Interaction between toothpaste abrasivity and toothbrush filament stiffness on the development of erosive-abrasive lesions in vitro

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Running title: Interactions in Erosion/Abrasion

Key words: erosion, abrasion, toothbrushing, filament stiffness, enamel, dentin

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Objectives: To investigate the enamel and dentin surface loss caused by the interaction between abrasives in toothpaste and toothbrush filament stiffness.

Methods: The study followed a 2 (high/low-level abrasive; silica) × 3 (filament stiffness; soft/medium/hard) × 2 (cycling time; 3/5d) factorial design. Polished bovine enamel and dentin specimens (n=8 each per group) were subjected to 5d of erosion/abrasion cycling: erosion (5min, 4×/d, 0.3% citric acid, pH 3.75), abrasion (15s, 2×/d, 45 strokes each, 150g load, automated brushing machine), fluoride treatment (15s with abrasion and 45s without abrasion; 275ppm F as NaF in abrasive slurry) with exposure to artificial saliva between erosion and abrasion/F exposure (1h) and all other times (overnight). Non-contact profilometry was used to determine surface loss (SL) after 3 and 5d of cycling. Data were analyzed using three-way ANOVA (factors: abrasive/filament stiffness/time) with separate analyses conducted for enamel and dentin.

Results: For enamel, only 'cycling time' was found to affect surface loss with 5>3d. Overall, there was little SL (mean range: 0.76-1.85µm). For dentin (mean SL range: 1.87-5.91µm), significantly higher SL was found for 5 vs. 3d, with particularly large differences for hard stiffness/high-level abrasive, and medium stiffness/medium abrasive. Hard stiffness resulted in significantly higher SL than medium stiffness for high abrasive after 5d, with no other significant stiffness differences. High abrasive had significantly higher SL than medium abrasive overall with strong effects for all combinations, except medium stiffness after 5d.

Conclusion: The interplay between abrasivity and filament stiffness appears to be more relevant for dentin than enamel.

INTRODUCTION

Tooth brushing is considered the most common method to maintain good oral hygiene. The general consensus is that the use of toothpastes and toothbrushes in line with guidelines of governmental and professional bodies does not cause significant wear of enamel and dentin over the course of life[1,2]. However, both toothpastes and toothbrushes have been shown to play a crucial role in the manifestation of erosive tooth wear[3,4]. While a brief acid challenge leads to no significant surface loss per se, it softens the hard tissue structure making it more vulnerable to abrasion[5]. Longitudinally, this can amount to clinically significant wear of the dentition, loss of form and function of the teeth and, ultimately, costly restorative procedures. Furthermore, dentin hypersensitivity has been considered an erosive tooth wear phenomenon[6], with more conclusive evidence about their association emerging recently[7].

There is a multitude of individual and often additive or synergistic but rarely mitigating factors that may potentially impact the severity of wear of the dental hard tissues. A significant body of literature exists on these factors which include the type of abrasive and its concentration, slurry viscosity, brushing force, frequency and duration, type of toothbrush and its geometry and age, filament stiffness, and the condition of the substrate as modified by the severity, duration and timing of the acid challenge and remineralization phase[8-910111213141516]. Based on these data it has been postulated that the abrasivity of the toothpaste is the most important parameter that affects the abrasion process of the dental hard tissues, with the toothbrush acting as carrier, thereby merely modifying the effects of the toothpaste abrasives[1]. This conclusion has been derived predominantly from a range of *in vitro*[13,15] studies with, however, conflicting results regarding the role of filament stiffness for enamel and dentin[13,15].

