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ORIGINAL ARTICLE

Laboratory evaluation of toothbrush/toothpaste abrasion resistance after smooth enamel surface sealing

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Abstract
Objectives The use of dental sealants has been extended to smooth enamel surfaces. The present study was conducted to test the in vitro performance of four sealants with different characteristics (highly and lowly filled, self-etching features).
Materials and methods Eighty human teeth (lower incisors and premolars) were randomly divided into following sealant test groups: ProSeal™, LightBond™, OrthoSolo™, and Seal&Protect®. Twenty untreated teeth served as a control group. Tooth brushing was conducted for a period of time simulating 12, 18, and 24 months. During the toothbrush abrasion protocol, the specimens were subjected to thermal and acidic challenge. Sealant thickness was determined with μCT imaging, and qualitative and quantitative surface effects were investigated using stereo microscopy and raster electron microscopy, respectively. Data were subjected to *t* test or Kruskal–Wallis/Mann–Whitney tests (alpha, 5 %).
Results The wear behavior and film integrity of highly filled sealants were superior to lowly filled sealants. Even after 1 year of tooth brushing, significant surface deterioration with deleterious loss of enamel and discoloration was observed in all tested materials (χ^2=15.349; P=0.004). The size of the observed defects increased over time.
Conclusion These results suggest that the application of sealants on smooth enamel surfaces should be limited to special indications, and their usefulness has to be revisited.
Clinical relevance Based on the results of this in vitro study, the general overall application of enamel sealants needs to be questioned.

Keywords Sealant · Enamel · Surface analysis · Abrasion · REM · Smooth surfaces

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Introduction

Enamel sealants have been developed with the aim of shielding dental pits and fissures from deteriorating agents like acid or bacterial plaque. They have been shown to prevent the development of new demineralization and to arrest the further progression of active lesions without the need for irreversible tooth preparation [1]. Based on the good clinical results with fissure sealing, approaches have been made to extend this preventive concept to smooth enamel surfaces. Among these, special attention is given to smooth enamel areas with increased caries risk such as interproximal or vestibular surfaces in patients with fixed orthodontic appliances [2–7].

Studies on toothbrush abrasion demonstrated that the surface of the tested materials was affected by different factors such as acidic challenge, filler features of the used composite, polymerization source, occlusal loading, and thermocycling. Although many comparative studies on toothbrush abrasion of restorative and prosthetic material exist [8–14], only limited information is available concerning toothbrush abrasion of sealants applied on smooth enamel [2–15].

Therefore, the aim of this study was to evaluate the sealant thickness and the surface effects of different enamel sealants applied on vestibular surfaces after being exposed to simulated mechanical, acidic, and thermal stressing in vitro. Two hypotheses were proposed: (1) toothbrush abrasion in combination with acidic and thermal stressing has effects on the sealants and (2) highly filled sealants show less effects than lowly filled sealants.

Materials and methods

Preparation of enamel specimens

One hundred caries-free human incisors and premolars of the lower jaw were stored for a maximum of 3 months in an aqueous 1 % chloramine-T solution. All teeth were extracted due to orthodontic or periodontal reasons at the Department of Oral and Maxillofacial Surgery of the University Hospital Hamburg-Eppendorf and affiliated clinical departments. The selected teeth revealed no cracks on the buccal surfaces as confirmed by stereomicroscopic evaluation (magnification ×25). After detachment of two thirds of the root and elimination of all soft tissue structures attached to the teeth, the teeth were embedded in chemically cured resin Palavit G® (Heraeus Kulzer, Wehrheim, Germany) with their vestibular surfaces pointing upward and parallel to the surface of the plastic tube. Finally, the teeth were pumiced with a non-fluoride-containing polishing paste.

Tested materials

Teeth were randomly divided into five groups of 20 teeth each.

Group 1: served as a control group. No enamel treatment was conducted.The application of the sealants of groups 2–5 was conducted according to the manufacturer's instructions by a trained orthodontist (HMKS). A detailed overview in terms of main composition and application modalities for each sealant is given in Table 1. In all groups, light polymerization was conducted with a halogen light (Ortholux™ XT, 3M Unitek). The power of the curing light was 600 mW/cm^2.

Group 2: Pro Seal™ (Reliance, Reliance Orthodontic Products, Itasca, IL, USA) is a newly introduced highly filled fluoride-releasing sealant. After 30 s etching time with 35 % phosphoric acid, the enamel surface was rinsed and dried thoroughly. A thin uniform layer was applied onto the etched enamel surface. The sealant was cured at close range for 15 s per tooth.

