

FLORIAN J. WEGEHAUPT¹

JANA SCHLEICH¹

BLEND HAMZA¹

DANIEL WIEDEMEIER²

THOMAS ATTIN¹

¹ Clinic of Preventive Dentistry, Periodontology and Cariology, Center of Dental Medicine, University of Zurich

² Center of Dental Medicine, Statistician, University of Zurich

CORRESPONDENCE

PD Dr. Florian J. Wegehaupt
Klinik für Präventivzahn-
medizin, Parodontologie und
Kariologie, Zentrum für Zahn-
medizin, Universität Zürich
Plattenstrasse 11
CH-8032 Zürich
Tel. +41 44 634 33 54
Fax +41 44 634 43 08
E-mail: florian.wegehaupt@
zsm.uzh.ch

SWISS DENTAL JOURNAL SSO 128:
790–797 (2018)
Accepted for publication:
27 March 2018

Performance of a newly developed mineral gel system on erosive and erosive/abrasive enamel loss

An in vitro study

KEYWORDS

Abrasion
Erosion
Enamel loss
Mineral gel

SUMMARY

We compared the prevention of erosive and erosive/abrasive enamel loss by a medical minerals gel system (R.O.C.S.) to that by an anti-erosive toothpaste. Seventy-two bovine enamel samples were randomly allocated to six groups (E1–E3 and EA1–EA3; n = 12). Per day, samples were eroded (2 min) 9 times using HCl (pH 2.6). Between erosive challenges and over night samples were stored in artificial saliva. Per day, samples were stored (100 s; groups E1–E3) or additionally brushed (20 brushing strokes; groups EA1–EA3) in/with slurries prepared from artificial saliva plus: no additional toothpaste (control groups E1 and EA1), elmex erosion protection toothpaste (groups E2 and EA2), or R.O.C.S. toothpaste (groups E3 and EA3). Once per day, samples of groups E3 and EA3 were additionally treated (40 min) with a slurry prepared from artificial saliva and R.O.C.S. medical minerals gel. After 7,

14 and 21 days enamel loss was measured by surface profilometry and analysed by Kruskal–Wallis tests and Conover post-hoc tests. Under erosive conditions only (groups E1–E3), at each time point of measurement the significantly lowest enamel loss was observed in group E2. Substance loss in group E3 was significantly higher, but significantly lower compared to that of group E1. Under erosive/abrasive conditions (groups EA1–EA3), at each time point of measurement the significantly lowest enamel wear was observed in group EA2. Wear in group EA3 was significantly higher even compared to that of group EA1. The tested R.O.C.S. medical minerals gel system was able to reduce erosive enamel loss but not erosive/abrasive enamel wear, and it was less effective than the elmex erosion protection toothpaste.

Introduction

With increased awareness of both the public and dentists on the issue of dental erosion, many products claiming to possess anti-erosive properties were developed and introduced into the market. Various combinations of fluorides were investigated regarding their anti-erosive properties or their ability to remineralise erosively demineralised dental hard tissues. Those combinations included sodium fluoride (NaF), amine fluoride (AmF), titanium tetrafluoride (TiF₄), zinc fluoride (ZnF₂) and stannous (tin II) fluoride (SnF₂) or chloride (SnCl₂) (WEGEHAUPT & ATTIN 2010; YU ET AL. 2010A; WIEGAND ET AL. 2010; GANSS ET AL. 2010; RAMOS-OLIVEIRA ET AL. 2017). Beside these solely fluoride- or at least partial fluoride-based products also other components such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (RANJITKAR ET AL. 2009; WANG ET AL. 2011; WEGEHAUPT ET AL. 2012), synthetic minerals composed of calcium, sodium, phosphorous and silica (WANG ET AL. 2011) and polymers (GANSS ET AL. 2011; GANSS ET AL. 2012; BUZALAF ET AL. 2014) have been investigated with regard to their anti-erosive properties or ability to remineralise or re-harden erosively softened dental hard tissues. The assumed mode of action to prevent erosive or erosive/abrasive dental hard tissue loss is either to increase the acid resistance of dentine and enamel (mainly fluoride and TiF₄ or SnF₂ containing products), to provide increased amounts of minerals, namely calcium and phosphate, in the close surrounding or on top of the dental hard tissues to protect or to enhance the mineral uptake from the respective products (mainly CPP-ACP containing products).

Based on the idea to provide an increased amount of minerals with the respective products, R.O.C.S. (R.O.C.S. Trading GmbH, Munich, Germany) is a newcomer in the field of prevention and treatment of caries and non-carious lesions by means of remineralisation. R.O.C.S. is a system consisting of toothpaste and a medical minerals gel. The manufacturer claims that the mechanism of action of R.O.C.S.'s system is based on the release of bioavailable calcium, phosphate and magnesium and is indicated to treat early carious and erosive lesions. The remineralising effect of R.O.C.S. mineral gel was tested in a study by Kunin et al. (KUNIN ET AL. 2014). It was observed that R.O.C.S. mineral gel was effective in remineralising white spots, reducing hypersensitivities and, anecdotally, improving teeth appearance. To our knowledge, no data from studies investigating the anti-erosive and/or anti-erosive/abrasive effect of the R.O.C.S. system are available.

Therefore, the aim of the present study was to investigate the anti-erosive and anti-erosive/abrasive effect of the R.O.C.S. toothpaste and medical minerals gel system and compare it with that of a toothpaste (elmex erosion protection toothpaste), which has been proved to be effective as an anti-erosive agent. Two null hypotheses were proposed: (1) the use of R.O.C.S. toothpaste and medical minerals gel system will result in no significant difference in the erosive enamel loss compared with elmex erosion protection toothpaste, and (2) the use of R.O.C.S. toothpaste and medical minerals gel system will result in no significant difference in the erosive/abrasive enamel wear compared with elmex erosion protection toothpaste.

