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REVIEW

Lipids in preventive dentistry

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Abstract
Objectives There is still a great demand for the improvement of oral prophylaxis methods. One repeatedly described approach is rinsing with edible oils. The aim of the present review paper was to analyze the role of lipids in bioadhesion and preventive dentistry.
Materials and methods Despite limited sound scientific data, extensive literature search was performed to illustrate possible effects of lipids in the oral cavity.
Results It is to be assumed that lipophilic components modulate the process of bioadhesion to the oral hard tissues as well as the composition and ultrastructure of the initial oral biofilm or the pellicle, respectively. Thereby, lipids could add hydrophobic characteristics to the tooth surface hampering bacterial colonization and eventually decreasing caries susceptibility. Also, a lipid-enriched pellicle might be more resistant in case of acid exposure and could therefore reduce the erosive mineral loss. Furthermore, anti-inflammatory effects on the oral soft tissues were described. However, there is only limited evidence for these beneficial impacts. Neither the lipid composition of saliva and pellicle nor the interactions of lipids with the initial oral biofilm and the pellicle layer have been investigated adequately until now.
Conclusion Edible oils might qualify as mild supplements to conventional strategies for the prevention of caries, erosion, and periodontal diseases but further research is necessary.
Clinical relevance Against the background of current scientific and empirical knowledge, edible oils might be used as oral hygiene supplements but a decisive benefit for the oral health status is questionable.

Keywords Lipids · Oral cavity · Pellicle · Bioadhesion · Erosion

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Introduction

Over the past decades, the prevention of oral diseases has become an essential component of dentistry and dental research [1]. But despite the great improvements in the field of prophylaxis, caries and periodontitis remain two challenging diseases [2, 3].

Recent publications call attention to a global increase in dental caries prevalence, affecting children as well as adults worldwide [4]. Even though many countries exhibit a promising decrease of decayed, missing, filled teeth values throughout the last years [5, 6], further analyses reveal a skewed distribution of caries prevalence occurring due to shifts in populations [2, 7, 8] whereby the status of oral health seems to be affected by the socioeconomic situation [6]. This, in turn, implies the urgent need for reasonable and worldwide accessible preventive methods.

But not only caries can lead to loss of tooth tissue. Dental erosion, induced by the presence of intrinsic and extrinsic acids originating from the environment, the diet, eating disorders, and others, is a severe problem of nonbacterial origin, widely spread throughout the population [9–12]. And there are more challenges to be faced in the future.

As a result of advanced medical knowledge and refined therapies, life expectancy increases, causing a demographic change. According to the Federal Statistical Office, the percentage of people in Germany over 65 will raise to 23 % in 2020 and 33 % in 2050 [13, 14]. Elderly people can be physically restricted, which affects their mechanical oral hygiene and additional systemic diseases could aggravate the risk of developing oral health complications [15]. Additionally, the *Periodontal Country Profiles* by the World Health Organization present further information regarding the occurrence of periodontitis in different age cohorts worldwide. Taking the middle aged and old people in Germany, there are around 50 % with probing depths over 4 mm and 21 % or else 41 % with pocket depths of 6 mm and more [3]. Alteration of oral tissue following severe operations, chemotherapy, and radiotherapy often evokes special requirements. Induced by hyposalivation, the mucosa can be very dry and inflamed [16], the healing of wounds is restricted and there is a high risk of developing radiation caries [17]. Several mouthwashes containing antiseptic or analgesic agents have been developed; however, they are often slightly efficient and the acceptance of conventional chemical solutions is low as they are associated with mouth burn, bad taste, a tainting effect, and dental stains [18–20]. Therefore, the establishment of biological preventive solutions as adjuvant methods could be a sensible attempt to reduce the incidence of oral diseases.

Considering the impact of microbial interaction and physicochemical dynamics onto the health of hard and soft oral tissues, it would be desirable to either strengthen the tissue against extrinsic degradation or modulate the process of bioadhesion. With that said, the characteristics of lipids appear promising as they could influence the microbial interaction, modulated oral surfaces might impede bacterial adherence [21] and a hydrophobic layer could protect against tooth demineralization or dry mouth [5]. Therefore, the aim of the present review was to investigate the value of lipid-containing mouthwashes and edible oils in the context of bioadhesion and preventive strategies.

Bioadhesion on the dental hard tissues

Pellicle formation

In the oral cavity, bacterial adhesion evolves on the basis of a proteinaceous layer, the acquired pellicle [22, 23], which is the first step of bioadhesion on solid surfaces exposed to the oral fluids (Figs. 1 and 2).

As a tenaciously absorbed bacteria-free coating of the tooth surface, the pellicle is the result of a highly selective adsorption of proteins, glycoproteins, lipids, and other macromolecules from the oral fluids (Figs. 1, 2, and 3) [24]. The initial formation process is determined by ionic interactions between the enamel surface and certain salivary proteins like statherin, histatin, and proline-rich proteins as well as by thermodynamically driven forces such as van der Waals forces and hydrophobic interactions [5, 22]. Subsequently, the composition becomes more complex by the adsorption of heterotypic structures and protein aggregates [24]. The pellicle serves as a protective lubricant and a diffusion barrier for the tooth, a nonshedding surface [21, 24]. Thereby, the electron-dense basal pellicle layer is especially resistant against acid attack and its semipermeable properties allow ion exchange of calcium and phosphate at the tooth surface (Figs. 2 and 3) [25].

Bacterial colonization

Nevertheless, components of the pellicle also serve as bacterial receptors and promote microbial biofilm formation [22, 24]. Microorganisms are transported passively to the tooth surface by the saliva flow where an initially nonspecific reversible attraction provoked by physicochemical forces occurs [26]. Several studies have proven the relevance of hydrophobic interactions and cell hydrophobicity for bacterial adherence. The experimental extraction of lipids from pellicles in vitro resulted in an increase of *Streptococcus mutans* numbers [27] and a reduction of biofilm formation was observed on hydrophobic surfaces such as dental materials in vivo [28, 29]. Bacterial attachment becomes irreversible if bacterial adhesins such as lipoteichoic acid or lectins interact with receptors of the pellicle components [22, 24, 30]. Several pellicle molecules, including some lipid components [27], are considered as bacterial binding sites. In consequence, systematic bacterial adhesion occurs, which eventually results in the development of an exceedingly organized solid biofilm [5, 22, 31].

Conventional biofilm management

The regular and consequent removal of oral plaque, as well as the retardation of biofilm formation are the decisive targets of dental prevention methods. A significant reduction of caries incidence can be achieved by fluoride application, especially since there are no noticeable side-effects if used properly [32]. However, the persistent need for caries treatment confirms that the protective effects of fluorides nonetheless are limited and require optimization. For example, the oral sustainability of fluorides is restricted and

