Erosive loss of tooth substance is dependent on enamel surface structure and presence of pellicle - An in vitro study

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Abstract: OBJECTIVE Aim of this in vitro study was to investigate erosive tooth loss in dependence of the enamel surface structure and presence of an acquired pellicle. METHODS Enamel specimens from 19 bovine incisors (4 specimens/incisor) were allocated to four experimental groups (n = 19). The surfaces of half of the specimens were polished (two groups), while the other half was left native (two groups). Specimens of one polished and one native group were placed in pooled human saliva (30 min) for the formation of an acquired pellicle. Thereafter, all specimens were demineralized by superfusion with hydrochloric acid (17 min, pH 2.3) with collection of the superfluent. Erosive substance loss was determined by measuring the dissolved calcium content using a colorimetric assay with Arsenazo III reagent. Differences in erosive substance loss were statistically analyzed with respect to enamel surface and pellicle. A linear mixed effects model was fitted to the data and pairwise differences between groups were evaluated (significance level = 0.05). RESULTS Enamel surface structure ($p < 0.001$) and presence of pellicle ($p = 0.01$) had a significant effect on erosive substance loss. Polished surfaces with pellicle showed the lowest cumulative calcium release [nmol Ca/mm^2] (means ± standard deviation: 48+/-5), followed by polished specimens without (51+/-9) and native specimens with pellicle (54+/-10). No significant differences were found between these groups. Highest cumulative calcium release was found for native specimens without pellicle (61+/-9; $p < 0.05$). CONCLUSIONS Both enamel surface structure and the acquired pellicle are important determinants of the susceptibility to erosive tooth loss.

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Erosive loss of tooth substance is dependent on enamel surface structure and presence of pellicle – an in vitro study

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Running title: Erosion depending on enamel surface & pellicle

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Abstract

Objective

Aim of this *in vitro* study was to investigate erosive tooth loss in dependence of the enamel surface structure and presence of an acquired pellicle.

Methods

Enamel specimens from 19 bovine incisors (4 specimens/incisor) were allocated to four experimental groups (n =19). The surfaces of half of the specimens were polished (two groups), while the other half was left native (two groups). Specimens of one polished and one native group were placed in pooled human saliva (30 min) for the formation of an acquired pellicle. Thereafter, all specimens were demineralized by superfusion with hydrochloric acid (17 min, pH 2.3) with collection of the superfluent. Erosive substance loss was determined by measuring the dissolved calcium content using a colorimetric assay with Arsenazo III reagent. Differences in erosive substance loss were statistically analyzed with respect to enamel surface and pellicle. A linear mixed effects model was fitted to the data and pairwise differences between groups were evaluated (significance level $\alpha = 0.05$).

Results

Enamel surface structure ($p < 0.001$) and presence of pellicle ($p = 0.01$) had a significant effect on erosive substance loss. Polished surfaces with pellicle showed the lowest cumulative calcium release [nmol Ca/mm^2] (means±standard deviation: 48+/-5), followed by polished specimens without (51+/-9) and native specimens with pellicle (54+/-10). No significant differences were found between these groups. Highest cumulative calcium release was found for native specimens without pellicle (61+/-9; $p < 0.05$).

Conclusions

Both enamel surface structure and the acquired pellicle are important determinants of the susceptibility to erosive tooth loss.

Keywords

Erosion; demineralization; native; hydrochloric acid; calcium

Main text

Introduction

Erosive tooth wear is on the rise, affecting between 30% and 50% of deciduous and 20% and 45% of permanent teeth (Schlueter & Luka, 2018). The pathogenesis of this common phenomenon is multifactorial. Over the last decades an increasing consumption of acidic foods and drinks could be observed (Lussi, Hellwig, Zero, & Jaeggi, 2006). Furthermore, individual behavioral criteria such as mode and timing of beverage consumption are important determinants in the development of erosive tooth wear (Edwards, Ashwood, Littlewood, Brocklebank, & Fung, 1998). Another relevant source for erosive tooth damage is gastric juice entering the oral cavity. Gastro-esophageal reflux disorder, one of the major causes of exposure to intrinsic acid, is a relatively common condition. Other possible reasons for the inflow of gastric juices include eating disorders, chronic alcoholism and pregnancy (Moazzez & Bartlett, 2014).

