. For this purpose a well-established combination of microbiological and microscopical methods was used which has already proven success in studies on polyphenolic beverages [25].

Methods

Subjects and specimens

A number of eight subjects participated in the study (aged 23–37 years). Visual oral examination was carried out by an experienced dentist. The subjects showed no signs of caries or gingivitis (plaque indices close to zero). Subjects with untreated carious lesions, infectious diseases, pregnancy or ingestion of antibiotics within the last 3 months were not included in the study. The participants were all non-smokers. The study was conducted at the university hospital of Dresden; the subjects were members of the laboratory staff or students, respectively. Informed written consent had been given by the subjects about participation in the study. The study design was reviewed and approved by the Ethics Committee of the Freiburg University (#222/08). Cylindrical enamel slabs (diameter 5 mm, 19.63 mm^2 surface area, height 1.5 mm) were prepared from the facial surfaces of bovine incisors of BSE-negative 2-year old cattle. The surfaces were polished by wet grinding with abrasive paper (400–4000 grit). The smear layer on the slabs was removed by ultrasonication with NaOCl for 3 min [12]. Then they have been washed two times in distilled water for 5 min also by ultrasonication (US). Afterwards, the samples were disinfected in ethanol (70 %) for another 10 min (US), washed in distilled water and stored in aqua dest. for 24 h before exposure in the oral cavity.

Initial biofilm formation and application of the edible oils

For in situ bioadhesion, individual upper jaw splints were vacuum-formed from 1.5-mm thick methacrylate foils. Cavities were prepared in the buccal aspects of the splints at the sites of the premolars and the 1st molar on the left and on the right side (n=6/splint). The slabs were fixed on the splints with polyvinyl siloxane impression material (Aquasil light body, Dentsply DeTrey, Konstanz, Germany).

Before insertion of the splints the subjects brushed their teeth without toothpaste and rinsed thoroughly with tap water; the splints were exposed to the oral fluids for 1 min

to allow pellicle formation on the surfaces. Thereafter, the subjects rinsed for 10 min with 8 ml of the different edible oils on different days. The application of the different rinses was conducted in a randomized order. Safflower oil (Brölio Diestelöl, Brökelmann+Co Ölmühle GmbH+Co, Hamm, Germany), linseed oil (Alnatura Leinöl (Alnatura GmbH), Germany) and olive oil (Bertolli Olivenöl—Extra Vergine Original, Unilever, Hamburg, Germany) were considered. The temperature of the oils was 20°C. Chlorhexidine rinses over 1 min served as positive controls (0.2 % chlorhexidin-digluconate, meridol med CHX 0.2 %, GABA, Lörrach, Germany). After the rinse, the splints were kept in the oral cavity for another 8 h overnight. Then the enamel slabs were removed immediately from the splints and thoroughly rinsed with running tap water for 5 s. Samples carried in the oral cavity for 8 h without application of any oil or rinsing agent served as controls.

One pass of the experiments was performed per subject and oil. The six enamel slabs per pass and subject were tested for the amount of adherent bacteria with DAPI, BacLight and the CFU method (colony forming units), each with two samples. Several additional passes were carried out to gain samples for FISH and DAPI/concanavalin A staining, lipid staining and transmission electron microscopy (TEM).

Total bacterial count (DAPI)

DAPI staining was conducted as described previously [12]. DAPI (4′,6-diamidino-2-phenylindole) stains DNA unspecifically by binding to the AT-rich regions of double stranded DNA. [26] Upon binding to DNA, the DAPI molecule fluoresces intensely. First the samples were rinsed with sodium chloride. For staining, the samples were covered with 1 ml DAPI solution (Merck, Darmstadt, Germany) in a dark chamber. After 15 min the DAPI solution was removed, and the samples were covered with methanol for 4 min. In the following, the specimens were dried at room temperature and coated with Citifluor (Citifluor Ltd., London, UK) and analysed by epifluorescence microscopy (Axioskop II, Zeiss, Oberkochen, Germany). The initial biofilms were analysed at 1000-fold magnification using the light filter for DAPI (BP 365, FT 395, LP 397). The number of cells observed in ten randomized microscopic ocular grid fields per sample was counted. The area of ocular grid (0.0156 mm^2) allowed calculating the numbers of bacteria per square centimeter. DAPI staining was combined with concanavalin A (Invitrogen, Molecular probes, Darmstadt, Germany) for visualization of glucan formation. The stock solution was 5 mg/ml Alexa Fluor 594 conjugate in 0.1 M NaH_2PO_4 buffer, pH 8.3. The stock solution was stored at −20°C. The working solution was a 10-µl stock solution in 490 µl PBS (1 mM $CaCl_2$, 1 mM $MnCl_2$, 1 mM $MgCl_2$).

BacLight^{TM} viability assay

The LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen, Molecular probes, Darmstadt, Germany) adopts two nucleic acid stains: green-fluorescent SYTO® 9 stain and red-fluorescent propidium iodide stain[27]. The BacLight kit was used for staining of enamel samples exposed to the oral fluids for visualization of vital and dead bacteria in the adherent state. Similar amounts of component A (Syto9 1.67 mM/propidium iodide 1.67 mM, 300 µl DMSO) and B (Syto9 dye 1.67 mM/ propidium iodide 18.3 mM, 300 µl DMSO) were mixed; 2 µl was added to 1 ml of saline solution. The samples were rinsed with sodium chloride. Afterwards the enamel slabs were incubated with this solution in a dark chamber for 10 min. Finally, the samples were rinsed with saline solution and evaluated immediately with a fluorescence microscope using the FDA filter and the ethidium bromide filter.