Group 3: Light Bond™ Filled Sealant (Reliance, Reliance Orthodontic Products, Itasca, IL, USA) is formulated with a patented fluoride-releasing monomer and contains up to 40 % micro particle size filler. After etching for 30 s with phosphoric acid, the etched enamel surfaced was rinsed and dried thoroughly. A thin uniform layer of sealant was applied to the etched enamel using a brush. The coat was cured for 10 s.

Group 4: OrthoSolo™ (Kerr Corporation, Orange, CA, USA) is a lowly filled (2–10 % fumed silica) fluoride-releasing universal primer for enamel condition. After 30 s of etching with a 35 % phosphoric acid, the tooth surface was rinsed thoroughly with air–water spray. The etched enamel was dried with clean, dry air. A small amount of OrthoSolo™ was applied to the etched enamel using the brushes provided. A thin, uniform coating covered the etched surface, which was then cured with the curing light for 30 s.

Group 5: Seal & Protect® (Dentsply, De Trey GmbH, Konstanz, Germany) is a light-curing, self-adhesive sealant. It is claimed to have antibacterial properties resulting from the incorporation of Triclosan™. In Seal & Protect®, nano fillers (7 nm) are incorporated. The percentage of silica in the overall composition is less than 5 %. After drying the cleaned tooth surface and dispensing Seal & Protect® to the tooth surface with the applicator tip, the tooth surface was left undisturbed for 20 s. Excess solvent was removed by blowing gently with air from the dental syringe for a few seconds. The remaining sealant was cured for 10 s with the curing light. A second layer of sealant was applied. Excess solvent from the second layer was removed by blowing gently with air from the dental syringe. Another curing phase for 10 s followed. The oxygen-inhibited layer was removed with a cotton roll.

After the application of the sealants, all teeth were stored in distilled water at 37 °C for 24 h.

Toothbrush–dentifrice abrasion

Abrasion testing was carried out in a newly developed toothbrush abrasion device (Fig. 1). Vertical load and abrasive slurry were based on the study designs of former studies in the field of restorative and prosthetic dentistry. The used power toothbrush (Oral-B® Professional Care 7000 Series, Oral-B Laboratories, Delmont, CA, USA; toothbrush head: Oral-B® Ortho, Oral-B Laboratories, Delmont, CA, USA)

Table 1 Main composition and application modalities of the different sealants

	Manufacturer	Main composition	Application modalities		Group
			Enamel treatment	Curing time	
ProSeal™	Reliance, Itasca, IL, USA	Ethoxylated bisphenol A diacrylate (10–50 %) Urethane acrylate ester (10–40 %) Polyethyleneglycol diacrylate (10–40 %) Fluoride-containing glass frit (5–40 %)	30 s H_3PO_4+rinse and dry	15 s	2
LightBond™	Reliance, Itasca, IL, USA	Glass filler (20–50 %) Urethane dimethacrylate (10–30 %) Triethyleneglycol dimethacrylate (10–30 %) Hydrofluoride methacrylate (1–3 %)	30 s H_3PO_4+rinse and dry	10 s	3
OrthoSolo™	Kerr, Orange, CA, USA	Alkyl dimethacrylate resins (60–80 %) Barium aluminoborosilicate glass (14–24 %) Silicon dioxide (2–10 %) Sodium hexafluorosilicate (1–5 %) Ethyl alcohol (1–5 %)	30 s H_3PO_4 + rinse and dry	30 s	4
Seal&Protect®	Dentsply De Trey, Konstanz, Germany	Acetone (25–50 %) Di- and trimethacrylate resins (25–50 %) PENTA (2.5–10 %) Triclosane (2.5–10 %)	Dispensing S&P for 20 s+second layer	10 s+10 s	5

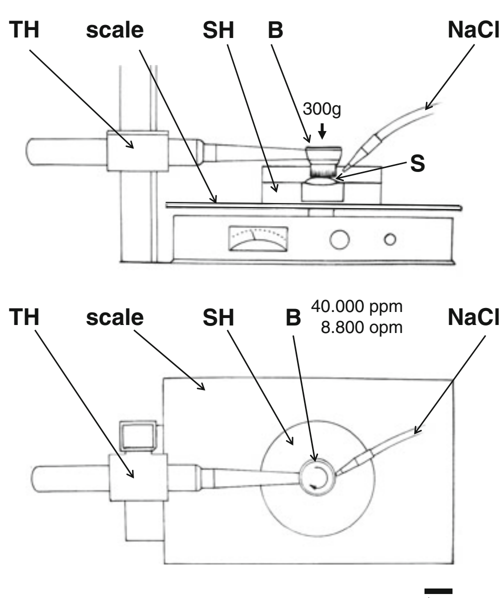

Fig. 1 A schematic diagram of the toothbrush abrasion testing device: Each specimen (*S*) was fixed on a specimen holder (*SH*). A power toothbrush (*B*) conducting 40,000 pulsations/min and 8,800 oscillations/min was fixed in a toothbrush holder (*TH*) and was set to give the specimen (*S*) 300 g vertical load (controlled by a scale). *Scale bar* indicates 1 cm