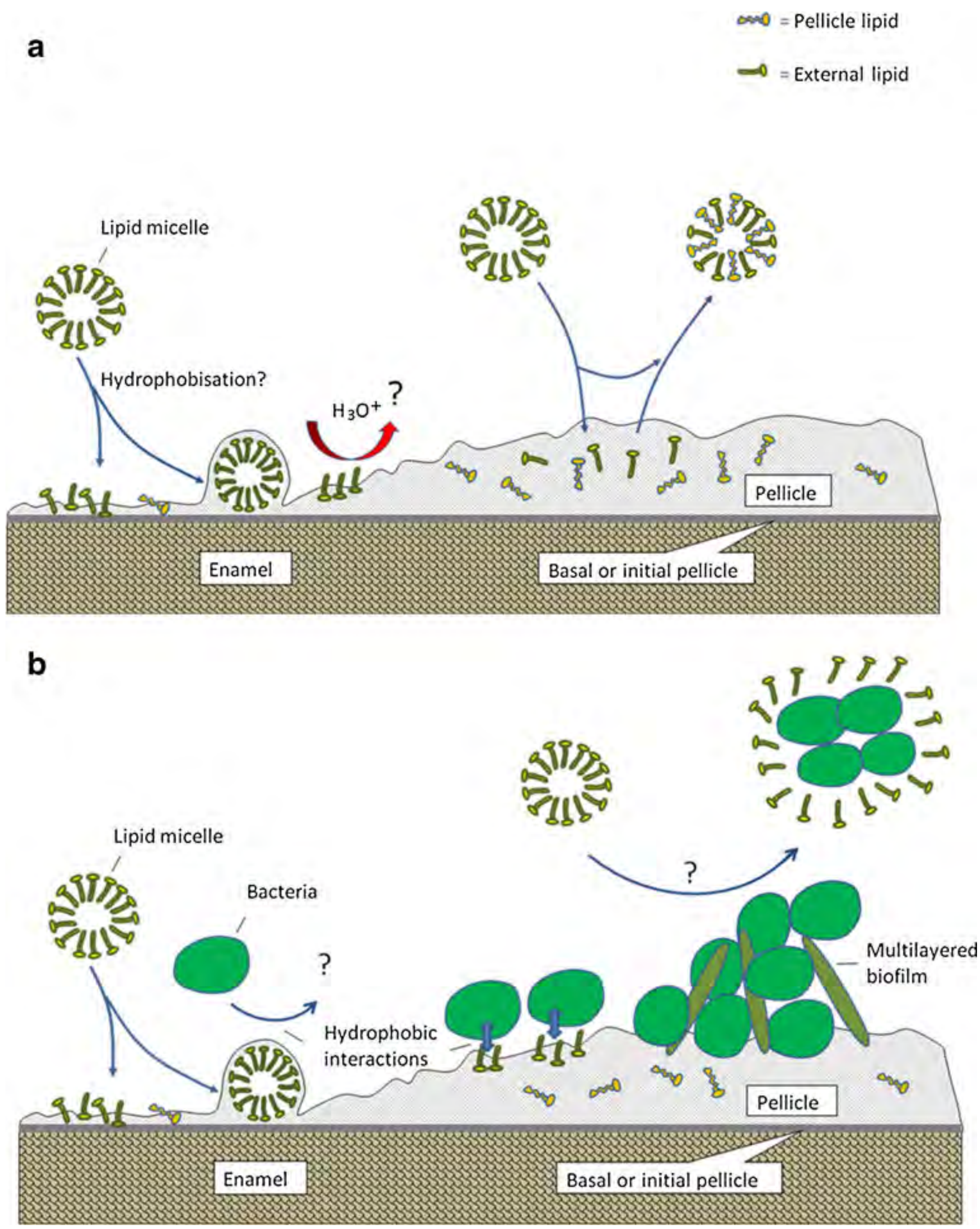

Fig. 1 Conceivable mechanisms of pellicle modification by lipid components and their impact on bacterial adhesion. **a** Initial and mature pellicle under influence of lipid constituents. TEM confirmed the attachment of lipid micelles to the pellicle surface directly after rinses with vegetable oil (consult Fig. 3). The sustainable integration of these substances could hydrophobize the pellicle and therewith prevent acidic ion diffusion and enamel demineralization. Nevertheless, there is some evidence that externally added lipid constituents might also facilitate pellicle degradation by detracting relevant protective lipids from the pellicle. **b** Bacterial adhesion. Hydrophobic interactions partially influence bacterial adhesion. A hydrophobic pellicle surface might hamper irreversible bacterial attachment. In contrast, lipids incorporated into the pellicle structure could also serve as bacterial nutrients and binding sites. Yet, bacterial affinity to hydrophobic substances could be advantageous if lipid application can remove biofilm bacteria. Further research will be required to verify the existing hypotheses

although they reduce bacterial viability, an inhibiting effect on bacterial adhesion is less clear [32, 33]. Additionally, chlorhexidine is a common active substance for chemical plaque control but electron microscopic studies have shown a limited efficacy of chlorhexidine against adherent bacterial biofilms [34]. Moreover, the side effects like discolorations, a tainting effect, and a shift of the common oral flora do not qualify it as a daily supplement of oral hygiene [35, 36].

Modern concepts try to protract biofilm formation at the start. This includes a wide variety of different approaches like nanomaterials, immunization, or bacterial replacement therapy [37–41]. However, additional research clarifying the effectiveness of those expensive methods will be necessary and a negative impact on the ecology of the oral cavity cannot fully be eliminated. Simultaneously natural, biological products come more and more into focus. They are often easily obtainable worldwide, generally biocompatible, and undesirable side effects are not to be expected. Several foods, such as polyphenolic beverages, are recognized for their wholesome effect [42]. Polyphenols are regarded as strong antioxidants with potential health benefits [43–45] and they exhibit a wide range of antibacterial, antifungal, and anti-inflammatory effects which also qualify them for the prevention of caries and periodontitis [46]. Another promising approach in dental prevention methods can be to reinforce the protective pellicle properties by modifying its composition. In this context, the enrichment of lipids in the pellicle has come into focus as they might help prevent erosion, have antibacterial effects, and be efficient in the modification of pellicle's physicochemical properties to delay bacterial adhesion [47–50].

