



OPEN

Anti-demineralizing protective effects on enamel identified in experimental and commercial restorative materials with functional fillers

Matej Par^{1,2}, Andrea Gubler², Thomas Attin², Zrinka Tarle¹ & Tobias T. Tauböck²

The aim of this study was to investigate whether experimental and commercial dental restorative materials with functional fillers can exert a protective anti-demineralizing effect on enamel that is not immediately adjacent to the restoration. Four experimental resin composites with bioactive glass and three commercial restorative materials were investigated. Enamel blocks were incubated in a lactic acid solution (pH = 4.0) at a standardized distance (5 mm) from cured specimens of restorative materials. The lactic acid solution was replenished every 4 days up to a total of 32 days. Surfaces of enamel blocks were periodically evaluated by Knoop microhardness measurements and scanning electron microscopy. The protective effect of restorative materials against acid was identified as enamel microhardness remaining unchanged for a certain number of 4-day acid addition cycles. Additionally, the pH of the immersion medium was measured. While enamel microhardness in the control group was maintained for 1 acid addition cycle (4 days), restorative materials postponed enamel softening for 2–5 cycles (8–20 days). The materials capable of exerting a stronger alkalizing effect provided longer-lasting enamel protection. The protective and alkalizing effects of experimental composites improved with higher amounts of bioactive glass and were better for conventional bioactive glass 45S5 compared to a fluoride-containing bioactive glass. Scanning electron micrographs evidenced the protective effect of restorative materials by showing a delayed appearance of an etching pattern on the enamel surface. A remotely-acting anti-demineralizing protective effect on enamel was identified in experimental composites functionalized with two types of bioactive glass, as well as in three commercial ion-releasing restorative materials.

As secondary caries represents one of the main reasons for the failure of composite restorations¹, bioactive glasses (BGs) have been investigated as interesting dopants for restorative resin composites due to their potential for releasing remineralizing ions^{2,3}, neutralizing acid^{4,5}, and precipitating hydroxyapatite^{6,7}. BGs encompass various formulations with adjustable relative ratios of constituent elements. The composition of a particular BG determines its network structure, which in turn reflects on its properties⁸. In this way, subtle compositional adjustments can be made to tailor reactivity, solubility, and ion release for a desired therapeutic effect⁹. Among various BG compositions that have been investigated as functional additives for methacrylate-based restorative composites, fluoride-modified BGs appear especially promising due to their ability for releasing fluoride ions when exposed to an aqueous medium². These ions can be incorporated into the enamel to render it more resistant to demineralization¹⁰. An additional potential benefit of fluoride-modified BGs is their capability to form a fluorapatite layer on the composite surface¹¹, which can be envisioned as a possible sealing agent for marginal gaps¹². Besides experimental BG formulations containing various therapeutic elements, the conventional Hensch's BG 45S5 formulation has also been extensively investigated as a possible filler for resin composites^{3–6,13}. Notwithstanding the unavoidable trade-offs between its high reactivity and stability of mechanical properties¹⁴ and the

¹Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb, Gundulićeva 5, Zagreb, Croatia. ²Department of Conservative and Preventive Dentistry, Center for Dental Medicine, University of Zurich, Plattenstrasse 11, Zurich, Switzerland. ✉email: mpar@sfgz.hr

	Bioactive glass 45S5	Experimental fluoride-containing bioactive glass	Inert barium glass	Silica
Particle size (d50)	3 μm	3 μm	1 μm	5–50 nm
Composition (wt%)	45.0% SiO ₂ 24.5% CaO 24.5% Na ₂ O 6.0% P ₂ O ₅	33.5% SiO ₂ 33.0% CaO 10.5% Na ₂ O 11.0% P ₂ O ₅ 12.0% CaF ₂	55.0% SiO ₂ 25.0% BaO 10.0% Al ₂ O ₃ 10.0% B ₂ O ₃	>99.8% SiO ₂
Silanization (wt%)	None	None	3.2	4–6
Manufacturer	Schott, Mainz, Germany	Schott, Mainz, Germany	Schott, Mainz, Germany	Evonik, Hanau, Germany
Product name / LOT	G018-144 / M111473	Experimental batch	GM27884 / Sil13696	Aerosil R 7200 / 157,020,635

Table 1. Bioactive glass and reinforcing fillers used in experimental composites.

Material designation	Filler composition (wt%)			Total filler ratio (wt%)
	Bioactive glass 45S5	Experimental fluoride-containing bioactive glass	Reinforcing fillers (inert barium glass : silica = 2:1)	
Control	0	0	70	70
C-10	10	0	60	70
C-20	20	0	50	70
E-10	0	10	60	70
E-20	0	20	50	70

Table 2. Composition of experimental composites.

lack of fluoride release, the traditional BG 45S5 composition represents a promising candidate for functionalizing resin composites^{4,6,15,16}.

BG embedded within resin composites dissolves when exposed to an aqueous medium, releasing its constituent elements into the solution². This ion release is accompanied by an alkalinizing effect¹¹. The release of remineralizing ions and the increase of the solution pH can be employed to protect dental hard tissues against demineralization caused by bacterial acids. Such a protective effect has been demonstrated in experimental BG-functionalized composites intended for use as orthodontic adhesives; these materials showed the potential for protecting enamel adjacent to bonded brackets against the formation of white spots^{17–21}. To investigate whether the protective effect of BG-functionalized composites can extend beyond the dental hard tissues immediately adjacent to the material²², the present in vitro study used enamel blocks incubated in a lactic acid solution at a standardized distance from cured specimens of restorative materials. Experimental composites functionalized with 10 or 20 wt% of conventional BG 45S5 and an experimental fluoride-modified BG were compared to three commercial restorative materials capable of exerting an anti-demineralizing protective effect. Microhardness (MH) was chosen as a convenient indicator of structural integrity of the enamel surface²³ exposed to simulated cycles of acid attack. Additionally, enamel surface morphology and pH changes in the immersion solution were evaluated. The null hypotheses were that the evaluated parameters (enamel MH, enamel surface morphology, and pH of the immersion solution) would: (1) remain unchanged during the simulated acid attack; and (2) not differ among the investigated restorative materials.