The presence of saliva plays a critical role in the prevention of erosive tooth loss. It offers protection through dilution, buffering, neutralization, and elimination of the dietary acid, as well as through providing minerals for the remineralization of the eroded tooth surface (Mandel, 1987; Zero, 1996). Furthermore it enables the formation of the acquired pellicle through adsorption of salivary proteins and glycoproteins, which constitutes an essential factor in the prevention of erosive tooth loss (Nieuw Amerongen, Oderkerk, & Driessen, 1987; Amaechi, Higham, Edgar, & Milosevic, 1999). This protein-containing layer is formed within minutes on all solid surfaces of the oral cavity and is considered an anti-erosive barrier and buffer zone (Hannig & Joiner, 2006; Hannig, Berndt, Hoth-Hannig, & Hannig, 2009).

Most studies on erosive tooth loss rely on *in vitro* experimentation because of better standardization of the experimental conditions, as well as financial and ethical limitations that need to be considered. In these studies, generally individual human or bovine enamel specimens are submitted to the necessary experiments. This holds true for most *in situ* studies as well, which also apply enamel specimens embedded in individualized splints that can be worn in the oral cavity.

The potential of the acquired pellicle to reduce erosive substance loss and to prevent an acid-induced decrease in microhardness has been studied comprehensively (Hannig & Balz, 1999; Hannig & Balz, 2001; Nekrashevych, & Stösser, 2003; Hara et al., 2006; Wiegand, Bliggenstorfer, Magalhaes, Sener, & Attin, 2008). For these kinds of studies mainly polished enamel specimens were applied out of methodological reasons, irrespective of the fact if they were of bovine or human origin. It was shown, however, that erosive attacks affect native and

polished enamel surfaces differently (Meurman & Frank, 1991; Ganss, Klimek, & Schwarz, 2000; Lin, Kitasako, Nakashima, & Tagami, 2017; Mullan, Bartlett, & Austin, 2017; Mullan, Austin, Parkinson, & Bartlett, 2018). Superficial enamel layers differ from underlying regions in terms of degree of mineralization, water content and level of trace elements like fluoride (Sullivan, 1954; Hallsworth & Weatherell, 1969). Polished enamel specimens expose deeper surface regions and may therefore present with altered dissolution characteristics. Furthermore, the formation of the acquired pellicle, and thus its protective effect might differ between native and polished enamel surfaces. It is possible that native enamel surfaces are more acid-resistant than artificially polished surfaces, and at the same time more restrictive for pellicle acquisition which further results in less susceptibility to erosive action.

Earlier publications investigated either the influence of the tooth structure or the influence of the salivary pellicle on the extent of a subsequent erosion. Therefore, the aim of the present study was to investigate the combination of these factors, i.e. to investigate erosive tooth loss in dependence of the enamel surface (polished vs. native) and presence or absence of an acquired pellicle. It was hypothesized that least erosive substance loss is found in native specimens which are covered by an acquired pellicle.

Materials & Methods

Experimental procedure

Figure 1 schematically illustrates the experimental procedure.

Enamel specimen preparation

A water-cooled, diamond-coated core drill was used to prepare cylindrical enamel specimens (diameter 3 mm) out of bovine anterior teeth (n=19). Four enamel specimens were obtained out of each bovine tooth, which were then distributed to four groups, respectively. In this manner one specimen from the same bovine tooth was included in each group in order to ensure evenly distributed baseline conditions. Thereafter, specimens were embedded in acrylic resin blocks (Paladur, Heraeus Kulzer, Hanau, Germany). For half of the specimens the surface was polished with water-cooled silicon carbide paper discs (# 1000, # 2000, # 4000; Struers, Birmensdorf, Switzerland). All specimens were stored in tap water.

Pellicle formation

No ethics committee vote was necessary to carry out the present study (Declaration of assurance by the Cantonal Ethics Committee of Zurich No. 58-2015). Half of the polished as well as half

of the native specimens were treated with human saliva for the formation of an acquired pellicle (n = 19 each). For this purpose, they were swirled in 1% NaOCl solution for 1 min, then dried and rinsed with distilled water. Thereafter, specimens were placed in individual micro test tubes containing fresh human saliva for the formation of an acquired pellicle for 30 min at room temperature, and were pivoted at regular intervals. At the end of the incubation period, specimens were rinsed with demineralized water.