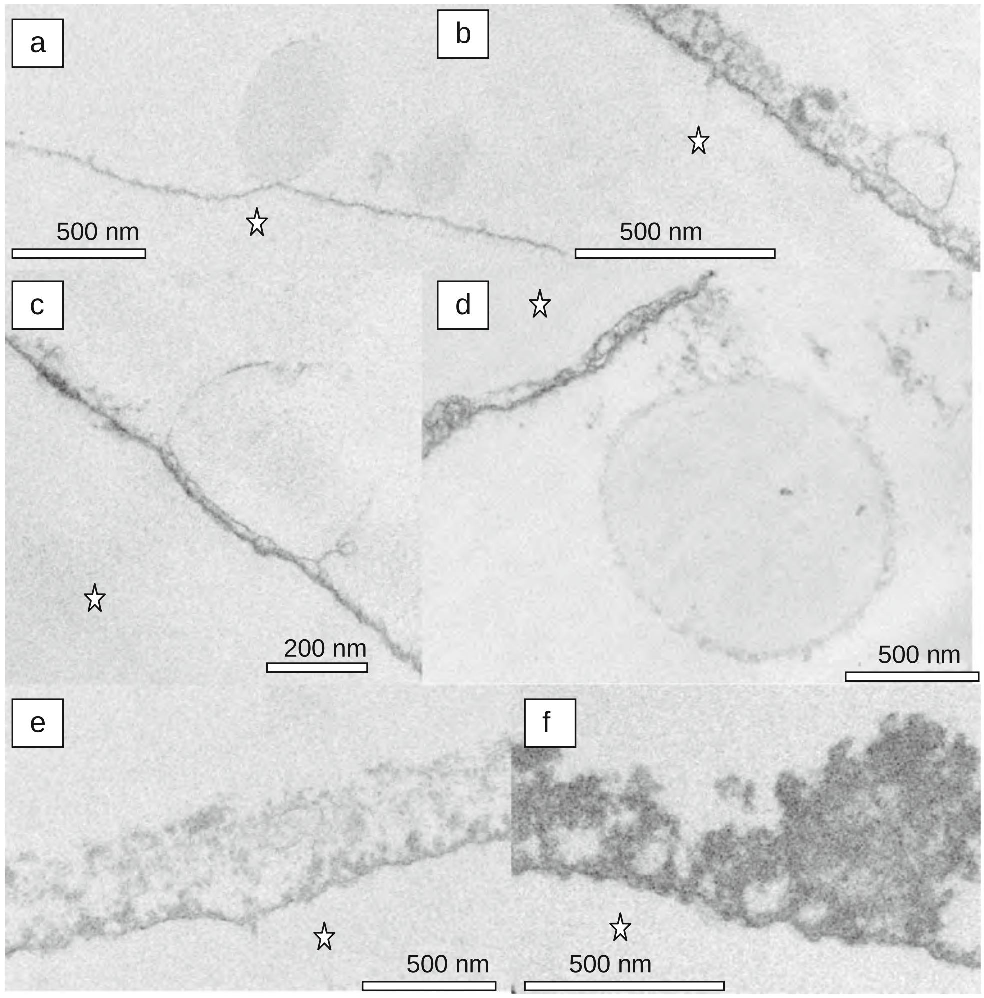

Fig. 2 Transmission electron microscopy: effect of rinses with safflower oil on the initial pellicle layer. After formation of a 3-min pellicle in situ, the subject rinsed with the edible oils for 10 min. Directly after the rinse, oil micelles and droplets were visible, either attached to the pellicle (**a**–**c**) or near the pellicle surface (**d**). Another 110 min later, the 120 min pellicle (**e**) was of lower density than the respective control (no rinse, **f**). Please note that the enamel was removed during preparation of the samples; the former enamel side is marked with an *asterisk*. The interaction of the lipids with the pellicle requires further research. Original magnification, 30,000-fold. Please compare with Fig. 1

Lipids and hydrophobic fluids and their effects on bioadhesion

Lipids play a vital role in all organisms, not only regarding the storage of energy (e.g., neutral lipids), or as structural elements of cell membranes (e.g., phospholipids). They are also heavily involved in signal transduction processes (e.g., isoprenoides/steroids). Thus, the term “lipid” comprises a diverse range of compounds, varying in characteristics, structure, and functionality. For this reason, there is no widely accepted definition of what is considered a lipid. Traditionally, lipids are described as nonpolar compounds insoluble in water but readily soluble in organic solvents such as alcohols, ethers, hydrocarbons, and chloroform. However, a definition of this kind excludes many substances that are widely regarded as lipids and are as soluble in water as in organic solvents. Therefore, Christie [51] introduced another definition, which describes lipids as fatty acids and their derivatives, and compounds biosynthetically or functionally related to them. In the last decades, many attempts regarding a comprehensive classification system for lipids were made [52–55]. Figure 3 gives a brief overview of different lipid classes. Due to their amphiphilic and hydrophobic properties, most of these substances are of potential interest for the purpose of biofilm management from a theoretical point of view.

When solid substrates are exposed to some aqueous solution, biofilm formation naturally occurs. It is an ubiquitous phenomenon whose delaying modification challenges medicine as well as industrial technologies [28, 56, 57]. Ionic, electrostatic, and hydrophobic interactions are important determinants for the adherence of microorganisms to a variety of surfaces and materials [58, 59]. It could be postulated that making a solid surface more hydrophobic might decrease its wettability and hamper biofilm accumulation in a moist environment [22]. A slight reduction of protein adsorption and bacterial adhesion was indeed observed on glass surfaces that had been hydrophobized in vitro by plasma polymerization of a low molecular weight siloxane [60, 61]. Furthermore, less biofilm formation was also observed in vivo on hydrophobic surfaces of voice prostheses in comparison to hydrophilized ones [62]. Similarities apply to the oral cavity as comparable findings were generated by an in vivo experiment that compared plaque formation on

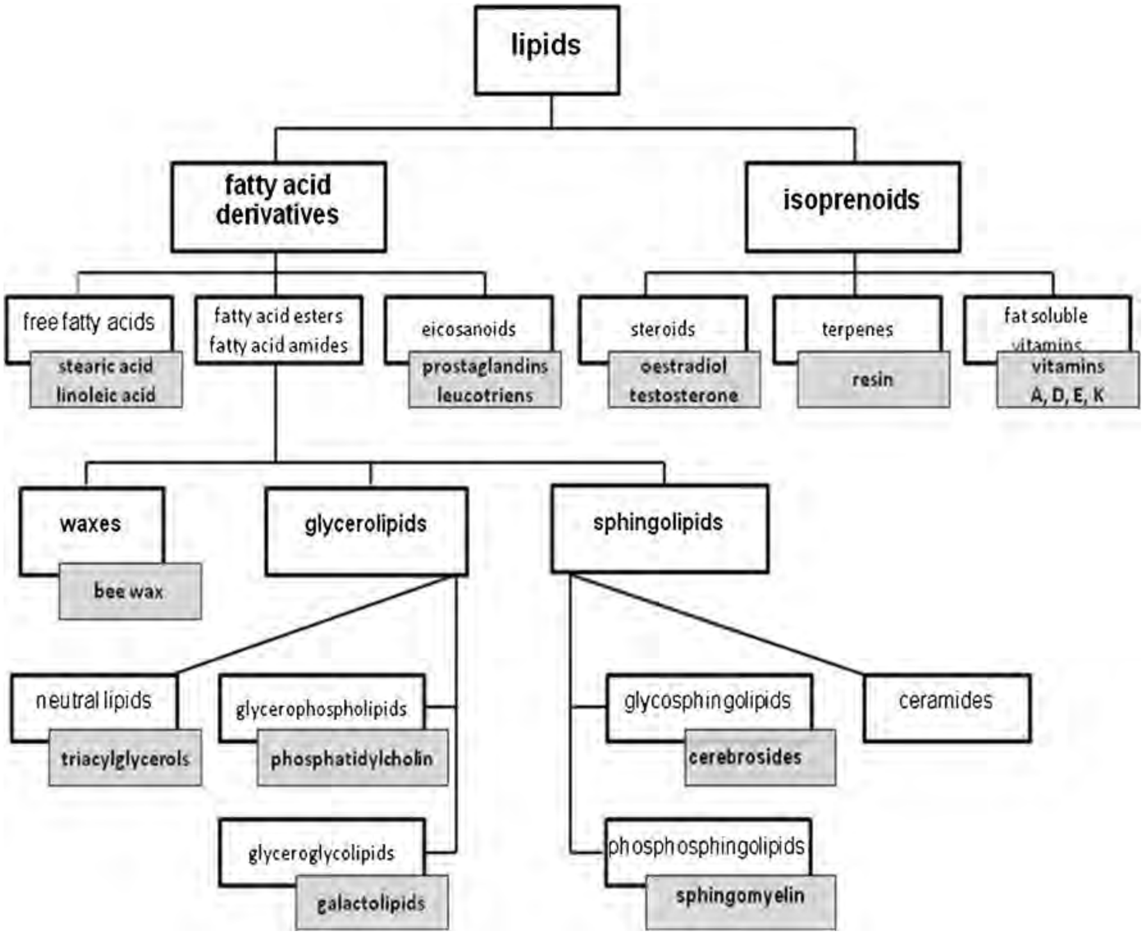

Fig. 3 Overview of various lipid classes with examples. Figure summarizes data presented in textbooks by Lottspeich [158] and Stryer [159]

enamel and intraorally worn material strips with different surface free energy levels such as parafilm and fluorethylenepropylene (Teflon) [63]. The results of the study showed remarkably less plaque formation on surfaces with low surface-free energy which the authors explained by a decreased binding force between bacteria and substrata of low surface-free energy. According to investigations of biofilm formation on different dental restorative and implant materials, considerably less bacteria adhere to supragingival hydrophobic surfaces in the oral cavity than to hydrophilic ones [28]. The same was observed on polysiloxane pretreated crowns in vivo [60, 61].