provided two simultaneous brushing motions: pulsations and oscillations. We used vertical loads of 300 g (2.94 N) [16]. The abrasive slurry was prepared by mixing distilled water and dentifrice (Pearls & Dents; Dr. Liebe, Leinfelden-Echterding, Germany, RDA value 45) in a ratio of 1:1 by weight at room temperature. Before testing, 100 ml of abrasive slurry was dispersed on each specimen. During the tooth brushing cycle, NaCl solution was continuously applied on the specimen to prevent abnormal heat development. In order to come close to in vivo conditions, toothbrush cycles were calculated according to the following formula (seconds tooth brushing of the buccal surface/tooth/day):

$$\frac{3 \times \text{toothbrushing/day} \times 180\text{s}}{28 \text{ teeth} \times 3 \text{ tooth surfaces}}$$

After 15 min of wet polishing, the toothbrush head was changed.

Acidic and thermal stressing

After 39 min (in vitro brushing time imitating 12 months) and 78 min (in vitro brushing time imitating 24 months) of tooth brushing, the specimens were immersed in a dark soft drink with low pH (pH=3.0) (Coca Cola, Coca Cola Company, Atlanta, GA, USA) for 12 h.

The thermal stressing regime was set up as follows: after 15 min of tooth brushing at room temperature, the specimens were immersed in 70 °C distilled water for 10 min following further 10 min at 5 °C in distilled water. Figure 2 illustrates the sequences of thermal and acidic challenges.

μCT analysis

For the three-dimensional visualization, the teeth were scanned in a μCT 40 (Scanco Medical, Bassersdorf, Switzerland) at a resolution of 12 μm (40 kV/114 μA). For the assessment of the sealant thickness, teeth surfaces were scanned at a midshaft at a resolution of 10 μm. The raw data were manually segmented and analyzed with the μCT Evaluation Program V4.4A (Scanco Medical, Bassersdorf, Switzerland). The thicknesses of the sealants were measured with the Distance 3D tool of the μCT Program V4.4A.

Stereomicroscopic evaluation

Sealant integrity was studied on the brushed surfaces using a stereo light microscope. Computer-aided image analysis (UTHSCSA ImageTool, San Antonio, USA) was used to morphometrically quantify the visible defects on the sealant surface. The whole tooth surface was investigated. The affected area of the sealant coating was calculated to the overall area of the coated enamel and was allocated to the following groups:

1 ≤1 % of the overall surface was affected
2 >1 and ≤10 % of the overall surface was affected
3 >10 and ≤30 % of the overall surface was affected
4 >30 and ≤50 % of the overall surface was affected
5 >50 % of the overall surface was affected

REM analysis

A raster electron microscope (REM) was used to analyze the surface microstructure of specimens at T0 and T3. For this purpose, the teeth were air-dried and sputtered with gold in a sputter coater 108 auto device (Cressington, Watford, UK). They were analyzed in a Leo 435vp raster electron microscope (Leo, Oberkochen, Germany) at a magnification of ×500.

Sampling operation

The evaluation of the film integrity was conducted directly after the application, after simulation of 1 year of tooth brushing, after simulation of 1.5 years, and after the simulation of 2 years. At each study point, five specimens of each group were collected, and evaluation was conducted as described. All measurements were blindly performed by one evaluator (AS).

Statistics

The statistical analysis of the data was performed with SPSS® 10.0 for Windows (Lead Technologies, Haddonfield, NJ, USA). All groups of specimens were analyzed for means and standard deviations. Changes of the sealant thickness which were detected with the μCT analysis were identified using the unpaired *t* test. To determine the significance of differences in the deterioration of the sealant film integrity, Kruskal–Wallis and Mann–Whitney nonparametric tests were used ($P \leq 0.05$). With the help of the Kruskal–Wallis test, the performance of the sealants in comparison to the control was evaluated. The Mann–Whitney was used to analyze the specific sealant group that revealed significant differences.