While there is little doubt that toothpaste abrasivity, measured as either relative dentin or enamel abrasivity (RDA or REA)[17], is positively correlated with wear of initially sound[Error! Bookmark not defined.] or eroded dentin[9,15], and sound[10] or eroded enamel[9,13], the role of filament stiffness is somewhat unclear. Filament stiffness is controlled by filament diameter; so-called 'hard toothbrushes' have filaments with a larger diameter than 'soft toothbrushes', with the most common 'medium toothbrushes' in between. It is often postulated that hard toothbrushes cause more wear than soft ones which has led to recommendations of soft toothbrushes for patients diagnosed with erosive tooth wear[18,19]. Mechanistic laboratory studies, however, revealed the opposite as soft toothbrushes were found to accelerate the wear process because of their greater ability to carry abrasive particles across the surface[13,15,16]. Nonetheless, these studies were somewhat limited in their approach as acid challenges were mimicking intrinsic erosion[13,15] or abrasion periods were too long[16], therefore likely exaggerating effects. The present study aimed to address these shortcomings and resolve the interaction between toothbrush filaments and abrasives used in toothpastes using a clinically relevant erosion/abrasion cycling model[11,14]. The aim of this *in vitro* study was therefore to investigate the interaction between two distinct abrasive levels commonly found in toothpastes and soft, medium and hard toothbrushes on the development of erosive/abrasive lesions in enamel and dentin under pH cycling conditions.

MATERIALS AND METHODS

Study design

The factorial design study investigated the interaction between abrasivity at two levels (high/low; silica) and toothbrush filament stiffness at three levels (soft/medium/hard) over time (cycling time; 3/5 d). Polished bovine enamel and dentin specimens (n=8 each per group) were subjected to 5 d of erosion/abrasion cycling: erosion (5 min, 4×/d, 0.3% citric acid, pH 3.75), abrasion (15 s, 2×/d, 45 strokes each, 150g load, automated brushing machine), fluoride treatment (15 s with abrasion and 45 s without abrasion; 275 ppm F as sodium fluoride [NaF] in abrasive slurry) with exposure to artificial saliva between erosion and abrasion/F exposure (1 h) and all other times (overnight). Non-contact profilometry was used to determine surface loss (SL) after 3 and 5 d of cycling.

Specimen preparation

Enamel and dentin specimens ($4 \times 4 \times 2$ mm^3) obtained from bovine incisors, stored in 0.1% thymol solution, were prepared. Bovine teeth were obtained from Tri State Beef Co. (Ohio, USA), from cattle with an average age of three years (range: 18 months to five years). The bottom and top of the enamel and dentin sides of the slabs were sequentially ground flat using silicon carbide grinding papers (Struers, USA; Struers RotoPol 31/RotoForce 4 polishing unit, USA). Enamel and dentin specimens were embedded, side by side with a small space in between, in acrylic resin (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicone mold, leaving the enamel and dentin surfaces exposed. The embedded blocks were then serially ground and polished up to 4000-grit grinding paper followed by a 1-μm diamond polishing suspension (Struers, USA). UPVC tapes were placed on the surface of the specimens, leaving an area of 1 × 4 mm exposed in the center of the each enamel/dentin specimen. Specimens were selected based on the quality of enamel and dentin: those exhibiting surface scratches, cracks, hypomineralized areas or a non-uniform surface polish were excluded. Specimens were then randomized into six experimental groups with eight specimens per group.

Erosive and remineralizing solutions

A solution of 0.3% (w/v) anhydrous citric acid (Sigma C1857) in deionized water (pH 3.75) was used as an erosive challenge in this study[14]. Artificial saliva (1.45 mM Ca, 5.4 mM PO_4, 0.1 M Tris buffer, 2.2 g/l porcine gastric mucin, pH 7.0) was used as the remineralization medium[14].

Abrasive slurries, toothbrushes and brushing abrasion

Two aqueous abrasive slurries were prepared using precipitated silica abrasives: “high” (15% [w/w] Zeodent 103, Huber Engineered Materials, USA) and “low” (5% [w/w] Zeodent 113). Slurries also contained 275 ppm F as NaF (mimicking 1100 ppm F toothpaste after 1:3 dilution), 0.5% (w/w) carboxymethylcellulose (Blanose 7MF) and 10% (w/w) glycerol. The relative dentin and enamel abrasivity (RDA and REA), determined according to ISO 11609, of the abrasive slurries were: low – REA = 4.0/RDA=69; high – REA = 7.1/RDA = 208.

Table 1 provides information about the study toothbrushes (Lactona, The Netherlands). Filament diameter was determined using a calibrated light reflection microscope (2100 HT; Wilson Instruments, USA).

Specimens were positioned in an automated brushing machine and brushed for 15s (45 strokes) with one of three test toothbrushes (load of 150 g) with their respective abrasive slurries.