Materials and methods

Sample preparation and allocation

For the study, 24 bovine lower incisors were used. From each tooth, three samples were obtained from the buccal surface of the crown using a cylinder-shaped diamond coated trephine

mill with an inner diameter of 3 mm. After preparation of the enamel samples, the specimens were marked with the respective tooth number (1–24) and sample number (I–III). Samples were then embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany) in a silicon mould with an inner diameter of 6 mm, so that only the enamel surface was free of resin. The acrylic resin was cured in a laboratory polymerizer (Palamat elite, Heraeus Kulzer, Germany) at 45 °C and 3 bar for 13 min. The enamel surface of the samples was gradually smoothed under water cooling, using 1,000-, 2,000- and 4,000-grit carborundum paper (waterproof silicon carbide paper, Stuers, Tegamin-30, Germany) for 5, 35 and 90 s, respectively. The grinding process was performed in an automatic grinding machine. During this grinding process, about 200 µm of superficial enamel was removed.

All samples I from teeth 1–12 were allocated to group E1, samples II to group E2 and samples III to group E3. The samples I of teeth 13–24 were allocated to group EA1, samples II to group EA2 and samples III to group EA3. This results in a total of 12 enamel samples per group (n = 12). The samples of groups E1–E3 were used for the erosion only part of the study while the samples EA1–EA3 were used for the erosion plus abrasion part of the study.

The samples of groups E1 and EA1 served as control groups and received no further treatment while samples of groups E2 and EA2 were treated with elmex erosion protection toothpaste (GABA, Therwil, Switzerland) and samples of groups E3 and EA3 were treated with R.O.C.S. toothpaste and medical minerals gel (R.O.C.S. Trading GmbH, Munich, Germany). Sample allocation and the experimental procedure are presented in a flow chart (Fig. 1).

The composition of the used toothpastes and the medical mineral gel is presented in table I.

Until final use, the samples were stored in tap water.

Erosive and erosive/abrasive cycling

The following de-/remineralisation cycling was performed for 21 days:

Each day, samples of groups E1–E3 were covered (20 ml/sample) with slurries prepared from artificial saliva (KLIMEK ET AL. 1982) and the respective toothpastes at a ratio of 1:3 for 120 s. Samples of groups EA1–EA3 were also covered with the respective slurries for 100 s and later brushed in an automatic brushing machine with reciprocating movements at a frequency of 60 brushing strokes (BS) per minute for 20 s, resulting in an application of 20 BS. For each sample, a specific medium bristle stiffness toothbrush was used (ParoM43, Esro AG, Thalwil, Zurich, Switzerland). A constant brushing force of 2.5 N was applied by fixing a respective weight on the toothbrush. Samples of groups E1 and EA1 were only covered (group E1) or covered and brushed (group EA1) with artificial saliva. Afterwards, samples were rinsed thoroughly with distilled water to remove all remnants of the respective products. Then, samples were stored in artificial saliva for 1 h. Afterwards the first erosive attack was performed by immersing the samples in HCl (pH 2.6, 2 ml per sample) for 2 min. The acid was kept under constant motion. After the erosive attack, samples were again rinsed thoroughly with distilled water and stored in artificial saliva. A total of nine erosive attacks per day were performed. In the mean time, samples were stored in artificial saliva. One hour after the last erosive attack per day, samples were again either only covered (120 s; groups E1–E3) or covered (100 s) and brushed (20 s)

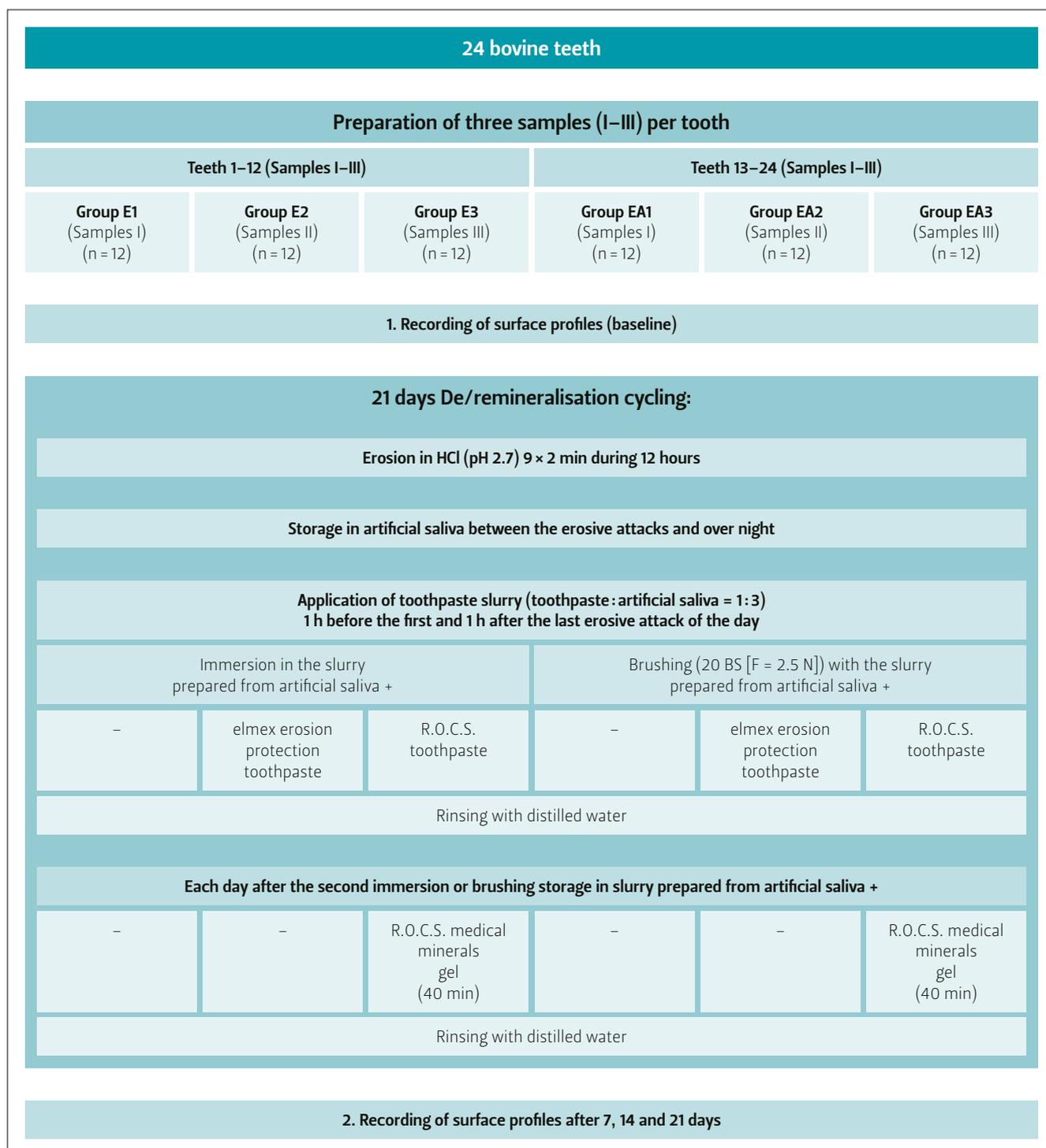


Fig. 1 Sample allocation and experimental procedure

Tab. I Composition of the used toothpastes/gel according to manufactures information.