Nevertheless, the few studies investigating the relevance of hydrophobic interactions on oral bioadhesion obtained divergent results regarding the effect of hydrophobic surfaces on bacterial colonization, as there is indications that they either reduce or enable bacterial adhesion [22, 28, 48, 64, 65].

Furthermore, the different investigations underline the distinct impact of the oral environment including saliva and in vivo pellicle formation [22, 66]. According to a study published by van der Mei et al., intraorally formed pellicles naturally tend to be more hydrophobic than salivary pellicles formed in vitro [66]. Although no analysis of the pellicles' specific lipid content was carried out, it was shown by the authors that the application of dietary lipids from salad oil in vitro increases the hydrophobicity of intraoral conditioning films. Due to these considerations, not only permanent hydrophobization of dental materials but also the application of mouth rinses for the purpose of transient surface hydrophobization might be of interest. Yet, several hypotheses concerning the effects of those expectable modifications so far remain unresolved (Fig. 1). A less recent publication by Rykke and Rölla described a notably retarded pellicle formation on enamel specimens in situ after extraoral silicon oil pretreatment [64]. Due to hydrophobization, less protein seemed to adhere to the hydroxyapatite surface and a different amino acid composition was determined for the resulting basal pellicle. The modulated pellicle structure as well as specific lipophilic components of the pellicle could either hamper but possibly also facilitate the attachment of certain microorganisms due to enforced hydrophobic interactions (Fig. 1) [28, 48, 67]. Latest information gained by transmission electron microscopy is illustrated in Fig. 1 and indicates modified, less electron-dense pellicle structures after rinses with vegetable oils, compared with controls [48]. The effects of lipid application on the biochemical compositions of the pellicle as well as the resulting impact on bioadhesion have not been clarified entirely until now. A loosened ultrastructure could evolve optimized or weakened protective properties. Yet, the said study determined no significant impact of rinses with three different vegetable oils on the bacterial colonization of enamel over 8 h [48]. As well as having an impact on pellicle formation and its susceptibility against acids and bacterial adhesion, it is also conceivable that applied lipids extract relevant protective lipophilic components from the pellicle and provide bacterial receptors or even substrates for microorganisms. Among others, bacterial adherence depends on the attraction resulting of hydrophobic interactions [22, 68]. Cell surface hydrophobicity is derived by the bacterial membrane composition, however, distributing bacteria in aqueous liquids reduces their affinity to hydrophobic surfaces considerably and influences microbial surface thermodynamics [27, 69]. It is noteworthy that intraoral rinses with silicon oil led to the embedding of oil vesicles full of viable bacteria into the biofilm as shown in Fig. 4. This indicates that bacteria could indeed prefer a hydrophobic environment. Last but not least, publications suggest the effectiveness of specific lipids as antimicrobial agents [47, 70, 71]. For example, oleic acid and linoleic acid were tested positive on the growth inhibition of either *group-A-streptococcus beta-hemolytic-non-A-streptococcus* or *Candida* in vitro [72]. This has to be differentiated from the adoption of triglycerides. Yet, there are also indications that bacterial enzyme activities might be increased by pellicle lipids [67].

Within the context of the observations described above, lipids deserve to be investigated thoroughly for their potential role as antiplaque agents in vivo, considering the physiology of lipid in the oral environment.

Physiology of lipids in the oral cavity

Lipids in human saliva

Saliva is an important protective system of the oral cavity, where it serves as a lubricant and buffer; several of its components were proved to have an anti-inflammatory or antimicrobial effect and it contains a diversity of inorganic substances important for the protection and repair of hard oral tissue [73].

Despite the extensive work done on some salivary constituents, only a few attempts have been made to examine the significance and quantification of its lipid components and data predominantly refer to the scientists around Slomiany. Their findings revealed essential aspects about the correlation between the individual lipid constitution of saliva and pellicle and the susceptibility to caries and periodontal diseases [74, 75]. Measurements regarding the total amount of lipids in whole human saliva vary from 8 to 10 mg/100 ml, mostly originating of glandular secretion. Only a small amount is believed to be the result of serum diffusion and cell exfoliation [76].

Studies based on saliva secreted by the major salivary glands identified 70–95 % of all lipids as nonpolar molecules varying from free fatty acids to cholesterol, cholesteryl esters, and mono-, di-, and triacylglycerols [77]. These neutral components are complemented by polar lipids such as glycolipids and 2–5 % phospholipids [17, 76]. Glycolipids mainly consist of glyceroglucolipids and even though they only account for 20–30 % of all salivary lipids, they appear to participate in several protective functions of human saliva [17, 78]. A summary of the major lipids contained in parotid and

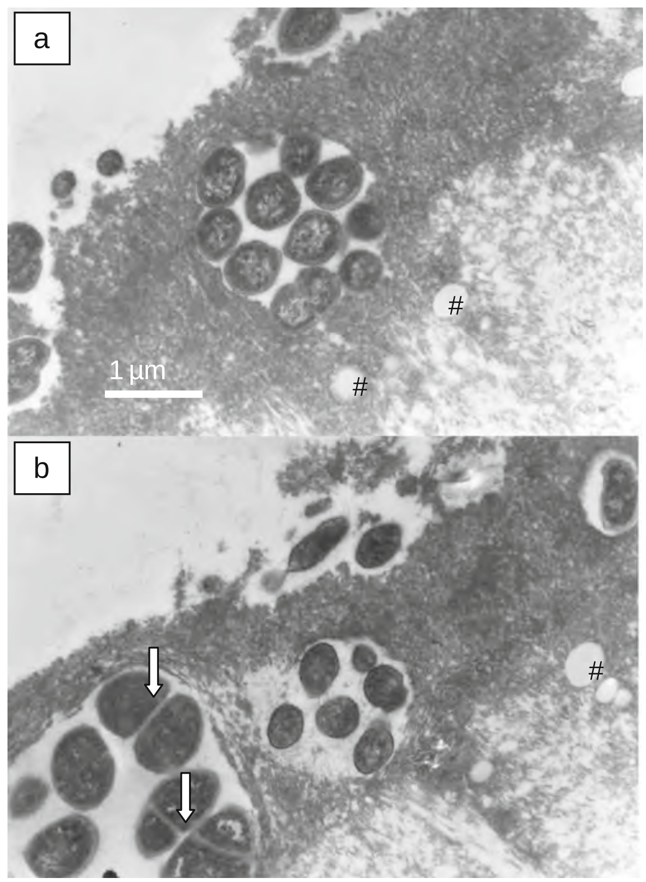

Fig. 4 Biofilm formation in situ over 6 h on pre-etched enamel after initial application of silicone oil. Silicone oil was used as a model for a hydrophobic and inert substance. The lacunae of the etched surface were filled with protein structures, covered by a typical mature pellicle layer. Please note the included oil droplets surrounded by ordinary pellicle structures (*number sign*). The general ultrastructure of the pellicle was not altered. Interestingly, groups of bacteria were detected in silicone oil drops surrounded by pellicle structures. It seems as if the bacteria preferred the hydrophobic milieu; inside these lipophilic drops, the bacteria are intact and viable, mitosis can be seen in some cases (*arrows*). The residual enamel structures were removed during preparation of the sample. Original magnification, 10,000-fold

submandibular saliva is given in Tables 1 and 2. Labial saliva contains four to five times more lipids per milliliter of saliva and exhibits significantly higher percentages of phospholipids and glycolipids than saliva secreted from parotid and submandibular glands [17, 79]. This might serve as a lubricant protecting the lips from dehydration.