Fluorescence in situ hybridization (FISH)

FISH was conducted according to Amann et al. and was adapted on bovine enamel slabs as described previously [12, 28]. Biofilms formed on enamel slabs were fixed in 4 % paraformaldehyde in phosphate-buffered saline (PBS, 1.7 mM KH_2PO_4, 5 mM Na_2HPO_4 with 0.15 M sodium chloride, pH 7.2) for 8 h or overnight at 4°C. After fixation, all specimens were washed twice with phosphate-buffered saline and incubated again in a solution containing ethanol (50 % in PBS, v/v) for another 3 h. Subsequently, the specimens were washed once with PBS, followed by incubation in a solution containing 7 mg of lysozyme (hen egg white, 105,000 U/mg, Fluka, Buchs, Switzerland) per milliliter of 0.1 M Tris–HCl and 5 mM EDTA (pH 7.2) for 9 min at 37°C. Afterwards, the samples were dehydrated with a series of ethanol washes each for 3 min. Specimens were then incubated with the oligonucleotide samples at a concentration of 50 ng per 20 ml of hybridization buffer (0.9 M NaCl, 20 mM Tris–HCl (pH 7.2), 25 % formamide (v/v) and 0.01 % sodium dodecyl sulphate (w/v)) for 90 min at 46°C covered with aluminium foil and parafilm in a water bath/quench. Following probe hybridization, specimens were incubated for 15 min in buffer (20 mM Tris–HCl (pH 7.5), 5 mM EDTA, 159 mM NaCl and 0.01 % sodium dodecyl sulphate (w/v)) again at 46°C in a water bath/quench. After that, the samples were dried at room temperature and analysed by epifluorescence microscopy (Axioskop II, Zeiss, Oberkochen, Germany) at a magnification of 1000-fold.

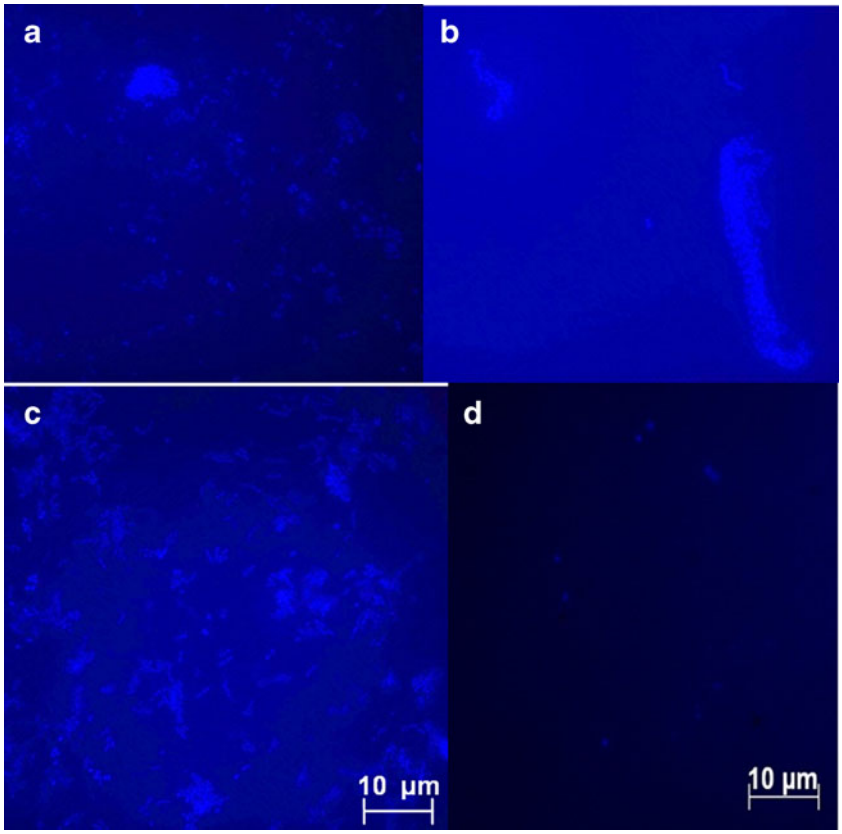


Fig. 1 DAPI staining, typical examples. Bacteria were distributed randomly in small aggregates on the enamel surfaces. The rinses with vegetable oils had no effect on the structure of the bacterial aggregates or on their distribution. Application of chlorhexidine led to very sparse bacterial adherence. **a** Control. **b** Olive oil. **c** Linseed oil. **d** Chlorhexidine

Lipid staining

In addition, samples were stained with Sulfan-red after oral exposure and evaluated light microscopically.

Colony forming units (CFU)

For determination of the CFU, the specimens were rinsed in 0.9 % sodium chloride after exposure in the oral cavity [12]. The samples were transferred into sterile tubes with 1 ml 0.9 % sodium chloride, vortexed and kept for 4 min in an ultrasonic bath on ice. This solution was serially diluted afterwards up to $1{:}10^3$ in physiological sodium chloride solution and plated on Columbia blood agar (CBA, aerobic bacteria and facultative anaerobic bacteria) or on yeast–cysteine–blood agar, respectively (HCB, anaerobic bacteria). The HCB plates were incubated for 7 days in anaerobic jars (Merck) at 37°C using BBL GasPak Anaerobic System Envelopes

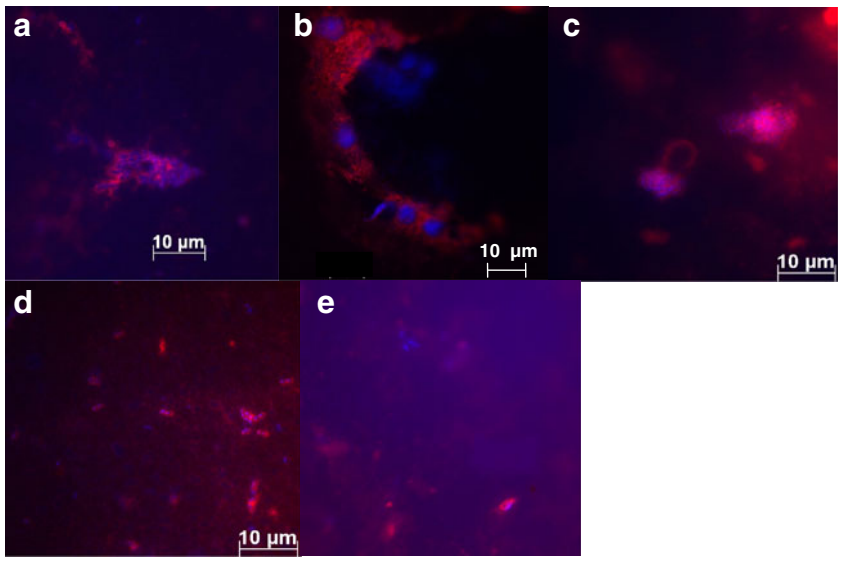


Fig. 2 DAPI staining (*blue*) combined with visualization of the glucans (*red*). Dense glucan structures surrounded the bacteria. Furthermore, slight glucan layers were observed on the enamel free of microorganisms. The rinses with vegetable oils had no effect on the structure of the bacterial aggregates or on their distribution. Following application of chlorhexidine, very sparse glucan formation was observed. **a**, **b** Olive oil. **c**, **d** Unrinsed control. **e** Chlorhexidine