Product	Ingredients
elmex erosion protection toothpaste	Aqua, Glycerin, Sorbitol, Hydrated silica, Hydroxyethylcellulose, Aroma, Cocamidopropyl Betaine, Titanium dioxide, Olafur, Sodium gluconate, Stannous chloride, Alumina, Chitosan, Sodium Saccharin, Sodium fluoride, Potassium hydroxide, Hydrochloric acid
R.O.C.S. toothpaste	Aqua, Silica, Glycerin, Xylitol, Sodium lauryl sulfate, Xanthan Gum, Aroma, Calcium glycerophosphate, Bromelain, Sodium Saccharin, Methylparaben, Titanium dioxide, Propylparaben magnesium chloride
R.O.C.S. medical minerals gel	Aqua, Glycerin, Xylitol, Hydroxyethylcellulose, Calcium glycerophosphate, Polysorbate-20, Aroma, Methylparaben, Magnesium chloride, Hydroxypropyl guar

(groups EA1–EA3) with the respective slurries. Finally, samples of groups E1, E2, EA1 and EA2 were stored in artificial saliva over night. Samples of groups E3 and EA3 were covered (20 ml/sample) with a slurry (ratio of 1:0.5) prepared from R.O.C.S. medical minerals gel and artificial saliva for 40 min, followed by rinsing with distilled water and storage in artificial saliva over night.

Measurement of erosive and erosive/abrasive enamel loss

The amount of erosive or erosive/abrasive enamel loss was measured profilometrically with a stylus profilometer (Perthometer S2, Mahr, Göttingen, Germany). From each sample, five surface profiles were recorded with a distance of 50 µm at baseline (before any acid attack was conducted). Further measurements were carried out after 7, 14 and 21 days. To ensure an exact re-positioning of the samples, the profilometer and the samples were equipped with a jig. The erosive or erosive/abrasive enamel loss was calculated with a custom-made software allowing an automatized superimposition of the baseline profiles with the respective profiles after 7, 14 and 21 days. If the calculated loss per profile was below the measurement limit of the profilometer of 0.105 µm (ATTIN ET AL. 2009), the value for this profile was set as 0.000 µm (WEGEHAUPT ET AL. 2016).

Statistical analysis

The enamel loss data were encoded into a Microsoft Excel file. The statistical analysis was then performed using R statistic software (R Foundation for Statistical Computing, Vienna, Austria).

Mean, standard deviation, median, interquartile range and 95% confidence intervals were calculated.

As the data of groups E1–E3 and groups EA1–EA3 were not normally distributed, according to the Kolmogorov–Smirnov

and Shapiro–Wilk tests, the non-parametrical Kruskal–Wallis tests and Conover post-hoc tests were used to disclose differences between the enamel loss in the different groups (E1–E3 and EA1–EA3, separately) after 7, 14 and 21 days of cycling.

P-values ≤ 0.05 were interpreted as statistically significant.

Results

Erosive enamel loss (groups E1–E3)

The amount of erosive enamel loss after 7, 14 and 21 days for each group 1–3 is presented in table II.

At all three time points of measurement (after 7, 14 and 21 days), the significantly highest enamel loss (median [IQR]: 2.19 [0.49], 3.59 [0.74] and 4.66 [0.88], respectively) was observed for group E1 (control) ($p < 0.01$, respectively).

The enamel loss for group E2 (elmex erosion protection toothpaste) was significantly lower after 7, 14 and 21 days of erosive cycling compared with the loss for groups E1 and E3 ($p < 0.01$, respectively).

At all time points of measurement (7, 14 and 21 days), the enamel loss (median [IQR]: 1.45 [0.32], 2.16 [0.47] and 3.15 [0.55], respectively) for group E3 (R.O.C.S. toothpaste and medical minerals gel) was significantly higher than the loss of group E2 (elmex erosion protection toothpaste) ($p < 0.01$, respectively).

Erosive/abrasive enamel wear (groups EA1–EA3)

The amount of erosive/abrasive enamel wear after 7, 14 and 21 days for each group EA1–EA3 is provided in table III.

After 7, 14 and 21 days of erosive/abrasive cycling, the significantly lowest enamel wear (median [IQR]: 0.32 [0.26], 0.50 [0.65] and 0.81 [0.80], respectively) was observed for group EA2 (elmex erosion protection toothpaste) ($p < 0.001$, respectively).

At all time points of measurement (7, 14 and 21 days), the sig-

Tab. II Median (interquartile range) of erosive enamel loss (µm) in each group (used products) for each time point of measurement (after 7, 14 and 21 days).

		Group E1 (no toothpaste, control)	Group E2 (elmex erosion protection toothpaste)	Group E3 (R.O.C.S. toothpaste and medical minerals gel)
Time point of measurement	7 days	2.19 (0.49) A	0.12 (0.03) B	1.45 (0.32) C
	14 days	3.59 (0.74) A	0.14 (0.04) B	2.16 (0.47) C
	21 days	4.66 (0.88) A	0.13 (0.04) B	3.15 (0.55) C
Values within the same time point of measurement for the three groups that are not statistically significantly different are marked with same capital letters (read horizontally within the respective line).				

Tab. III Median (interquartile range) of erosive/abrasive enamel wear (µm) in each group (used products) for each time point of measurement (after 7, 14 and 21 days).

