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Development of a Remineralizing Saliva Substitute and Effects of Various Saliva Substitutes in Combination with Fluorides on Enamel and Dentin

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“Der Grund dafür, daß unser fühlendes, wahrnehmendes und denkendes Ich in unserem naturwissenschaftlichen Weltbild nirgends auftritt, kann leicht in fünf Worten ausgedrückt werden: Es ist selbst dieses Weltbild. Es ist mit dem Ganzen identisch und kann deshalb nicht als ein Teil darin enthalten sein.”

Erwin R. J. A. Schrödinger
Physiker, 1887 - 1961

TABLE OF CONTENTS

1. INTRODUCTION	1
2. SCIENTIFIC BACKGROUND	3
3. OBJECTIVES OF THE PRESENTED STUDIES	4
4. PRESENTATION OF ORIGINAL MANUSCRIPTS	5
5. DISCUSSION	6
5.1. DISCUSSION OF MATERIALS AND METHODS	6
5.1.1. Human versus Bovine Teeth	6
5.1.2. Hard Tissue Substrates: Enamel and Dentin	6
5.1.3. Artificial Caries-Like Lesions	7
5.1.4. Study Duration	7
5.1.5. Fluoride Application Protocols	7
5.1.6. Transversal Microradiography	8
5.2. DISCUSSION OF THE RESULTS	9
5.2.1. Basic Storage Solutions	9
5.2.2. Mouthrinses versus Gel versus Toothpaste Application	10
5.2.3. Erosions Observed with Fluoride Gels	11
5.2.4. BMC Oral Health Discussion	11
6. CONCLUSION	13
7. SUMMARY	14
8. ZUSAMMENFASSUNG	15
9. REFERENCES	16
10. ACKNOWLEDGMENTS	21
EIDESSTATTLICHE ERKLÄRUNG NACH § 4 ABS. 3 (K) DER HABOMED DER CHARITÉ	22

ABBREVIATIONS

CaF ₂	calcium fluoride
CMC	carboxymethylcellulose
DCPD	dicalcium phosphate dihydrate
D/DP	Duraphat toothpaste
EG	Elmex gelée
ES	Elmex sensitive mouthrinse
FA	fluoroapatite
G	Glandosane
HA	hydroxyapatite
LD	lesion depth
M	Meridol mouthrinse
OCP	octacalcium phosphate
PS	ProSchmelz fluoride gel
S	saturation
SL	surface layer
SN	modified Saliva natura
TMR	Transversal microradiography
w	water
ΔLD	change in lesion depth
ΔZ	mineral loss
ΔΔZ	change in mineral loss

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1. INTRODUCTION

Human saliva influences various reactions in the oral cavity, such as caries protection, digestion and immunological processes. Therefore, physiological amounts of salivary secretion are essential for oral health [Brosky, 2007; Kielbassa and Meyer-Lueckel, 2002]. The ability to promote remineralization and to reduce demineralization makes saliva a major player in caries protection [Dowd, 1999]. Moreover, this fluid is implicated in a wide variety of digestive events, including lubrication of mucosa, bolus formation and enzymatic digestion of food [Pedersen et al., 2002]. Saliva's protective role in humans is exhibited by the delivery of antimicrobial peptides and proteins to the oral epithelium [Abiko et al., 2003; Tschoppe and Kielbassa, 2011]. The objectively decreased flow of saliva is known as hyposalivation, which can be caused by water or metabolite loss, damage of salivary glands and interference with neural transmission. Furthermore, common reasons for decreased salivary secretion are chronic inflammation of the salivary glands, Sjögren's syndrome, radiation treatment, dehydration, psychological factors and medications [Mese and Matsuo, 2007; Nederfors, 2000; Tschoppe et al., 2010]. In addition, in the presence of reduced salivary flow, oral functions (e.g., chewing, speech, and swallowing) are hampered because the wetting and lubrication of mucosal surfaces and the moistening of food items are insufficient [Atkinson et al., 2005]. A reduced salivary flow inhibits the transport and solubility of gustatory stimulants, leading to decreased gustatory stimuli and reduced excitability of taste buds.

Patients suffering from hyposalivation frequently experience high caries activity. As most patients suffering from hyposalivation are the elderly, gingival recession and subsequently exposed dentin surfaces are very common within this group of patients. Because the solubility of dentin is higher than that of enamel, earlier and more severe demineralization can be expected [Saunders and Meyerowitz, 2005]. The relationship between higher caries prevalence and hyposalivation has been demonstrated in patients irradiated for tumors in the head and neck region [Atkinson et al., 2005; Beetz et al., 1999; Kielbassa, 1999; Kielbassa et al., 2006]. The increased caries incidence in these patients is associated with the side effects of hyposalivation, such as reduced antibacterial function, impaired remineralization of dental hard tissues, lowered pH and reduced buffering capacity, as well as compromised self-cleaning effects. In addition to the consumption of soft foods with high carbohydrate content and the use of cariogenic saliva stimulants, these alterations stimulate the rapid onset and progression of caries [Dreizen et al., 1977; Kielbassa et al., 2006; Vissink et al., 2003].