Results

μCt analysis

The μCt images and the calculated film thicknesses of all sealants at baseline and after 2 years of tooth brushing are presented in Fig. 3a, b. The initial sealant thickness of the different sealants showed a wide range (Seal & Protect® 33.15 ±6.05 μm, OrthoSolo™ 53.63±3.73 μm, LightBond™

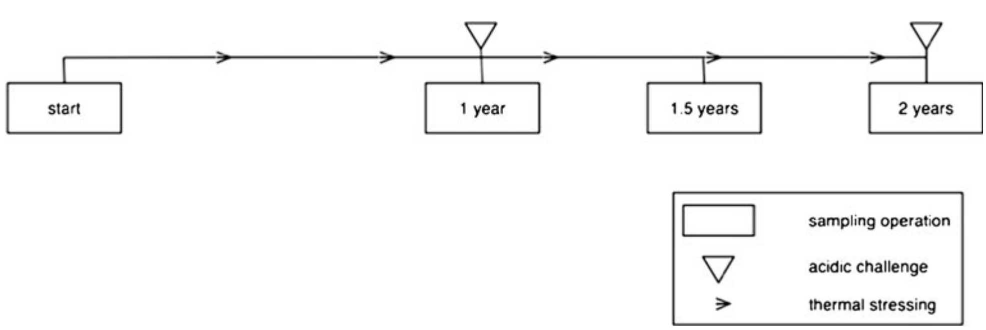

Fig. 2 Flow chart of the acidic and thermal stressing regime

81.68±9.98 µm, and ProSeal™ 123.5±22.24 µm). After 2 years of simulated tooth brushing with acidic and thermal stressing, the overall thickness was unchanged in all sealants except for ProSeal™, which revealed a significant decrease (P=0.022). The decreased coating of ProSeal™ after 2 years of tooth brushing was still significantly higher than the initial film thicknesses of the tested, lower filled sealants.

Stereomicroscopic evaluation

As demonstrated by Fig. 3c, the surfaces of all sealants deteriorated during the 2 years of tooth brushing. At baseline in all sealants, the surfaces had a smooth and glossy appearance. Light-reflecting parts were seen in all sealants. Seal & Protect® demonstrated in comparison to the other sealant groups a less smooth surface with patchy staining. After 2 years, we observed decreased gloss and patchy areas of discoloration in all sealant groups, but not on the unsealed enamel that was subjected to the same toothbrush procedure. In order to quantify our observations, we calculated the extension of the defects in comparison to the overall surfaces (Fig. 4). At baseline, the Kruskal–Wallis test indicated that there were no differences among the control and sealant groups (χ^2=5.821; P=0.213). After 1 year of toothbrush abrasion, significant differences were detected (χ^2=15.349; P=0.004). The Mann–Whitney test showed that all sealant films were significantly affected in comparison to the control ($P \leq 0.05$). After 18 months of tooth brushing, all