		Group EA1 (no toothpaste, control)	Group EA2 (elmex erosion protection toothpaste)	Group EA3 (R.O.C.S. toothpaste and medical minerals gel)
Time point of measurement	7 days	2.36 (0.35) A	0.32 (0.26) B	4.96 (0.50) C
	14 days	4.47 (0.89) A	0.50 (0.65) B	8.25 (1.10) C
	21 days	6.63 (1.46) A	0.81 (0.80) B	12.37 (1.40) C
Values within the same time point of measurement for the three groups that are not statistically significantly different are marked with same capital letters (read horizontally within the respective line).				

nificantly highest enamel wear (median [IQR]: 4.96 [0.50], 8.25 [1.10] and 12.37 [1.40], respectively) was observed for group EA3 (R.O.C.S. toothpaste and medical minerals gel) ($p < 0.001$, respectively).

The wear in group EA1 (control) was at all time points significantly lower compared to that of group EA3 (R.O.C.S. toothpaste and medical minerals gel), but significantly higher than that of group EA2 (elmex erosion protection toothpaste).

Discussion

The present in vitro study showed that the combination of R.O.C.S. toothpaste and medical minerals gel was able to reduce enamel erosion only compared with the untreated control. However, under erosive/abrasive conditions, the combination of R.O.C.S. toothpaste and medical minerals gel showed a significantly higher wear compared with the untreated control.

The size of a bovine incisor allows obtaining more than one sample from each tooth. The allocation of one sample from each tooth to one of the three groups E1–E3 or EA1–EA3 reduces possible differences of reacting with de- and remineralisation cycles performed in this study. Bovine teeth are generally easy to obtain and do not have a history of either caries or fluoridation, which might affect their mechanical or chemical properties. Furthermore, bovine enamel was shown to be a reliable substitution to human enamel (TURSSI ET AL. 2010; LAURANCE-YOUNG ET AL. 2011) and was used in numerous previous in vitro and in situ studies (HANNIG & BALZ 1999; AMAECHI & HIGHAM 2001; RIOS ET AL. 2006; VIEIRA ET AL. 2006; YU ET AL. 2010B; HAMBAL ET AL. 2011; OLIVEIRA ET AL. 2015) investigating erosive and erosive/abrasive enamel loss to substitute human enamel. However, bovine enamel showed a higher susceptibility to erosion and erosion/abrasion compared to human enamel (ATTIN ET AL. 2007). Nevertheless, as in the present study, only the relative values (within the respective groups of the present study) were interpreted, the use of bovine enamel is acceptable.

Artificial saliva prepared following the formulation given by Klimek et al. (KLIMEK ET AL. 1982) was used in this study. This formulation was found to be the most used formula in in vitro studies in the literature, which is quite expected as it is the first formula of artificial saliva that was suggested for such type of studies (BATISTA ET AL. 2016). The usage of the same artificial saliva for all samples provided a unitary medium to create slurries and to store the samples overnight or in between the acidic attacks. However, Batista et al. (BATISTA ET AL. 2016) observed in a recent study that neither artificial saliva nor human saliva – when used in vitro – could reflect the in situ situation perfectly. Furthermore, one has to consider that the artificial saliva here used does not form an acquired pellicle when applied on the enamel samples as it happens when whole human saliva is used. As the present study was performed during 21 days, a consistent collection of the needed amount of human saliva for such a long time seemed to be difficult. Therefore, to keep the composition consistent artificial saliva was used. However, the above mentioned aspects have to be taken into consideration when interpreting the results of the present study.

Acid attacks were performed in this study using hydrochloric acid simulating the clinical condition of patients suffering from GERD or bulimia where gastric juice comes in contact with the dental hard tissues. The acidic component of gastric juice is hydrochloric acid (HUNT 1951). Hydrochloric acid is a strong monovalent acid which – at a pH value of 2.6 – completely dissociates to hydrogen and chloride ions and dissolves tooth

minerals (ATTIN ET AL. 2013). The same acid was used to erode dental hard tissue in previous studies (GANSS ET AL. 2009; YU ET AL. 2010B; AUSTIN ET AL. 2011; ATTIN ET AL. 2013; OLIVEIRA ET AL. 2015; WEGEHAUPT ET AL. 2017). It is important to take in consideration that the buffer capacity of HCl is very small. Therefore, the volume used to simulate the in vivo situation is important and will influence the results. In the present study, 2 ml acid was used per erosive attack per sample. Samples had a diameter of 3 mm resulting in a surface area of about 7 mm². Taking in consideration that 2 ml acid equals about 2,000 mm³, one can assume that a sufficient amount of acid per sample was provided and the duration of the acidic attack was rather short. Bartlett et al. (BARTLETT & COWARD 2001) found the mean pH of seven gastric acid samples to be pH 2.92. Therefore, the here used pH of 2.6 is slightly more acidic. However, Bartlett et al. (BARTLETT & COWARD 2001) recorded that the pH of gastric acid might vary from as low as 1.2 up to nearly neutral pH of 6.78. The samples were eroded for 2 min, which is within the acid-attack duration recommended by Wiegand and Attin (WIEGAND & ATTIN 2011) for in vitro studies. Nine erosive attacks were performed each day in the present study. This is in accordance with an observations by Ganss et al. (GANSS ET AL. 2009) reporting vomiting frequencies of 6–10 times per day for patients in their surgery.

Enamel erosive loss and erosive/abrasive wear was measured using surface profilometry. This method has been widely used to assess dental hard tissue loss and proved to be of high precision and reproducibility (ATTIN ET AL. 2009; ATTIN & WEGEHAUPT 2014).

The enamel samples were treated (immersed or brushed) with the respective slurries 1 h before the first acid attack and 1 h after the last one. A waiting time of at least one hour after an acid attack and before tooth brushing was once recommended and significantly less erosion wear was noticed when more time elapsed after the acid attack (JAEGGI & LUSSI 1999; ATTIN ET AL. 2001). However, recent studies showed that the waiting time before brushing is a minor factor (GANSS ET AL. 2007) and not closely associated with the amount of dental erosive tooth wear (O'TOOLE ET AL. 2017). Furthermore, a large epidemiological study (BARTLETT ET AL. 2013) concerning factors associated with tooth wear on buccal/facial and lingual/palatal tooth surfaces found no evidence that waiting after breakfast before tooth brushing has any effect on the degree of tooth wear.