Moistening of the oral mucosa with saliva substitutes is a widely used alternative for relieving hyposalivation symptoms [Atkinson et al., 2005; Hahnel et al., 2009; Nieuw Amerongen and Veerman, 2003]. A number of artificial saliva products have been developed for the palliative treatment of patients with salivary hypofunction. These products, which are based on hydrophilic polymers, act to replace or boost salivary film [Urquhart and Fowler, 2006]. In this regard, artificial saliva has proven to be at least partially effective in relieving the subjective symptoms

of hyposalivation [Hahnel et al., 2009; Momm et al., 2005]. From a dental point of view, it would seem reasonable to expect that saliva substitutes will not damage sound dental hard tissues. Moreover, because patients with extensive mouth dryness are particularly susceptible to caries, a remineralizing effect of the artificial saliva on initial caries lesions would be most preferable. However, some saliva substitutes have displayed the capacity to demineralize both dental hard tissues (enamel and dentin), which may be disadvantageous for dentate patients suffering from hyposalivation [Joyston-Bechal and Kidd, 1987; Kielbassa and Shohadai, 1999; Meyer-Lueckel et al., 2002; Smith et al., 2001]. Glandosane, a carboxymethylcellulose (CMC)-based solution, is a widely used commercially available product that has shown detrimental demineralizing effects on both enamel and dentin [Kielbassa et al., 2001; Meyer-Lueckel et al., 2002; Smith et al., 2001; Tschoppe et al., 2007].

The use of fluoride-containing products is considered an important requirement for caries prevention in patients with hyposalivation. From a practical point of view, it might be advantageous to use demineralizing saliva substitutes in combination with fluoride products that are capable of preventing demineralization and/or remineralization of dental hard tissues. So far, fluoride gels or mouthrinses that are applied several times daily are recommended in addition to the use of traditional toothpastes (e.g., 1,500 µg F/g) to prevent carious lesions in patients with hyposalivation [Kielbassa et al., 1997a; Kielbassa et al., 1998; Meyerowitz et al., 1991; Papas et al., 2008; Spak et al., 1994]. In particular, for patients with hyposalivation resulting from radiation to the head and neck region, long-term compliance with two-step regimens (i.e., toothpaste and fluoride gel) is quite poor [Epstein et al., 1996; Horiot et al., 1983]. Recently, toothpastes with fluoride levels up to 5,000 µg F/g have been developed for “high-risk individuals”. Therefore, the use of toothpastes without the application of additional mouthrinses or fluoride gels may increase patient compliance. Preferably, a remineralizing saliva substitute could address both outcomes (i.e., dental caries and hyposalivation) while avoiding the additional use of highly concentrated fluoride agents (e.g., mouthrinse, gel or highly fluoridated toothpaste). Nevertheless, only limited information exists in the literature regarding the effects of saliva substitutes in combination with fluoride products on the remineralization of early enamel and dentin lesions.

The aims of the postdoctoral thesis are a) to evaluate the effect of a new commercially available saliva substitute (Saliva natura) on enamel and dentin, b) to assess the effects of various modifications of Saliva natura with respect to calcium phosphates on both dental hard tissues and c) to analyze the treatment effects of different fluoride agents (e.g., highly concentrated fluoride toothpaste, mouthrinses and fluoride gels) in combination with de- and remineralizing saliva substitutes, as well as with water. Furthermore, a design for a randomized controlled double-blind crossover clinical trial to assess the effects of saliva substitutes on bovine enamel and dentin *in situ* is presented.

The present work represents a cumulative habilitation thesis. First, an overview is given of the scientific background. Next, the formulation of the main objectives and hypotheses of the included studies, as well as the studies behind these ideas, are outlined.

2. SCIENTIFIC BACKGROUND

Tschoppe P, Wolgin M, Pischon N, Kielbassa AM

Etiologic factors of hyposalivation and consequences for oral health.

Quintessence Int 2010;41:321-333.

3. OBJECTIVES OF THE PRESENTED STUDIES

Study A - The purpose of this study was to evaluate the effects of a newly developed saliva substitute (Saliva natura; medac, Hamburg, Germany) on mineral loss and lesion depths of demineralized (subsurface lesions) bovine enamel and dentin *in vitro*. Glandosane (cell pharm, Hanover, Germany) was used for comparative reasons, and an aqueous remineralizing solution served as positive control. The null hypothesis (H_0) was that the two saliva substitutes would not differ significantly in their remineralizing capacities as compared to the positive control.

Studies B1 and B2 - These two studies were undertaken to assess the effects of modified solutions with respect to the degree of saturation of calcium phosphates (main focus on precursors of remineralization: OCP and DCDP) of a commercially available saliva substitute on mineral loss and lesion depth of demineralized bovine enamel (B1) and dentin (B2) *in vitro*. The null hypothesis (H_0) was that saliva substitutes with varying degrees of saturation with respect to calcium phosphates do not differ significantly in their remineralizing capacities compared to the positive control.

Studies 1 and 3 - These *in vitro* studies aimed to determine whether daily applications of different fluoride products (e.g., mouthrinses and/or fluoride gels) could influence the de- and remineralization of subsurface bovine enamel and dentin lesions stored in two saliva substitutes. Non-carbonated mineral water was used as a control. The null hypotheses (H_0) tested were that daily applications of fluoride products in combination with 1) a known demineralizing saliva substitute (Glandosane) would not result in significantly less pronounced demineralization, and 2) a possibly remineralizing artificial saliva (supersaturated with respect to OCP and DCPD) would not result in significantly enhanced remineralization.