Materials and methods

Experimental resin composites. Experimental resin composites were prepared as described in previous studies^{11,24}. The resin system contained bisphenol-A-glycidyl dimethacrylate (Bis-GMA, Merck, Darmstadt, Germany) and triethylene glycol dimethacrylate (TEGDMA, Merck) in a ratio of 60:40 wt%. Camphorquinone (0.2 wt%; Merck) and ethyl-4-(dimethylamino) benzoate (0.8 wt%; Merck) were added to photoactivate the resin system. The components of the resin system were mixed using a magnetic stirrer for 48 h.

The composition of fillers used to prepare experimental composites is given in Table 1. BG 45S5, inert barium glass, and silica were obtained from commercial vendors. The experimental fluoride-modified experimental BG with theoretical network connectivity similar to that of BG 45S5 (2.1)⁸ was prepared on-demand by Schott (Mainz, Germany) via melt-quench route. By using similar preparation and grinding procedures, comparable particle sizes were obtained for both BG types.

Experimental composites with a total filler ratio of 70 wt% were prepared by replacing 0, 10, or 20 wt% of silanized reinforcing fillers (barium glass and silica) with two types of unsilanized BG (Table 2). The experimental composite containing only reinforcing fillers was used as an inert control. The composites were prepared by mixing the resin system and the fillers for 5 min in a dual asymmetric centrifugal mixing system (Speed Mixer TM DAC 150 FVZ, Hauschild & Co. KG, Hamm, Germany) at 2,000 rpm, and deaerating in vacuum for 48 h.

In addition to the experimental composites, three commercial restorative materials with an acid-protective capability were used as references: a reinforced glass ionomer restorative (ChemFil Rock, Dentsply Sirona,

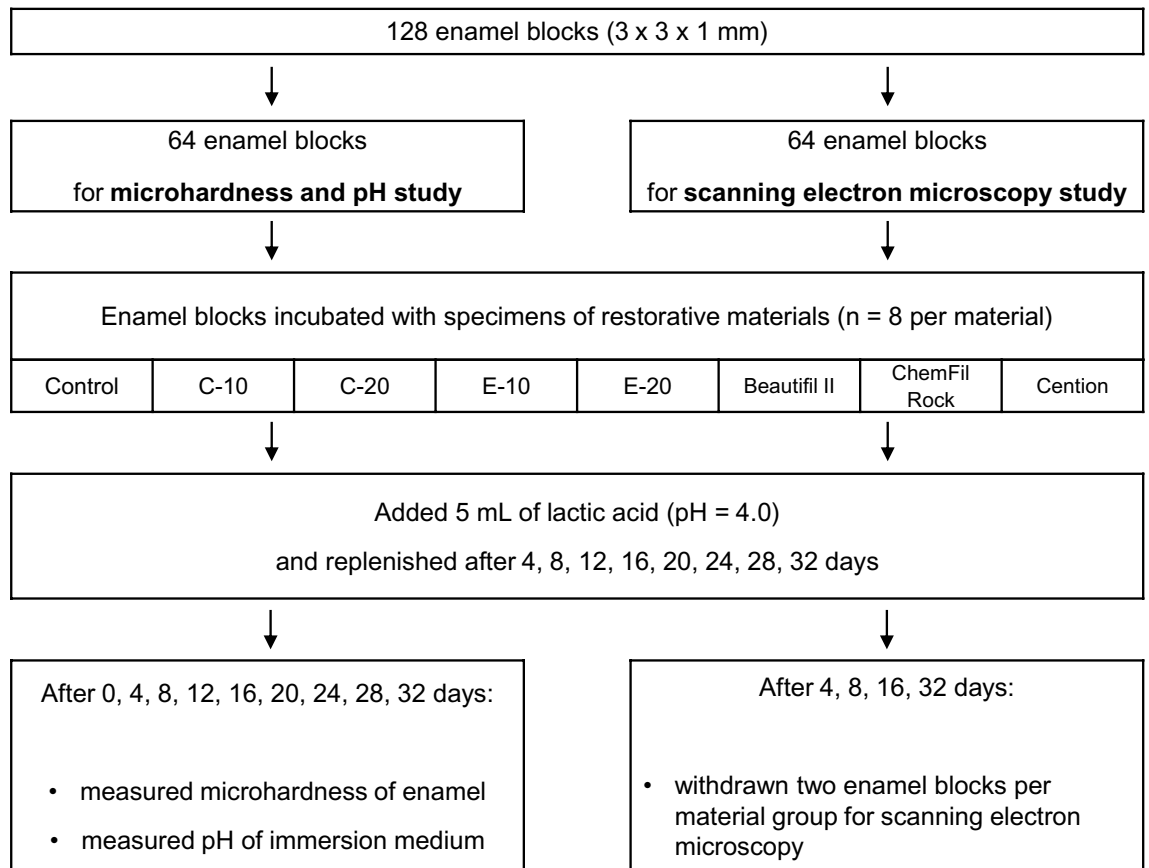


Figure 1. Experimental design.

Konstanz, Germany; shade: A2, LOT: 1903000819), a giomer (Beautifil II, Shofu, Kyoto, Japan; shade: A2, LOT: 041923), and a resin-based “alkasite” material (Cention, Ivoclar Vivadent, Schaan, Liechtenstein; shade: universal, LOT: XL7102). The alkasite material contains two types of reactive fillers: an ionomer glass based on a calcium barium alumino-fluoro-silicate, and a calcium fluoro-silicate glass^{25,26}.

Enamel blocks. One hundred twenty-eight intact human third molars were collected as by-products of regular dental treatment. Patients had given written informed consent to use the teeth for research purposes, and all teeth were irreversibly anonymized immediately after extraction. Under these terms, the research complied with the use of anonymized biological material and, consequently, authorization from the local ethics committee was not required (Federal Act on Research involving Human Beings (Human Research Act; article 2, paragraph 2)). The teeth were stored at 8 °C in 0.1% thymol solution and used within 6 months of extraction.

Enamel blocks (1 block per tooth; 3 × 3 × 1 mm) were prepared from buccal surfaces using a low-speed precision cutting machine (IsoMet, Buehler, Lake Bluff, IL, USA). The buccal sides of enamel blocks were ground using P4000 silicon carbide paper²⁷ (Buehler; 2 min at 30 rpm, median particle size = 2.5 μm). After preparation, the enamel blocks were stored in a phosphate-buffered saline solution and used within five days of preparation. From the total of 128 enamel blocks, 64 were used for the MH and pH measurements, while 64 were used for the scanning electron microscopy (SEM) study, as described in the schematic representation of the study design in Fig. 1.