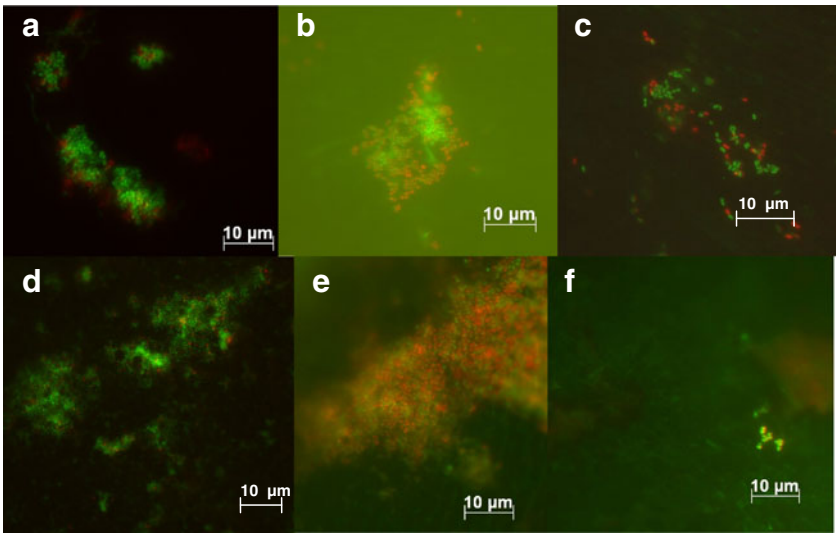


Fig. 3 BacLight™ staining, typical examples. Viable (*green*) and dead (*red*) bacteria were distributed randomly in small aggregates on the enamel surfaces. The rinses with vegetable oils had no effect on the structure of the bacterial aggregates or on their distribution; application of chlorhexidine led to very sparse bacterial adhesion. **a** Safflower oil. **b** Safflower oil. **c** Olive oil. **d** Linseed oil. **e** Unrinsed control. **f** Chlorhexidine

(Becton Dickinson, New Jersey, USA); the CBA plates were under aerobic conditions with 5 % CO_2 for 2 days [12].

Transmission electron microscopy (TEM)

Immediately after oral exposure, the enamel slabs were fixed in glutaraldehyde for 2 h (2.5 % glutaraldehyde, 1.5 % formaldehyde in phosphate buffer, pH 7.4). Afterwards, the specimens were washed five times in phosphate buffer. Postfixation for visualization of organic structures took place in 1 % osmium tetroxide for 2 h. The specimens were dehydrated in increasing concentrations of alcohol and embedded in Araldite M (Serva, Darmstadt, Germany). The dentine was removed from the samples with a diamond bur, and the samples were decalcified in 1 M HCl for preparation of the biofilm. Re-embedding was performed with Araldite. Ultrathin sections of the pellicle samples were cut in series with an ultramicrotome (Ultracut E, Reichert, Bensheim, Germany), using a diamond knife. The ultrathin sections were mounted on mesh grids (Plano, Wetzlar, Germany) and contrasted with uranylacetate and lead citrate. Transmission electron microscopic investigation took place at 3,000–50,000-fold magnification in a TEM TECNAI 12 Biotwin (FEI, Eindhoven, The Netherlands).

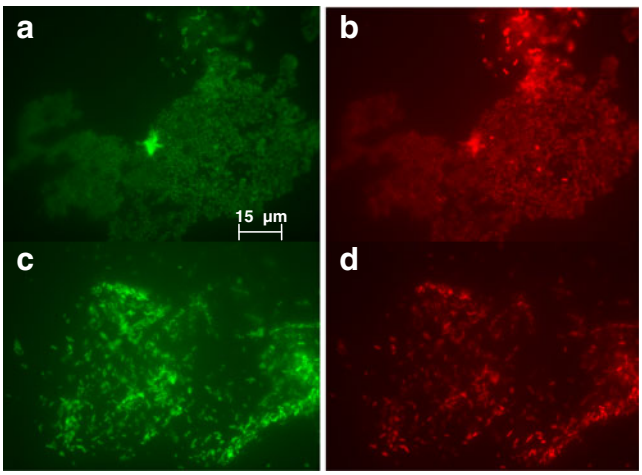


Fig. 4 FISH: nearly all bacteria were identified as streptococci irrespective of the adopted rinse. **a** and **b** as well as **c** and **d** show the same area of the samples; *green*: eubacteria, *red*: streptococci (rinse: safflower oil)

Statistics

Statistical evaluation was performed by Kruskal–Wallis test followed by the Mann–Whitney *U* test ($p<0.05$). The Kruskal–Wallis test was adopted to check within one exposure time and within one method if the rinses had any effect; the additional Mann–Whitney *U* test was used for pairwise comparisons, if the Kruskal–Wallis test yielded significant results. The software used was SPSS statistics 17.0 (IBM, Ehningen, Germany).