Daily treatment regimen

The daily treatment regimen is presented in *Table 2*. The experiment was conducted at room temperature. Erosion was performed under static conditions, whereas the artificial saliva was stirred at 100 rpm. After each cycling procedure, specimens were rinsed with deionized water for 10 s.

Profilometry

After the 3rd and 5th day of cycling enamel and dentin surface loss (SL) were measured by non-contact profilometry (Proscan 2000, Scantron, United Kingdom). The UPVC tapes were removed from the specimens and an area of 1×4 mm^2 in the center of the specimen (including both exposed and previously tape-covered areas) was scanned. Dedicated software (Proscan 2000, Scantron) was used to analyze SL using a three-point height tool.

Statistical analysis

The effects of cycling time, slurry abrasiveness, and toothbrush filament hardness on surface loss were examined using ANOVA. Separate analyses were performed for enamel and dentin. The ANOVA included main effect terms for each of the three factors, all interactions among the factors, and a random effect to correlate the results from the two cycles within a sample. Fisher's Protected Least Significant Differences were used to control the overall significance level of the tests. A 5% significance level was used.

Based on a prior study the within-group standard deviation of the surface loss was expected to be 1.5 µm. With a sample size of eight specimens per abrasivity-hardness combination, the study had 80% power to detect differences of 2.3 µm between any two abrasivity-hardness combinations for each cycling time, assuming two-sided tests conducted at a 5% significance level.

RESULTS

Enamel

Figure 1 shows the enamel SL data (mean ± standard error) by filament stiffness, abrasivity level and cycling time. The three-way interaction abrasivity × stiffness × cycling time was not significant ($P = 0.48$), neither were any of the two-way interactions ($P = 0.86 – 0.98$). Both abrasivity ($P = 0.24$) and filament stiffness ($P = 0.62$) did not affect SL. Only cycling time affected SL significantly ($P = 0.0003$) with day 5 (SL = 1.68 ± 0.16 µm) being proportionally higher than day 3 (0.95 ± 0.10 µm).

Dentin

Figure 2 shows the dentin SL data (mean ± standard error) by filament stiffness, abrasivity level and cycling time. The three-way interaction abrasivity × stiffness × cycling time was significant ($P = 0.0464$). However, the data did not show significant interaction between the two main factors (abrasivity and filament stiffness; $P = 0.1948$). Cycling time affected SL ($P < 0.0001$) but not proportionally (day 3: 3.07 ± 0.17 µm vs. day 5: 4.25 ± 0.21 µm), with particularly large differences for hard toothbrush/high abrasive ($P < 0.0001$) and medium toothbrush/low abrasive ($P = 0.0001$). Hard toothbrush had significantly higher SL than medium toothbrush for high abrasive at day 5 ($P = 0.0088$), with no other significant toothbrush differences ($P > 0.18$). High abrasive had significantly higher SL than low abrasive overall ($P < 0.0001$) with strong effects for all combinations, except medium toothbrush at day 5. SL was directionally but disproportionately correlated with RDA values (SL: 4.46 vs. 2.86 µm – ratio of 1.56:1; RDA: 208 vs. 69 – ratio of 3.0:1).

DISCUSSION

The present study was aimed at investigating SL of eroded enamel and dentin resulting from the interaction between toothpaste abrasivity and toothbrush filament stiffness using an established five-day erosion/abrasion pH cycling model[11,14]. The often recommended brushing time of 2 min per brushing equates to approx. 15 s per surface[20] which was simulated presently as each specimen was brushed for 15 s or 45 strokes per cycle. This equates to a total of 450 brushing strokes for the entire study duration of 5 d (90 strokes/d) and is considerably less than that used in prior *in vitro* (strokes/d: 450[11]; 160[13,15]; 450-1500[14]) and *in situ* (daily brushing duration: 2 min[9]; 1 min[10]) studies on this topic. These levels of brushing abrasion are all justifiable as brushing duration does not only vary between individuals but also between surfaces within individuals[21]. Not considering behavioral aspects, there is little increase in SL of sound enamel with increasing brushing duration; SL of previously eroded enamel, however, increases with the number of brushing strokes, although not in a linear fashion[22]. This is due to gradual loss of affected surface enamel which behaves differently than the underlying bulk tissue. Similar results were obtained for dentin[23], although wear of sound dentin can become significant during a lifetime of toothbrushing abuse.

