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SHORT COMMUNICATION

Impact of storage conditions on profilometry of eroded dental hard tissue

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Abstract The aim of the present study was to analyze in how far drying of eroded dentin and enamel surfaces influence the results of profilometrical determinations with a stylus profilometer. Each five dentin and enamel samples were eroded with HCl (pH2.6, 2 min). Surface profiles of the samples were recorded with a stylus profilometer in three series. In series 1, the samples were measured while stored in water and in series 2, under ambient conditions (21°C, 35% humidity). In series 3, samples were completely desiccated and then rewetted. Profilometry was conducted at various time intervals for a period of up to 181 min (series 1 and 2) and 72 h (series 3). Only the dentin samples were affected by the storage conditions. Stable profilometrical readings for the eroded dentin samples were only feasible when the specimens were stored in water during the complete period of the experiment, including the profilometrical measurement. Thus, for erosion experiments using profilometrical analysis with a stylus profilometer, it is advised to store and measure dentin samples under wet conditions.

Keywords Profilometry · Dentin · Enamel · Erosion

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Introduction

Erosive attacks induced by acidic substances lead to surface loss of dental hard tissues. This loss could be determined by profilometrical scanning of specimens with a laser beam or a contact stylus (metal or diamond; diameter, ca. 2–20 µm) [1, 5, 7, 13, 14]. The contact stylus is loaded with a force of few millinewtons with a resolution in height of about 10 nm. Own studies revealed that in enamel, the acidic attack does also lead to a roughening of the surface of about 0.4 µm [unpublished data]. Thus, reliable detection of losses below 1 µm are generally difficult to accomplish, although Hooper et al. [6] used profilometry to distinguish between different abrasivities of toothpastes creating hard tissue loss of about 0.5 µm. For such precise measurements with low variations, meticulous flattening of sample surface is decisive. With dentin, another aspect might impact accuracy of the profilometrical determination. In dentin, the acidic attack leads to a loss of the anorganic minerals, leaving back the exposed organic matrix at the surface. It was recently shown that the contact stylus of a profilometer cave into the exposed matrix resulting in different values than an optical stylus [4]. This study gave a hint that drying of eroded dentin might affect profilometrical readings. In adhesive dentistry, dentin is pre-treated with phosphoric acid, when the so-called etch and rinse technique is used. Investigations of dentin after application of phosphoric acid showed that the organic matrix shrinks when the dentin is dried [10]. This effect might also have an impact on the records performed by profilometry.

The intention of the present study was (1) to analyze in how far drying of the eroded dentin and enamel surface might influence the results of profilometrical determination

and (2) if recommendations could be given with regard to the best time point and storage conditions using profilometry after an erosion. Also, influence of rewetting of dried eroded dentin and enamel should be evaluated.

Materials and methods

A stylus profilometer (Mahr Perthometer S2/GD 25; Mahr, Göttingen, Germany) placed on a pneumatic stone desk was used (stylus tip, 2 µm in diameter; force during measurements, about 0.7 mN). The device is equipped with a custom-made jig for repositioning of samples for successive measurements.

Determination of background noise Background noise of the profilometer as located in our laboratory was recorded. Vertical displacement of the stationary stylus during 20 s showed a mean of 0±0.035 µm. Thus, according to the guidelines of bioanalytical analysis, the lower limit of measurements (mean+3×standard deviation) is 0.105 µm [11]. Therefore, readings and differences below 0.105 µm are not distinguishable from "zero" and were marked as "below detection limit".

Determination of reproducibility For determination of reproducibility of the profilometrical measurements, a bovine dentin sample (3 mm in diameter) was embedded in a di-acrylate ring (Paladur, Heraeus Kulzer, Hanau, Germany) and polished as for the main experiment. The sample was kept wet during the following assessments. With a custom-made device, scratched line marks were placed on the surface of the mold and the dentin. The line marks on the mold and on the dentin surface were arranged in an angle of 45° (Fig. 1, left), allowing determination of the precision of the movement of the *xy* table of the profilometer. Thus, movement of 100 µm (d*y*) of the table in the *y*-axis for recording two profiles at an interval of 100 µm should result in a distance of also 100 µm (d*x*) of the two scratches on the dentin of these two profiles (Fig. 1, right). In other words, the distance between these two scratches represents the distance between two profiles on the *y*-axis. Firstly, we checked reproducibility of profiles, when the dentin sample was repositioned in the special holder of the profilometer for ten times. After each repositioning, the sample was profilometrically scanned with a single profile. On these profiles, the ten central scratches on the dentin surface were located in an interval of 109 µm with a mean of 0±34 µm. Secondly, we checked the reproducibility of repeated measurement by performing ten profiles of the sample without removing the sample from the holder in between the determinations. The vertical difference of each profile with regard to the first profile was recorded, obtaining a range of 0±0.031 µm.