Results

Bacterial colonization was traceable on all enamel slabs exposed to the oral environment. With all methods, no effect of the rinses with vegetable oils on the distribution pattern or on the amount of adherent bacteria was observed as compared with unrinsed controls (Figs. 1, 2, 3 and 4). After 8 h of oral exposure following the application of the oils, the adherent bacteria were distributed randomly on the enamel surfaces; mono-layered chains and aggregates were visible. Nearly all bacteria had a more or less coccoid shape; FISH analysis of selected samples indicated that most bacteria were streptococci (Fig. 4). Furthermore, considerable glucan formation was visualized (Fig. 2). The adherent bacteria were surrounded by dense glucan structures; the whole

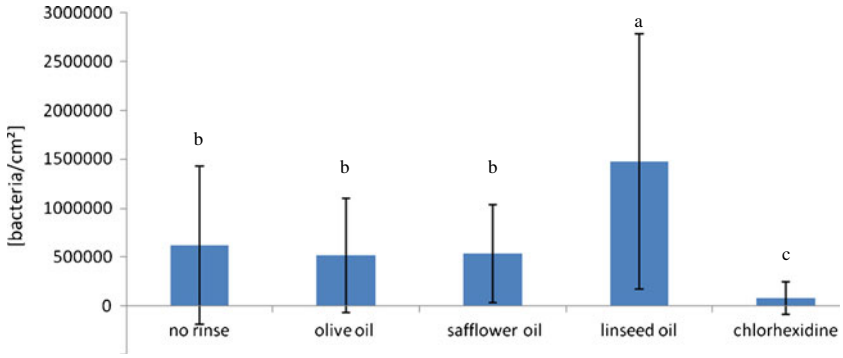


Fig. 5 DAPI staining for detection of adherent bacteria after rinses with edible oils or chlorhexidine. Exposition of enamel slabs at buccal sites of the upper 1st and 2nd premolar and 1st molar for 8 h, MV± S.D., $n=16$ samples per subgroup ($n=8$ subjects, two samples per subject, and oil). Kruskal–Wallis test: $p<0.001$. Data significantly different from each other are marked with different *letters* (pairwise comparison, Mann–Whitney U test, $p<0.05$)

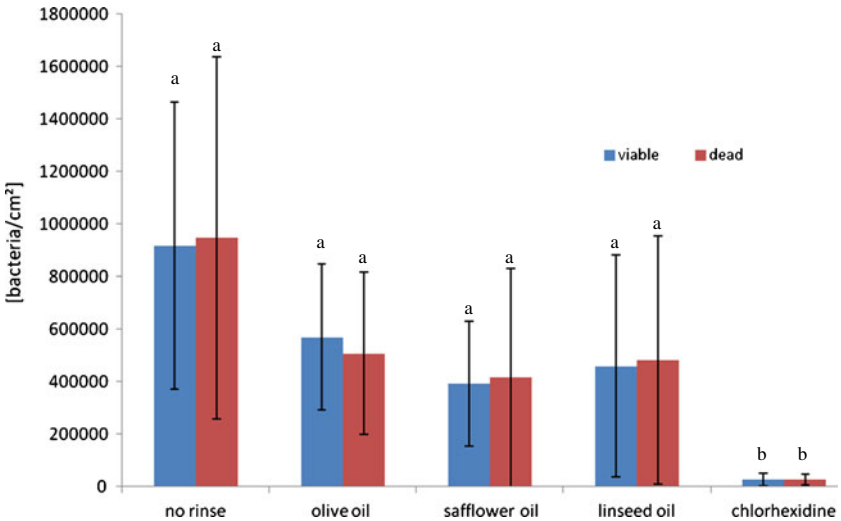
enamel surface was covered by a hazy shade of glucans. As compared with controls or slabs rinsed with vegetable oils, application of chlorhexidine had considerable effects on the amount of adherent bacteria; also, glucan formation was diminished distinctly (Figs. 1, 2, 3 and 4).

Quantification of the bacteria in the adherent state was performed with DAPI and BacLight staining (Figs. 5 and 6). The number of adherent bacteria on control samples was $6.1\pm8.1\times10^5/cm^2$ after 8 h as recorded with DAPI. A considerable interindividual and intraindividual variability was observed. Nevertheless, with both methods a significant reduction of the bacterial adherence by more than 85 % was recorded following initial application of chlorhexidine, whereas the rinses with the vegetable oils had no significant impact on the amount of adherent microorganisms. After adoption of linseed oil, there was even an elevated number of bacteria as shown with DAPI. The proportion of viable and dead bacteria was affected neither by the vegetable oils nor by chlorhexidine.

The highest variability of the data was recorded with the CFU method (Fig. 7). Nevertheless, for aerobia and anaerobia, Kruskal–Wallis test and Mann–Whitney U test indicated a significant impact of the chlorhexidine rinse on the amount of culturable bacteria. The determination of the colony forming units confirmed the lacking effect of vegetable oils on bacterial adhesion within 8 h.

Light microscopic images indicated an initial oil accumulation at the enamel surface following the rinses with the edible oils (Fig. 8). In addition, TEM analysis was carried out. Interestingly, adherent lipid micelles were observed at the pellicle's surface directly after the rinse, but large areas of the pellicle showed a rather unmodified ultrastructure (Fig. 9). Furthermore, 109 min after application of the rinse, the ultrastructure of the pellicle differed considerably from the control sample, whereas the pellicle's thickness was quite similar (Fig. 10). Following the oil rinse, the pellicle was of lower density and showed a rather inhomogeneous structure as compared with the 120-min

Fig. 6 Determination of viable and dead bacteria (BacLight assay) for detection of adherent bacteria after rinses with edible oils or chlorhexidine. Exposition of enamel slabs at buccal sites of the upper 1st and 2nd premolar and 1st molar for 8 h, MV±S.D., $n=16$ samples per subgroup ($n=8$ subjects, two samples per subject, and oil). Viable bacteria: Kruskal–Wallis test $p<0.001$; dead bacteria: $p<0.001$. Data significantly different from each other are marked with different *letters* (pairwise comparison, Mann–Whitney U test, $p<0.05$)