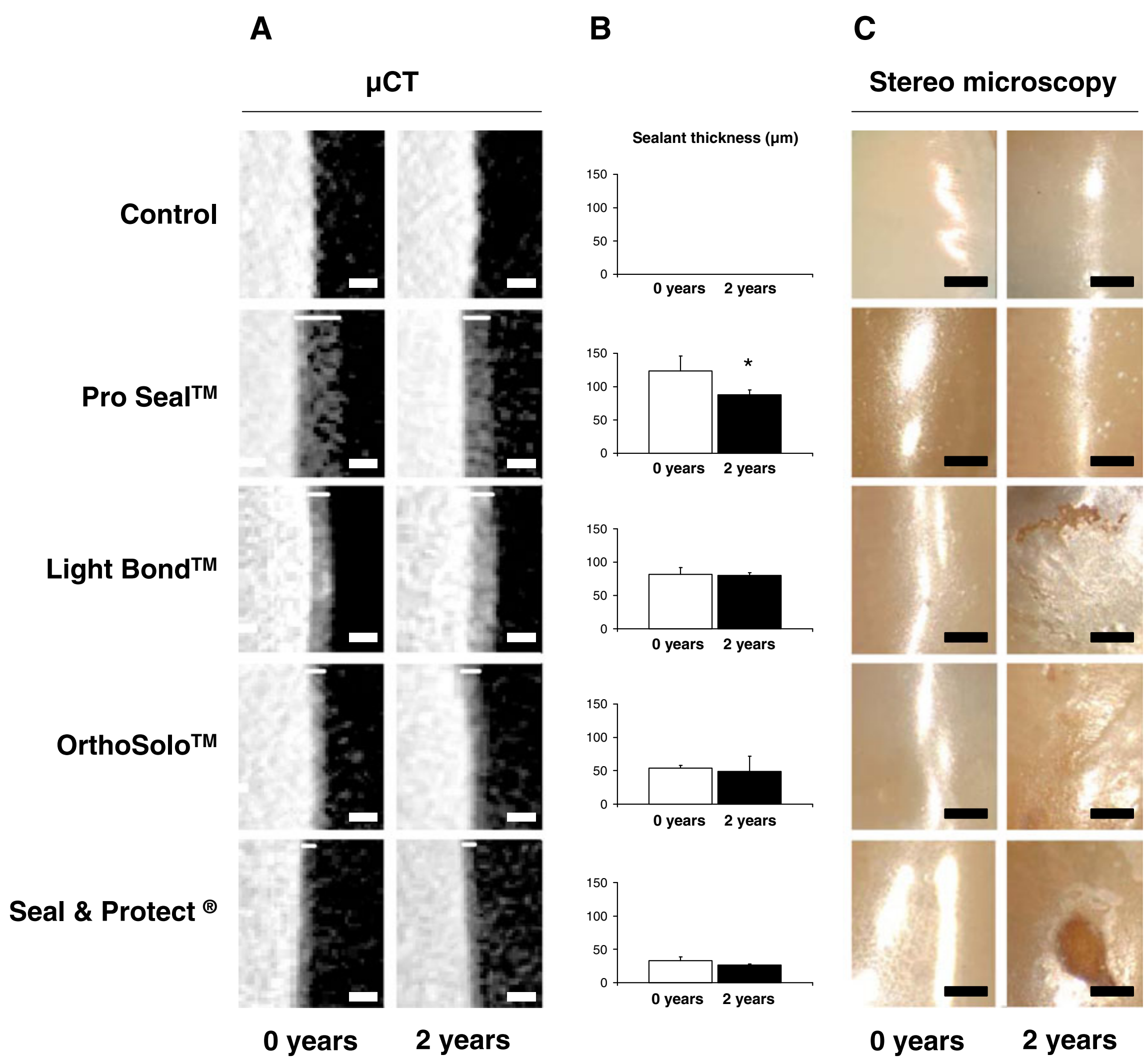

Fig. 3 **a** µCT evaluation of sealant thickness: on the cross-sectional image, the teeth on the *left hand side* (*white*) can easily be separated from the sealant (*gray*) *Scale bar* indicates 100 µm. **b** Pro Seal™ had the highest sealant thickness (*white bar*) at baseline and was the only sealant that deteriorated over the studied time (*P<0.05). **c** At baseline, all teeth show a glossy appearance. Only teeth treated with Seal & Protect® displayed a patchy surface. After 2 years of simulated tooth brushing, the surface of untreated control teeth was unchanged. Pro Seal™ retained the glossy appearance; however, a speckled pattern of small circular defects was visible. Light Bond™, OrthoSolo™, and Seal & Protect® lost the gloss and show large discolored defects. (Light microscopy, ×20). *Scale bar* indicates 500 µm