Study 2 - Toothpastes with up to 2,800 $\mu\text{g F}^-/\text{g}$ are widely used in the prevention of dental caries [Biesbrock et al., 2001]. Recently, toothpastes with fluoride levels up to 5,000 $\mu\text{g F}^-/\text{g}$ have been developed for "high-risk individuals," which are now available with prescriptions. In particular, for patients with hyposalivation resulting from radiation to the head and neck region, long-term compliance with two-step regimens (i.e., toothpaste and fluoride gel) is quite poor [Epstein et al., 1996; Horiot et al., 1983]. Therefore, the use of toothpastes without the application of additional fluoride gels may increase patient compliance. Thus, study 2 evaluated the effect of daily application of highly concentrated fluoride toothpaste and/or fluoride gel in combination with de-/remineralizing saliva substitutes on enamel subsurface lesions. Non-carbonated mineral water was used as a control. It was hypothesized that the additional use of a fluoride agent in combination with a demineralizing saliva substitute would not result in a significantly less pronounced demineralization effect and that use of the remineralizing saliva substitute would not result in significantly enhanced remineralisation. These null hypotheses were tested against the alternative hypotheses that assumed differences.

Study 4 - The last paper involved a study protocol of a randomized controlled double-blind crossover phase II/III clinical trial to assess the effects of saliva substitutes on bovine enamel and dentin *in situ*. In a detailed report, the characteristics, challenges and limitations of an *in situ* study are explained.

4. PRESENTATION OF ORIGINAL MANUSCRIPTS

- Study A:** P. Tschoppe, H. Meyer-Lueckel, R. Toll, A. M. Kielbassa
[*In vitro* analysis of a new saliva substitute (Saliva natura on enamel and dentin)]
Laryngo-Rhino-Otologie 2007; 86(10):723-727
- Study B1:** P. Tschoppe, A. M. Kielbassa, R. Toll, H. Meyer-Lueckel
[Modification of the mineralizing capacity of a saliva substitute (Saliva natura) on enamel *in vitro*]
Laryngo-Rhino-Otologie 2009; 88(11):717-722
- Study B2:** P. Tschoppe, A. M. Kielbassa, H. Meyer-Lueckel
Evaluation of the remineralizing capacity of modified saliva substitutes *in vitro*
Arch Oral Biol 2009; 54(9):810-816
- Study 1:** H. Meyer-Lueckel & P. Tschoppe
Effect of fluoride gels and mouthrinses in combination with saliva substitutes on demineralised bovine enamel *in vitro*
J Dent 2010 38(8):641-647
- Study 2:** P. Tschoppe, A. Siegel, H. Meyer-Lueckel
Saliva substitutes in combination with highly concentrated fluorides and brushing.
In vitro effects on enamel subsurface lesions
Caries Res 2010 44(6):571-578
- Study 3:** P. Tschoppe & H. Meyer-Lueckel
Mineral distribution of artificial dentinal caries lesions after treatment with fluoride agents in combination with saliva substitutes
Arch Oral Biol 2011 56(8):775-784
- Study 4:** P. Tschoppe, O. Wolf, M. Eichhorn, P. Martus, A. M. Kielbassa
A randomized controlled double blind crossover clinical trial to assess the effects of saliva substitutes on bovine dentin and enamel *in situ*
BMC Oral Health 2011 11:1-13

5. DISCUSSION

5.1. DISCUSSION OF MATERIALS AND METHODS

5.1.1. HUMAN VERSUS BOVINE TEETH

Bovine teeth were used in the present *in vitro* and *in situ* studies. Regarding the origin of the substrates, human teeth can be regarded as the most appropriate source from a clinical perspective. However, their composition is variable due to genetic influences, environmental conditions (e.g., diet, fluoride exposure and previous caries challenges) and age (e.g., post-eruptive maturation and dentin sclerosis). These differences lead to large variations in their test responses under cariogenic challenges [Mellberg, 1992; Ogaard and Rolla, 1992]. Bovine teeth are more readily available and have a more uniform composition than human teeth, which provides a less variable response to both cariogenic challenges and anti-caries treatments such as fluoride dentifrices [Lynch, 2006; Mellberg, 1992]. Additionally, bovine teeth have a larger surface area, which enables easily experimental manipulation. Furthermore, bovine teeth present higher levels of porosity, which facilitate a faster diffusion of ions into the demineralized areas [Featherstone and Mellberg, 1981; Lynch et al., 2006]. However, these differences result in quantitative rather than not qualitative differences in behavior [Edmunds et al., 1988]. Additionally, the artificial carious lesions produced from bovine teeth have mineral distributions and structures that resemble lesions produced from human teeth, both for enamel and dentin [Featherstone and Mellberg, 1981; Hara et al., 2003; Mellberg, 1992]. Thus, bovine teeth can be considered an acceptable alternative to human teeth in cariology research [Mellberg, 1992].