Flat-trim manual toothbrushes bearing tufts of filaments with round-ended tips were chosen as they represent the most commonly used toothbrush. Likewise, the brushing load of 150 g was selected in line with recommendations by the International Standards Organization (ISO11609), for toothbrushing abrasivity tests. Slurries of the most commonly used abrasives (conventional and whitening precipitated silicas) rather than actual toothpastes were used to minimize the influence of formulation parameters and excipients which can modify the abrasion process (e.g. viscosity, pH, anti-tartar agents).

The high abrasive slurries caused more SL of eroded dentin than the low abrasive one. These results were expected and are in agreement with previous findings[8,9,15]. From the present data, it can be assumed that low abrasive toothpastes may only abrade the superficial layer of softened dentin whereas their high abrasive counterparts are likely to affect deeper parts of the challenged dentin structure. However, eroded dentin is susceptible to wear even under mild erosive/abrasive conditions and much more so than enamel.

This may be related to the fact that dentin is a more vulnerable tissue with little tendency to remineralize once its structural backbone (collagen) has been affected by physical insult[24].

The use of toothpastes with low RDA is part of recommendations for patients with signs of erosive tooth wear[18,19]. While this is undoubtedly a 'common sense' recommendation, its implementation presents issues as manufacturers are not legally required to declare RDA (or REA) data for their products, leaving patients in the dark. Furthermore, RDA and REA are determined under conditions atypical of *in vivo* tooth wear and do not necessarily correlate linearly with clinical or laboratory observations. The presently found discrepancy between RDA and SL data for dentin can be explained by various aspects, such as the presence of fluoride in the abrasive slurries which would have allowed for subsequent remineralization, and the study design (continuous brushing abrasion vs. cycling to allow for relaxation and remineralization of the tissue). Therefore, RDA data should be seen solely as guidance.

Previous research established that nylon toothbrushes alone have negligible effects on the dental hard tissues[Error! Bookmark not defined.], but may indirectly influence the abrasion process by modulating the action of toothpaste abrasives. This is related to the previous indication that different types of toothbrushes likely differ in their capacity to carry toothpaste abrasives across the surface, which may result in differences in abrasion of the dental substrate. Filament stiffness, density of the brush, and filament area of the brush head were shown to modulate this process[8].

In two previous studies of similar design[15,16], wear of eroded dentin increased with decreasing filament diameter. However, the present data suggests that filament stiffness is likely to be a secondary factor in wear of eroded dentin, behind abrasivity. Surprisingly, our data showed that neither toothbrush by itself or its interaction with abrasives were significant factors in dentin SL. Only when combined with cycling time, the interaction became significant. Previous studies[15,16] employed stronger acid challenges, higher brushing loads and longer brushing durations than tested presently, which taken together may explain as to why prior studies were able to demonstrate filament effects. It is likely that the brushing abrasion was too mild in the present study, therefore not allowing for potential differences between filaments to be observed. However, it must be borne in mind that the present study was designed to mimic day-to-day life rather than to artificially demonstrate an effect of a variable implicated in erosive tooth wear.

Enamel did not respond in the same manner as dentin. Neither differences in abrasivity nor filament stiffness were implicated in the wear process. These findings are somewhat in agreement with previous investigations. Hooper *et al.*[9] found no correlation between REA and SL; however, there was directionality between RDA and SL, indicating that surface-softened enamel may behave more like dentin than enamel, although no such observation was made presently. In contrast, Wiegand *et al.*[13] concluded that SL is positively correlated with REA and that filament stiffness does play a minor role in wear, which was confirmed by other investigators[12]. It is likely that differences in the brushing abrasion combined with the severity of the erosive challenge are responsible for the discrepancy between studies. Furthermore, when considering all previous and the present findings it must be borne in mind that enamel wears slowly in comparison to dentin (ratio of 1:2.5 in the present study) which suggests that in patients with gingival recession, non-carious cervical lesions are more likely to manifest themselves than coronal wear.