The first and second null hypothesis of the present study, namely that the use of R.O.C.S. toothpaste and medical minerals gel system will result in no significant difference in (1) the erosive enamel loss and (2) the erosive/abrasive enamel wear compared with elmex erosion protection toothpaste, have to be rejected. For both conditions (erosion and erosion/abrasion) and at all time points of measurement, enamel loss in the R.O.C.S. toothpaste and medical minerals gel system groups was significantly higher than that in the respective elmex erosion protection toothpaste group. Compared to the control group, the R.O.C.S. system presented a significant anti-erosive effect. However, under erosive/abrasive conditions, no such preventive effect compared with the control group could be observed. On the contrary, the erosive/abrasive enamel wear of the R.O.C.S. system group was even significantly higher than that of the untreated control group. It might be speculated that this finding can be attributed to differences in the REA-values of the used toothpastes. One might assume that the R.O.C.S. toothpaste has a higher REA-value than the

elmex erosion protection toothpaste. However, as the present study was intended to evaluate possible anti-erosive and anti-erosive/abrasive effects of the R.O.C.S. toothpaste and medical minerals gel system as postulated by the manufacturer an exclusive testing of the two products of the R.O.C.S. toothpaste and medical minerals gel system and the REA-value would not result in a different result concerning the above-mentioned question.

Both R.O.C.S. toothpaste and R.O.C.S. medical minerals gel do not contain any kind of fluoride. Their claimed remineralising effect therefore depends solely on the effect to provide calcium, phosphate and magnesium ions to fill up defects in the enamel crystals. Calcium and phosphate compounds seemed to form precipitates on the surface of previously eroded dental hard tissues, however, a remineralisation was not observed (BUZALAF ET AL. 2014). It is unclear if these precipitates provide a protective effect against later applied mechanical forces such as tooth brushing. On the other hand, it is conceivable that the released ions might act as a reservoir providing an increased amount of minerals on the dental hard tissues surface and therefore prevent the demineralisation if applied before an erosive or carious attack.

Beside calcium, phosphate and magnesium, R.O.C.S. toothpaste and medical minerals gel contain xylitol and bromelain (protein extract derived from the stems and fruits of pineapple plant [*Ananas comosus*] [ROWAN ET AL. 1990]). In toothpastes, bromelain is added to support stain removal (KALYANA ET AL. 2011; CHAKRAVARTHY & ACHARYA 2012). Xylitol in combination with fluorides has demonstrated to be able to support remineralisation of carious lesions (ARENDS ET AL. 1990; AMAECHI ET AL. 1999; MIAKE ET AL. 2003; CARDOSO ET AL. 2014). Concerning an anti-erosive effect, it has been shown that the incorporation of xylitol into erosive beverages (AMAECHI ET AL. 1998; CHUNMUANG ET AL. 2007), mouth rinses (CHUNMUANG ET AL. 2007) or toothpastes (ROCHEL ET AL. 2011) results in a reduced erosive or erosive/abrasive dental hard tissue loss. However, a distinct anti-erosive effect was observed only in combination with fluorides (AMAECHI ET AL. 1998; CHUNMUANG ET AL. 2007; ROCHEL ET AL. 2011).

The AmF/NaF/SnCl₂ combination (elmex erosion protection toothpaste) showed a significant reduction in erosive and erosive/abrasive enamel loss compared to the untreated control, where samples were only stored in or brushed with artificial saliva. Also compared to the samples treated with R.O.C.S. toothpaste and medical minerals gel, samples treated with elmex erosion protection toothpaste showed a significantly reduced enamel loss. It has been postulated that the protective effect of AmF/NaF/SnCl₂ might be due to the precipitation of the stannous salts (Sn₂OHPO₄, Sn₃F₃PO₄, Ca(SnF₃)₂), which act as a protective layer against acidic attacks (BABCOCK ET AL. 1978; SCHLUETER ET AL. 2009). This anti-erosive and anti-erosive/abrasive effect of AmF/NaF/SnCl₂ is in accordance with the findings of various previous studies (GANSS ET AL. 2010; YU ET AL. 2010B; AYKUT-YETKINER ET AL. 2014; WIEGAND & ATTIN 2014; RAMOS-OLIVEIRA ET AL. 2017).

Under erosion only conditions the R.O.C.S. system showed lower enamel loss than the untreated control group, but under erosive/abrasive conditions the same system showed a higher enamel wear than the untreated control. Taking this finding in consideration, further studies are needed to evaluate if this might be caused by a high abrasivity of the R.O.C.S. toothpaste.

Conclusion

Within the limitation of the present study, it can be concluded that the tested R.O.C.S. toothpaste and medical minerals gel were able to reduce erosive enamel loss compared with the untreated control, but not compared to the elmex erosion protection toothpaste. However, under erosive/abrasive conditions, R.O.C.S. toothpaste and medical minerals gel cause a significantly higher erosive/abrasive enamel wear compared to both the elmex erosion protection toothpaste and the negative control (artificial saliva).

Acknowledgment

The current study is part of and in parts identical with the master's theses "Comparison of anti-erosive effect between R.O.C.S. and Elmex Erosionsschutz, an in-vitro study" by B. Hamza and "Vergleich des Schutzes vor Erosion/Abrasion von ROCS und Elmex Erosionsschutz Zahnpaste" by J. Schleich, both performed at the University of Zurich, Switzerland, under the supervision of F. Wegehaupt and T. Attin.

Zusammenfassung

Einleitung

Dentale Erosionen und die mit dem Säureangriff einhergehende Erweichung der Zahnhartsubstanzen sind neben Karies die häufigste Ursache für Zahnhartsubstanzverlust. Zur Prävention von Erosionen sind aktuell neue Produkte (R.O.C.S.-Zahnpaste und Mineralgel) auf den Schweizer Markt gebracht worden. Um Patienten adäquat über das mögliche präventive Potenzial dieser Produkte zu informieren, war es das Ziel der vorliegenden Studie, den erosiven und erosiv/abrasiven Schmelzverlust bei Verwendung des R.O.C.S.-Systems zu bestimmen und mit dem einer Zahnpaste mit bekannten antierosiven Eigenschaften (Elmex-Erosionsschutz-Zahnpaste) zu vergleichen.