The dynamic interaction between lipids, salivary proteins, and glycoproteins is regarded as determinant for many physical, chemical, and functional properties of saliva [17]. It was confirmed in several studies carried out by Slomiany that elevated levels of lipids in saliva are often associated with a higher incidence of caries and periodontal diseases [17, 74, 75, 80]. These observations might be derived by a facilitated bacterial adherence involving hydrophobic bonds or high levels of lysophosphatidylcholine might enhance glucosyltransferase activity promoting glucan formation and bacterial adhesion [67]. Glucoglycerolipids as well as phospholipids interact with proteins, particularly low and high molecular weight glycoproteins, either by hydrophobic forces or covalent linkage [17]. The function of those lipid–protein complexes is not fully clarified yet, but they seem to influence the viscoelastic and hydrophobic properties of the secretions. Additionally, salivary mucins have an important impact on the pellicle formation and epithelial integration of mucous tissue, interact with inorganic ions but might also serve as bacterial receptors [16, 81–83]. Compositional differences in lipid distribution referring to caries activity were observed in different studies and are summarized in Table 1.

The saliva of caries-resistant individuals contains smaller amounts of total lipids with a significantly lower percentage of neutral lipids and phospholipids whereas only minor differences occurred in the content of glyceroglucolipids. Although caries susceptibility does not directly refer to a certain group of associated fatty acids, Table 2 reveals a tendentially frequent appearance of elevated levels of stearic acid and docosanoic acid in salivary lipids of caries-susceptible individuals [74].

Interestingly, saliva of caries-susceptible individuals exhibits more lipids associated with mucous glycoproteins and although the mucins of both groups show similar protein and carbohydrate content, several differences were detected for their lipid distribution. Neutral lipids derived from mucus glycoproteins of saliva of caries-resistant individuals contain about 50 % more cholesterol, 38 % more cholestryl ester, and 32 % less triacylglycerols than the associated neutral lipids of salivary mucous glycoproteins of caries-susceptible individuals [17]. The phospholipids associated with glycoproteins of caries-resistant individuals have a higher content of phosphatidylethanolamine while those associated with mucus glycoproteins of caries-susceptible individuals are rich in sphingomyelin and phosphatidylcholine [17]. And also, the glycoproteins of caries-susceptible individuals contain significantly more bound fatty acids [84, 85] which make them less prone to peptic degradation and exhibit a lower buoyant density and more viscosity than those of caries-resistant individuals [16, 17]. Besides, it was reported that the saliva of heavy calculus formers contained about 50 % more lipids, larger amounts of free fatty acids in general, more cholesterol esters and glyceroglucolipids, whereas light calculus formers exhibit higher levels of triglycerides and free cholesterol [75].

In summary, it can be stated that elevated levels of specific lipids in saliva are associated with a higher incidence of caries and periodontal disease. As emphasized earlier, bacterial adherence is, among others, initiated by hydrophobic interactions [24], which might be facilitated by a lipid-rich environment (Fig. 1).

Lipids in the pellicle

There are considerable distinctions between the lipid composition of saliva and the pellicle [74, 86]. First of all, the amount of lipids incorporated in the pellicle is significantly higher. Although they account for 22–23 % of the pellicle's

Table 1 Average content and standard deviation of proteins and lipids in the parotid and submandibular saliva of caries-resistant (CR) and caries-susceptible (CS) individuals based on data provided by Slomiany et al. [160]

Constituent (mg/100 ml of saliva)	Parotid		Submandibular	
	CR	CS	CR	CS
Proteins	180.36±60.20	221.30±78.92	102.92±49.32	132.36±44.35
Total lipids	4.81±0.28	7.63±0.57	5.20±0.37	8.01±0.32
Neutral lipids	2.89±0.34	5.35±0.58	3.23±0.40	5.64±0.52
Free fatty acids	1.32±0.22	2.33±0.41	1.39±0.11	2.34±0.30
Mono- and diglycerides	0.09±0.02	0.11±0.03	0.12±0.03	0.19±0.04
Tricglycerides	0.58±0.11	0.98±0.14	0.60±0.15	1.34±0.19
Cholesterol	0.44±0.07	0.51±0.15	0.50±0.10	0.51±0.14
Cholesterol esters	0.46±0.08	1.42±0.35	0.62±0.13	1.26±0.37
Glycolipids	1.27±0.08	1.21±0.13	1.46±0.23	1.56±0.29
Phospholipids	0.09±0.02	0.12±0.03	0.10±0.02	0.15±0.03

Table 2 Fatty acid composition of parotid saliva lipids from caries-resistant (CR) and caries-susceptible (CS) subjects as measured by Slomiany et al. [160]

Fatty acid	Neutral lipids		Glycolipids		Phospholipids	
	CR	CS	CR	CS	CR	CS
Percent of total lipids						
16:0	23.1	12.0	9.8	14.9	29.1	13.8
16:1	3.3	3.8	1.2	3.3	2.5	2.3
18:0	26.9	27.2	20.5	36.5	23.3	32.3
18:1	23.2	9.9	15.9	14.9	19.3	16.8
18:0α-OH	2.7	2.7	2.2	2.6	1.3	2.8
20:0	1.9	–	9.2	11.4	5.5	5.7
20:1	–	–	3.8	–	–	–
22:0	8.0	36.8	4.9	11.1	10.7	16.4
24:0	4.1	2.4	–	–	–	4.1
24:1	3.6	2.7	4.7	3.0	4.8	4.0
24:0α-OH	1.7	–	–	–	–	–
26:0	–	–	10.9	–	–	–
26:1	–	–	12.3	–	–	–
Unidentified	1.5	2.5	4.6	2.3	3.5	1.8

dry weight little is known about their individual distribution and precise function [24]. The presence of phospholipids is suggested to have an important impact on the tenacity of the pellicle [87]. Therefore, compositional differences and the extent of interactions with proteins and glycoproteins of the pellicle could modulate bacterial adhesion [88]. Table 3 summarizes the distribution of different pellicle lipids. As shown, glycolipids are the major lipid fraction of the pellicle and might be a significant source of pellicle glucose [16, 88]. Very isolated studies indicate that inter-individual differences in the lipid composition of the pellicle might reflect differences in the caries activity of the individuals [87, 88]. In comparison to caries-susceptible subjects, the pellicle of caries-resistant subjects contained 42 % less neutral lipids and 31 % less phospholipids but had a higher proportion of cholesterol, cholesterol esters, and sphingomyelin, which might have a caries-protective effect [87]. Once more, advances concerning the value of pellicle lipids for the protection of the tooth tissue refer to the working group around Slomiany. They conducted an in vitro study which proved a considerable reduction of lactic acid retardation after extracting lipids out of the experimental pellicles of caries-resistant and caries-susceptible individuals by chloroform methanol followed by thin layer chromatography with hexane-diethyl ether acid [87]. In conclusion, lipid removal caused 50–52 % less lactic acid retardation capacity for the pellicles of caries-resistant subjects and 30–32 % less for the caries-susceptible ones [87]. Accordingly, a generally protective effect of the hydrophobic pellicle constituents against acidic noxae could be hypothesized. Yet, first experiments suggest that although rinses with safflower oil modulate the ultrastructural pellicle composition by a loose attachment of lipid micelles, they have a rather negative effect on the susceptibility of the pellicle against acids [49]. Electronmicroscopic investigations showed that the modified pellicle is of lower density than in controls and exposing these pellicle samples to hydrochloric acid with low pH values led to a remarkable pellicle degradation. But since all indications regarding the protective function of lipids in human saliva as well as in the formation of the acquired pellicle only refer to a few studies, additional research based on modern analytical techniques is required. This applies for the physiological lipid composition of the pellicle as well as for the impact of edible oils on the initial oral biofilm.