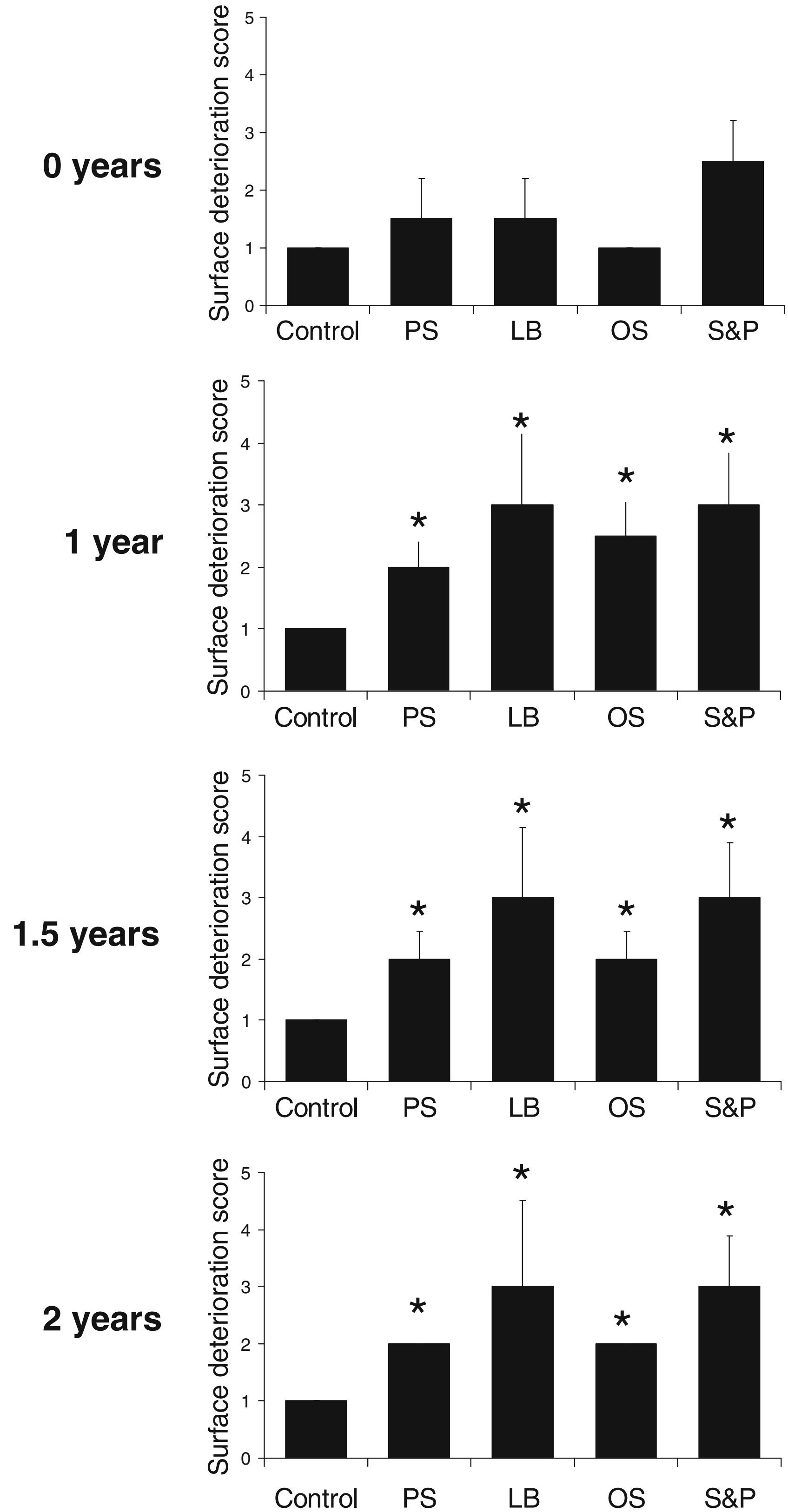

Fig. 4 Quantification of surface discoloration. Surface deterioration was classified from 1 to 5 (*1* ≤1 % of the total surface, *2* >1 and ≤10 %, *3* >10 and ≤30 %, *4* >30 and ≤50 %, *5* >50 %). (*$P \leq 0.05$)

sealants surfaces were significantly affected in comparison to the control. The Kruskal–Wallis test detected significant changes within the sealant groups ($\chi^2=0.029$; $P=9.041$). As proven by the Mann–Whitney test, Seal & Protect® demonstrated significantly more extension of surface deterioration compared to all other used sealants. As demonstrated by the

Kruskal–Wallis test after 2 years, significant surface deterioration was observed in all groups in comparison to the control (χ^2=15.349; P=.004). The sealant film was significantly more affected by Seal & Protect® when compared to OrthoSolo™ (P=0.033) and ProSeal™ (P=0.033).

REM

Representative REM images of enamel surfaces at baseline and after the simulation of 2 years of tooth brushing are shown in Fig. 5. In the control group, the untreated enamel texture remained unchanged during the investigation. At 2 years of simulated tooth brushing, the surface was smooth and homogenous.

ProSeal™ displayed small defects at baseline with an extent of 30–50 μm in which the fillers were visible. At 2 years, the size of these defects had increased (up to 400 μm in diameter). The particles of the fillers became obvious. In some areas, uncoated enamel was visible.

Light Bond™ had an uneven surface texture already at baseline. Shallow elevations with a diameter of 10–50 μm were visible. At 2 years, the sealant film was still detectable, but erosive areas were obvious, and uncoated enamel was visible. The defects had a size of up to 300 μm in diameter.

At baseline, OrthoSolo™ showed a smooth surface with small defects (30–100 μm in diameter) probably due to air inclusion. Within these air included pits the rough matrix of the filled composite was visible. At 2 years, the sealant film showed large defects (up to 700 μm in diameter). Etched enamel was revealed at the bottom of these defects.

Seal & Protect® demonstrated an uneven texture already at baseline. Cracks and defects of up to 500 μm in diameter were observed. Uncoated enamel was visible. At 2 years, the peeling and cracking of the coating were advanced. In some areas, the sealant was virtually undetected.