Material und Methoden

Es wurden 72 bovine Schmelzproben hergestellt und auf sechs Gruppen (E1-E3 und EA1-EA3; n=12) aufgeteilt. Die Proben wurden anschliessend einem erosiven (E1-E3) oder erosiv/abrasiven (EA1-EA3) Zyklus zugeführt. Pro Tag wurden die Schmelzproben neunmal zwei Min. mit Salzsäure (pH 2,6) erodiert. Zwischen jeder erosiven Attacke und über Nacht wurden die Proben in künstlichem Speichel gelagert. Zweimal am Tag (morgens und abends) wurden die Proben der Gruppen E1-E3 für 100 Sek. in Zahnpastenslurry gelagert oder zusätzlich mit 20 Bürststrichen mit dem entsprechenden Slurry gebürstet (EA1-EA3). Die Zahnpastenslurries wurden aus künstlichem Speichel und den folgenden Pasten hergestellt: Keine Zahnpaste (Kontrollgruppe E1 und EA1), Elmex-Erosionsschutz-Zahnpaste (E2 und EA2) oder R.O.C.S.-Zahnpaste (E3 und EA3). Zusätzlich wurden die Proben der Gruppen E3 und EA3 einmal am Tag für 40 Min. in ein Slurry aus künstlichem Speichel und R.O.C.S.-Medical-Minerals-Zahngel eingelegt.

Nach 7, 14 und 21 Tagen wurde der resultierende Schmelzverlust mit einem Kontaktprofilometer bestimmt und mit Kruskal-Wallis-Tests und Conover-post-hoc-Tests verglichen.

Resultate

Unter rein erosiven Bedingungen (Gruppen E1-E3) wurde zu allen Messzeitpunkten der geringste Schmelzverlust in der Gruppe E2 (Elmex-Erosionsschutz-Zahnpaste) beobachtet. Der Schmelzverlust in Gruppe E3 (R.O.C.S.) war signifikant höher als in Gruppe E2, aber signifikant geringer als in Gruppe E1 (Kon-

trolle). Unter erosiv/abrasiven Bedingungen wurde erneut zu allen Messzeitpunkten der signifikant geringste Schmelzverlust in der Elmex-Erosionsschutz-Zahnpasten-Gruppe (EA2) beobachtet. Der Schmelzverlust in Gruppe EA3 (R.O.C.S.) war sogar höher als der Schmelzverlust der Kontrollgruppe (EA1).

Diskussion

Innerhalb der Limitationen der vorliegenden Studie kann geschlossen werden, dass die Kombination aus R.O.C.S.-Zahnpaste und Medical-Minerals-Zahngel in der Lage ist, den erosiven Schmelzverlust zu reduzieren. Allerdings wurde für die Kombination aus R.O.C.S.-Zahnpaste und Medical-Minerals-Zahngel ein signifikant höherer erosiv/abrasiver Schmelzverlust als für die unbehandelte Kontrolle beobachtet.

Résumé

Introduction

L'érosion dentaire et le ramollissement du tissu dentaire dur associé à l'attaque acide sont la cause la plus commune pour la perte du tissu dentaire dur à côté de la carie. Pour la prévention des érosions, de nouveaux produits (pâte dentaire et gel minéral R.O.C.S.) ont été récemment lancés sur le marché suisse. Afin d'informer adéquatement les patients sur le potentiel préventif potentiel de ces produits, le sujet de l'étude présente était d'évaluer la perte de l'émail érosif et érosif/abrasif utilisant le système R.O.C.S. et de le comparer à celui d'un dentifrice aux propriétés antiérosives connues (dentifrice antiérosion Elmex).

Matériel et méthodes

72 échantillons d'émail bovin ont été préparés et subdivisés en six groupes (E1-E3 et EA1-EA3, n = 12). Les échantillons ont ensuite été introduits dans un cycle érosif (E1-E3) ou érosif/abrasif (EA1-EA3). Par jour, les échantillons d'émail ont été érodés neuf fois deux minutes avec de l'acide chlorhydrique (pH 2,6). Entre chaque attaque d'érosion et pendant la nuit, les échantil-

lons ont été stockés dans de la salive artificielle. Deux fois par jour (matin et soir), les échantillons des groupes E1-E3 ont été stockés dans une pâte de dentifrice pendant 100 secondes ou en plus brossés avec 20 coups de brossage avec la suspension correspondante (EA1-EA3). Les pâtes dentifrices ont été préparées à la base de salive artificielle et des dentifrices suivants: pas de dentifrice (groupe témoin E1 et EA1), dentifrice Elmex contre l'érosion (E2 et EA2) ou R.O.C.S. Dentifrice (E3 et EA3). De plus, les échantillons des groupes E3 et EA3 ont été placés une fois par jour pendant 40 minutes dans une suspension de salive artificielle et de R.O.C.S. Minéraux médicaux gel dentaire. Après 7, 14 et 21 jours, la perte d'émail résultante a été déterminée avec un profilomètre de contact et comparée aux tests de Kruskal-Wallis et aux tests post-hoc de Conover.

Résultats

Dans des conditions purement érosives (groupes E1-E3), à tous les temps de mesure, la perte la plus faible a été observée dans le groupe E2 (dentifrice Elmex contre l'érosion). La perte d'émail dans le groupe E3 (R.O.C.S.) était significativement plus élevée que dans le groupe E2 mais significativement plus faible que dans le groupe E1 (témoin). Dans des conditions érosives/abrasives, de nouveau à tous les temps de mesure, la perte d'émail significativement la plus faible a été observée dans le groupe de dentifrice Elmex protection contre l'érosion (EA2). La perte d'émail dans le groupe EA3 (R.O.C.S.) était même plus élevée que la perte d'émail du groupe témoin (EA1).

