Dentin Protection of Different Desensitizing Varnishes During Stress Simulation: An *In Vitro* Study

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Clinical Relevence

In treatment of dentin hypersensitivity, light-curing desensitizing varnishes might be able to avoid dentin loss. Consequently, these materials could be a promising preventive approach and may be preferred for clinical use.

SUMMARY

Objective: The aim of this study was to investigate dentin protection of different desensitizing varnishes (light- and self-curing) during

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acid action/abrasion stress and thermocyclic loading in vitro.

Methods: Dentin discs of 2 mm thickness were cut from 120 human molars, embedded, and polished. Specimens were randomized into five groups (n=24): A, negative control; B, Gluma Desensitizer; C, Cervitec plus (self-curing); D, Seal&Protect; and E, Admira Protect (lightcuring). In groups B-E, varnish was applied on two-thirds of the dentin surface, and one-third acted as internal control. Stress cycle (2 cycles/ day) for specimens were as follows: 1, acid action (pH: 2.9: five minutes); 2, remineralization (synthetic saliva: 60 minutes); 3, brushing (100 strokes); 4, thermocycling (five cycles); and 5, remineralization (synthetic saliva: six hours) for each group (n=12) for 30 (15 days) or 60 times (30 days). Specimens were analyzed using an incident light microscope. Substance loss was measured in micrometers. Statistical analysis was performed with the multiple contrast test (p < 0.05).

Results: Groups B and C had a significantly lower dentin loss than A (p<0.01). After 30 days, group A showed the highest dentin loss (p<0.01), whereas the other groups lacked a significant difference regarding their substance loss (dentin and/or varnish; p>0.05).

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Varnish layer loss was shown for groups D and E with a remaining protective layer; groups A-C showed dentin removal.

Conclusion: All four varnishes are protective compared with an untreated control. Lightcuring varnishes might provide higher dentin protection than self-curing materials.

INTRODUCTION

In a current review, Splieth and others highlighted the importance of dentin hypersensitivity (DHS), with a prevalence range between 3% and 98%. The crucial problem of DHS is the exposed dentin surface, whereby, based on the current scientific opinion, the short and sharp pain is explained by Brännström's hydrodynamic theory: nociception as a result of nerve stimulation induced by fluid movements in dentin tubules.

Furthermore, the different etiologic factors including dentin exposure because of gingival recession during periodontal disease, traumatic loss of the tooth surface, and erosion and abrasion must be considered. In this context, erosive food and drink, as well as tooth-brushing using abrasive toothpaste, are important.³

Considering DHS as a painful and frequently occurring problem, several therapeutic approaches are available. One is the use of fluoride-containing toothpastes and varnishes.^{5,6} Other ingredients, such as potassium, could help manage the pain caused by hypersensitive dentin. Varnishes in different application forms as self-curing and lightcuring materials are also available to treat DHS. For self-curing varnishes like Gluma Desensitizer and Cervitec plus, a positive clinical effect was reported.^{8,9} These clinical benefits are consistent for lightcuring materials, such as Seal&Protect and Admira Protect. 10,11 Additionally, an intervention, such as laser irradiation and the combination of laser and desensitization varnishes, could be a possible approach. 12,13

Although a clinical benefit was shown for both light- and self-curing varnishes, different results were found. The light-curing materials appeared to show a higher effectiveness compared with self-curing materials, especially over a period of a few months. An important point regarding this issue might be the wear resistance of the desensitizing varnishes. Therefore, their stability against erosive and abrasive stress could be a decisive factor for their ability to reduce DHS sufficiently over a prolonged time. Moreover,

protection of exposed dentin appears to be preferable to avoid further dentin loss. Data regarding dentin protection of desensitizing varnishes are rare, showing higher protective potential of light-curing varnishes.¹⁸

Accordingly, the current study investigated the resistance of different self- and light-curing desensitizing varnishes during erosion, abrasion, and thermocyclic loading to draw conclusions on their potential of dentin protection. The aim of this study was to investigate the dentin protection with light-curing and self-curing varnishes *in vitro*. It was hypothesized that light-curing materials protect dentin better than self-curing varnishes.