Restorative material specimens. Discoid specimens (diameter = 7 mm, thickness = 2 mm) were prepared by casting the restorative materials into custom-made polyoxymethylene molds, covering their surfaces with Mylar foils, and flattening using glass plates. The glass ionomer material was left to set in the mold for 15 min, while the light-curable materials (giomer and experimental composites) and the dual-curable alkasite material were illuminated using a violet-blue LED curing unit (Bluephase PowerCure, Ivoclar Vivadent, Schaan, Liechtenstein; emission wavelength range: 390–500 nm, radiant exitance: 1,340 mW/cm²) for 20 s, resulting in a total radiant exposure of 26.8 J/cm². To simulate a clinically realistic scenario, the specimens were exposed to the immersion medium within 15 min after preparation. Per material, 16 specimens were prepared (8 for the MH and pH study; and 8 for the SEM study).

Time	No. of acid addition cycles	Material							
		Control	C-10	C-20	E-10	E-20	Beautifil II	ChemFil	Cention
Initial	0	243.8 (38.8) a	243.7 (39.2) ab	255.2 (42.9) a	262.4 (20.5) a	233.8 (45.0) a	252.7 (33.9) a	267.6 (22.2) a	261.9 (28.6) ab
4 days	1	228.3 (29.8) a	254.2 (32.6) a	267.6 (38.1) a	259.3 (25.1) a	263.8 (21.5) a	245.9 (27.2) a	260.5 (22.2) ab	276.4 (29.8) a
8 days	2	174.9 (32.3) b	207.2 (27.3) bc	256.6 (33.9) a	231.1 (34.1) a	245.4 (40.3) a	203.1 (33.1) ab	244.8 (23.8) abc	269.9 (20.5) a
12 days	3	145.8 (26.8) bc	178.5 (29.8) bc	239.7 (40.4) a	220.6 (22.1) b	221.0 (35.1) ab	201.6 (42.7) ab	221.4 (32.7) bc	239.3 (27.8) ab
16 days	4	107.1 (33.0) cd	136.1 (23.4) d	230.2 (51.8) a	167.2 (32.5) c	174.9 (37.5) bc	175.3 (33.1) b	203.5 (39.5) bc	212.8 (28.2) bc
20 days	5	81.8 (13.7) de	113.9 (32.6) d	207.7 (49.7) ab	123.5 (32.4) d	136.1 (40.0) c	156.9 (36.8) bc	198.5 (47.6) c	175.0 (50.1) c
24 days	6	40.7 (20.3) ef	47.1 (11.6) e	147.4 (51.6) bc	55.5 (30.7) e	69.4 (27.9) d	99.3 (36.5) d	129.4 (44.0) d	98.2 (50.3) d
28 days	7	29.4 (10.6) f	38.6 (14.8) e	145.1 (48.1) bc	43.0 (23.2) e	59.2 (19.1) d	106.0 (29.1) cd	112.4 (49.6) d	70.5 (30.6) d
32 days	8	25.3 (5.3) f	30.8 (9.2) e	122.3 (41.5) c	36.0 (11.4) e	50.0 (16.0) d	88.9 (35.3) d	113.4 (48.6) d	63.8 (19.5) d

Table 3. Knoop microhardness of enamel blocks (mean values with standard deviations in parentheses). Same letters denote statistically similar microhardness values within a material.

Immersion in lactic acid. The enamel blocks and restorative material specimens were immersed in closed vials (Eppendorf; Hamburg, Germany) containing 5 mL of lactic acid solution (pH = 4.0). This experimental setup was adopted and modified according to previous studies^{28–30}. Each vial contained one enamel block and one restorative material specimen, which were held at a standardized distance of 5 mm. The immersion solution was agitated using a horizontal laboratory shaker at a speed of 30 revolutions per minute. The environmental temperature ranged between 23–24 °C. Surface MH of enamel blocks and pH of the immersion solution were measured at the following time points: 4, 8, 12, 16, 20, 24, 28, and 32 days. At each time point, the immersion medium was replenished with 5 mL of fresh lactic acid solution (pH = 4.0).

Microhardness measurements. Knoop MH measurements were performed on the buccal sides of enamel blocks using a digital hardness tester (model no. 1600–6106; Buehler). Indentations were made under a load of 100 g and a dwell time of 20 s at random positions. The indentations were evaluated within 2 min after preparation, with a resolution of 0.015 μm^3 ³¹. Per each specimen and time point, five replicate indentations were made and their mean values were considered as a statistical unit. Eight specimens were used per material (n = 8).

pH measurements. A calibrated pH electrode (780 pH Meter, Metrohm, Herisau, Switzerland) was immersed in the solution, and pH values were recorded with a resolution of 0.01 pH units. Per each specimen and time point, three replicate measurements were made and their mean values were considered as a statistical unit. Eight specimens were used per material (n = 8).

Scanning electron microscopy. Enamel blocks for the SEM study (n = 8 per material) were subjected to the same acid exposure protocol as the specimens for the evaluation of MH and pH. Upon reaching the time points of 4, 8, 16, and 32 days, two enamel blocks per material were withdrawn, rinsed with distilled water, dried, and sputtered with 5 nm of gold. A scanning electron microscope (SEM; GeminiSEM 450, Zeiss, Oberkochen, Germany) was used at 10 kV and 10,000 \times magnification to evaluate surface morphology of enamel blocks.

Statistical analysis. Normality of distribution and homogeneity of variances were checked using Levene's and Shapiro–Wilk's tests, respectively. Within each restorative material, MH and pH values were compared among time points using repeated-measures ANOVA with Bonferroni correction for multiple comparisons. The statistical analysis was performed using SPSS (version 20, IBM, Armonk, NY, USA) at an overall level of significance of $\alpha = 0.05$.

Results

MH values of enamel blocks measured after acid addition cycles are shown in Table 3. Repeated additions of acid solution significantly decreased enamel MH in all groups. The protective effect of restorative materials against acid was identified as enamel MH remaining statistically similar to initial MH values for a certain number of acid additions. The enamel MH in the control group was maintained for up to 1 acid addition and showed a statistically significant decrease thereafter. In all other groups, enamel MH was maintained for more acid addition cycles, remaining statistically similar to initial MH values for up to 2 cycles (E-10 and ChemFil), 3 cycles (C-10, E-20, and Beautifil II), 4 cycles (Cention) and 5 cycles (C-20). The number of acid additions over which enamel MH remained statistically similar to the initial values is summarized in Fig. 2.