Methods for quantification of lipids in the saliva and in the oral biofilm

Analytical methods for determination of lipids are as various as the lipid classes themselves. Besides recently published books dealing with this topic [51, 89], there are also several journal reviews available [90–94]. Therefore, only a rough survey of the most important techniques for lipid analysis is given in Table 4 providing widely accepted references.

Table 3 Average lipid content and standard deviation of pellicles formed on enamel and cementum in vitro from saliva of caries-resistant (CR) and caries-susceptible (CS) subjects, based on data provided by Slomiany et al. [161]

Constituent (mg/100 mg)	Enamel		Cementum	
	CR	CS	CR	CS
Protein	36.68±4.21	44.64±5.83	34.73±5.11	47.62±5.24
Total lipids (determined gravimetrically)	22.36±3.80	24.61±3.65	19.20±3.07	23.43±3.47
Free fatty acids	2.24±0.21	4.67±0.58	1.79±0.25	5.16±0.64
Cholesterol	0.75±0.06	0.93±0.10	0.56±0.07	1.13±0.15
Cholesteryl esters	0.55±0.08	0.67±0.09	0.41±0.05	0.86±0.09
Triglycerides	0.60±0.07	1.06±1.20	0.49±0.06	1.13±1.24
Glycolipids	16.12±2.41	14.37±1.72	14.17±1.95	12.63±1.91
Phospholipids	2.10±0.32	2.90±0.31	1.78±0.20	2.52±0.22

Table 4 General survey of common, well-established analytical methods for lipid analysis. Journal reviews and books are listed as references

Analytical Method	Principle	Analytes	Reference
Gas chromatography (GC; GC/MS)	Separation by partition between a solid/liquid stationary phase and a gaseous mobile phase; detection most commonly with a FID or coupled with MS	Volatile compounds, determination of fatty acids after derivatisation	[51, 162, 163]
High-performance liquid chromatography (HPLC)	Separation between a solid stationary phase and a liquid mobile phase under high pressure; various detection possibilities including MS, UV, RI	Depending on the broad range of lipid classes depending on the detector	[91]
Thin-layer chromatography (TLC)	Separation between a solid stationary phase and a liquid mobile phase; detection most commonly via staining	Quick and inexpensive separation, identification of individual lipid classes	[92]
Mass spectrometry (MS) Soft ionization methods MALDI and ESI	Mass spectrometry converts molecules to ions. Resulting ions and their characteristic fragments are separated by their mass to charge ratio (m/z); ESI often coupled with HPLC	Direct analyte detection; multiple lipid classes; screening of biological tissue	[93, 100, 164, 165]
Nuclear magnetic resonance (NMR)	Nondestructive spectroscopic technique that gives detailed structure information	Basically all lipid classes; location of the position of double bonds; isomeric lipid analysis	[94, 166]

Independent of the specific analytical method, the first and crucial step of lipid analysis is the extraction of lipids from the matrix combined with cleanup by removing any nonlipid contaminants from the extract (e.g., proteins and polysaccharides). The importance of sample preparation is often underestimated and therefore carried out hurriedly and incorrectly [89]. It should be kept in mind that, in case of errors occurring during the extraction procedure, even the best analytics are worthless. A comprehensive summary of this sophisticated topic can be found in [95]. Several extraction procedures are found in scientific literature. The best described and most commonly used procedures were introduced by Folch et al. [96] and Bligh and Dyer [97]. As lipids are naturally occurring substances, there is a high risk of cross-contamination. Thus, various precautions concerning sample handling, solvents, and glassware have to be taken [89]. This is especially important for lipid analysis of pellicle samples, which represents a multistep procedure of minute quantities from specimen generation to the final chemical analytical determination.

Although salivary analysis has been considered important in terms of oral health for a long time [98], and the fact that there are notable indications that lipids play a significant role in this context, there is still a paucity of data on the nature and amounts of lipids in human saliva. Analytical methods predominantly used for this task are thin layer chromatography and gas chromatography coupled with flame ionization detector (GC-FID). Most of the data derive from studies carried out in the 1980s and refer almost exclusively to the workgroup around Slomiany [17, 75, 80, 86].

The analytical procedures used in all these salivary studies are basically the same. Samples are extracted with chloroform/methanol (2:1, v/v) and filtered to remove insoluble protein residue. The lipids dissolved in the extract are fractionated on silica columns into neutral lipids, phospholipids, and glycolipids, which are separated into their individual lipids by TLC, respectively. For identification, authentic standards were used. Fatty acid composition is determined by GC-FID after acidic methanolysis.

Regarding the nature, function, and composition of lipids in the acquired pellicle, the current state of research gives only preliminary information. Studies on pellicle composition are hampered by the fact that only limited amounts of pellicle material can be harvested and recovered from human teeth in vivo for analytical investigation [24]. In vitro pellicle samples on the other hand give only limited insight in the process of bioadhesion in the oral cavity. Accordingly, in situ approaches are most convenient. They are usually based on enamel slabs exposed to the oral fluids with aid of individual splints [48, 49]. Due to the high tenacity of the pellicle, extensive desorption procedures based on EDTA, sodium hypochloride, and ultrasonication are required to harvest the complete pellicle for purpose of analysis [99]. The existing knowledge of the lipids' protective properties for the pellicle is solely based on two publications [87, 88]. The methodology used for lipid determination is similar to that described above for salivary samples. Therefore, additional research with current analytical methods that are more specific and more sensitive such as gas chromatography coupled with mass spectrometry (GC/MS) are necessary to provide a more thorough understanding of the lipids involved in pellicle formation [100].

Vegetable oils—a natural source of lipids

The following considerations regarding the value of lipids in preventive dentistry will deliberately be focused on vegetable oils, as they are a natural, biocompatible, inexpensive, and worldwide accessible source of these substances [101].

Although health-promoting effects were also described for marine oils or essential oils their ubiquitous application is naturally restricted by the elaborate extraction. Last but not least, there are also concerns about the medical use of animal fats questioning their biocompatibility and religious as well as cultural aspects.

Vegetable oils are extracts obtained from oil plants and seeds, which have been diversely used in many cultures for centuries. Not only foods, but cosmetics, medical products, and technologies cannot be imagined without their incorporation.

In general, vegetable oils are composed of triglycerides and their fatty acids consist of 8–24 carbon atoms with saturation levels determined by the amount of double bonds [101]. Valuable oil is rich in mono- and polyunsaturated fatty acids, which are considered to have a health-promoting effect [102, 103]. Further important components of vegetable oils are liposoluble vitamins, lecithin, phytosterols, minerals, and trace elements [104]. The stability of vegetable oils against oxidation processes not only depends on the amount of unsaturated fatty acids but is also determined by the presence of antioxidants like phenols, chelating agents, and oxygen quenchers [105]. Different types of vitamin E, tocopherol α, -β, -γ, -δ, can be distinguished, acting as radical quenchers, preventing oxidation and stabilizing cell membranes of the human body [106, 107]. Additionally, polyphenols as secondary plant products are believed to have anti-inflammatory and antimicrobial effects [108–110]. Despite the similar basic elements, there are specific differences concerning the chemical composition of various vegetable oils.