5.1.2. HARD TISSUE SUBSTRATES: ENAMEL AND DENTIN

Considering the different types of mineralized dental tissues, enamel and dentin have very different structures and compositions, which in turn influence their susceptibilities to dental caries. Basically, permanent enamel is composed of minerals (85% of the total volume) in the form of hydroxy- or fluoroapatite crystals organized in prisms [Goldberg et al., 1995]. Upon a cariogenic challenge ($\text{pH} \leq 5.5$), hydroxyapatite crystals dissolve from the subsurface, while fluoroapatite crystals are deposited at the surface, which produces a subsurface lesion. Hence, the dissolution process is merely a chemical event [Featherstone, 2004].

In contrast, permanent dentin contains 47% apatite, 33% organic components (90% collagen and 10% non-collagenous proteins) and 20% water according to volume. The mineral phase is hydroxyapatite, which is similar to enamel, but the crystallites have much smaller dimensions [Goldberg et al., 1995]. This results in a much larger surface area to crystallite volume ratio and, therefore, a more reactive mineral phase. The organic matrix is composed mainly of collagen. The dentinal demineralization rate decreases when the amount of degradable collagen increases, whereby the demineralized matrix may hamper ionic diffusion both into and out

of the demineralizing areas [Klont and ten Cate, 1991]. One should note that during carious lesion formation *in vivo*, teeth with vital pulpo-dentinal organs respond to most exogenous stimuli through the deposition of minerals along and within the dentinal tubules [Frank and Voegel, 1980]. This phenomenon, coupled with the outward flow of dentinal fluid from the pulp, may significantly reduce the rate of *in vivo* lesion progression in dentin compared to an *in vitro* situation [Shellis, 1994]. Furthermore, periodontal diseases occur predominantly in elderly patients and often lead to gingival recessions [Albandar and Kingman, 1999]. Additionally, progressive attrition/abrasion during prolonged utilization of teeth cause dentinal exposure [Van't Spijker et al., 2009]. Because dentin is not as resistant to acid exposure as enamel, earlier and more severe demineralization can be expected [Saunders and Meyerowitz, 2005]. As such, carious lesions easily develop at the cervical areas of teeth [Kielbassa, 2000; Kielbassa et al., 1999a]. Therefore, in the present study, both enamel and dentin were assessed.

5.1.3. ARTIFICIAL CARIES-LIKE LESIONS

A subsurface type of lesion with a well-mineralized surface layer is one of the most specific enamel and dentinal characteristics that can be clinically observed in initial carious lesions [Arends and Christoffersen, 1986; Schupbach et al., 1989]. Therefore, specimens were demineralized to create subsurface lesions with mineral profiles similar to typical natural carious lesions. However, artificial caries-like lesions were separately created for each study. In this context, it is important to consider that the slightly different levels of surface layer thickness may have influenced the demineralization and remineralization processes [Lynch et al., 2007] and, subsequently, may have also influenced the results.

5.1.4. STUDY DURATION

After demineralization (i.e., creation of artificial subsurface lesions), specimens were stored for five weeks (with an additional evaluation after two weeks) in different solutions (i.e., control solutions or different saliva substitutes). This period can be considered as an intensive contact period that is not predictable under clinical conditions. However, saliva substitutes are administered *ad libitum*, and generally, a maximum daily dose is not recommended for patients with hyposalivation [Beetz et al., 1999; Meyer-Lueckel and Kielbassa, 2002]. Thus, similar observations might be expected after long periods *in vivo*. Furthermore, evaluating the effect after two weeks enables the possibility of investigating mineral changes over time. Therefore, a more detailed insight into the mechanism of de- and remineralization is possible [Damen et al., 1997].

5.1.5. FLUORIDE APPLICATION PROTOCOLS

To investigate the possible benefits of fluoride application in combination with saliva substitutes on enamel and dentin mineralization, specimens with subsurface lesions were stored in different

saliva substitutes (i.e., CMC-based experimental saliva substitute/Glandosane and modified Saliva natura) or water and additionally treated either with Meridol mouthrinse (250 µg F/g; GABA, Lörrach, Germany), Elmex sensitive mouthrinse (250 µg F/g; GABA), Elmex gelée (12,500 µg F/g; GABA), ProSchmelz fluoride gel (12,500 µg F/g; GSK, München, Germany) and/or Duraphat toothpaste (5,000 µg F/g, Colgate, Hamburg, Germany). To simulate the *in vitro* clinical application of each product closely, three different protocols for fluoride application were used twice daily. For patients undergoing post-radiation therapy, rinsing with a fluoride mouthrinse solution or the application of a fluoride gel with a customized mouth-tray for longer times than that used in persons without salivary flow diseases is recommended [Spak et al., 1994; Wei and Yiu, 1993]. Even longer periods may be advised until normal salivation rates are restored [Epstein et al., 1996b; Papas et al., 2008]. Mimicking this scenario, specimens were either stored in the mouthrinses (10 min), or gels were applied on top of the specimens without any force for 10 min. According to the third protocol, specimens were brushed with a homogeneous toothpaste/saliva substitute slurry (ratio 1:3) for 10 s, resulting in a total slurry contact time of 130 s. This ratio is in accordance with the European standards for preparing artificial saliva/toothpaste slurries (EN ISO 11609). The specimens were gently brushed with an electric toothbrush by the same operator. Although the study did not utilize a completely standardized procedure (i.e., using a brushing machine), the investigation involved different forces that likely averaged out during the study period of 35 days.