The pH changes in the immersion solution are shown in Fig. 3. The initial pH (measured before incubating enamel blocks and restorative materials) was 4.0 in all groups. Statistically significant pH changes were observed after periodic acid additions, leading to the following three patterns in the pH curves:

1. A significant initial pH increase, followed by a plateau at pH = 6–7. Materials: Control, E-10, Beautifil II, and ChemFil.

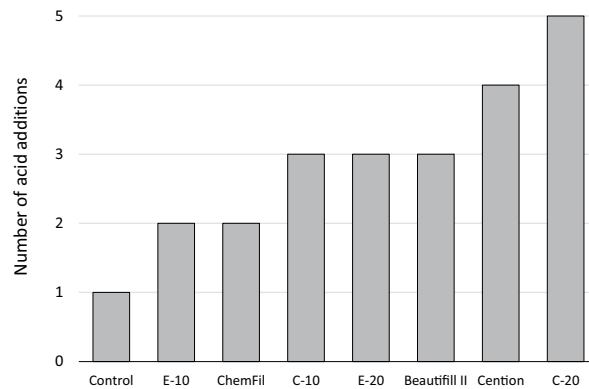


Figure 2. Number of acid addition cycles for which enamel surface MH remained statistically similar to initial values.

2. A transient peak at pH = 8–10 after 4 days, followed by a pH drop and stabilization at pH = 6–7. Materials: C-10, E-20, and Cention.
3. A significant initial pH increase, followed by a plateau at pH = 9–10. Material: C-20.

The pH changes in patterns (1) and (2) converged to a plateau at pH = 6–7; this was the most common behavior identified in 7 out of 8 tested materials. Pattern (3) was observed for only one material (C-20) capable of maintaining alkaline pH over the whole observation period.

SEM images of enamel surfaces after 4, 8, 16, and 32 days of immersion in the acid solution are shown in Fig. 4 for the experimental materials and Fig. 5 for the commercial materials. After 4 days, enamel surfaces in all groups showed a scratch pattern from grinding, while enamel blocks immersed with Cention additionally showed a precipitate on their surface. Over the subsequent time points (8, 16, and 32 days), the scratch patterns gradually became less visible and were replaced with etching patterns. For C-20, Beautifil II, and Cention, the scratch pattern remained visible up to 16 days, whereas for other materials they were replaced by etching patterns at earlier times (4–8 days). After 32 days, etching patterns were clearly observable for all materials.

Discussion

This study showed that restorative materials with functional fillers exert an anti-demineralizing effect when incubated with enamel blocks in an acid solution. This effect was evidenced by MH of enamel blocks remaining unimpaired over a material-dependent number of acid attack cycles. To demonstrate that the protective effect does not depend on the close proximity of restorative materials to dental hard tissues, enamel blocks and restorative material specimens were separated by a distance of 5 mm. The protective effect was material-dependent and identified for the experimental BG-containing composites, as well as for three commercial restorative materials, leading us to reject both null hypotheses.

The composition of experimental composites with 10 and 20 wt% of BG followed a previous study in which these materials were shown to neutralize acid similarly to the alkaSite material and better than a giomer and a glass ionomer¹¹. These results motivated the present study, which aimed to investigate whether the acid-neutralizing and ion-releasing activities of experimental BG-composites can protect remote enamel surfaces against demineralization caused by lactic acid.

Notwithstanding the complex pathophysiology of tooth caries, the initial damage to the enamel surface stems from an imbalance in the dissolution and precipitation processes³². While various sophisticated approaches are beneficial for in-depth evaluations of caries progression²³, simple measurements of enamel surface MH indicating initial structural changes due to acid exposure have proven useful for evaluations of the anti-demineralizing effect of restorative materials^{33–35}. Therefore, our study employed periodic replenishing of the lactic acid solution and evaluated the number of acid addition cycles that enamel blocks withstood before showing signs of surface softening. The initial enamel MH in all experimental groups was within the usual MH range for sound enamel³⁶. Statistical comparisons of those initial MH values and MH measured after successive acid additions enabled identifying the point of significant MH decrease, which was considered to signify initial enamel softening.

The capability of the investigated materials for protecting enamel against demineralization is based on two main effects, namely the neutralization of acid and the release of remineralizing ions^{2,11}. The latter mechanism shifts the dissolution/precipitation dynamics between the enamel hydroxyapatite and the immersion solution towards precipitation. Sufficiently saturating the immersion solution with calcium and phosphate ions can prevent enamel demineralization even in moderately acidic environments³². A strong alkalinizing effect is therefore not necessary for a protective effect if sufficient concentrations of remineralizing ions are released. This consideration can explain the finding that E-10, Beautifil II, and ChemFil demonstrated a better protective effect than the control composite by maintaining enamel MH unchanged for a longer time (8–12 days vs. 4 days for the control composite) despite showing pH curves similar to that of the control composite. A comparatively better protective effect (MH unchanged for 12–20 days) was identified for the materials with a pronounced alkalinizing