As predicted, cycling time was positively correlated with SL of both enamel and dentin. While enamel SL was proportional; i.e. day 5 SL was approx. 5/3× that of day 3, this was not the case for dentin. Structural differences between tissues may explain this finding. Enamel contains approx. 96% (w/w) mineral which is also responsible for its structural backbone. Hence, SL is expected to be proportional over time. Dentin, however, contains 70% (w/w) mineral which is embedded in a collagen matrix (20%; w/w). Erosion affects in large the mineral content of dentin, leaving behind collagen which cannot be removed completely through abrasion (at least under the presently chosen conditions). Depending on the severity of erosive vs. abrasive challenges, the measured SL may therefore not necessarily correlate with mineral loss. Several methodological considerations were brought forward recently to address differences between mineral and SL[25], however also highlighting the need for longitudinal clinical observations to provide better recommendations for *in vitro* research and model development.

‘Soft’, ‘medium’ and ‘hard’ toothbrushes from the same brand vary in their filaments, number of filaments per tuft but not in tuft diameter. However, the effective contact area of the filaments in each tuft with the tooth surface varies as filaments are packed more closely in soft than hard toothbrushes. These differences affect how abrasive particles are carried across the surface – while wider filaments can drag more particles in the tip contact area than narrower filaments, the larger number of narrower filaments can compensate for this difference. However, how effective a filament can drag abrasive particles across the tooth surface depends on a variety of other factors and most importantly on the brush load, and consequently the degree of filament deflection, as well as the particle size[26]. The present study employed silica abrasives which have similar particle size distribution but different surface geometry (personal communication with J.M. Huber Corporation). Future studies on a variety of other commonly used abrasives with different particle size distributions and/or hardness (e.g. calcium carbonate, aluminium oxide) may be able to provide more definite recommendations to patients at risk of erosive tooth wear. Undoubtedly, laboratory studies have their limitations. Only a subset of commercially available toothbrushes and abrasives can be evaluated. Abrasives vary considerably between types as, for example, precipitated silica and calcium carbonate (chalk) have inherently different properties (e.g. particle size, hardness, concentration used). Likewise, a different brush design or filaments from different manufacturers may impact SL, especially when combined with varying abrasives. And as pointed out earlier, differences in brushing load or experimental design per se can lead researchers to different conclusions.

CONCLUSION

For enamel, neither abrasive nor filament stiffness affected SL of softened enamel under the conditions of the present study. However, SL of dentin was mainly affected by abrasivity, with some minor modulating effect of filament stiffness.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author contributions

Revised and reviewed the paper: FL, GJE and ATH. Conceived and designed the experiments: FL, MA and ATH. Performed the experiments: MA. Analyzed the data: FL, GJE and ATH. Wrote the paper: FL and MA.

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Table 1 Properties of the study toothbrushes

Parameter	Soft	Medium	Hard
Filament Diameter [µm]	212.8	228.6	310.4
Bristle Length [mm]	11	11	11
Tufts	43	43	43
Bristles per tuft	50	36	16

Table 2 Daily Treatment Schedule

Treatment	Duration
Erosion (1/4)	5 min
Remineralization (1/6)	60 min
Treatment/abrasion (1/2)	Brushing: 15s (45 stk) + 45s slurry exposure
Remineralization (2/6)	60 min
Erosion (2/4)	5 min
Remineralization (3/6)	60 min
Erosion (3/4)	5 min
Remineralization (4/6)	60 min
Erosion (4/4)	5 min
Remineralization (5/6)	60 min
Treatment/abrasion (2/2)	Brushing: 15s (45 stk) + 45s slurry exposure
Remineralization (6/6)	Overnight