5.1.6. TRANSVERSAL MICRORADIOGRAPHY

Microradiography is a well-known and widely accepted tool for the quantification of mineral loss based on the attenuation of X-ray irradiation that transmits dental hard tissues. X-ray photons transmitting a dental hard tissue specimen can be recorded by an X-ray sensitive film. The mineral mass can be calculated by determining photographic density measurements calibrated by an aluminum step-wedge [de Josselin de Jong et al., 1988]. Subsequently, microradiography has been frequently used in studies determining mineral changes due to both de- and remineralization in terms of caries. In the presented studies, transversal microradiography (TMR) was used as an analytical technique to measure mineral loss and lesion depths. TMR is a tool for the quantitative assessment of the mineral content as a function of depth from the surfaces of caries and caries-like lesions. From the in-depth profiles, lesion depth and mineral loss integrated over the entire depths (ΔZ) of lesions can be calculated. Lesion depth usually is defined up to the point where the mineral content reaches 95% of the mineral content of sound enamel or dentin [Arends and ten Bosch, 1992; Damen et al., 1997]. Generally, various other experimental methods are available for analyzing artificial caries-like lesions. These include polarized microscopy [Crabb and Darling, 1956; Kielbassa and Shohadai, 1999], microhardness testing [Buchalla et al., 2008; Kielbassa et al., 1999b], electric caries monitoring [Petersson and Kambara, 2004; Wolinsky et al., 1999], transversal wavelength-independent microradiography [Thomas et al., 2006], optical coherence tomography [Holtzman et al., 2010], and scanning

electron microscopy [Arends et al., 1987; Kielbassa et al., 1997; Shellis and Hallsworth, 1987]. With the exception of transversal microradiography, all of the abovementioned technologies are associated with certain shortcomings with regard to accuracy when specimens are analyzed according to mineral loss and lesion depth. In contrast, TMR allows a direct measurement of the longitudinal mineral distribution as a profile in a subsurface lesion, and it has long been established and recognized as the gold standard for analyzing mineral content changes over time [Damen et al., 1997]. The mineral loss ΔZ (vol% \times μm) has been used mostly for quantitative purposes in *in vitro* studies. However, the ΔZ value does not take the profile difference (of mineral in the lesion) into account. Therefore, the effect of the distribution profile on demineralization and remineralization has not been studied in depth [Kawasaki et al., 2000; Tschoppe and Kielbassa, 2011; Tschoppe et al., 2008]. Although gaining full understanding of the remineralization process may be difficult, both physical and chemical processes may be relevant. The site and the amount of mineral deposition are probably determined by the physical conditioning (e.g., mineral distribution profile and transport mechanism) and by the chemical process (e.g., deposition). Recently, it was demonstrated that changes in diffusion patterns strongly affected the progress of demineralization in the depth direction in both enamel and dentin [Ruben et al., 1999]. This implies that the diffusion (or transport) process and geometry of the lesion are important factors in the mineralization process. Therefore, the mineral distribution profile of the lesion should be considered one of the most important parameters in the study of remineralization. Subsequently, the evaluation of surface layers and inner lesion areas have been conducted in both study B2 and study 3, which have provided more detailed insight into the de- and remineralization processes.

5.2. DISCUSSION OF THE RESULTS

5.2.1. BASIC STORAGE SOLUTIONS

Non-carbonated mineral water was used as a control solution because moistening of the mouth with water is a simple and inexpensive technique frequently used by many patients with hyposalivation to alleviate their oral symptoms. In all of the conducted studies as well as in another study [Zandim et al., 2010], mineral water showed a neutral effect due to its pH value (7.0) and its undersaturation state with respect to OCP and DCPD ($S_{\text{OCP}} = 0.7$ and $S_{\text{DCPD}} = 0.2$). The commercially available saliva substitute Glandosane (cell pharm, Hanover, Germany) demonstrated remarkable demineralizing effects in all of the conducted studies. Similar effects have been previously observed [Kielbassa et al., 2001; Meyer-Lueckel et al., 2002; Smith et al., 2001; Zandim et al., 2010]. Glandosane is a carboxymethylcellulose-based solution with a pH value (5.2) that is lower than the critical value of demineralization for both dentin (6.0 - 6.5) and enamel (5.2 - 5.7). The pH value, the unspecified amount of titrable acids (sorbic acid and hydrochloric acid) and, consequently, the low saturation with respect to OCP and DCPD ($S_{\text{OCP}} = 0.3$ and $S_{\text{DCPD}} = 0.2$) could shed light on the progressive mineral loss induced by Glandosane. In contrast, specimens stored in modified Saliva natura revealed considerable mineral gain and lesion depth reduction. After five weeks, the mineral gain

induced by modified Saliva natura was higher in comparison with the other solutions (with the exception of study 1) as well as with the baseline values after demineralization. A similar study on dentinal subsurface lesions corroborates these results [Zandim et al., 2010]. These results could be explained by this saliva substitute's supersaturation with respect to calcium phosphates ($S_{\text{OCP}} > 1.9/S_{\text{DCPD}} > 1.3$).