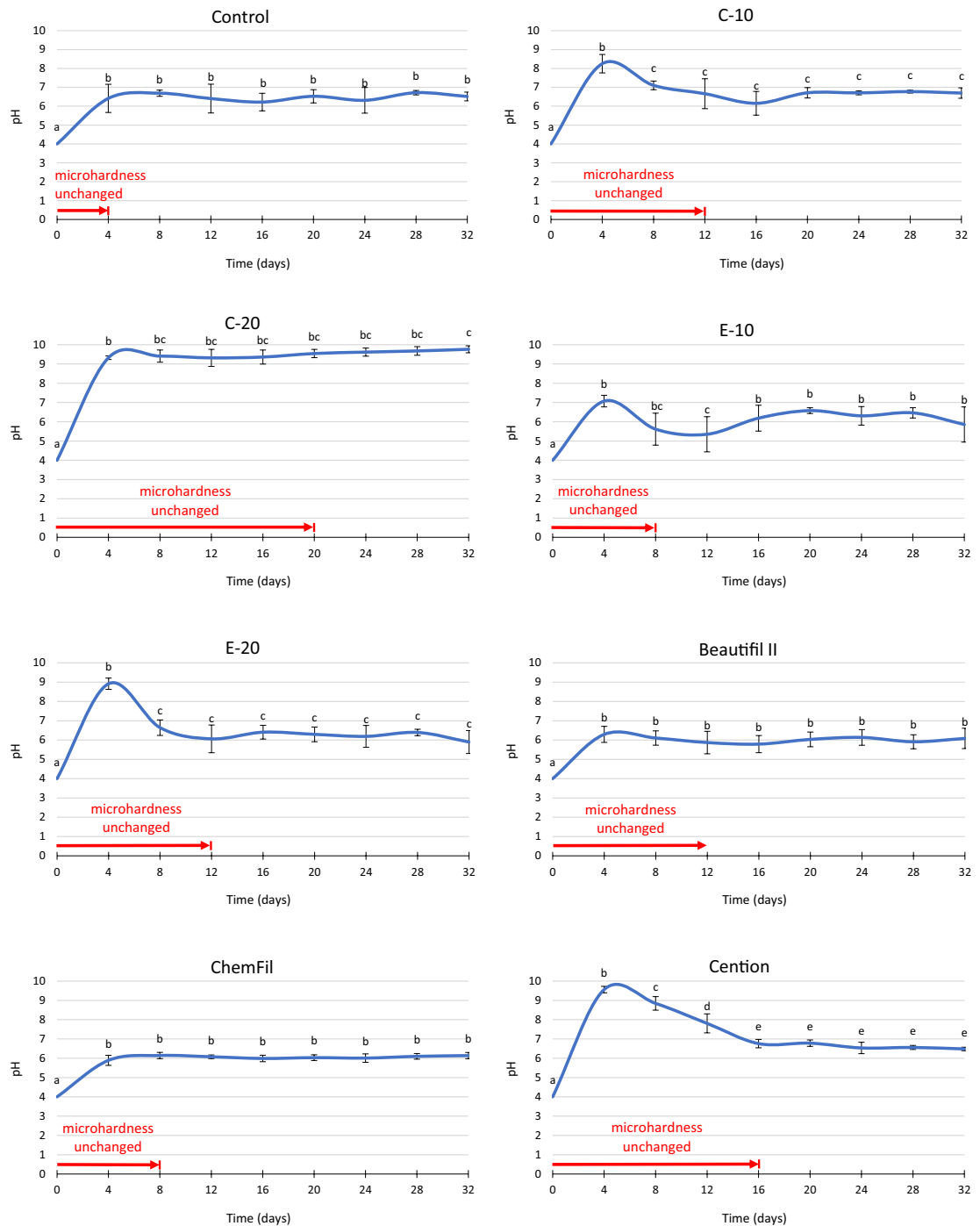


Figure 3. pH changes (mean values \pm standard deviations) of the immersion medium and time periods for which enamel microhardness remained unchanged compared to initial values. Same letters denote statistically similar pH values within a material.

capability, namely C-10, C-20, E-20, and Cention. For these materials, the synergistic effect of the alkalinization and ion release led to a longer-lasting protective capability.

The pH increase observed for the control group can be attributed to the dissolution of enamel³⁷ which led to acid neutralization, as previously reported in studies that incubated enamel blocks in lactic acid solution^{28,30}. As the control group showed a plateau at the pH = 6–7 throughout the whole observation period, the alkalinizing effect in other groups had to be sufficiently pronounced in order to become distinguishable from the acid neutralization by enamel blocks alone. Such an effect was observed for C-10, E-20, and Cention showing a transient alkalinization, and for C-20 which showed a more notable alkalinization by maintaining a plateau at pH = 9–10. The observation of the alkalinizing effect of E-10, Beautifil II, and ChemFil being similar to that of the control composite does not

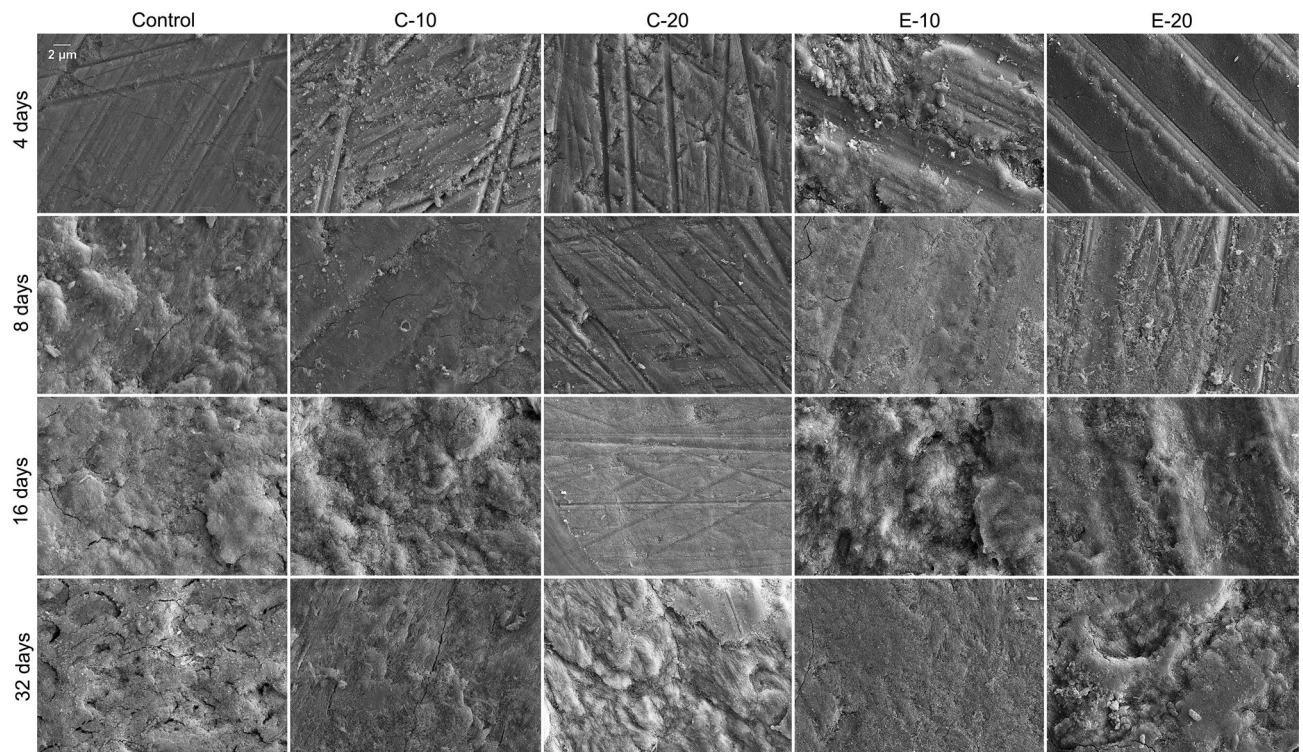


Figure 4. Scanning electron microscopy images of the surfaces of enamel blocks immersed with the control composite and the composites containing conventional (C-10 and C-20) and experimental (E-10 and E-20) bioactive glass.

necessarily imply that these materials lack an acid-neutralizing effect. These materials indeed demonstrated a moderate acid-neutralizing effect in a previous study¹¹; however, by measuring pH at the end of four-day acid addition cycles in the present study, their contributions to the pH increase were indiscernible from the acid-neutralizing effect of the dissolution of enamel blocks.