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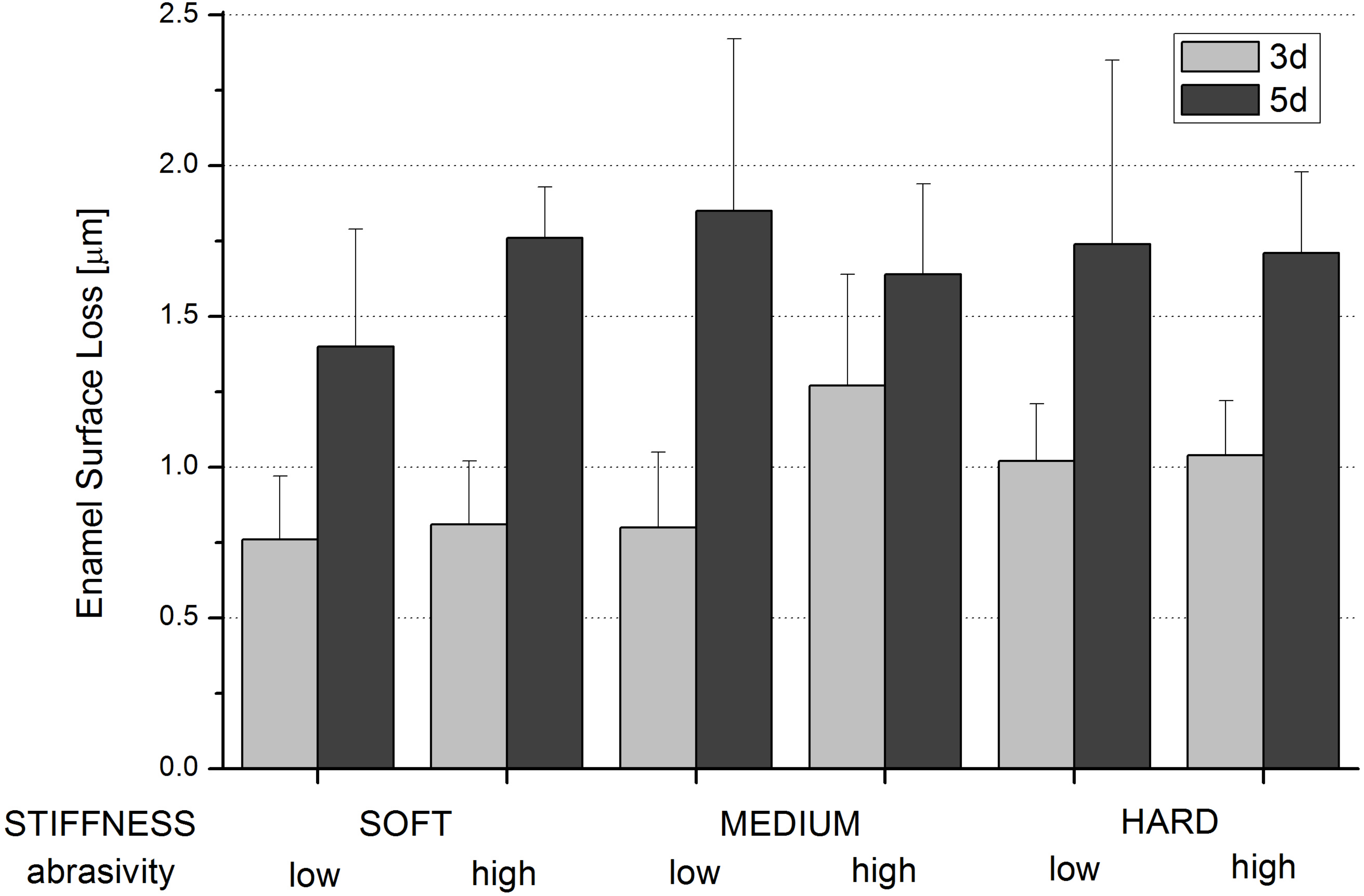
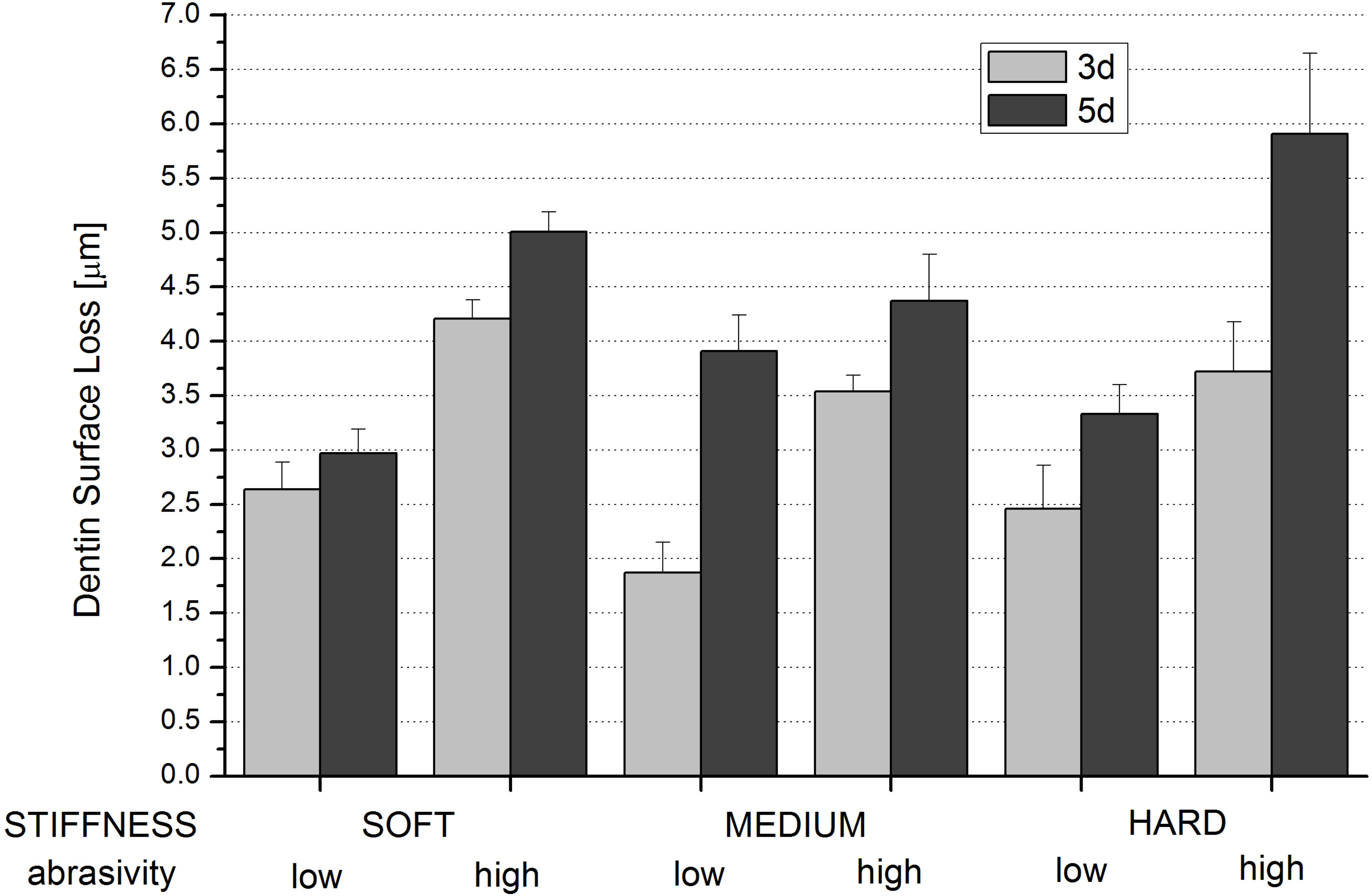
Erosion (2/4)	5 min
Remineralization (3/6)	60 min
Erosion (3/4)	5 min
Remineralization (4/6)	60 min
Erosion (4/4)	5 min
Remineralization (5/6)	60 min
Treatment/abrasion (2/2)	Brushing: 15s (45 stk) + 45s slurry exposure
Remineralization (6/6)	Overnight

Figure 1 Enamel surface loss as a function of abrasive and bristle stiffness during the pH cycling period.

Figure 2 Dentin surface loss as a function of abrasive and bristle stiffness during the pH cycling period.



Enamel Surface Loss [μm]
0.0
0.5
1.0
1.5
2.0
2.5
3d
5d
STIFFNESS
abrasivity
SOFT
MEDIUM
HARD
low
high
low
high
low
high


Dentin Surface Loss [µm]
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3d
5d
STIFFNESS
SOFT
MEDIUM
HARD
abrasivity
low
high
low
high
low
high