METHODS AND MATERIALS

Study Design

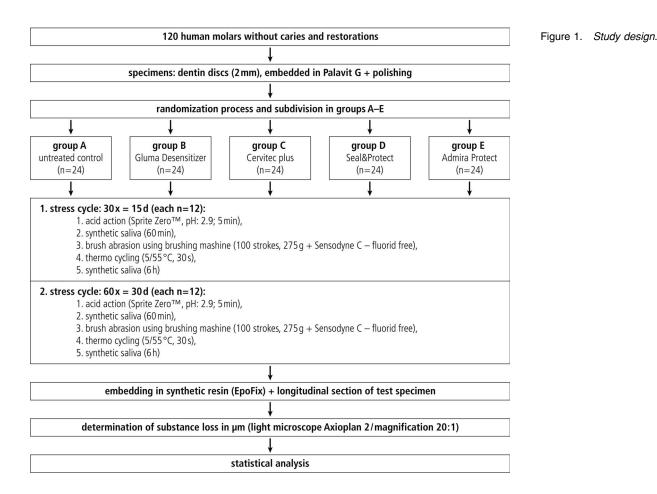
A randomized, five-arm *in vitro* study was performed on extracted caries-free human molars. The teeth were not extracted for this study, but for periodontal or orthodontic reasons (third molars). The use of teeth for *in vitro* studies was approved by the ethics committee (number 16/6/09); patients were informed and gave written informed consent. The resistance of two light- and two self-curing dentin varnishes against abrasion and erosion was investigated compared with an untreated control (Figure 1).

Test Specimen Preparation

A total of 120 freshly extracted caries-free human molars were cleaned and stored in physiologic saline solution. Discs of 2 mm thickness were cut from upper and middle dentin in the transverse direction (Exakt Apparatebau GmbH, Norderstedt, Germany). Discs were embedded (Palavit G, Heraeus Kulzer GmbH, Hanau, Germany) avoiding contamination of the upper dentin surface as well as possible. Finally, the test specimens were polished with water-cooled sandpaper discs at a grain size of 1200/4000 (Struers GmbH, Willich, Germany).

Test Material and Group

Self-curing varnishes (groups B and C) included Gluma Desensitizer (Heraeus Kulzer GmbH, Hanau, Germany) and Cervitec Plus (Ivoclar Vivadent GmbH, Ellwangen, Germany). Light-curing materials (groups D and E) were Admira Protect (Voco GmbH, Cuxhaven, Germany) and Seal&Protect (Dentsply DeTrey GmbH, Konstanz, Germany). Group A without a varnish layer was used as a negative control (Table 1).



Randomization Procedure and Varnish Application

Test specimens were randomly divided into five groups (A-E) with 24 probes each. A further selection of the groups in A1-E1 and A2-E2, in accordance with the cycle length (30 or 60 cycles), was performed afterward (n=12). Test specimens from groups B1 and B2 to E1 and E2 were taped to cover one third of the dentin using sticky tape number 1 (thickness 0.15 mm, Coroplast, Wuppertal, Germany), protecting them from loading. Groups B1 and B2 to E1 and E2 were treated according to the manufacturer's instructions, with the corresponding varnishes (Table 1). Light-curing materials (groups D and E) were light cured with an LED polymerization lamp (Bluephase [1200 mW/ cm²], Ivoclar Vivadent, Schaan, Lichtenstein). Groups A1 and A2 remained untreated. Additionally, tape number 2 was applied to one half of the varnished dentin specimens to obtain a reference surface, which was also protected from loading. The specimens from groups A1 and A2 were only taped on one half because there was no varnish layer (Figure 2).

Stress Cycles: Abrasion, Erosion, and Thermocyclic Loading

For stress simulation, a repetitious cycle was conducted, consisting of acid action, remineralization, brush abrasion, and thermocycling (Figure 1). A soft drink with a pH of 2.9 (Sprite Zero, Coca Cola GmbH, Berlin, Germany) was used, followed by a remineralization step with synthetic saliva. Abrasion was performed with slurry of synthetic saliva and fluoride-free toothpaste (Sensodyne C, GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Hamburg, Germany).

Acid action lasted for five minutes, followed by remineralization for 60 minutes in synthetic saliva. ¹⁸ Brush abrasion was performed with an automatic brushing machine (University Medical Centre Goettingen) with a loading mass for the brushes on specimens of 275 g. The brushing machine operated at 100 strokes/min for one minute and used 20 mL