Discussion

Dans les limites de l'étude présente, on peut conclure que la combinaison de R.O.C.S. Toothpaste et Medical Minerals Tooth Gel est capable de réduire la perte érosive de l'émail. Cependant, la combinaison de R.O.C.S. Toothpaste et Medical Minerals Tooth Gel n'offrent aucune protection contre la perte d'émail érosif/abrasif.

References

- AMAECHI B T, HIGHAM S M: In vitro remineralisation of eroded enamel lesions by saliva. *J Dent* 29: 371-376 (2001)
- AMAECHI B T, HIGHAM S M, EDGAR W M: The influence of xylitol and fluoride on dental erosion in vitro. *Arch Oral Biol* 43: 157-161 (1998)
- AMAECHI B T, HIGHAM S M, EDGAR W M: Caries inhibiting and remineralizing effect of xylitol in vitro. *J Oral Sci* 41: 71-76 (1999)
- ARENDS J, SMITS M, RUBEN J L, CHRISTOFFERSEN J: Combined effect of xylitol and fluoride on enamel demineralization in vitro. *Caries Res* 24: 256-257 (1990)
- ATTIN T, BECKER K, ROOS M, ATTIN R, PAQUE F: Impact of storage conditions on profilometry of eroded dental hard tissue. *Clin Oral Investig* 13: 473-478 (2009)
- ATTIN T, BECKER K, WIEGAND A, TAUBÖCK T T, WEGEHAUPT F J: Impact of laminar flow velocity of different acids on enamel calcium loss. *Clin Oral Investig* 17: 595-600 (2013)
- ATTIN T, KNOFEL S, BUCHALLA W, TUTUNCU R: In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. *Caries Res* 35: 216-222 (2001)
- ATTIN T, WEGEHAUPT F, GRIES D, WIEGAND A: The potential of deciduous and permanent bovine enamel as substitute for deciduous and permanent human enamel: Erosion-abrasion experiments. *J Dent* 35: 773-777 (2007)
- ATTIN T, WEGEHAUPT F J: Methods for assessment of dental erosion. *Monogr Oral Sci* 25: 123-142 (2014)
- ATTIN T, ZIRKEL C, HELLWIG E: Brushing abrasion of eroded dentin after application of sodium fluoride solutions. *Caries Res* 32: 344-350 (1998)
- AUSTIN R S, STENHAGEN K S, HOVE L H, DUNNE S, MOAZZEZ R, BARTLETT D W, TVEIT A B: A qualitative and quantitative investigation into the effect of fluoride formulations on enamel erosion and erosion-abrasion in vitro. *J Dent* 39: 648-655 (2011)
- AYKUT-YETKINER A, ATTIN T, WIEGAND A: Prevention of dentine erosion by brushing with anti-erosive toothpastes. *J Dent* 42: 856-861 (2014)
- BABCOCK F D, KING J C, JORDAN T H: The reaction of stannous fluoride and hydroxyapatite. *J Dent Res* 57: 933-938 (1978)
- BARTLETT D W, COWARD P Y: Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. *J Oral Rehabil* 28: 1045-1047 (2001)
- BARTLETT D W, LUSSI A, WEST N X, BOUCHARD P, SANZ M, BOURGEOIS D: Prevalence of tooth wear on buccal and lingual surfaces and possible risk factors in young European adults. *J Dent* 41: 1007-1013 (2013)
- BATISTA G R, ROCHA GOMES TORRES C, SENER B, ATTIN T, WIEGAND A: Artificial Saliva Formulations versus Human Saliva Pretreatment in Dental Erosion Experiments. *Caries Res* 50: 78-86 (2016)
- BUZALAF M A, MAGALHAES A C, WIEGAND A: Alternatives to fluoride in the prevention and treatment of dental erosion. *Monogr Oral Sci* 25: 244-252 (2014)
- CARDOSO C A, DE CASTILHO A R, SALOMÃO P M, COSTA E N, MAGALHÃES A C, BUZALAF M A: Effect of xylitol varnishes on remineralization of artificial enamel caries lesions in vitro. *J Dent* 42: 1495-1501 (2014)
- CHAKRAVARTHY P, ACHARYA S: Efficacy of extrinsic stain removal by novel dentifrice containing papain and bromelain extracts. *J Young Pharm* 4: 245-249 (2012)
- CHUNMUANG S, JITPUKDEEBODINTRA S, CHUENARROM C, BENJAKUL P: Effect of xylitol and fluoride on enamel erosion in vitro. *J Oral Sci* 49: 293-297 (2007)