The demineralizing and remineralizing effects observed in study 1, which involved the basic storage solutions (i.e., experimental CMC-based saliva substitute and modified Saliva natura), were lower than in all of the other presented studies. Two differences might have induced these effects. A CMC-based citric acid-buffered storage solution with a pH level that is one unit higher than that of Glandosane was used in study 1 (pH 6.3) in contrast to studies A, 2 and 3 in which Glandosane revealed a pH of 5.3. Moreover, the CMC-based solution contained 2 $\mu\text{g F}^-/\text{g}$. The addition of fluorides also might have played some role in reducing the demineralizing potential [Featherstone, 2004]. Subsequently, less demineralization occurred in study 1 than in all of the other studies. In study 1, neutral effects were observed with modified Saliva natura, whereas remineralizing effects were seen in studies B1, B2, 2 and, 3. This can be explained by the lower pH of 5.85 and the consequently lower S_{OCP} of the present Saliva natura modification (study 1) as compared with other modifications (pH 5.95-6.0) that were used in studies B1, B2, 2, and 3. Because Saliva natura is a product of natural origin (that is, a plant extract), the pH can range from 5.2 to 5.6 according to the manufacturer's specifications. This could not be balanced with the current addition of calcium and phosphate to induce the remineralization of enamel specimens in study 1.

5.2.2. MOUTHRINSES VERSUS GEL VERSUS TOOTHPASTE APPLICATION

All of the fluoride treatments displayed the capacity to prevent the further demineralization of specimens stored in the CMC-based solution or Glandosane. The fluoride treatments with mouthrinse, gel and/or toothpaste should have resulted in a distinct calcium fluoride-like layer on specimen surfaces [Christoffersen et al., 1988; Ogaard, 2001], which should have dissolved over time as a result of the demineralizing saliva substitute. These precipitates on the specimen surface might have acted as a fluoride reservoir, thus hampering the demineralization caused by the demineralizing saliva substitute [Ogaard, 2001]. The contact between the specimens and neutral mineral water (S_{HA} of 4.9) should not have affected the above mentioned layer. Consequently, the available fluorides in the layer were capable of remineralizing the specimens [Ogaard, 2001], resulting in a more pronounced rate of remineralization than from the use of mineral water alone.

The additional use of a fluoride product did not enhance the remineralization of enamel specimens stored in modified Saliva natura, with $S_{\text{OCP}} > 1.9/S_{\text{DCPD}} > 1.3$ (study 2). However, in study 1, modified Saliva natura ($S_{\text{OCP}} = 1.6/S_{\text{DCPD}} = 1.2$) showed a neutral effect, while additional applications of ProSchmelz fluoride gel revealed a remineralizing effect. Because the surface layer was not highly mineralized through storage in modified Saliva natura, the

“phosphate-contaminated calcium fluoride layer” that was built by ProSchmelz fluoride gel application should have been more easily transformed into fluoroapatite [Chander et al., 1982]. Consequently, remineralization of the deeper parts of lesions and surfaces could be observed. The chemical differences between enamel and dentin also influenced the effects of modified Saliva natura in combination with fluorides. In study 3, the remineralizing effect observed with modified Saliva natura ($S_{\text{OCP}} = 1.9/S_{\text{DCPD}} = 1.3$) was increased by the additional application of ProSchmelz fluoride gel, whereas this was not the case for the corresponding group in study 2 (enamel). Subsurface lesions in the bovine enamel revealed a surface layer peak at approximately 60 vol% (sound: 87 vol%), whereas for dentin, this peak could be observed at about 40 vol% (sound: 50 vol%). One of the determining steps in both de- and remineralization is the rate of ion transport through the surface layer pores. As such, low mineralized surface layers can be accessed easily [Klont and ten Cate, 1991], thus explaining the observed differences between enamel and dentin with respect to remineralization.

5.2.3. EROSIONS OBSERVED WITH FLUORIDE GELS

The application of both fluoride gels (i.e., Elmex gelée and ProSchmelz fluoride gel) in combination with storage in water yielded less pronounced erosions than in combination with storage in CMC-based solution and Glandosane. This was due to the neutral and demineralizing nature of CMC-based solution and Glandosane versus that of water, respectively. Generally, the erosive effects of fluoride gels are induced by their polymers that adhere to calcium on specimen surfaces [Gebauer et al., 2009]. This temporarily bound layer was rinsed off after each of the gel treatments, resulting in surface erosion. Moreover, the erosive character observed with Elmex gelée should have been favoured by the low pH value [Kielbassa et al., 2005]. In contrast, no erosions could be observed in combination with modified Saliva natura. Supersaturation with regard to the calcium phosphates of modified Saliva natura probably led to a pronounced remineralizing effect after each fluoride gel application, preventing the erosive effect. This result also corroborates the effects of acidified fluoride gels in clinical settings involving patients without hyposalivation. Patients with normal salivary flow rate, saliva composition and buffering capacity are resistant against erosions through the protective effects of human saliva [Lussi and Hellwig, 2001; Lussi et al., 2006; ten Cate, 1997]. Therefore, the use of modified Saliva natura for patients with hyposalivation might be useful not only for the remineralization of artificial carious lesions but also for the prevention of erosions.