As the acid-neutralizing effect was eventually exhausted for all materials except C-20, their pH curves converged to the plateau values of the control composite at pH = 6–7. Although C-20 maintained a high pH of 9–10 throughout the whole observation period, MH measurements showed that its protective effect on enamel was exhausted after 20 days. This finding suggests that an apparently long-lasting alkalizing capability of C-20 was not sufficient to protect enamel against demineralization. Although the pH = 9–10 was consistently measured at the end of each four-day acid addition cycle, repeatedly exposing the enamel to the low-pH solution undersaturated with calcium and phosphate ions at the beginning of each cycle produced cumulative enamel damage, which was not remineralized over the rest of the cycle despite the alkaline pH being attained towards the end of each cycle. This reasoning also implies that the commonly used approach of measuring the alkalizing effect of restorative materials after particular time intervals^{2,4,6,38} does not necessarily indicate protection against demineralization because it does not take into account the demineralization that occurs before reaching a particular time point.

Whereas all investigated commercial restorative materials showed a better protective effect than the control composite, the best performance among them was identified in a recently launched “alkasite” restorative material (Cention). In addition to showing a transient alkalizing capability, this material also showed the longest protective effect among the commercial materials. It is interesting to note that the other two commercial materials protected enamel better than the control composite, even though their pH curves indicated an acid neutralization capability similar to that of the control. Their protective effect was apparently more reliant on ion release, particularly of fluoride ions which can be incorporated into demineralized enamel over a 4-day acid exposure cycle, thereby rendering enamel more acid-resistant to the acid attack in the next cycle³⁹.

The results for the BG-functionalized composites show that (I) the protective and alkalizing effects were dose-dependent, and (II) the protective and alkalizing effects were better for composites functionalized with the conventional BG 45S5 compared to the experimental fluoride-containing BG. Since these differences imply various extents of material degradation due to the dissolution of functional fillers¹¹, the ion release profiles vs. the stability of mechanical properties of experimental composites with these two BG types are being addressed in ongoing studies. These studies will also include the commercial material Cention since the evidence of its high reactivity (protective and alkalizing effects better than all other tested materials except C-20) makes it an interesting candidate for studying long-term degradation of mechanical properties.

Although Cention lacks true self-adhesive properties, it was initially marketed as capable of being placed without an adhesive system due to the assumed capability for sealing marginal gaps via hydroxyapatite precipitation. These recommendations have recently been revised by the manufacturer, indicating that an adhesive system (preferably the proprietary product Cention Primer) should be applied before restoring the cavity using

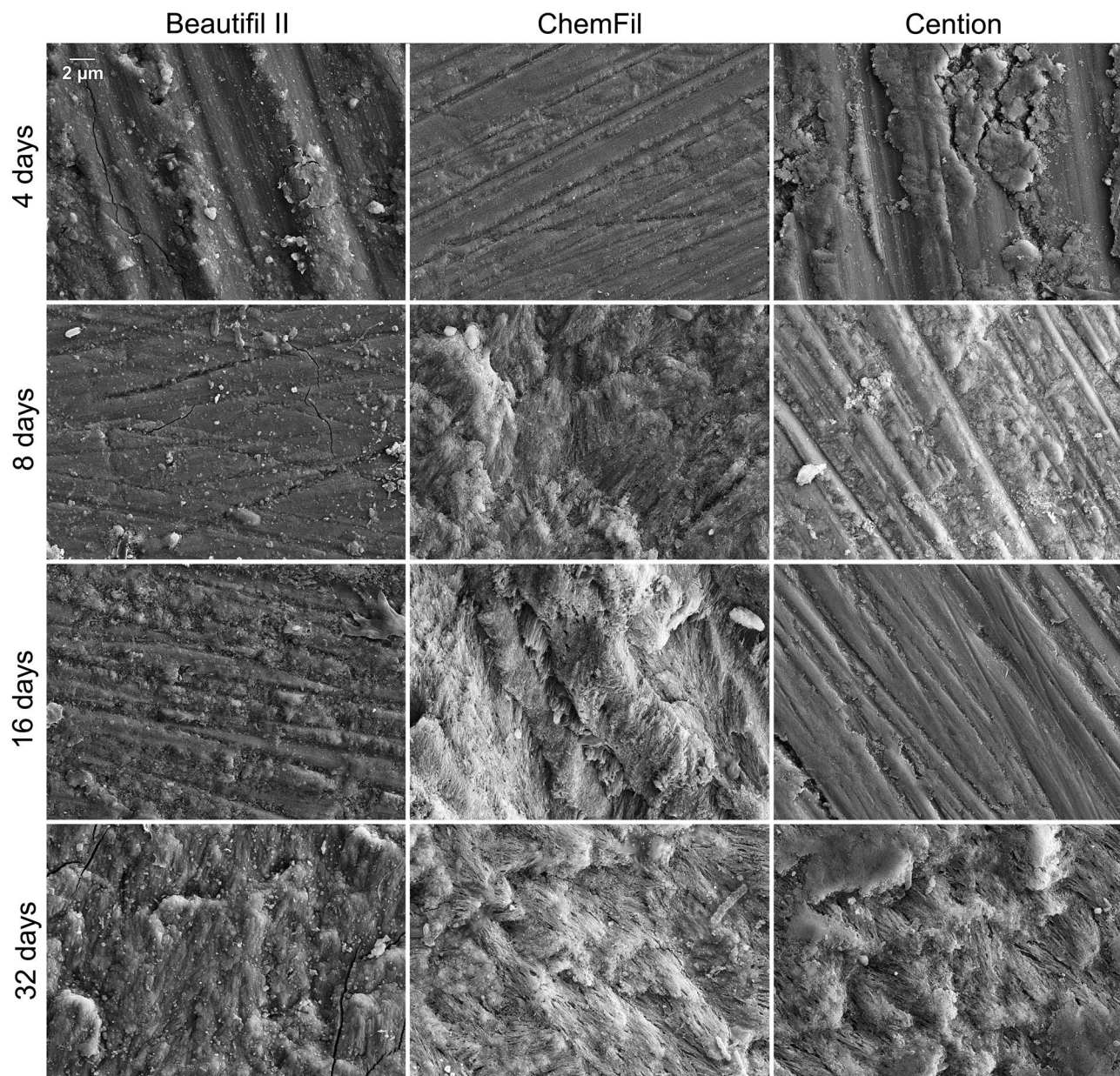


Figure 5. Scanning electron microscopy images of the surfaces of enamel blocks immersed with commercial reference materials.