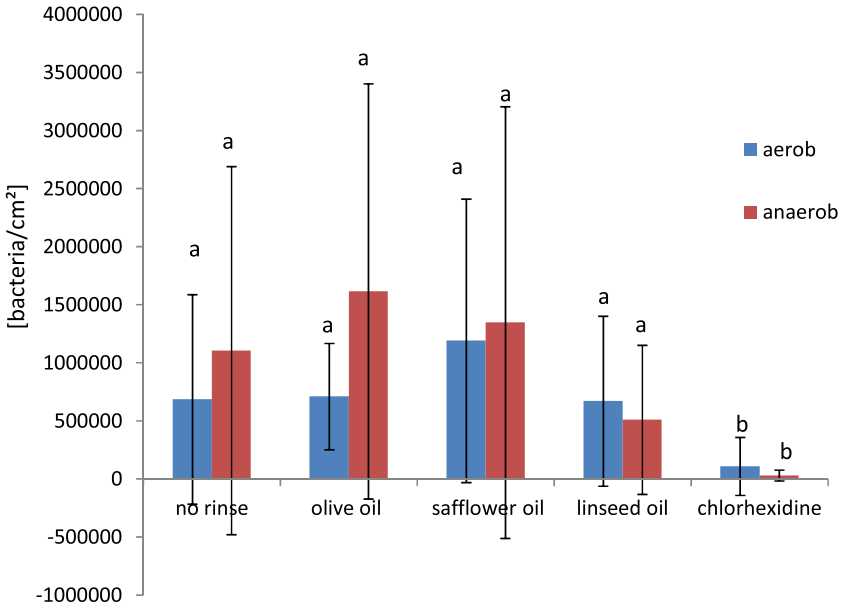


Fig. 7 Determination of CFU after desorption of adherent bacteria following rinses with edible oils or chlorhexidine. Exposition of enamel slabs at buccal sites of the upper 1st and 2nd premolar and 1st molar for 8 h, MV±S.D., n=16 samples per subgroup (n=8 subjects, two samples per subject, and subgroup). Aerob: Kruskal–Wallis test $p<0.001$; anaerob: $p<0.001$. Data significantly different from each other are marked with different *letters* (pairwise comparison, Mann–Whitney U test, $p<0.05$)

control pellicle which was of a homogenous fine granular structure.

Discussion

The present in situ study aimed to investigate the effect of edible oils on initial bacterial adhesion—no reduction of the microbial colonization of the enamel was observed. The methodical approach based on a combination of modern fluorescence microscopic techniques adapted to enamel specimens with the conventional culture plate method has been used in several studies for investigation of the initial bacterial adherence occurring in situ. In particular, the combination of the in situ exposure with the fluorescence microscopic techniques allows investigation of initial bacterial colonization without any desorption procedure [27, 29–33]. The mode of application over 10 min was chosen to simulate the oil pulling [5, 6, 34]. However, chlorhexidine was only adopted for 1 min according to generally accepted recommendations and due to the taste of the substance. After the rinses, the slabs were carried overnight to avoid disturbance of bioadhesion and to achieve the greatest possible standardization under in situ or in vivo conditions. Despite these precautionary measures, a considerable inter-individual and intraindividual variability was observed which seems to be common for intraoral bacterial colonization, especially for the number of colony forming units [12, 25]. With the CFU method only 50 % of the oral bacterial strains can be cultivated; direct comparison with the fluorescence microscopic methods is not suitable [35]. However, it offers insight into the biological activity and viability of the bacteria; as expected, a significant reduction of the CFU by application of chlorhexidine was observed.

Nonetheless, the study based on several methods and data gained from eight subjects gave clear insight into the lacking effects of edible oils on initial bacterial adhesion. Despite this observation, many anti-fouling strategies are based on hydrophobic surface properties, and low bacterial adhesion was recorded on hydrophobic surfaces exposed to the oral cavity [16, 36–40]. Several studies suggest the efficacy of lipids and vegetable oils as antimicrobial agents [5, 6, 41, 42]. It was postulated that oils could add hydrophobic and therewith anti-adhesive properties of high substantivity to the pellicle or the enamel surface, respectively, hampering bacterial adhesion [5, 6, 16]. The experimental extraction of lipids from in vitro pellicles in vitro resulted in an increase of *Streptococcus mutans* numbers [43]. For effective

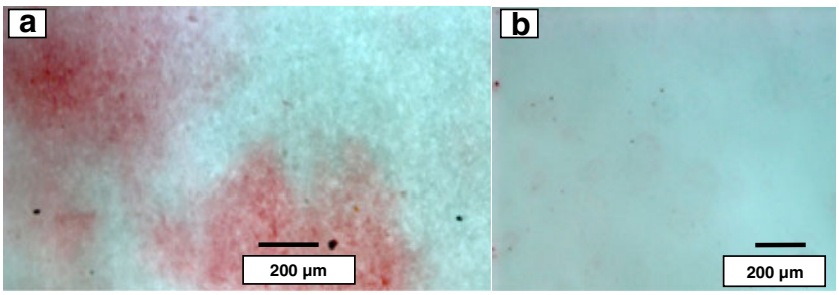


Fig. 8 Sulfan III staining of the pellicle. **a** After 1 min of pellicle formation in situ, the subject rinsed with safflower oil for 10 min; large areas of the sample were stained extensively. **b** Control: 11-min pellicle, nearly no staining