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Group	Product name	Manufacturer	Mechanism of action	Ingredients	Application
A1/A2	Untreated control	_	_	_	_
B1/B2	Gluma Desensitizer	Heraeus Kulzer	Self-curing	(2-hydroxyethyl) methacrylate, glutaraldehyde, purified water	Clean the dentin surface with a pumice slurry. Rinse off with water. Apply the smallest possible amount of GLUMA Desensitizer required for treatment to the dentinal surface using brush and then leave for 30-60 seconds. Dry the surface carefully by applying a stream of compressed air until the fluid film has disappeared and the surface is no longer shiny. Then rinse thoroughly with water.
C1/C2	Cervitec plus	Ivoclar Vivadent	Self-curing	Ethanol, water, acrylate copolymer, vinyl acetate copolymer, and chlorhexidine diacetate 1%, Thymol 1%	Clean the tooth surfaces thoroughly. Dry with cotton rolls and an air syringe. Apply a thin coat of varnish using a brush. Let the varnish dry. Dry again with compressed air for 30 seconds (no water rinse).
D1/D2	Seal&Protect	Dentsply	Light-curing	Di- and Trimethacrylate-resins, PENTA (dipentaerythritol pentacrylate- phosphoric acid - monomer), functionalized amorphous silicium dioxide, photoinitiators, butylated hydroxytoluol, cetylaminhydrofluoride, Triclosan, acetone	Clean the dentin surface with a rubber cup and a prophy paste. Remove prophylaxis paste with an air/water spray. Dry clean the area with a two-second blow of air free of oil or water contamination; avoid desiccating the dentin, leave a moist, but not wet, glistening surface. Two to three drops are required per surface to be treated. Apply with an applicator tip. Leave the dentin surface undisturbed for 20 seconds. Remove excess solvent by blowing gently with air for a few seconds. Cure Seal&Protect for 10 seconds using a curing light. Apply a second layer of Seal&Protect. Remove excess solvent from the second layer by blowing gently with air. Cure Seal&Protect for 10 seconds using a curing light. Remove oxygen-inhibited (soft surface) layer with a cotton pellet or cotton roll.
E1/E2	Admira Protect	Voco	Light-curing	Mixture of different dimethacrylates, acetone, catalysts, ormocers, auxiliaries	Clean teeth with fluoride-free cleaning paste on a rubber cup. Remove excess moisture with an oil-free airjet; the dentin surface should be slightly moist. Apply Admira Protect with a disposable brush on dentin surfaces. Disperse Admira Protect with a faint airjet. Light cure for 10 seconds. Apply a second layer of Admira Protect, disperse it with a faint airjet and light-cure for 10 seconds. Remove the oxygen-inhibited layer (soft surface) with a cotton pellet.

slurry for each brushing procedure. ¹⁸ Thermocycling was conducted with five cycles between 5°C and 55°C in tempered water for 30 seconds each with a changeover time of 15 seconds (Haake DC10, Thermo Fisher Scientific GmbH, Schwerte, Germany). This cycle was conducted 30 times for groups A1-E1 and 60 times for groups A2-E2 to simulate 15 or 30 days of loading with two cycles per day. Between cycles, specimens were stored in synthetic saliva for six hours.

Microscopic Analysis

After air drying for 60 seconds and the removal of the sticky tapes, the specimens were embedded in epoxy resin (EpoFix, Struers GmbH, Willich, Germany) and separated vertically using a cutting disc. Thicknesses of the varnish layer and substance loss were imaged and measured in micrometers by an incident light microsope (Axioplan, Carl Zeiss Jena GmbH, Jena, Germany; magnification 20×) in combination with a digital camera (AxioCam HRc,

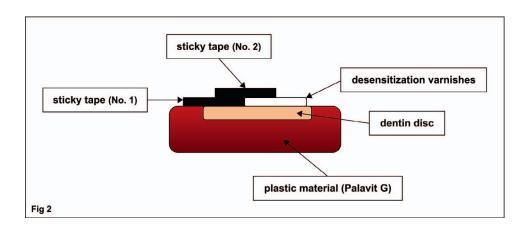


Figure 2. Graphic presentation of test specimen with varnish application and placement of sticky tape.

Software AxioVision 4.7, Carl Zeiss Jena GmbH). The measurement was executed every 50 μ m using a digital ruler. The untreated, unstressed dentin surface served as an internal reference. Three parameters were measured: layer thickness, varnish layer loss, and/or dentin loss.

Statistical Analysis

Mean values of layer thickness and varnish/dentin loss of both halves of each probe were summarized to a total value of the test specimen. Mean values of groups were generated out of the total values from the specimen. Group differences were assessed by the nonparametric multiple contrast test. Calculation was conducted with "nparcomp" with the help of the Software "R GUI" (www.r-project.org). The significance level was set at $\alpha = 0.05$.

RESULTS

Results are given in Table 2 and Figure 3.

References and Negative Control

The untreated groups A1 and A2 lacked a varnish layer. Although at the reference surface (under tape no. 2; Figure 2) for self-curing materials (groups B1/B2 and C1/C2) no varnish layer could be measured, light-curing groups D1 and D2 and groups E1 and E2 showed a varnish layer of 65.27 (D1), 59.89 (D2), 42.28 (E1), and 41.07 μ m (E2).