Main experiment From each bovine incisor, five dentin and enamel cylinders (3 mm diameter) were prepared from the root and the crown, respectively. The samples were adhesively fixed in ceramic rings (Degussit; height, 3 mm). Therefore, the ceramic was etched with 9.5% HF gel for 1 min (Porcelain Etch, Ultradent, Cologne, Germany), followed by application of a silane coupling agent (Monobond S, Ivoclar Vivadent, Schaan, Liechtenstein). The enamel was etched with 35% phosphoric acid (Ultra-Etch, Ultradent). Enamel and dentin samples were then treated with primer and adhesive of Syntac classic (Ivoclar Vivadent). Heliobond (Ivoclar Vivadent) was used for cementation of the samples into the rings using light-polymerization for 60 s (Bluephase, Ivoclar Vivadent). All specimens were ground flat and polished with water-cooled carborundum discs (1,200, 2,400, and 4,000 grit, Water Proof Silicon

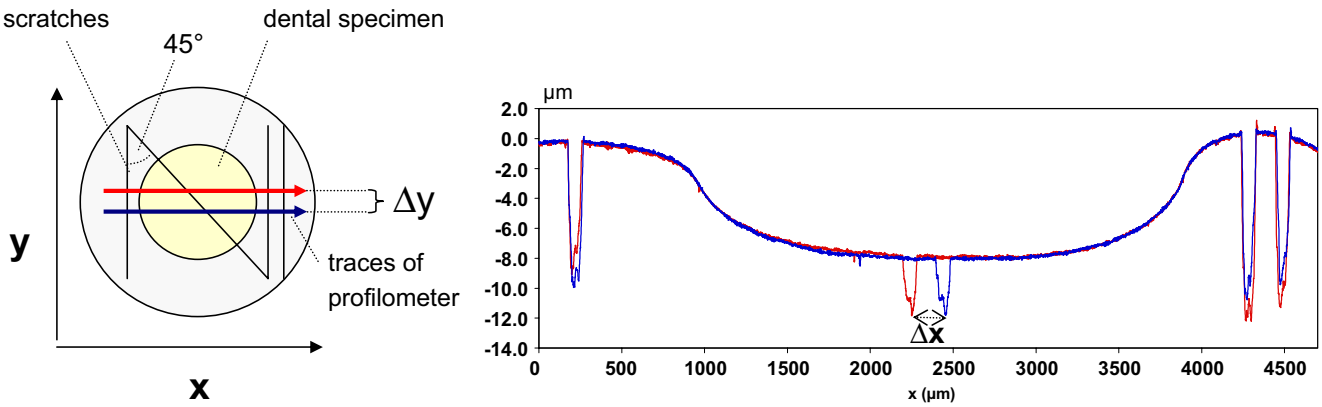

Fig. 1 *Left* Schematic drawing of a specimen with intentionally placed scratches used to determine reproducibility of profilometric assessment. *Right* Exemplary surface profile gained from a specimen with respective scratches and an intentional movement, with Δ*y*= 100 µm=Δ*x*

carbide Paper, Stuers, Erkrat, Germany). The samples were stored in tap water for 1 week and afterwards immersed in HCl (pH2.6) for 2 min. The samples were removed from the acidic solution, rinsed with distilled water, and were not dried. They were then fixed in a special adapter with a rim of 1 cm in height, allowing to keep the samples immersed in water during the following profilometrical determination. In the first series ("wet specimens"), the samples were scanned while the adaptor was filled with water. The stylus of the profilometer moved across the center of the specimen, including the ceramic surface within about 20 s. The ceramic surface was later taken as reference for the depth of the groove created by the erosion. Each one profile was recorded for each specimen at baseline and at different intervals (1.0, 1.4, 2.0, 2.8, 4.0, 5.7, 8.0, 11.3, 16.0, 22.6, 32.0, 45.3, 64.0, 90.5, 128.0, 181.0 min). In another series ("ambient specimens"), the same samples were measured under ambient conditions without drying the samples in between the two series. The baseline determination was performed while the samples were still covered with water. Then, the water was removed from the adapter, and the samples were carefully dapped off with absorbent tissue. The following determinations were done under ambient conditions (21°C, 35% air humidity) without further drying or wetting at the same intervals as described for the wet samples. For the third series ("rewetted specimens"), the samples were desiccated in an exsiccator for 7 days. Then, they were fixed in the profilometer and the adaptor was filled with water. Profiles were recorded at the following time points (baseline, 0.27, 0.53, 1, 2, 4, 8, 24, 48, and 72 h). In series 1 and 2, the samples were not removed from the profilometer during the respective interval of measurements. In series 3, specimens were removed after the first hour of the interval and repositioned for the next determinations.