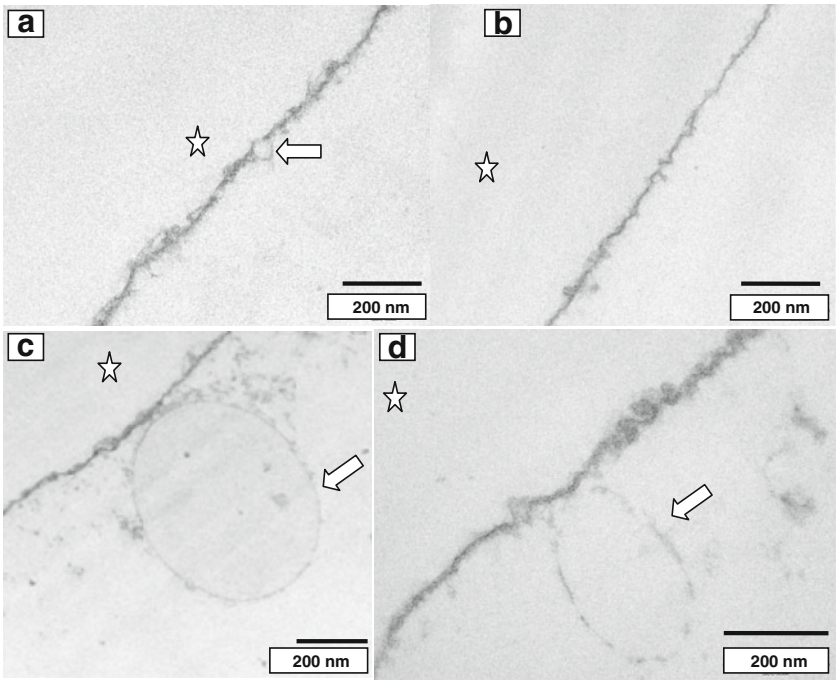


Fig. 9 TEM images indicated formation of lipid micelles at the surface of the pellicle directly after rinsing with safflower oil (**a**–**d** 1-min pellicle, 10-min rinse with safflower oil). The lipid micelles were of different size (*arrows*). However, large areas of the pellicle showed an unmodified ultrastructure (**b**). Please note that the enamel was removed during the preparation of the samples; the former enamel side is marked with an *asterisk* (original magnification: 30,000-fold)

prevention of bacterial adherence to the enamel surface by edible oils, accumulation and smooth distribution of the lipids in the pellicle layer is required. It was to be expected that lipids in oils interact with lipophilic components of the pellicle. The present TEM micrographs indicate adherence of lipid micelles to the pellicle surface but suggest limited integration of lipids into the initial pellicle layer. Large areas of the pellicle showed an unmodified ultrastructure directly after rinsing with oil. Nevertheless, the ultrastructure of the 120-min pellicle following a rinse with safflower oil was of a lower density than the control pellicle and appeared to be very inhomogeneous. Further research is necessary to investigate the lipid composition of the pellicle with and without application of different edible oils; rinses with oils might change the composition of the pellicle and could even remove or substitute certain lipophilic components relevant for protective effects.

Besides these considerations, the hydrophobic character of the oils allows direct interactions with the bacterial cell membrane [44]. This could lead to permeabilization and depolarisation of the membrane and reduce the activity of membrane associated enzymes [44]. Bacterial membrane composition determines cell surface hydrophobicity. Some bacteria in aqueous environments have a reduced affinity to hydrophobic surfaces [43]; the thermodynamic properties of the bacteria are

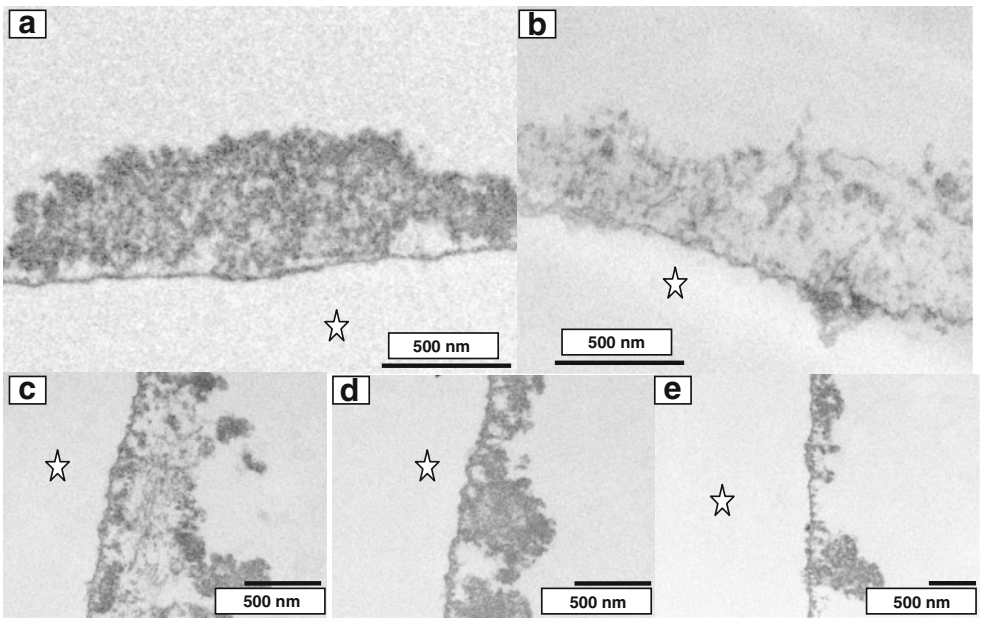


Fig. 10 TEM images of 120-min pellicles. The 120-min control pellicle was of a fine granular structure; an electron dense basal layer was visible (**a**). After an initial 10-min rinse with safflower oil followed by a 109-min oral exposure, a distinctly different ultrastructure was recorded (**b**–**e**). The pellicle was of lower density and showed a loosened and heterogeneous ultrastructure. Inhomogeneous fibrillar networks and electron dense structures were observed (**b**–**e**). Please note that the enamel was removed during the preparation of the samples; the former enamel side is marked with an *asterisk* (original magnification: 30,000-fold)

altered [45]. Many bacterial species present in the oral biofilm such as streptococci have high cell hydrophobicity [17, 18]. Accordingly, rinses with lipids could aggregate the respective microbial species in the semi-planctonic state for improved clearance or even detract bacteria from the pellicle layer. Due to the high clearance in the oral cavity, this effect can be neglected in the present study. On the other hand, effective and tenacious accumulation of lipids in the pellicle could mean increased bacterial colonization of the tooth surface due to the hydrophobic interactions which have been identified as relevant promoters of bioadhesion and microbial colonization in several in vitro experiments [17, 18, 46, 47]. In addition, the oils could serve as substrate for some bacteria; their growth might be enhanced.

Moreover, glucan formation was visualized successfully in the present experiments [27]. Water insoluble glucans are the product of glucosyltransferases, a main virulence factor of *S. mutans*; they promote bacterial biofilm formation considerably [48]. Apparently, the oils had no impact on glucan formation indicating that glucosyltransferase activity was not affected whereas chlorhexidine diminished it distinctly. This corresponds to in vitro data on the inhibiting effect of chlorhexidine on glycosyltransferase [49].