Erosion

All specimens were eroded with hydrochloric acid (HCl 2.5 mmol/l, pH 2.6) in a custom-built superfusion chamber under controlled conditions (Wiegand et al., 2008). HCl was fed separately to each specimen with a peristaltic pump (Ismatec®, IPC 12, Wertheim, Germany). The first 20 s of acid flow were discarded to avoid initial nonlinearities due to air bubbles and possible contamination with rinsing water. Thereafter, specimens were eroded for a total of 17 min at a rate of 1 µl/s. For the first 5 min the effluent acid with its dissolved calcium was collected every minute into a collection dish (Microplate, 96 well, PS, F-Bottom, Clear, Greiner Bio-One, Germany), while for the following 12 min collection took place in 2 min-intervals.

Calcium dissolution measurement

To quantify the erosive enamel loss, the collected acidic solutions were colorimetrically examined for dissolved calcium (Attin, Becker, Hannig, Buchalla, & Hilgers, 2005). 10 µl of each of the resulting acid fractions were transferred to a microplate and mixed with 100 µl of an indicator reagent solution consisting of 100 mmol/l Imidazol buffer (pH 6.5) and 0.12 mmol/l of Arsenazo III (Fluitest®, CA AIII, Analyticon Biotechnologies AG, Lichtenfels, Germany). Spectrometrical absorbance measurements were performed at 650 nm to determine the calcium content after generation of standard curves.

Data and Statistical analysis

Cumulative calcium release was calculated with respect to the two factors presence of pellicle and surface characteristics (native/polished). A linear mixed model was fitted to the data to account for paired measurements, with "cumulative calcium release" as the target variable, the explanatory variables "pellicle" and "polish" as fixed factors, and the tooth ID as a random factor. Subsequently the four groups were compared pairwise based on marginal means and p-values were corrected for multiple testing. The software R (Team, 2015) and the package lme4

(Bates, Maechler, Bolker, & Walker, 2015; Bates et al., 2015) and emmeans (Lenth, 2018) were used. The significance level was set to $\alpha = 0.05$.

Results

Means and standard deviations of the cumulative calcium release data [nmol/mm^2] over the entire erosion period (17 min) are presented in table 1. Both factors "pellicle" and "polishing" significantly influenced the calcium release ($p = 0.01$ and $p < 0.001$) without interaction ($p = 0.25$). Significantly highest erosive substance loss was observed for native specimens without pellicle. Lowest calcium release was found for polished specimens with pellicle, followed by polished specimens without pellicle and native specimens with pellicle, but no significant differences were found between these groups.

Discussion

The results of the present investigation confirm the findings of multiple prior studies that the acquired pellicle displays a protective effect against erosive challenge (Hannig & Balz, 1999) (Hannig, Hess, Hoth-Hannig, & De Vrese, 2003; Hannig et al., 2004; Nekrashevych & Stösser, 2003). When it comes to the impact of the surface structure, however, native surfaces were less resistant against erosive challenge than polished enamel. Therefore, the null-hypothesis that least erosive substance loss is found in native pellicle-covered enamel surfaces had to be rejected.

It is known that the enamel structure may influence the progression of *in vitro* caused erosion, in particular in human tooth specimens (Meurman & Frank, 1991). Prior studies generally (Meurman & Frank, 1991; Ganss et al., 2000; Lin et al., 2017) found that polished enamel was more prone to erosion than native enamel, which is contrasted by the outcome of the present study. One can speculate about the causes for these contradicting results. One possible explanation lies in the methodology of the presented studies. The cited studies used scanning electron microscopy (Meurman & Frank, 1991), profilometry (Ganss et al., 2000), or focus variation 3D scanning microscopy (FVM) (Lin et al., 2017), and did not include the factor acquired pellicle. The present study measured dissolved calcium as proximate of erosive substance loss while also incorporating the factor pellicle. Since natural enamel provides a larger surface for acid contact, it also allows for the dissolution of more calcium, which could explain the higher tissue loss values found in the present study. Furthermore, due to the lack of polishing native surfaces may have also presented with a larger overall curvature, resulting in an overall bigger surface. Another aspect could be the use of bovine teeth in the present study,

which are not subjected to iatrogenic fluoride sources. Therefore, the typical solubility differences between surface and inner enamel layers due to fluoride apatite formation might not have applied. Lastly there might be an inherently different erosion rate in native and polished enamel; an *in vitro* study, which however did not directly compare the erosion rate of polished and native enamel, observed a large variation in the erosion susceptibility of native human enamel specimens (Rakhmatullina, Bossen, Bachofner, Meier, & Lussi, 2013).