Discussion

The idea of using sealants for prevention of demineralization is not a new one: In the late 1970s, silanes, coupling agents, and unfilled resins were claimed to reduce demineralization and to seal etched enamel [17]. Subsequent research dispelled these beliefs [5, 18–21].

The tested new protective sealant materials were selected to mirror the different types of composites available on the market: lowly and highly filled composites with self-adhesive features or with a need for prior etching. The power toothbrush was chosen since plaque removal and control of gingivitis were shown to be significantly greater with the new power toothbrush when compared with an ADA reference manual toothbrush [22]. The exposure to the acidic soft drink and the thermal stress have been shown to affect the integrity of tooth surfaces [23–28]. Therefore, we challenged the sealants in our study with a combination of thermal and acidic stress in addition to toothbrush abrasion.

The abrasion testing method and the chosen stressing regime of the study protocol were intended to simulate in vivo conditions. All in vitro testing of toothbrush abrasion (including our study) differ from the in vivo situation in that the protective salivary pellicle is missing, and the enamel surfaces are in continuous contact with the erosive challenge. Furthermore, the successful application of sealants in vivo is complicated by wetting problems that may cause some uneven sealing patterns.

The measured film thickness showed a range from 30 μm (Seal & Protect®) to 120 μm (ProSeal™). The sealant films of the lowly filled composites were thinner than those of the highly filled composites. The thinnest coating is observed in the self-adhesive, lowly filled Seal & Protect®. Significant decrease in the sealing over time was only observed in the ProSeal™ group. The observed changes of sealant thickness might be explained by the conducted abrasion testing regime that simulates severe oral rigors. Although decreased, the sealant thickness of ProSeal™ after 2 years of simulated toothbrush abrasion was still higher than the thickness of the lowly filled composites tested at baseline.

The surface analysis indicated that toothbrush–dentifrice abrasion in conjunction with acidic and thermal stressing resulted in surface deterioration in all sealants. The integrity of the sealant film seemed to be the major problem. Even after 1 year of tooth brushing, the sealant film showed inhomogeneous surfaces textures, which are reported to be predilection sites for surface deterioration of dental materials by toothbrush–dentifrice abrasion [13]. Indeed, the size of the observed defects increased over time. At 2 years, the observed surface effects led to discoloration after being subjected to dark acidic buffer solution.

The acidic challenge contributes to the abrasion. It has been demonstrated that wear resistance of dental material is reduced under acidic conditions [28]. Van Eygen et al. [24] showed that acidic soft drink intake, even of short duration, can cause reductions in enamel micro hardness. In contrast to this observation, our control teeth, which were subjected to the same regimen with additional tooth brushing, showed a homogenous surface without discolorations. Thus, tooth brushing might smooth the effects caused by acidic stress on the surface roughness of enamel, while the sealant films were continuously affected and became heterogeneously rough over time.

Less severe but similar observations as noted in the sealant groups were described in an in vitro study on the resistance of light-cured sealants to acidic soft drinks [23]. After being soaked in acidic soft drinks, the sealant surfaces were inhomogeneous with the majority of the sealant being removed by the chemical attack and revealing only small islands of sealants that withstood the acidic stress.