Cention. Therefore, clinical application of this new “alkasite” material type can be regarded as being similar to other dual-curing bulk-fill composites with the additional benefit of protective effect on surrounding dental hard tissues through ion release and alkalization.

The SEM micrographs support the finding of the protective effect of restorative materials on enamel. The anti-demineralizing effect was identified as the grinding scratch pattern on the enamel surface remaining visible over a material-dependent period of acid immersion³⁰. As the protective effect was exhausted by the periodical replenishing of the acid solution, the scratch pattern progressively became shallower and was eventually replaced by etching patterns. An illustrative example of such a gradual transition was observed for C-20, which showed a gradual thinning of the scratch pattern over 4–16 days, followed by an emergence of the etching pattern after 32 days.

Distinctive etching patterns of Type 1 and 2 according to Silverstone et al.⁴⁰ were observed on SEM micrographs. For example, Type 1 etching pattern characterized by enamel dissolution being more pronounced in prism cores was observed after 32 days for the control group, while Type 2 etching pattern in which dissolution occurs preferentially on prism peripheries was observed after 32 days for C-20 and ChemFil. Another notable finding is a layer of mineral precipitate identified after 4 days on enamel blocks immersed with Cention. Although no detailed characterization of this precipitate was performed, it was likely a hydroxyapatite layer that formed

under the conditions of alkaline pH at the time point of 4 days²⁵. As the pH dropped over subsequent time points, the precipitate was dissolved, revealing the underlying scratch pattern on the enamel surface.

A simple demineralization model using periodically replenished acid solution was employed in this study because it was a convenient and cost-effective means for demonstrating the protective effects of experimental composites³⁰. As such a model does not simulate clinically realistic slowly-advancing subsurface lesions, a multispecies biofilm model⁴¹ is planned to be used in a future study that will evaluate the capability of experimental bioactive glass-containing composites for preventing the progression of carious lesions along restoration margins.

Conclusions

Restorative materials functionalized with reactive fillers showed an anti-demineralizing effect on enamel blocks that were 5 mm away from material specimens. The remotely-acting protective effect was identified in experimental composites functionalized with two types of bioactive glass, as well as in three commercial restorative materials. Although the materials which showed pronounced alkalizing capability also showed a longer-lasting protective effect, the protection of enamel against demineralization was also attainable without a pronounced alkalization.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 9 March 2021; Accepted: 25 May 2021

Published online: 03 June 2021

References

- Nedeljkovic, I. *et al.* Secondary caries: Prevalence, characteristics, and approach. *Clin. Oral Investig.* **24**, 683–691 (2020).
- Al-eesa, N. A., Wong, F. S. L., Johal, A. & Hill, R. G. Fluoride containing bioactive glass composite for orthodontic adhesives—ion release properties. *Dent. Mater.* **33**, 1324–1329 (2017).
- Tezvergil-Mutluay, A. *et al.* Effects of composites containing bioactive glasses on demineralized dentin. *J. Dent. Res.* **96**, 999–1005 (2017).
- Odermatt, R. *et al.* Bioactivity and physico-chemical properties of dental composites functionalized with nano- vs. micro-sized bioactive glass. *J. Clin. Med.* **9**, 772. <https://doi.org/10.3390/jcm9030772> (2020).
- Yang, S.-Y. *et al.* Acid neutralizing, mechanical and physical properties of pit and fissure sealants containing melt-derived 45S5 bioactive glass. *Dent. Mater.* **29**, 1228–1235 (2013).
- Tauböck, T. T. *et al.* Functionalizing a dentin bonding resin to become bioactive. *Dent. Mater.* **30**, 868–875 (2014).
- Al-eesa, N. A., Johal, A., Hill, R. G. & Wong, F. S. L. Fluoride containing bioactive glass composite for orthodontic adhesives—Apatite formation properties. *Dent. Mater.* **34**, 1127–1133 (2018).
- Jones, J. R. Review of bioactive glass: From Hench to hybrids. *Acta Biomater.* **9**, 4457–4486 (2013).
- Jones, J. R., Ehrenfried, L. M., Saravanapavan, P. & Hench, L. L. Controlling ion release from bioactive glass foam scaffolds with antibacterial properties. *J. Mater. Sci. Mater. Med.* **17**, 989–996 (2006).
- Weir, M. D., Chow, L. C. & Xu, H. H. K. Remineralization of demineralized enamel via calcium phosphate nanocomposite. *J. Dent. Res.* **91**, 979–984 (2012).
- Par, M., Attin, T., Tarle, Z. & Tauböck, T. T. A new customized bioactive glass filler to functionalize resin composites: Acid-neutralizing capability, degree of conversion, and apatite precipitation. *J. Clin. Med.* **9**, 1173. <https://doi.org/10.3390/jcm9041173> (2020).
- Khvostenko, D., Hilton, T. J., Ferracane, J. L., Mitchell, J. C. & Kruzic, J. J. Bioactive glass fillers reduce bacterial penetration into marginal gaps for composite restorations. *Dent. Mater.* **32**, 73–81 (2016).
- Par, M., Spanovic, N., Tauböck, T. T., Attin, T. & Tarle, Z. Degree of conversion of experimental resin composites containing bioactive glass 45S5: the effect of post-cure heating. *Sci. Rep.* **9**, 17245. <https://doi.org/10.1038/s41598-019-54035-y> (2019).
- Par, M., Tarle, Z., Hickel, R. & Ilie, N. Mechanical properties of experimental composites containing bioactive glass after artificial aging in water and ethanol. *Clin. Oral Investig.* **23**, 2733–2741 (2019).
- Par, M. *et al.* Curing potential of experimental resin composites with systematically varying amount of bioactive glass: Degree of conversion, light transmittance and depth of cure. *J. Dent.* **75**, 113–120 (2018).
- Par, M. *et al.* Curing potential of experimental resin composites filled with bioactive glass: A comparison between Bis-EMA and UDMA based resin systems. *Dent. Mater.* **36**, 711–723 (2020).
- Nam, H.-J. *et al.* Enamel surface remineralization effect by fluorinated graphite and bioactive glass-containing orthodontic bonding resin. *Materials* **12**, 1308 (2019).
- Nam, H.-J. *et al.* Fluorinated bioactive glass nanoparticles: Enamel demineralization prevention and antibacterial effect of orthodontic bonding resin. *Materials* **12**, 1813 (2019).
- Manfred, L., Covell, D. A., Crowe, J. J., Tufekci, E. & Mitchell, J. C. A novel biomimetic orthodontic bonding agent helps prevent white spot lesions adjacent to brackets. *Angle Orthod.* **83**, 97–103 (2013).
- Firzok, H. *et al.* Sol-gel derived fluoridated and non-fluoridated bioactive glass ceramics-based dental adhesives: Compositional effect on re-mineralization around orthodontic brackets. *J. Non-Cryst. Solids* **521**, 119469 (2019).
- Kohda, N. *et al.* Inhibition of enamel demineralization and bond-strength properties of bioactive glass containing 4-META/MMA-TBB-based resin adhesive. *Eur. J. Oral Sci.* **123**, 202–207 (2015).
- Attin, T. *et al.* Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site. *Clin. Oral Investig.* **11**, 83–88 (2007).
- Lippert, F. & Lynch, R. J. M. Comparison of Knoop and Vickers surface microhardness and transverse microradiography for the study of early caries lesion formation in human and bovine enamel. *Arch. Oral Biol.* **59**, 704–710 (2014).
- Par, M., Mohn, D., Attin, T., Tarle, Z. & Tauböck, T. T. Polymerization shrinkage behaviour of resin composites functionalized with unsilanized bioactive glass fillers. *Sci. Rep.* **10**, 15237 (2020).
- Tiskaya, M., Al-eesa, N. A., Wong, F. S. L. & Hill, R. G. Characterization of the bioactivity of two commercial composites. *Dent. Mater.* **35**, 1757–1768 (2019).
- Kelić, K., Par, M., Peroš, K., Šutej, I. & Tarle, Z. Fluoride-releasing restorative materials: The effect of a resinous coat on ion release. *Acta Stomatol. Croat.* **54**, 371–381 (2020).
- Wiegand, A., Credé, A., Tschammler, C., Attin, T. & Tauböck, T. T. Enamel wear by antagonistic restorative materials under erosive conditions. *Clin. Oral Investig.* **21**, 2689–2693 (2017).