In general, bovine teeth are a popular substitute for human tooth material because of their wide availability, lack of caries and large tooth surfaces. In this investigation the great surface area allowed for the distribution of one specimen of the same bovine tooth into each of the four experimental groups. In this way similar experimental baseline conditions could be ensured (Wiegand & Attin, 2011). The calcium loss found in this study, however, cannot directly be compared to experiments using human specimens, where slightly lower values would be expected. Due to increased porosity and slightly lower calcium and phosphate contents acidic dissolution is accelerated in bovine material (Featherstone & Mellberg, 1981). This fact was also demonstrated in cyclic erosion/abrasion models (Attin, Wegehaupt, Gries, & Wiegand, 2007). Furthermore, calcium loss of the specimens was variable, which might in part be explained by variation of the natural susceptibility to erosive attacks present in all biologic material (Attin, Weiss, Becker, Buchalla, & Wiegand, 2005).

Erosive conditions were chosen to mimic an intrinsic acid attack by using hydrochloric acid with a pH adjusted to 2.6 (2.5 mmol/l). This value is commonly found in the gastric juices of patients with reflux disease (Bartlett & Coward, 2001). Pooled stimulated saliva was used for the pellicle formation on half of the native and polished specimens. The pellicle formation time of 30 min was chosen because of data published by Hannig et al. (Hannig et al., 2003; Hannig et al., 2004), who investigated the influence of different pellicle-formation times on its anti-erosive function. In these studies, no significant differences in calcium release were found after pellicle formation times of 2, 6, 12 and 24 h followed by treatment with citric acid (Hannig et al., 2003). In a similar set-up with even shorter pellicle formation times, namely 3 min, 60 min and 120 min, also no significant differences with regard to microhardness loss or amount of dissolved calcium were detected, while in pellicle-free surfaces significantly more signs of erosion were found (Hannig et al., 2004). Therefore, the 30 min of pellicle formation in this study seemed sufficient for the study intent.

The use of native enamel surfaces in this study is an aspect that has hardly been investigated with regard to pellicle formation and its anti-erosive effect. Since pellicle adsorption may be different on native and polished enamel, this possibility needs to be further investigated in

future studies. The common use of polished, often even highly polished enamel surfaces can be explained by widely applied methods for assessing erosive tooth loss. For example, when applying microhardness and indentation measurements or profilometry, smoothly polished surfaces are needed to accurately register the occurred erosive substance loss (Attin & Wegehaupt, 2014). In the oral cavity, however, native enamel surfaces are encountered. The results of the present study, i.e. the significant difference in the susceptibility to erosion found between native surfaces with and without pellicle suggest, that surface-related differences in the susceptibility to erosive damage need to be tested in further experimental set ups. If future studies come to similar conclusions, however, the experimental use of native surfaces needs to be considered in studies whenever absolute values of erosive tooth loss are of interest. Furthermore, measuring methods that allow for the assessment of native tooth surfaces should be given preference if possible.

Conclusion

Both enamel surface structure and the acquired pellicle are important determinants of the susceptibility to erosive tooth loss.

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References

Amaechi, B. T., Higham, S. M., Edgar, W. M., & Milosevic, A. (1999). Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. *J Dent Res*, *78,* 1821-1828. https://doi.org/10.1177/00220345990780120901.

Attin, T., Becker, K., Hannig, C., Buchalla, W., & Hilgers, R. (2005). Method to detect minimal amounts of calcium dissolved in acidic solutions. *Caries Res*, *39,* 432-436. https://doi.org/10.1159/000086852.