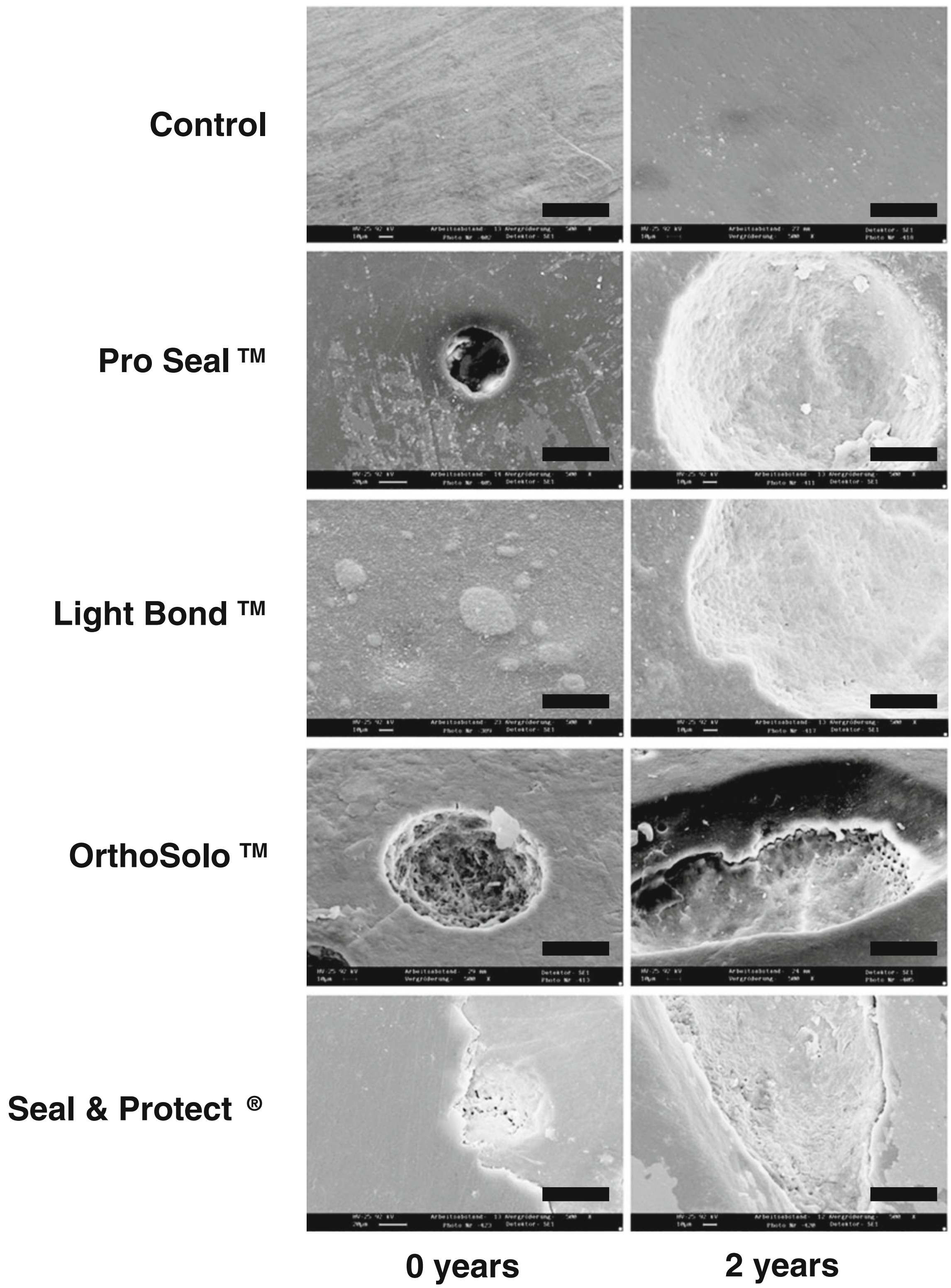

Fig. 5 Raster electron microscopic evaluation of the surface at a magnification of ×500. The surface of control teeth stayed smooth and unchanged over 2 years of simulated tooth brushing. All studied sealants showed small surface defects already at baseline. At 2 years of simulated tooth brushing, the defects increased in size and depth. Uncoated surfaces of etched enamel were visible. *Scale bar* indicates 50 μm

Filler features need be taken into account as a factor that influences wear. The results of our study suggest that the wear behavior and the sealant film integrity of highly filled composites are superior to lowly filled sealants: OrthoSolo™ as a lowly filled universal primer showed the greatest surfaces effects in the group of primers with prior etching. Seal & Protect®, a lowly filled self-adhesive composite, showed a less homogenous surface texture when compared with the highly filled Light Bond™ and ProSeal™. This is in line with previous reports that showed that the presence of small filler particles enhances the wear resistance of composites [10, 15]. Unfilled resin has been proven to show an inadequate resistance to erosive challenges [3].

In addition to filler features and prior preparation, the surface effects of the tested composites—except for ProSeal™ which is claimed to have no oxygen inhibition layer—can be explained by oxygen inhibition. The application of sealants on smooth enamel surfaces leads to oxygen inhibition and air inclusion of the curing reaction preventing the creation of a sufficient homogenous film surface. As demonstrated by the current μCT investigation, the initial thicknesses of the sealant films were sufficient. But initial lesions of the film due to oxygen inclusion during the application expanded over the long-term treatment and led to increased surface deterioration.

Conclusion

Toothbrush abrasion and acidic and thermal stressing under function must be taken into consideration when using enamel sealants in clinical practice. Due to patchy defect formation, measurement of film thickness is not sufficient to assure satisfying performance. In the in vitro testing, sealants with highly filled filler features performed better than lowly filled sealants. However, we observed significant discolorations in all sealant groups. In summation, toothbrush abrasion combined with acidic and thermal stressing has an impact on the sealant film integrity and thickness.

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

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