5.2.4. BMC ORAL HEALTH DISCUSSION

The number of older adults who have retained their natural teeth is increasing [Nicolau et al., 2000]. Thus, clinicians now face a new caries-related challenge in older dentate patients [Griffin et al., 2004; Saunders and Meyerowitz, 2005]. Physiological (or pathological) gingival recession usually observed in these patients will increase the risk of the development of root surface caries [Joshi

et al., 1993; Shay, 1997]. Moreover, several determinant factors may influence the caries risk at the individual level, such as oral hygiene, frequency of fermentable carbohydrates consumption and salivary aspects (e.g., flow, remineralizing potential, buffer capacity, cleaning properties and defensive factors) [Curzon and Preston, 2004]. More than half of the elderly who are dentate are affected with either coronal or root caries, while caries is the primary cause of tooth loss in this population [Saunders and Meyerowitz, 2005]. Although fluoride is considered to be effective in reducing caries levels, it seems to be insufficient in overcoming high carious challenges in certain individuals, like those with reduced salivary function. In these patients, new approaches should be identified to enhance the remineralization process [Featherstone, 2009]. Therefore, the aim of this study protocol is to describe the design of an *in situ* study with the potential to evaluate the effects of different saliva substitutes for the remineralization of enamel and enamel subsurface lesions.

6. CONCLUSION

Within the limitations of the studies A, B1, and B2, it can be concluded that Glandosane revealed a demineralizing potential on enamel as well as on dentin lesions. Saliva natura showed a demineralizing effect on dentin lesions, but revealed a neutral effect on enamel. Therefore, Saliva natura was modified by the addition of calcium, phosphate, and fluoride. Following, modifications of Saliva natura with various saturations with respect to calcium phosphates were evaluated on their remineralizing capacities. Here, slightly supersaturated Saliva natura modifications with an S_{OCP} of 2 and S_{DCPD} of 1.4 showed the highest remineralizing potential.

From a practical point of view, it might be advantageous to use demineralizing products (e.g., Glandosane) in combination with fluoride products that are capable of preventing demineralization and/or of promoting remineralization of dental hard tissues. So far, fluoride gels or mouthrinses that are applied several times daily are recommended in addition to the use of traditional toothpastes (e.g., 1,500 $\mu\text{g F/g}$) to prevent carious lesions in patients with hyposalivation [Nieuw Amerongen and Veerman, 2003]. In particular, for patients with hyposalivation resulting from radiation to the head and neck region, long-term compliance with two-step regimens (i.e., toothpaste and fluoride gel) is quite poor [Epstein et al., 1996; Horiot et al., 1983]. Recently, toothpastes with fluoride levels up to 5,000 $\mu\text{g F/g}$ have been developed for “high-risk individuals” [Lynch and Baysan, 2001; Nordstrom and Birkhed, 2010]. Therefore, the use of toothpastes without the application of additional mouthrinses or fluoride gels may increase patient compliance. Preferably, a remineralizing saliva substitute could address both outcomes (dental caries and hyposalivation) in this patient group, eliminating the need for additional fluoride products (i.e., fluoride gels or mouthrinses).

Following, three further studies (1 - 3) were conducted, which addressed the questions mentioned above. Data from these studies highlight that the choice of saliva substitute should be carefully made to avoid the introduction of substances with potentially demineralizing effects on dental hard tissues. In general, Glandosane demonstrated a pronounced demineralizing effect on enamel and dentin specimens, which were inhibited by the daily application of fluoride products (e.g, mouthrinse, highly concentrated fluoride toothpaste, and fluoride gels). In contrast, modified Saliva natura ($S_{\text{OCP}} > 1.9/S_{\text{DCPD}} > 1.3$) appeared to have a remineralizing potential, which can only be increased by the additional application of fluoride gel (ProSchmelz fluoride gel) in dentin, but not in enamel. For this reason, the use of remineralizing artificial saliva (i.e., modified Saliva natura) is a promising approach for dentate patients suffering from hyposalivation in their management of both dental caries and hyposalivation. For patients with exposed dentinal surfaces additional ProSchmelz fluoride gel application might promote the remineralizing effect of modified Saliva natura and therefore, should be used as adjuvant. However, the results of these *in vitro* studies should be extrapolated with caution to the clinical conditions. Therefore, a detailed concept of an *in situ* study protocol completes this thesis, which could confirm the potential benefits of the use of modified Saliva natura compared to Glandosane in patients with hyposalivation.

7. SUMMARY

Statement of problem: Hyposalivation is the most common side effect of radiation therapy in the head and neck areas, but the reduction of salivary flow rates can also be associated with prolonged use of certain drugs, or some diseases (Sjögren's syndrome, diabetes mellitus). Moistening of the oral mucosa with saliva substitutes is the widely prescribed palliative treatment to alleviate oral complaints in patients with hyposalivation. However, some commercially available products have been shown to demineralize dental hard tissues. **Objectives:** Therefore, the general purpose of the present postdoctoral thesis was to develop a remineralizing saliva substitute, and, following, to evaluate the effects of saliva substitutes in combination with or without fluoride products on the remineralization of dentin and enamel subsurface lesions. **Material and Methods:** De- and remineralization of predemineralized enamel and dentin specimens (subsurface lesions) was microradiographically evaluated after application of different protocols. The saliva substitute Saliva natura was modified by the addition of different amounts of calcium and phosphate, and this resulted in various saturations levels with respect to octacalcium phosphate (S_{OCP}) and dicalcium phosphate dihydrate (S_{DCPD}). The treatment protocols included storage of specimens in control solutions or saliva substitutes for 5 weeks (37 °C). During this period, specimens were additionally treated with or without different fluoride products (mouthrinse, highly concentrated toothpaste, or fluoride gel). **Results:** The saliva natura modification with an S_{OCP} of 2 and an S_{DCPD} of 1.4 enabled the highest level of dentin and enamel remineralization. In contrast, storage in Glandosane (cell pharm) resulted in pronounced demineralizing effects on both dental hard tissues. This detrimental effect was reduced or inhibited by daily fluoride applications. The additional treatment with fluoride products enhanced the remineralizing effect of modified Saliva natura only for dentin specimens. **Conclusions:** Based on the results of these *in vitro* studies, it can be concluded that Glandosane is a demineralizing saliva substitute that should only be used in combination with frequently applied fluorides in dentate patients. Modified Saliva natura enables remineralization of enamel and dentin subsurface lesions, which could be raised with additional ProSchmelz fluoride gel application in dentin. Following, these promising results represent a sound basis for a *in situ* study, which evaluates the effects of saliva substitute on the dental hard tissues.