Attin, T., Wegehaupt, F., Gries, D., & Wiegand, A. (2007). The potential of deciduous and permanent bovine enamel as substitute for deciduous and permanent human enamel: Erosion-abrasion experiments. *J Dent*, *35,* 773-777. https://doi.org/10.1016/j.jdent.2007.07.007.

Attin, T., & Wegehaupt, F. J. (2014). Methods for assessment of dental erosion. *Monogr Oral Sci*, *25,* 123-142. https://doi.org/10.1159/000360355.

Attin, T., Weiss, K., Becker, K., Buchalla, W., & Wiegand, A. (2005). Impact of modified acidic soft drinks on enamel erosion. *Oral Dis*, *11,* 7-12. https://doi.org/10.1111/j.1601-0825.2004.01056.x.

Bartlett, D. W., & Coward, P. Y. (2001). Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. *J Oral Rehabil*, *28,* 1045-1047.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-9, <URL: https://CRAN.R-project.org/package=lme4>. *R package version 1.1-9*,

Edwards, M., Ashwood, R. A., Littlewood, S. J., Brocklebank, L. M., & Fung, D. E. (1998). A videofluoroscopic comparison of straw and cup drinking: the potential influence on dental erosion. *Br Dent J*, *185,* 244-249.

Featherstone, J. D., & Mellberg, J. R. (1981). Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res*, *15,* 109-114. https://doi.org/10.1159/000260508.

Ganss, C., Klimek, J., & Schwarz, N. (2000). A comparative profilometric in vitro study of the susceptibility of polished and natural human enamel and dentine surfaces to erosive demineralization. *Arch Oral Biol*, *45,* 897-902. https://doi.org/10.1016/s0003-9969(00)00041-8.

Hallsworth, A. S., & Weatherell, J. A. (1969). The microdistribution, uptake and loss of fluoride in human enamel. *Caries Res*, *3,* 109-118. https://doi.org/10.1159/000259575.

Hannig, C., Berndt, D., Hoth-Hannig, W., & Hannig, M. (2009). The effect of acidic beverages on the ultrastructure of the acquired pellicle--an in situ study. *Arch Oral Biol*, *54,* 518-526. https://doi.org/10.1016/j.archoralbio.2009.02.009.

Hannig, M., & Balz, M. (1999). Influence of in vivo formed salivary pellicle on enamel erosion. *Caries Res*, *33,* 372-379. https://doi.org/10.1159/000016536.

Hannig, M., & Balz, M. (2001). Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. *Caries Res*, *35,* 142-148. https://doi.org/10.1159/000047446.

Hannig, M., Fiebiger, M., Güntzer, M., Döbert, A., Zimehl, R., & Nekrashevych, Y. (2004). Protective effect of the in situ formed short-term salivary pellicle. *Arch Oral Biol*, *49,* 903-910. https://doi.org/10.1016/j.archoralbio.2004.05.008.

Hannig, M., Hess, N. J., Hoth-Hannig, W., & De Vrese, M. (2003). Influence of salivary pellicle formation time on enamel demineralization--an in situ pilot study. *Clin Oral Investig*, *7,* 158-161. https://doi.org/10.1007/s00784-003-0219-2.

Hannig, M., & Joiner, A. (2006). The structure, function and properties of the acquired pellicle. *Monogr Oral Sci*, *19,* 29-64. https://doi.org/10.1159/000090585.

Hara, A. T., Ando, M., González-Cabezas, C., Cury, J. A., Serra, M. C., & Zero, D. T. (2006). Protective effect of the dental pellicle against erosive challenges in situ. *J Dent Res*, *85,* 612-616. https://doi.org/10.1177/154405910608500706.

Lenth, R. (2018). Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.2.3. https://CRAN.R-project.org/package=emmeans.

Lin, W. T., Kitasako, Y., Nakashima, S., & Tagami, J. (2017). A comparative study of the susceptibility of cut and uncut enamel to erosive demineralization. *Dent Mater J*, *36,* 48-53. https://doi.org/10.4012/dmj.2016-239.

Lussi, A., Hellwig, E., Zero, D., & Jaeggi, T. (2006). Erosive tooth wear: diagnosis, risk factors and prevention. *Am J Dent*, *19,* 319-325.

Mandel, I. D. (1987). The functions of saliva. *J Dent Res*, *66 Spec No,* 623-627. https://doi.org/10.1177/00220345870660S203.