28. Kaga, M. *et al.* Inhibition of enamel demineralization by buffering effect of S-PRG filler-containing dental sealant. *Eur. J. Oral Sci.* **122**, 78–83 (2014).
29. Kaga, N. *et al.* Protective effects of GIC and S-PRG filler restoratives on demineralization of bovine enamel in lactic acid solution. *Materials* **13**, 2140 (2020).
30. Kakuda, S., Sidhu, S. K. & Sano, H. Buffering or non-buffering; an action of pit-and-fissure sealants. *J. Dent.* **43**, 1285–1289 (2015).
31. Par, M., Marovic, D., Attin, T., Tarle, Z. & Tauböck, T. T. The effect of rapid high-intensity light-curing on micromechanical properties of bulk-fill and conventional resin composites. *Sci. Rep.* **10**, 10560 (2020).
32. Dawes, C. What Is the critical pH and why does a tooth dissolve in acid?. *J. Can. Dent. Assoc.* **69**, 722–724 (2003).
33. Esteves-Oliveira, M. *et al.* Rehardening of acid-softened enamel and prevention of enamel softening through CO₂ laser irradiation. *J. Dent.* **39**, 414–421 (2011).
34. Hara, A. T. & Zero, D. T. Analysis of the erosive potential of calcium-containing acidic beverages: Erosive potential of calcium-containing beverages. *Eur. J. Oral Sci.* **116**, 60–65 (2008).
35. Maltz, M., Scherer, S. C., Parolo, C. C. F. & Jardim, J. J. Acid susceptibility of arrested enamel lesions: In situ study. *Caries Res.* **40**, 251–255 (2006).
36. Chuenarrom, C., Benjakul, P. & Daosodsai, P. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. *Mater. Res.* **12**, 473–476 (2009).
37. Attin, T., Becker, K., Wiegand, A., Tauböck, T. T. & Wegehaupt, F. J. Impact of laminar flow velocity of different acids on enamel calcium loss. *Clin. Oral Investig.* **17**, 595–600 (2013).
38. Balbinot, G., Leitune, V. C. B., Ogliaeri, F. A. & Collares, F. M. Niobium silicate particles as bioactive fillers for composite resins. *Dent. Mater.* **36**, 1578–1585 (2020).
39. Francois, P., Fouquet, V., Attal, J.-P. & Dursun, E. Commercially available fluoride-releasing restorative materials: A review and a proposal for classification. *Materials* **13**, 2313 (2020).
40. Silverstone, L. M., Saxton, C. A., Dogon, I. L. & Fejerskov, O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. *Caries Res.* **9**, 373–387 (1975).
41. Thurnheer, T., Rohrer, E., Belibasakis, G. N., Attin, T. & Schmidlin, P. R. Static biofilm removal around ultrasonic tips in vitro. *Clin. Oral Investig.* **18**, 1779–1784 (2014).

Author contributions

M.P. study conception, experimental design, funding acquisition, formal analysis and data interpretation, statistical analysis, visualization, wrote manuscript. A.G. performed experiments, data acquisition and interpretation, resources, revised and approved final manuscript. T.A. study conception, experimental design, funding acquisition, supervision, data interpretation, project administration, resources, revised and approved final manuscript. Z.T. study conception, supervision, data interpretation, project administration, revised and approved final manuscript. T.T.T. study conception, experimental design, funding acquisition, supervision, data interpretation, project administration, resources, revised and approved final manuscript.

Funding

This work was financially supported by the Research Fund of the Swiss Dental Association SSO (grant number 321-20). Matej Par's research stay at University of Zurich was funded by a grant from the Swiss Federal Department of Economic Affairs, Education and Research (2019.0221).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.P.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021