Analysis For each specimen, the differences of the profile taken at baseline of the respective series to the value measured at the various time points were calculated. Custom-made software allowing exact matching of the ceramic surfaces was used so that the differences between the profiles could be determined. These differences were calculated for the central 1 mm of the profile.

Statistical analysis Power analysis was done under the assumption that with a standard deviation of 0.14 µm, a two-sided significance level of 0.05 and a sample size equal to 5, a power of 99% exists to detect the smallest relevant effect of 0.6 µm. Linear mixed models were applied to investigate the changes of the profiles with time for the samples of the second series [3]. Data of the samples of the first and third series were not further evaluated either due to the heterogeneity of the results or due to fact that the majority of values gathered were below detection limit.

Results

The samples continuously stored in wet conditions did not show any change over time with respect to the profiles recorded. The results of the other storage conditions (series 1 and 2) are given in Tables 1 and 2. In Table 1, the mean changes of the profiles with respect to the respective baseline value are given for the enamel samples measured under ambient conditions. The dentin samples of this second series (=ambient specimens) were the only group with a significant change over time ($p<0.001$); the other groups did not change with time ($p>0.813$). The mixed model approach was then used to estimate the profile changes of the "ambient" dentin samples with time. The following model was found: Profile change=$-0.6-0.15\times$ time$+0.0005$ time^2. This would, for example, mean that at the time point 4 min after start of storage in ambient conditions, the change of the profile amounts to −1.19 µm.

Discussion

The background noise of the profilometer was determined to assess the detection limit of the device. It should be noticed that the value obtained is only representative for the profilometer, when located under the conditions given in our laboratory. Background noise does not only reflect the characteristic feature of the device itself but does also depend on ambient conditions such as vibration of the building or presence of people. Reproducibility of the measurements was high with low variations as described above when applying repeated measurements. However, it should be noticed that both the custom-made software for matching of profiles and the special jig for repositioning of samples might contribute to the precision of the repeated measurements. As in some previous studies, the samples were embedded in ceramic rings, acting as reference surfaces during profilometrical assessment [2]. This procedure was necessary in the present study in order to have an unchangeable reference not affected by shrinkage. Other investigators prefer the use of protected reference areas on the sample surface itself for determining surface loss of unprotected sites [4, 8, 9]. This approach might lead to problems in the profilometrical assessment when shrinkage of the sample occurs.

In demineralized dentin, a mechanical stylus will cave into the soft collagen structures while not reaching the mineral front of the underlying dentin. A possible solution

Table 1 Means and standard deviations (SD) of differences in profile (micrometer) at the respective time points as compared to baseline for the samples of series 1 (ambient conditions)

	Time (min)															
	1.0	1.4	2.0	2.8	4.0	5.7	8.0	11.3	16.0	22.6	32.0	45.3	64.0	90.5	128.0	181.0
Dentin																
Mean	−0.32	−0.45	−0.73	−0.65	−1.09	−1.43	−1.98	−2.48	−3.20	−3.99	−5.60	−6.73	−7.93	−9.24	−10.98	−10.88
SD	0.47	0.67	0.80	1.15	1.22	1.54	1.91	2.20	2.83	3.41	4.18	5.01	5.50	6.52	7.10	7.06
Enamel																
Mean	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	−0.12	_[a]	_[a]	_[a]	_[a]	_[a]	−0.23	−0.49
SD	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	0.24	_[a]	_[a]	_[a]	_[a]	_[a]	0.17	0.29

Negative values indicate shrinkage
[a] Below detection limit

Table 2 Means and standard deviations (SD) of differences in profile (μm) at the respective time points as compared to baseline for the samples of series 3 (rewetted)

Time (h)	0.27	0.53	1.00	2.00	3.00	4.00	5.00	8.00	24.00	32.00	48.00	72.00
Dentin												
Mean	12.83	14.34	15.06	16.26	16.94	17.99	19.81	21.15	24.10	25.47	24.95	23.82
SD	5.79	8.12	9.79	11.21	12.24	13.51	16.39	18.79	23.20	24.40	24.89	23.20
Enamel												
Mean	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	−2.74	_[a]	_[a]	−2.62
SD	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	4.06	_[a]	_[a]	3.96