Meurman, J. H., & Frank, R. M. (1991). Progression and surface ultrastructure of in vitro caused erosive lesions in human and bovine enamel. *Caries Res*, *25,* 81-87. https://doi.org/10.1159/000261348.

Moazzez, R., & Bartlett, D. (2014). Intrinsic causes of erosion. *Monogr Oral Sci*, *25,* 180-196. https://doi.org/10.1159/000360369.

Mullan, F., Austin, R. S., Parkinson, C. R., & Bartlett, D. W. (2018). An in-situ pilot study to investigate the native clinical resistance of enamel to erosion. *J Dent*, *70,* 124-128. https://doi.org/10.1016/j.jdent.2018.01.005.

Mullan, F., Bartlett, D., & Austin, R. S. (2017). Measurement uncertainty associated with chromatic confocal profilometry for 3D surface texture characterization of natural human enamel. *Dent Mater*, *33,* e273-e281. https://doi.org/10.1016/j.dental.2017.04.004.

Nekrashevych, Y., & Stösser, L. (2003). Protective influence of experimentally formed salivary pellicle on enamel erosion. An in vitro study. *Caries Res*, *37,* 225-231. https://doi.org/10.1159/000070449.

Nieuw Amerongen, A. V., Oderkerk, C. H., & Driessen, A. A. (1987). Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. *Caries Res*, *21,* 297-309. https://doi.org/10.1159/000261033.

Team, R. C. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/.

Rakhmatullina, E., Bossen, A., Bachofner, K. K., Meier, C., & Lussi, A. (2013). Optical pen-size reflectometer for monitoring of early dental erosion in native and polished enamels. *J Biomed Opt*, *18,* 117009. https://doi.org/10.1117/1.JBO.18.11.117009.

Schlueter, N., & Luka, B. (2018). Erosive tooth wear - a review on global prevalence and on its prevalence in risk groups. *Br Dent J*, *224,* 364-370. 10.1038/sj.bdj.2018.167.

Sullivan, H. R. (1954). The solubility of enamel surfaces. *J Dent Res*, *33,* 504-510. https://doi.org/10.1177/00220345540330041001.

Wiegand, A., & Attin, T. (2011). Design of erosion/abrasion studies--insights and rational concepts. *Caries Res*, *45 Suppl 1,* 53-59. https://doi.org/10.1159/000325946.

Wiegand, A., Bliggenstorfer, S., Magalhaes, A. C., Sener, B., & Attin, T. (2008). Impact of the in situ formed salivary pellicle on enamel and dentine erosion induced by different acids. *Acta Odontol Scand*, *66,* 225-230. https://doi.org/10.1080/00016350802183401.

Wiegand, A., Meier, W., Sutter, E., Magalhães, A. C., Becker, K., Roos, M., & Attin, T. (2008). Protective effect of different tetrafluorides on erosion of pellicle-free and pellicle-covered enamel and dentine. *Caries Res*, *42,* 247-254. https://doi.org/10.1159/000135669.

Zero, D. T. (1996). Etiology of dental erosion--extrinsic factors. *Eur J Oral Sci*, *104,* 162-177.

Figure Captions

Figure 1

Schematic illustration of the experimental procedure.

Tables

Table 1

Means ± standard deviations of the cumulative calcium release data [nmol/mm^2] of the four groups (n = 19).

19 bovine teeth
(4 specimens each, allocated to groups A-D)

Pel^{-}Pol^{+}
(n = 19)

Pel^{+}Pol^{+}
(n = 19)

Pel^{+}Pol^{-}
(n = 19)

Pel^{-}Pol^{-}
(n = 19)

Surface polished
(n = 38)

Surface native
(n = 38)

Pellicle formation
(treatment with saliva for 30 min)

17 min of erosion with HCl at pH 2.6

Determination of Ca^{2+} release by Arsenazo III method

Table 1

	Mean ± SD cumulative calcium release [nmol/mm2]
Group A: polished without pellicle	51.1 ± 9.2
Group B: polished with pellicle	48.4 ± 5.4
Group C: native with pellicle	53.5 ± 9.8
Group D: native without pellicle	60.5 ± 8.7