Characteristics of typical vegetable oils and their relevance in preventive medicine

Over the last years, further knowledge has been gained about the impact of lipid components on human health. Especially the noticeable linkage between the dietary intake of certain lipids and coronary heart diseases as well as chronic degenerative diseases has repeatedly been examined and might offer potential prophylactic measures [111, 112]. There is decisive evidence that vegetable oils rich in unsaturated fatty acids have a health promoting effect: an enhanced consumption of unsaturated fatty acids can lower blood cholesterol levels and reduces the risk of atherosclerosis [113]. Omega-3 and omega-6 fatty acids are essential fatty acids. Besides their protective effect on the cardiovascular system, omega-3 fatty acids might also prevent dementia and macular degeneration [114–116]. Other publications discuss a possible protection against certain cancer types such as prostate, colorectal, or lung cancer, but relating epidemiological studies achieved inhomogeneous results [117].

Sesame seeds are one of the oldest crops used for the production of vegetable oil and today they are predominantly cultivated in Asian developing countries. This resulted in a manifold use of sesame oil, which is rich in unsaturated fatty acids, as a nutrient as well as an important component of topical cosmetics and traditional medical procedures [118–120]. In consideration of possible antimicrobial effects, it is promising that sesame oil was used for the preservation of fish approximately 3,000 years ago in Mesopotamia and even today several oils are recommended for the preservation of fresh nutrients [121]. Interestingly, the traditional Indian method of oil pulling, described as an effective adjunct for the prevention of oral maladies, was also initially recommended with sesame oil as it contains the antioxidant lignans sesamin, sesamolin, and sesaminol serving as natural preservatives [122]. It is performed to prevent diseases in the upper respiratory area, of the locomotor system, chronic diseases, tiredness, and infections of the oral cavity [47, 70, 122]. For the procedure, a tablespoon of edible oil is taken into the mouth and sipped and sucked between the teeth for 10–15 min [122]. Although there is no definite scientific evidence for this method yet, it is expected to help the excretion of toxic compounds such as heavy metals by the saliva. More recently, sunflower oil, which is common in Europe, has been suggested as an efficient alternative [123].

There is extensive literature referring to health-promoting benefits of olive oil as a dietary ingredient, commonly described as the Mediterranean diet [124, 125]. About 70 % of the olive oil's fatty acids are mono-unsaturated fatty acids, with oleic acid as the major compound [104, 126]. Additionally, olive oil contains a diversity of secondary plant products such as phenolic components; squalen; vitamins A, E, and K; and phytosterols [104, 110]. These substances are suggested to have an antioxidative, immunomodulatory, and antimicrobial effect. Therefore, olive oil is presumed to prevent coronary heart diseases, neural degeneration, cell damage, and even oral mal-odor [125, 127, 128].

Sunflower oil and rapeseed oil are very standard cooking oils whereas linseed oil is known to have a fishy taste and low stability against oxidation. Nevertheless, the particularly high amount of linolenic acid makes linseed oil valuable for the treatment of high serum cholesterol and triglycerides and hypertonia [129]. Furthermore, linseed oil was promoted to have an antibacterial effect on *Staphylococcus aureus* and dermatologic poultices seem to facilitate cell regeneration [130].

Safflower oil is considered as particularly valuable and healthy for the human consumption (Fig. 2). Two types of the oil have to be distinguished: one which is rich in mono-unsaturated oleic acid and another rich in linoleic acid [104]. The high content of unsaturated fatty acids, especially linoleic acid qualifies the oil for the reduction of cholesterol levels and might even have a positive impact on diabetes [131, 132]. Due to the dominance of essential ω-6-fatty acids, safflower oil was also widely spread as a traditional component of parenteral nutrition solutions [133]. However, uncertainty has emerged if an excess supply of linoleic acid could increase the production of proinflammatory lipid mediators [134].

Efficacy of vegetable oils and their derivatives for the prevention of oral diseases

Besides the positive effects on the general health constitution, traditional oil pulling is also suggested to prevent tooth decay, bleeding gums, and halitosis [47, 50, 70, 135–137]. Patients repeatedly described a positive impact on their oral tissues and although there is no clear scientific explanation to this phenomenon yet, several popular scientific sources promote the procedure as a daily supplement of oral hygiene. It was to be expected that there are numerous research findings on the effects of edible oils in preventive dentistry but there are only a few scientific studies. Some mouth care preparations, predominantly in the field of natural cosmetics, contain vegetable oils as ingredients, varying from "mouth-oil" to toothpaste or saliva substitutes [138]. It is important to note that their distribution is rather based on an empirical application, as previous findings and literature regarding the efficacy of vegetable oils are controversial and require further investigation. Nevertheless, a few studies attempt to scientifically prove a potential of vegetable oils to inhibit oral biofilm accumulation and to have a protective impact on the oral hard and soft tissue [48, 49, 70, 135]. The following will point out beneficial results that were obtained in vitro as well as in vivo and are derived by lipid components; however, further scientific data is needed to comprehend molecular interactions. For instance, a positive correlation between dietary fats and the fatty acid composition of the few enamel lipids was noticed in vitro [139, 140]. Certain fatty acids were shown to disturb bacterial adhesion as an in vitro study revealed for *S. aureus* under the influence of oleic acid [141].

Caries

If similarities apply for cariogenic bacteria, mouth rinses containing lipids could delay biofilm maturation and prevent carious lesions. The adhesion and total count of *S. mutans* were inhibited repeatedly by vegetable oil-based products in vitro [142, 143]. For example, in order to determine an inhibiting effect of olive oil on bacterial growth, *S. mutans* solutions were incubated with olive oil overnight and total viable counts conducted afterwards. Additionally, oil pretreated microscope slides were likewise incubated with *S. mutans* and again total viable counts were determined. Although none of the experiments were conducted under clinical conditions, they still arouse interest owing to their promising *S. mutans*-reducing effects [143]. Furthermore, Green and Hartles examined the impact of dietary lipids on caries prevalence and reported that the addition of 5 % groundnut oil to a cariogenic diet of rats could reduce the incidence of caries significantly [144]. A few attempts were undertaken to investigate the effects of rinses with edible oils under in vivo conditions [48, 70, 135, 143]. According to their knowledge about oil pulling as a traditional folk remedy, Asokan et al. conducted studies to investigate its effect on the prevention of caries as well as gingival inflammation. Their findings suggest that vegetable oils as a supplement for oral hygiene reduce the concentration of *S. mutans* in human saliva and certain fatty acids (e.g., linoleic and oleic) might inhibit bacterial regrowth [70]. Similarities were reported in vivo for almond oil, which additionally had a positive impact on the buffer capacity of human saliva [145]. Asokan et al. also proposed the process of "saponification", owing to the alkali hydrolysis of fat, as one explanation for the cleansing effect of vegetable oils [70]. However, it is not clear which lipids or other components of the oils are relevant for these partially observed effects, the interactions with the pellicle and with the initial oral biofilm have not been investigated sufficiently until now. However, as pointed out earlier, recent experiments including modern analytical techniques and transmission electron microscopic investigations did not confirm any positive impact of rinses with different edible oils on the bacterial colonization of enamel samples in situ [48].