Despite all these considerations, the potential effects of edible oils or lipophilic rinses on bioadhesion in vitro seem to nullify each other under in vivo conditions in the oral cavity. Beside the lipid fraction, other components of the edible oils such as phenolic compounds and polyphenols could have added antibacterial effects [50, 51]. The most relevant phenolic components in olive oil are represented by tyrosol, hydroxytyrosol and lignanes. The antibacterial properties of these compounds have been shown [52, 53]. The relevant phenolic components in safflower oil are flavonoides with antimicrobial efficacy, but in general this oil is poor in polyphenolic compounds [54]. Linseed oil contains antioxidative lignanes [21, 22]. It seems as if their efficacy were hampered by lipid induced micelle formation. On the one hand, the oils failed any effect on bacterial colonisation as shown with different methods and they cannot be recommended for biofilm management; on the other hand, they had no promoting effect on microbial adhesion. Accordingly, patients are free to adopt these foodstuffs for care of oral soft tissues as they have been described to prevent gingival inflammation [55], and thus might serve as an adjuvant oral therapeutic agent on the oral soft tissues.

Conclusion

Rinses with edible oils have no significant impact on the initial pattern and amount of bacterial colonization on enamel over 8 h and modify the ultrastructure of the pellicle.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Longbottom, C, Ekstrand, K, Zero, D, Kambara, M (2009) Novel preventive treatment options. Monogr Oral Sci 155–163
2. Deng DM, ten Cate JM, Crielaard W (2007) The adaptive response of Streptococcus mutans towards oral care products: involvement of the ClpP serine protease. Eur J Oral Sci 115:363–70
3. Vissink A, Mitchell JB, Baum BJ, Limesand KH, Jensen SB, Fox PC, Elting LS, Langendijk JA, Coppes RP, Reyland ME (2010) Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys 78:983–91
4. Ship JA, McCutcheon JA, Spivakovsky S, Kerr AR (2007) Safety and effectiveness of topical dry mouth products containing olive oil, betaine, and xylitol in reducing xerostomia for polypharmacy-induced dry mouth. J Oral Rehabil 34:724–32
5. Asokan S, Emmadi P, Chamundeswari R (2009) Effect of oil pulling on plaque induced gingivitis: a randomized, controlled, triple-blind study. Indian J Dent Res 20:47–51
6. Asokan S, Rathan J, Muthu MS, Rathna PV, Emmadi P (2008) Effect of oil pulling on Streptococcus mutans count in plaque and saliva using Dentocult SM Strip mutans test: a randomized, controlled, triple-blind study. J Indian Soc Pedod Prev Dent 26:12–7
7. Ravikumar N, Suhas S (2009) Not recommended Br Dent J 207:518
8. Aguiar AA, Saliba NA (2004) Toothbrushing with vegetable oil: a clinical and laboratorial analysis. Braz Oral Res 18:168–73
9. Pretty IA, Gallagher MJ, Martin MV, Edgar WM, Higham SM (2003) A study to assess the effects of a new detergent-free, olive oil formulation dentifrice in vitro and in vivo. J Dent 31:327–32
10. Kozlovsky A, Goldberg S, Natour I, Rogatky-Gat A, Gelernter I, Rosenberg M (1996) Efficacy of a 2-phase oil: water mouthrinse in controlling oral malodor, gingivitis, and plaque. J Periodontol 67:577–82
11. Marsh P, Martin M (1999) Oral microbiology, 4th edn. Wright, Oxford
12. Hannig C, Hannig M, Rehmer O, Braun G, Hellwig E, Al-Ahmad A (2007) Fluorescence microscopic visualization and quantification of initial bacterial colonization on enamel in situ. Arch Oral Biol 52:1048–56
13. Slomiany BL, Murty VL, Mandel ID, Sengupta S, Slomiany A (1990) Effect of lipids on the lactic acid retardation capacity of tooth enamel and cementum pellicles formed in vitro from saliva of caries-resistant and caries-susceptible human adults. Arch Oral Biol 35:175–80
14. Slomiany BL, Murty VL, Zdebska E, Slomiany A, Gwozdzinski K, Mandel ID (1986) Tooth surface-pellicle lipids and their role in the protection of dental enamel against lactic-acid diffusion in man. Arch Oral Biol 31:187–91
15. Wiegand A, Gutsche M, Attin T (2007) Effect of olive oil and an olive-oil-containing fluoridated mouthrinse on enamel and dentin erosion in vitro. Acta Odontol Scand 65:357–61
16. Olsson J, van der Heijde Y, Holmberg K (1992) Plaque formation in vivo and bacterial attachment in vitro on permanently hydrophobic and hydrophilic surfaces. Caries Res 26:428–33
17. Doyle R, Rosenberg M (eds) (1990) Microbilal cell surface hydrophobicity. American Society for Microbiology, Washigton, DC