- GANSS C, HARDT M, BLAZEK D, KLIMEK J, SCHLUETER N:** Effects of toothbrushing force on the mineral content and demineralized organic matrix of eroded dentine. *Eur J Oral Sci* 117: 255–260 (2009)
- GANSS C, LUSSI A, GRUNAU O, KLIMEK J, SCHLUETER N:** Conventional and anti-erosion fluoride toothpastes: effect on enamel erosion and erosion-abrasion. *Caries Res* 45: 581–589 (2011)
- GANSS C, NEUTARD L, VON HINCKELDEY J, KLIMEK J, SCHLUETER N:** Efficacy of a tin/fluoride rinse: a randomized in situ trial on erosion. *J Dent Res* 89: 1214–1218 (2010)
- GANSS C, SCHLUETER N, FRIEDRICH D, KLIMEK J:** Efficacy of waiting periods and topical fluoride treatment on toothbrush abrasion of eroded enamel in situ. *Caries Res* 41: 146–151 (2007)
- GANSS C, VON HINCKELDEY J, TOLLE A, SCHULZE K, KLIMEK J, SCHLUETER N:** Efficacy of the stannous ion and a biopolymer in toothpastes on enamel erosion/abrasion. *J Dent* 40: 1036–1043 (2012)
- HAMBA H, NIKAIKO T, INOUE G, SADR A, TAGAMI J:** Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: a quantitative assessment using micro-computed tomography. *J Dent* 39: 405–413 (2011)
- HANNIG M, BALZ M:** Influence of in vivo formed salivary pellicle on enamel erosion. *Caries Res* 33: 372–379 (1999)
- HUNT J N:** The composition of gastric juice. *J Physiol* 113: 419–424 (1951)
- JAEGGI T, LUSSI A:** Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. *Caries Res* 33: 455–461 (1999)
- KALYANA P, SHASHIDHAR A, MEGHASHYAM B, SREEVIDYA K R, SWETA S:** Stain removal efficacy of a novel dentifrice containing papain and Bromelain extracts – an in vitro study. *Int J Dent Hyg* 9: 229–233 (2011)
- KLIMEK J, HELLWIG E, AHRENS G:** Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. *Caries Res* 16: 156–161 (1982)
- KUNIN A A, BELENOVA I S, KUPETS T V:** Evaluating the effectiveness of structural and metabolic tooth enamel reparation by magnesium-calcium remineralizing complex. *EPMA Journal* 5: A122 (2014)
- LAURANCE-YOUNG P, BOZEC L, GRACIA L, REES G, LIPPERT F, LYNCH R J, KNOWLES J C:** A review of the structure of human and bovine dental hard tissues and their physicochemical behaviour in relation to erosive challenge and remineralisation. *J Dent* 39: 266–272 (2011)
- MIAKE Y, SAEKI Y, TAKAHASHI M, YANAGISAWA T:** Remineralization effects of xylitol on demineralized enamel. *J Electron Microscop* (Tokyo) 52: 471–476 (2003)
- O'TOOLE S, BERNABÉ E, MOAZZEZ R, BARTLETT D:** Timing of dietary acid intake and erosive tooth wear: A case-control study. *J Dent* 56: 99–104 (2017)
- OLIVEIRA G C, BOTEON A P, IONTA F Q, MORETTO M J, HONORIO H M, WANG L, RIOS D:** In Vitro Effects of Resin Infiltration on Enamel Erosion Inhibition. *Oper Dent* 40: 492–502 (2015)
- RAMOS-OLIVEIRA T M, SILVA C V, NUNES P M, TURSSI C P, RECHMANN P, FREITAS P M:** AmF/NaF/SnCl₂ solution reduces in situ enamel erosion – profilometry and cross-sectional nanoindentation analysis. *Braz Oral Res* 31: e20 (2017)
- RANJITKAR S, RODRIGUEZ J M, KAIKONIS J A, RICHARDS L C, TOWNSEND G C, BARTLETT D W:** The effect of casein phosphopeptide-amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion. *J Dent* 37: 250–254 (2009)
- RIOS D, HONORIO H M, MAGALHAES A C, BUZALAF M A, PALMA-DIBB R G, MACHADO M A, DA SILVA S M:** Influence of toothbrushing on enamel softening and abrasive wear of eroded bovine enamel: an in situ study. *Braz Oral Res* 20: 148–154 (2006)
- ROCHEL I D, SOUZA J G, SILVA T C, PEREIRA A F, RIOS D, BUZALAF M A, MAGALHAES A C:** Effect of experimental xylitol and fluoride-containing dentifrices on enamel erosion with or without abrasion in vitro. *J Oral Sci* 53: 163–168 (2011)
- ROWAN A D, BUTTLE D J, BARRETT A J:** The cysteine proteinases of the pineapple plant. *Biochem J* 266: 869–875 (1990)
- SCHLUETER N, HARDT M, LUSSI A, ENGELMANN F, KLIMEK J, GANSS C:** Tin-containing fluoride solutions as anti-erosive agents in enamel: an in vitro tin-uptake, tissue-loss, and scanning electron micrograph study. *Eur J Oral Sci* 117: 427–434 (2009)
- TURSSI C P, MESSIAS D F, CORONA S M, SERRA M C:** Viability of using enamel and dentin from bovine origin as a substitute for human counterparts in an intraoral erosion model. *Braz Dent J* 21: 332–336 (2010)
- VIEIRA A, OVERWEG E, RUBEN J L, HUYSMANS M C:** Toothbrush abrasion, simulated tongue friction and attrition of eroded bovine enamel in vitro. *J Dent* 34: 336–342 (2006)
- WANG X, MEGERT B, HELLWIG E, NEUHAUS K W, LUSSI A:** Preventing erosion with novel agents. *J Dent* 39: 163–170 (2011)
- WEGEHAUPT F, JORGE F, ATTIN T, TAUBÖCK T:** Influence of Shortened Light-curing Duration on the Potential of Resin-based Surface Sealants to Prevent Erosion. *Oral Health Prev Dent* 15: 79–87 (2017)
- WEGEHAUPT F J, ATTIN T:** The role of fluoride and casein phosphopeptide/amorphous calcium phosphate in the prevention of erosive/abrasive wear in an in vitro model using hydrochloric acid. *Caries Res* 44: 358–363 (2010)
- WEGEHAUPT F J, LUNGI N, HOGGER V M, ATTIN T:** Erosive potential of vitamin and vitamin-mineral effervescent tablets. *Swiss Dent J* 126: 457–465 (2016)
- WEGEHAUPT F J, TAUBÖCK T T, STILLHARD A, SCHMIDLIN P R, ATTIN T:** Influence of extra- and intra-oral application of CPP-ACP and fluoride on re-hardening of eroded enamel. *Acta Odontol Scand* 70: 177–183 (2012)
- WIEGAND A, ATTIN T:** Design of erosion/abrasion studies – insights and rational concepts. *Caries Res* 45 Suppl 1: 53–59 (2011)
- WIEGAND A, ATTIN T:** Randomised in situ trial on the effect of milk and CPP-ACP on dental erosion. *J Dent* 42: 1210–1215 (2014)
- WIEGAND A, HIESTAND B, SENER B, MAGALHAES A C, ROOS M, ATTIN T:** Effect of TiF₄, ZrF₄, HfF₄ and AmF on erosion and erosion/abrasion of enamel and dentin in situ. *Arch Oral Biol* 55: 223–228 (2010)
- YU H, ATTIN T, WIEGAND A, BUCHALLA W:** Effects of various fluoride solutions on enamel erosion in vitro. *Caries Res* 44: 390–401 (2010a)
- YU H, WEGEHAUPT F J, ZARUBA M, BECKER K, ROOS M, ATTIN T, WIEGAND A:** Erosion-inhibiting potential of a stannous chloride-containing fluoride solution under acid flow conditions in vitro. *Arch Oral Biol* 55: 702–705 (2010b)