Clinical Significance: Data from the present studies demonstrated that the commercially available saliva substitute Glandosane might have demineralizing effects on dental tissues if not used in combination with fluoride products. Saliva natura supersaturated with respect to calcium phosphates should be an advantageous artificial saliva for dentate patients suffering from hyposalivation. Additional ProSchmelz fluoride gel application might promote the remineralizing effect of Saliva natura on dentin and therefore, could be used as adjuvant in daily oral care.

8. ZUSAMMENFASSUNG

Problemstellung: Die Hyposalivation kann bei verschiedenen systemischen Erkrankungen und zahlreichen Medikamenten als mögliche Nebenwirkung auftreten sowie nach einer tumortherapeutischen Bestrahlung im Kopf-Hals-Bereich beobachtet werden. Zur symptomatischen Linderung finden häufig Speichelersatzmittel Verwendung. In-vitro-Untersuchungen zeigten jedoch ein ausgeprägtes demineralisierendes Potenzial von handelsüblichen Speichelersatzmitteln auf Schmelz und Dentin. **Zielsetzung:** Das Ziel der vorliegenden kumulativen Habilitationsschrift war, ein remineralisierendes Speichelersatzmittel zu entwickeln und im Anschluss die Effekte von verschiedenen Speichelersatzmitteln in Kombination mit oder ohne zusätzliche Fluoridapplikation auf das De- und Remineralisationsverhalten von Schmelz- und Dentinläsionen zu untersuchen. **Material und Methoden:** Die De- und Remineralisation der demineralisierten Proben wurde mikroradiografisch im Anschluss an die unterschiedlichen Behandlungen evaluiert. Ein Speichelersatzmittel (Saliva natura) wurde durch die Zugaben von Kalzium und Phosphat modifiziert, hierdurch konnten verschiedene Sättigungen hinsichtlich Oktakalziumphosphat (S_{OKP}) und Dikalziumphosphatdihydrat (S_{DKPD}) erzielt werden. Die jeweiligen Behandlungen schlossen die Lagerung der Proben in Speichelersatzmittel und einer Kontrolllösung für 5 Wochen (jeweils bei 37 °C) ein. Während dieser Zeit wurden die Proben entweder mit oder ohne zusätzliche Fluoridprodukte behandelt. **Ergebnisse:** Es konnte gezeigt werden, dass das Speichelersatzmittel (Saliva natura) mit einer S_{OKP} von 2 und S_{DKPD} von 1,4 die ausgeprägtesten remineralisierenden Eigenschaften auf Schmelz- und Dentinläsionen besitzt. Im Gegensatz dazu führte die Lagerung in Glandosane zu einer ausgeprägten Demineralisation der Proben. Diese schädigende Auswirkung konnte durch die tägliche Applikation von Fluoridprodukten reduziert werden. Die zusätzliche Behandlung mit Fluoridprodukten konnte den remineralisierenden Effekt von Saliva natura nur bei den Dentinproben steigern. **Schlussfolgerungen:** Basierend auf den vorliegenden Ergebnissen kann geschlossen werden, dass das demineralisierende Potenzial von Glandosane bei gleichzeitiger regelmäßiger Verwendung von (hoch konzentrierten) Fluoridprodukten reduziert werden kann. Das modifizierte Produkt Saliva natura zeigte remineralisierende Effekte, welche nur bei Dentin durch eine zusätzliche Fluoridapplikation gesteigert werden konnte. Diese viel versprechenden Ergebnisse stellen eine gute Basis für eine In-situ-Studie dar, um die Effekte von Speichelersatzmitteln auf die Zahnhartsubstanzen klinisch zu überprüfen.

Klinische Bedeutung: Die Ergebnisse der vorliegenden Studien zeigen, dass das kommerziell erhältliche, demineralisierende Produkt Glandosane bei behafteten Patienten vornehmlich in Kombination mit Fluoridprodukten verwendet werden sollte. Das modifizierte Produkt Saliva natura dürfte für behaftete Patienten mit Hyposalivation von Vorteil sein. Die remineralisierende Wirkung auf Dentinproben kann durch die zusätzliche Applikation von ProSchmelz Fluoridgel verstärkt werden und kann deshalb als Adjuvans zur täglichen Mundpflege empfohlen werden.

9. REFERENCES

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