18. Rosenberg M, Judes H, Weiss E (1983) Cell surface hydrophobicity of dental plaque microorganisms in situ. Infect Immun 42:831–4
19. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol 23:622–9
20. Carluccio MA, Massaro M, Scoditti E, De Caterina R (2007) Vasculoprotective potential of olive oil components. Mol Nutr Food Res 51:1225–34
21. Prasad K (2005) Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis 179:269–75
22. Prasad K (2009) Flaxseed and cardiovascular health. J Cardiovasc Pharmacol 54:369–77
23. Carlen A, Borjesson AC, Nikdel K, Olsson J (1998) Composition of pellicles formed in vivo on tooth surfaces in different parts of the dentition, and in vitro on hydroxyapatite. Caries Res 32:447–55
24. Al-Ahmad A, Wunder A, Auschill TM, Follo M, Braun G, Hellwig E, Arweiler NB (2007) The in vivo dynamics of *Streptococcus* spp., *Actinomyces naeslundii*, *Fusobacterium nucleatum* and *Veillonella* spp. in dental plaque biofilm as analysed by five-colour multiplex fluorescence in situ hybridization. J Med Microbiol 56:681–7
25. Hannig C, Sorg J, Spitzmuller B, Hannig M, Al-Ahmad A (2009) Polyphenolic beverages reduce initial bacterial adherence to enamel in situ. J Dent 37:560–566
26. Schwartz T, Hoffmann S, Obst U (2003) Formation of natural biofilms during chlorine dioxide and u.v. disinfection in a public drinking water distribution system. J Appl Microbiol 95:591–601
27. Hannig C, Follo M, Hellwig E, Al-Ahmad A (2010) Visualization of adherent micro-organisms using different techniques. J Med Microbiol 59:1–7
28. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–69
29. Al-Ahmad A, Wiedmann-Al-Ahmad M, Faust J, Bachle M, Follo M, Wolkewitz M, Hannig C, Hellwig E, Carvalho C, Kohal R (2010) Biofilm formation and composition on different implant materials in vivo. J Biomed Mater Res B Appl Biomater 95:101–9
30. Jung DJ, Al-Ahmad A, Follo M, Spitzmuller B, Hoth-Hannig W, Hannig M, Hannig C (2010) Visualization of initial bacterial colonization on dentine and enamel in situ. J Microbiol Methods 81:166–74
31. Al-Ahmad A, Roth D, Wolkewitz M, Wiedmann-Al-Ahmad M, Follo M, Ratka-Kruger P, Deimling D, Hellwig E, Hannig C (2010) Change in diet and oral hygiene over an 8-week period: effects on oral health and oral biofilm. Clin Oral Investig 14:391–6
32. Al-Ahmad A, Follo M, Selzer AC, Hellwig E, Hannig M, Hannig C (2009) Bacterial colonization of enamel in situ investigated using fluorescence in situ hybridization. J Med Microbiol 58:1359–66
33. Hannig C, Sorg J, Spitzmuller B, Hannig M, Al-Ahmad A (2009) Polyphenolic beverages reduce initial bacterial adherence to enamel in situ. J Dent 37:560–6
34. Hannig C, Spies B, Spitzmuller B, Hannig M (2010) Efficacy of enzymatic mouth rinses for immobilisation of protective enzymes in the in situ pellicle. Arch Oral Biol 55:1–6
35. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 43:5721–32
36. Rosenberg M, Bar-Ness Greenstein R, Barki M, Goldberg S (1996) Hydrophobic interactions as a basis for interfering with microbial adhesion. Adv Exp Med Biol 408:241–8
37. Schultz MP, Bendick JA, Holm ER, Hertel WM (2011) Economic impact of biofouling on a naval surface ship. Biofouling 27:87–98
38. Stein J, Truby K, Wood CD, Gardner M, Swain G, Kavanagh C, Kovach B, Schultz M, Wiebe D, Holm E, Montemarano J, Wendt D, Smith C, Meyer A (2003) Silicone foul release coatings: effect of the interaction of oil and coating functionalities on the magnitude of macrofouling attachment strengths. Biofouling 19 (Suppl):71–82
39. Knörr W., DP, Grützmacher R., Höfer R. (1995) Development of new fields of application for linseed oil. European Journal of Lipid Science and Technology. 97:5
40. Goldberg S, Doyle RJ, Rosenberg M (1990) Mechanism of enhancement of microbial cell hydrophobicity by cationic polymers. J Bacteriol 172:5650–4
41. Kabara JJ, Vrable R (1977) Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. Lipids 12:753–9
42. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP (1972) Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother 2:23–8
43. Gibbons RJ, Etherden I (1983) Comparative hydrophobicities of oral bacteria and their adherence to salivary pellicles. Infect Immun 41:1190–6
44. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–53
45. Strevett KA, Chen G (2003) Microbial surface thermodynamics and applications. Res Microbiol 154:329–35
46. Doyle RJ, Rosenberg M (1995) Measurement of microbial adhesion to hydrophobic substrata. Methods Enzymol 253:542–50
47. Hannig C, Hannig M (2009) The oral cavity—a key system to understand substratum-dependent bioadhesion on solid surfaces in man. Clin Oral Investig 13:123–139
48. Bowen WH, Koo H (2011) Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res 45:69–86
49. Scheie AA, Kjeilen JC (1987) Effects of chlorhexidine, NaF and SnF2 on glucan formation by salivary and culture supernatant GTF adsorbed to hydroxyapatite. Scand J Dent Res 95:532–5
50. Medina E, Romero C, Brenes M, De Castro A (2007) Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J Food Prot 70:1194–9
51. Romero C, Medina E, Vargas J, Brenes M, De Castro A (2007) In vitro activity of olive oil polyphenols against Helicobacter pylori. J Agric Food Chem 55:680–6
52. Perez-Jimenez F, Alvarez de Cienfuegos G, Badimon L, Barja G, Battino M, Blanco A, Bonanome A, Colomer R, Corella-Piquer D, Covas I, Chamorro-Quiros J, Escrich E, Gaforio JJ, Garcia Luna PP, Hidalgo L, Kafatos A, Kris-Etherton PM, Lairon D, Lamuela-Raventos R, Lopez-Miranda J, Lopez-Segura F, Martinez-Gonzalez MA, Mata P, Mataix J, Ordovas J, Osada J, Pacheco-Reyes R, Perucho M, Pineda-Priego M, Quiles JL, Ramirez-Tortosa MC, Ruiz-Gutierrez V, Sanchez-Rovira P, Solfrizzi V, Soriguer-Escofet F, de la Torre-Fornell R, Trichopoulos A, Villalba-Montoro JM, Villar-Ortiz JR, Visioli F (2005) International conference on the healthy effect of virgin olive oil. Eur J Clin Invest 35:421–4
53. Cicerale S, Lucas L, Keast R (2010) Biological activities of phenolic compounds present in virgin olive oil. Int J Mol Sci 11:458–79
54. Cho SH, Lee HR, Kim TH, Choi SW, Lee WJ, Choi Y (2004) Effects of defatted safflower seed extract and phenolic compounds in diet on plasma and liver lipid in ovariectomized rats fed high-cholesterol diets. J Nutr Sci Vitaminol (Tokyo) 50:32–7
55. Haffajee AD, Roberts C, Murray L, Veiga N, Martin L, Teles RP, Letteri M, Socransky SS (2009) Effect of herbal, essential oil, and chlorhexidine mouthrinses on the composition of the subgingival microbiota and clinical periodontal parameters. J Clin Dent 20:211–7