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Effect of *Inula viscosa* on the pellicle's protective properties and initial bioadhesion *in-situ*

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ABSTRACT

Objectives: The present *in situ* study investigated the effect of *Inula viscosa* tea on the pellicle's acid protective properties and on initial oral biofilm formation.
Design: Biofilm formation was performed on bovine enamel slabs on individual maxillary splints. Following 1 min of pellicle formation, eight subjects rinsed for 10 min with *Inula viscosa* tea and the splints remained for 8 h intraorally. Samples carried after 1-min rinsing with CHX (0.2%) or without rinse served as controls. BacLight^{TM} staining, 4′,6-diamidino-2-phenylindole (DAPI)-staining and fluorescence *in situ* hybridization (FISH) were used for fluorescence microscopic detection of adherent bacteria. For investigation of acid protective properties, three subjects rinsed for 10 min with *Inula viscosa* tea after 1 min pellicle formation and kept the splints intraorally for further 19 min. Physiological 30-min pellicles and native enamel samples served as controls. After HCl incubation of the samples *ex-vivo* over 120 s (pH 2.0, 2.3, 3.0) calcium- and phosphate release were quantified photometrically. Potential influences on the pellicle's ultrastructure by *Inula viscosa* tea were evaluated by transmission electron microscopy (TEM).
Results: Application of *Inula viscosa* tea yielded a significant reduction of adherent bacteria on all enamel samples as detected by fluorescence microscopy. For calcium- and phosphate release no significant effect was recorded. TEM investigation indicated a modification of the pellicle's ultrastructure, but no enhanced protection against erosive noxae.
Conclusion: Rinsing with *Inula viscosa* tea influences the bacterial colonization on enamel *in situ* over 8 h but has no impact on acid protective properties of the pellicle.

1. Introduction

Despite the general global caries decline in the past decades, caries is and remains one of the greatest challenges in dentistry (Kassebaum et al., 2015). Furthermore, epidemiological studies indicate an increasing prevalence of dental erosion in all age groups (Jaeggi & Lussi, 2014). Starting point of caries initiation is the bacterial attachment to and further accumulation on the tooth surface with following sucrose metabolization to form organic acids in a three-dimensionally organized biofilm; whereas dental erosion is due to intrinsic (diseases as reflux esophagitis, bulimia nervosa) or extrinsic (eating and drinking habits) acids without bacterial involvement (Lussi, Schlueter, Rakhmatullina, & Ganss, 2011; Schlueter & Tveit, 2014). However, both diseases are finally leading to an irreversible loss of dental hard tissues. In view of the increasing prevalence of carious infections, especially in socially deprived people (Bagramian, Garcia-Godoy, & Volpe, 2009), adjuvant and cost-efficient strategies are desirable for dental prophylaxis. Researchers are currently focusing on natural substances present in foods and vegetables which have been shown to promote oral health and reduce risk for many common diseases (Karygianni et al., 2014; Zhang et al., 2014). In this context, polyphenolic compounds for example from the leaves of *Camilla sinensis* (catechins), the natural beehive product propolis

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(flavonoides and terpenoids), cacao bean husk extracts (oleic and linoleic acids), cranberry (proanthocyanidin) and traditional plants (*Melia azedarach*) have been exclusively studied for their antimicrobial activities against cariogenic bacteria, especially against *Streptococcus mutans* and *Streptococcus sobrinus* and, moreover, for their inhibitory effects against glucan synthesis through bacterial glucosyltransferases (Della Bona & Nedel, 2015; Koo et al., 2010; Koo, Pearson et al., 2002; Koo, Rosalen, Cury, Park, & Bowen, 2002; Ooshima et al., 2000; Osawa et al., 2001; Otake, Makimura, Kuroki, Nishihara, & Hirasawa, 1991). However, most of these studies have been performed *in vitro*; only few studies have been conducted *in vivo* using animal models of dental caries (Otake et al., 1991). Accordingly, there is a strong demand for additional studies to evaluate the clinical efficacy of these and other natural substances. It has been shown that selected polyphenolic plant extracts are suitable for intentional modification of the *in-situ*-pellicle, a proteinaceous layer formed immediately on the tooth surface that mediates the interactions between the tooth surface and the surrounding oral fluids (Hannig & Joiner, 2006; Joiner, Müller, Elofsson, Malmsten, & Arnebrant, 2003; Joiner, Müller, Elofsson, & Arnebrant, 2004). The pellicle contains structural components with antibacterial properties but also presents specific receptors for bacterial adherence (Hannig & Joiner, 2006). Furthermore, the pellicle serves in a limited scale as protection barrier during acid attacks (Hannig & Hannig, 2014). As demonstrated *in situ*, rinses with certain teas and watery plant extracts rich in polyphenols resulted in an enhanced resistance of the pellicle against erosive noxae and further, led to a significant reduction of bacterial colonization of the tooth surface (Hannig, Spitzmüller, Al-Ahmad, & Hannig, 2008; Weber, Hannig, Pötschke, Höhne, & Hannig, 2015).