Negative values indicate shrinkage
[a] Below detection limit

for the problem might be the use of an optical, non-contact stylus. A non-contact profilometer would be more appropriate for profilometry of dentin, but the problem of dentin shrinkage is existent irrespective of the kind of profilometer used. However, measurements with either of these two styluses do not reflect the mineral loss satisfactorily. For determination of mineral loss, other methods such as microradiography might be more promising, at least for assessment of mineral loss due to severe demineralizing conditions with an extensive loss of mineral. It should also be noticed that an optical stylus would not allow for surface profiling under wet conditions since parts of the light are already reflected by the liquid surface not reaching the sample surface. This might lead to artifacts.

The present study intended to evaluate the effect of storage conditions and shrinkage behavior of eroded enamel and dentin on the outcome of a profilometrical analysis. Shrinkage of a demineralized dentin sample is caused by both shrinkage of the dentin bulk and the exposed surface collagen. It might be speculated that these two parts might behave differently with respect to rewetting and the velocity of shrinkage. In the present study, no distinction between the behavior of these two parts and their impact on the profilometrical determination was made. The eroded enamel samples did not show significant changes over time irrespective of the storage conditions. This would mean that for this substrate, profilometrical analysis could be comparably performed under wet and ambient conditions. Also, the excessive desiccation and rehydration of the enamel specimens did not influence the performance. With the enamel samples, the values recorded after rewetting of the desiccated samples showed no (below detection limit) or only negligible effects. However, this was not true for the dentin samples, which exhibited at least for the first 24 h a continuous expansion. It was striking that the standard deviations in this group were very high, indicating that the amount of expansion was very inhomogeneous and not predictable. Also, with regard to the ambient dentin samples, the data revealed that shrinkage over time significantly influenced the outcome of the determination. These facts showed that profilomerical measuring under ambient conditions or after excessive drying should be avoided in order to gain reliable data. The observation that drying of dentin and the related shrinkage is critical was also made for other measurements such as the use of microradiography in dentin [12]. It should also be noticed that only a single profile was captured at each time point. Usually, when the loss due to erosion is determined, more profiles or even a complete mapping of the surface is done. These procedures take some time so that shrinkage of the eroded dentin under ambient conditions might affect the reliability and homogeneity of the data. Rewetting of the desiccated dentin samples caused partly debonding of the dentin from the ceramic ring resulting in a partial gap formation between the sample and the ceramic. These samples were still fixed in the mold, allowing profilometrical analysis. However, it is probable that this behavior affected the analysis and was responsible for the heterogeneity of the data.

The samples in series 1 and 2 were not removed from the profilometer during the measurement. This might have caused that the stylus traced the same sample surface carving into the surface. However, the data of series 2 (wet conditions) showed that no measurable difference between the various profiles with regard to baseline. This means that (relevant) carving due to the stylus might not have been a serious problem. The study by Ganss et al. [4] showed that shrinkage of eroded dentin during short drying episodes might be reversed by rewetting. In contrast to the present study, Ganss et al. [4] did not desiccate the dentin samples in a desssicator, but by keeping the samples under ambient conditions prior to rewetting. Although the exact duration of storage under ambient conditions is not mentioned in that study, it is assumed that the different dessication procedures might have led to different rewetting behavior of the samples in the two studies. Moreover, in the study by Ganss et al. [4], profilometrical measurements were only influenced by shrinkage during the first 10 min of storage under ambient conditions. Within the following 20 min, no significant shrinkage was detected. In the present study, a plateau was achieved after about 30–60 min. In contrast to the present study, Ganss et al. used reference surfaces for the profilometrical assessment, which were located on the dentin surface areas protected during erosion and not on unchangable sites. It might be speculated that within the first minutes under ambient conditions, a rapid loss of water especially occurred in the exposed collagen layer, leading to distinct and measurable shrinkage as referred to the non-demineralized reference areas. It is conceivable that, thereafter, shrinkage mainly occurred in the dentin bulk, leading to similar shrinkage of the demineralized and non-demineralized reference areas. Owing to the observations of these two studies, it is advisable to avoid desiccation and to store dentin samples during the experiment in a liquid, whenever possible. To generate profilometrical data unaffected by ambient conditions, it is advised to measure the profiles with the samples under water. This approach is only feasible with a stylus profilometer and not with an optical one since the liquid deflects the light beam of the latter. Nevertheless, it should be respected that profilometrical determinations do not satisfactorily reflect the mineral loss of eroded dentin samples.

Conflict of interest The authors declare that they have no conflict of interest.

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