Erosions

Research concerning demineralization derived by erosive processes indicated in vitro that vegetable oil mouth rinses might form a protective coating against acids as illustrated in Fig. 1. Since the extraction of lipids out of tooth tissue resulted in a faster progression of caries lesions in vitro, it was concluded that hydrophobic lipids act as a diffusion barrier [146]. Similarities were described earlier according to Slomiany et al. for the effects of the acquired pellicle [87]. Furthermore, vegetable oils might increase the lipid content in the outermost layer of hard tooth tissue or more likely in the pellicle [48, 140] (Fig. 2). In vitro studies, which questioned exactly those ideas, investigated the effect of olive oil-based emulsions on dentin demineralization [147, 148]. Once, bovine dentin samples were subjected to three demineralization cycles, each one lasting 8 h in a demineralization solution with an initial pH of 5.0 and the lesion depth as well as mineral loss were determined daily up to 9 days by microradiographs. In between, the samples were stored and pretreated in different olive oil emulsions (50 or 5 % olive oil), distilled water, or a 1,500 ppm fluoride solution. In summary, a small but significant decrease of mineral loss was observed for the samples pretreated by olive oil emulsions whereby the higher concentration showed a stronger protecting effect [148]. Comparable findings were presented for dentin and enamel samples by another in vitro study [147]: during 10 cycles, several concentrations of olive oil-containing preparations were applied to enamel and dentin samples before transfer to artificial saliva and 1 % citric acid exposure for 3 min. Afterwards, the samples were stored in artificial saliva for 30 min to enable remineralization and enamel and dentin loss were quantified using profilometric measurements. In conclusion, a significant reduction of mineral loss was noticed if samples were pretreated with 2 % olive oil. In turn, pure olive oil (100 %) was less effective which possibly relates to its low polarity and therefore less affinity to the tooth surface [147]. In contrast, first clear doubts regarding the protective effect of applied edible oils against erosive noxae were latest revealed [49]. For this purpose, enamel slabs were carried in the oral cavity for pellicle formation in situ and rinses with safflower oil were performed. Afterwards, the specimens were exposed to hydrochloric acid in vitro and the erosive mineral loss was determined by measurement of calcium and phosphate release. An even slightly increased loss of the respective minerals was recorded for the samples after application of the edible oil in situ as compared with unrinsed pellicles indicating diminished protective effects. The pellicles modified by rinsing with safflower oil were degraded faster in the acidic milieu [49]. It was postulated that the edible oil could have detracted relevant lipophilic pellicle components.

Periodontal diseases

In contrast to the dental hard tissues, the periodontal structures and especially the gingiva underlie a considerable turnover, lipids, and other components of edible oil can directly influence cellular structures, cell membranes, etc. Accordingly, the possible effects of oils have to be analyzed from a completely different point of view. In consequence of general microorganism removal, the use of vegetable oil as a mouth rinse might reduce gingival inflammation and therefore prevent tissue degradation [47]. Particularly for both *n*-3 and *n*-6 polyunsaturated fatty acids anti-inflammatory effects have been demonstrated in different in vitro models. According to their anti-inflammatory properties, the enrichment of certain fatty acids in oral care products might be useful [149] as clinical studies also observed benefits on periodontal diseases [150, 151]. In contrast, another study stated a less obvious impact on periodontitis and further data will be required [152]. Referring to an in vivo study, a daily mouth wash with sesame oil could decrease the plaque and gingival index and decline the total amount of aerobic microorganisms in the plaque within 10 days [47]. A gingivitis-reducing effect was also reported for other oils like sunflower oil, herbal oils, or essential oils [153]. Nevertheless, those findings currently lack a detailed scientific analysis as they primarily refer to the empirical observations of a folk remedy.

Mucosal infections

The increased utilization of plant cooking oils like perilla oil and soybean oil was reported to inhibit the incidence of minor recurrent aphthous stomatitis in vivo [154]. These promising findings mentioned above are of special interest for patients with xerostomia and pronounced oral inflammation, as such occur due to oral radiation therapy. Their mucosa appears exceedingly sensitive so that further irritation by sharp or burning mouth rinses should strictly be avoided [155]. In turn, microorganism removal is indispensible to prevent oral infections. An in situ study performed on irradiated rats examined the effect of sunflower oil on the manifestation of mucositis [156]. Although the side effect could obviously not be prevented it still appeared to emerge slightly retarded.

Discussion

Rinses with edible oils are a well-known practice in traditional folk medicine as well as in modern alternative medicine for the prevention and therapy of oral diseases. However, scientific evidence for the efficacy of these strategies is sparse and the physicochemical background of recorded effects has not been investigated adequately. This is surprising as in vivo or in situ studies with the safe/nonhazardous edible oils can be carried out easily [48, 49]. Despite modern ultrastructural investigations, most explanations for the effects of lipophilic substances in oral prophylaxis are rather theoretical than evidence based and unfortunately the few recent studies tend to vary a lot regarding their observations.

Typical examples are the suspected interactions with the tooth surface and the postulated hydrophobization, respectively, after rinses with vegetable oils. In vitro studies suggest the protection especially against acidic noxes, though the interactions with the ubiquitous pellicle layer have not been investigated completely until now [48, 49, 143, 147]. This applies especially for the lipid composition. Yet, latest in situ studies did not confirm the expected positive impact of oil rinses on oral diseases, especially with respect to the protection of dental hard tissues [48, 49]. There is no evidence that the lipids are bound tenaciously to the pellicle following rinses with oils. If so, it is uncertain whether they are really integrated into the ultrastructure of the pellicles or whether oil micelles only stick to them without providing any preventive effect. Figure 2 summarizes data gained by transmission electron microscopy that confirm the lipid accumulation at the pellicle's surface directly after rinses with vegetable oils [48]. Nevertheless, as pellicle formation is a highly selective process, tenacious and sustainable integration of relevant lipids in the pellicle seems doubtful and requires further research. It might also be postulated that rinses with oils could detract relevant lipophilic components from the pellicle or modulate the adsorption of other pellicle components as outlined in Fig. 2 [48, 64]. Until now, there are only two rather aged studies on the physiological lipid composition of the pellicle despite the potential relevance of this substance group [87, 88]. Modern analytical methods such as (GC; GC/MS) offer the opportunity to identify and to qualify the relevant lipid fractions in the pellicle layer though lipid analytics are still a considerable challenge [100]. Furthermore, it is not easy to deal with the small sample sizes which require distinct methodical approaches. Nevertheless, the effect of these interactions on erosion or bacterial adhesion also requires further research as potentially occurring lipid accumulation in the pellicle does not necessarily mean an improvement of protective properties [48, 49].

In situ studies based on enamel slabs exposed to the oral fluids are almost ideal to elucidate all these scientific questions. This allows harvesting of pellicle material for lipid analysis as well as fluorescence microscopic visualization and quantification of adherent bacteria to monitor the process of bioadhesion. Lipid accumulation at the pellicle's surface is not necessarily accompanied by a reduction of microbial adhesion as lipids could also promote hydrophobic interactions or even serve as substrates for certain bacteria [22]. Figures 3 and 4 show the discrepancy between lipid accumulation and their integration into the pellicle. Eventually, studies on lipids in the pellicle offer new insights into the process of bioadhesion in general.

Also the effects on the oral soft tissues and on the periodontal structures require further research based on prospective controlled clinical studies including adequate placebos. If protective effects are observed, it is necessary to identify the fractions of the edible oils relevant for the effects. Edible oils and foodstuffs rich in lipids contain a vast number of chemical substances with certainly different effects. Thereby, the phospholipids for example contained in soya milk are of special interest due to their interactions with mucins potentially making the pellicle more tenacious in contrast to triglycerides [87]. The identification of relevant groups of elements would allow the development of tailored biomimetic and biological oral healthcare preparations of high biocompatibility. Incidentally, comparable approaches are reported for dairy components that are thought to promote the anticariogenic properties of milk [41, 157]. All in all, only a few evidence based conclusions can be drawn with respect to the relevance and the efficacy of edible oils and lipids in general for preventive dentistry.

Conclusions

Lipophilic substances could be promising supplements to conventional dental prophylaxis for the prevention of gingivitis, periodontitis, and caries but the data so far available suggest very limited evidence. The modification of the pellicle structure and of bioadhesion processes seems to be the main target of rinses with edible oils. In any case, further research is required to understand the effect of oils and lipids on the composition and characteristics of the pellicle layer and on bioadhesion in the oral cavity.

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