The medicinal plant considered in the present study is *Inula viscosa (Dittricha viscosa)*, a flowering plant in the daisy family with narrow leaves and characteristic yellow heads. Due to its pronounced adaptation behavior the plant is found in Southern Europe, Turkey and Middle East and further as a neophyte in Belgium, United Kingdom and North America. Since ancient times *Inula viscosa* is an integral part of Mediterranean folk medicine and has antimicrobial, antifungal and anti-inflammatory properties which are attributed to the high content of phenolic acids and flavonoids (Karygianni et al., 2014; Talib, Zarga, & Mahasneh, 2012). In detail, *Inula viscosa* was used in treatment of wound supplies, bruises, bronchitis, diabetes, tuberculosis, malaria, scurvy and rheumatic complaints (Hernandez, Recio, Manez, Giner, & Rios, 2007; Manez, Hernandez, Giner, Rios, & Recio Mdel, 2007). In addition, antibacterial effects of *Inula viscosa* were described against *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus oralis*, *Porhyromonas gingivalis* and *Provotella intermedia* (Karygianni et al., 2014). Therefore, a promising potential in caries prevention can be assumed. Additionally, a modification of the pellicle with enhanced protective properties against erosion would be conceivable.

The aim of the present *in-situ* study was to evaluate whether the rinsing with *Inula viscosa* tea has an influence on the bacterial colonisation of enamel after 8 h. For this purpose, fluorescence microscopic approaches were used. Furthermore, the impact of *Inula viscosa* tea on the acid protective properties of the pellicle was investigated by determination of calcium and phosphate release during exposure to hydrochloric acid. Moreover, the potential modification of the pellice's ultrastructure was visualized by transmission electron microscopy.

2. Materials and methods

2.1. Subjects

Eight healthy volunteers (age 22–29) had agreed to participate in this study. According to the visual oral examination by an experienced dentist, oral diseases such as caries, periodontitis or erosions could be excluded. During examinations, physiological salivary flow rates of all subjects were recorded and good oral hygiene with plaque index scores close to zero was assessed. Informed written consent had been given by the volunteers about participation in the study. The study design was reviewed and approved by the Ethics Committee of the Medical Faculty, TU Dresden, Germany (Vote EK 147052013).

2.2. Tea preparation

The dried *Inula viscosa* leaves originated of plants from Syria. The tea was brewed by hand daily; a volume of 200 ml boiling hot water was added to 3 g of *Inula viscosa* leaves and infused for 10 min. The rinses were performed with 200 ml of naturally cooled tea (25 °C).

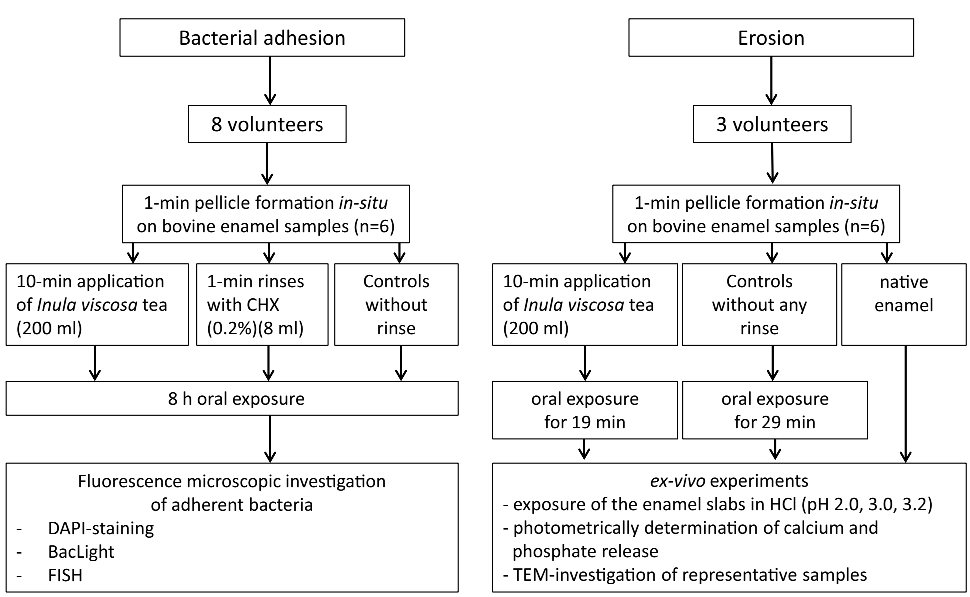

Fig. 1. Flow chart of the experiments.