Dentin and Material Losses After 15-day Simulation of Erosion/Abrasion and Thermocyclic Loading

There were significant differences of substance loss between groups A1-B1, B1-D1, B1-E1, C1-D1, and

Group	Varnish layer: reference (MV \pm SD in μ m)	Varnish layer: postcycle (MV \pm SD in μ m)	Substance loss (MV \pm SD in μ m)
Time 1 (after 15 days)			
A: untreated control	_	−11.1 ± 2.8	11.1 ± 2.8
B: Gluma Desensitizer	_	-7.9 ± 1.4	7.9 ± 1.4
C: Cervitec plus	_	-8.4 ± 1.8	8.4 ± 1.8
D: Seal&Protect	65.3 ± 21.7	50.2 ± 21.1 ^a	15.1 ± 5.5
E: Admira Protect	42.3 ± 13.7	21.6 ± 7.3 ^a	20.7 ± 11.7
Time 2 (after 30 days)			
A: untreated control	_	-63.2 ± 27.7	63.2 ± 27.7
B: Gluma Desensitizer	_	-21.5 ± 3.7	21.5 ± 3.7
C: Cervitec plus	_	−24.1 ± 6.0	24.1 ± 6.0
D: Seal&Protect	59.9 ± 19.8	41.2 ± 21.5^a	20.4 ± 7.6
E: Admira Protect	41.1 ± 13.8	12.0 ± 32.1 ^a	29.1 ± 24.6

Substance loss for groups A-C is pure dentin removal, whereas the substance loss for D and E indicates a loss of varnish layer. The missing material layer is expressed by missing values. MV, mean value; SD, standard deviation.

^a Remaining varnish layer on dentin.

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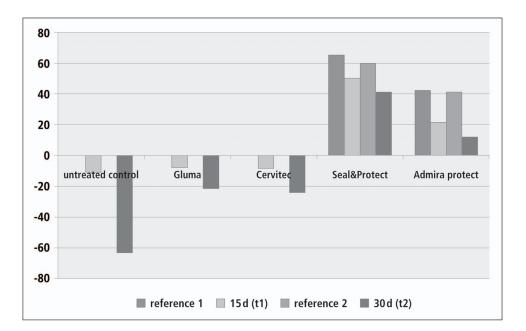


Figure 3. Layer thickness reference. at t1/t2 and substance loss (dentin) at t1/t2. (The average values [in micrometers] are illustrated at baseline 1 and 2, which shows the reference values for each group. It is worth noting that the first three groups show no values at baseline because there was no detectable layer. At t1 [after 15 days] and t2 [after 30 days], the first three groups had negative values, which shows dentin loss, whereas the positive values for Seal&Protect and Admira Protect represent the presence of a varnish layer.)

C1-E1 (p_i <0.01). Additionally, a trend was seen comparing groups A1 and C1 (p=0.051). After 15-day simulation of stress, a dentin loss was registered in groups A1, B1, and C1. The untreated group A1 had the highest dentin loss. Groups with self-curing varnishes showed less dentin loss compared with group A, with minor differences between groups B and C. In contrast, for the light-curing groups (D1 und E1), a limited varnish removal with a remaining layer was observed.

Dentin and Material Losses After 30-day Simulation of Erosion/Abrasion and Thermocyclic Loading

After 30-day stress simulation, there were significant differences between the groups A2-B2, A2-C2, A2-D2, and A2-E2 (p_i <0.01). The significantly highest dentin loss was detected in group A2. Substance losses of the self-curing desensitizers were not significantly different (p>0.05). The lightcuring varnishes had a remaining varnish layer with the highest remaining layer thickness in group D2.

Each specimen showed a substance loss at both times of measurement. Group A had a pure dentin loss, for groups B and C a complete varnish and dentin loss, and for groups D and E a pure varnish loss were registered. Accordingly, no dentin removal was measured for the light-curing varnishes.

DISCUSSION

The aim of this *in vitro* study was to investigate dentin protection with light-curing and self-curing

varnishes during abrasion, acid action, and thermocyclic loading under standardized conditions.

The main result of the study registered for self-curing varnishes a complete varnish and dentin loss, whereas for light-curing materials, a remaining varnish layer could generally be detected after 30 or 60 cycles simulating loading of 15 or 30 days. This suggests that light-curing materials are able to protect dentin, whereas self-curing varnishes showed no stable protective layer for the study period, which resulted in a measurable dentin loss. In this study setup, loading caused substance removal in every specimen. However, with light-curing materials, the dentin always remained undamaged.

Methods for stress simulation were chosen in accordance to Schneider and others. 18 Periods of acid action, storage in artificial saliva, and brush abrasion were standardized. The medium for erosive action in the earlier study mentioned above was Sprite Light with a pH of 2.9. This is almost identical to the measurements for Sprite Zero in the current study with a pH of 2.9. Other investigations with comparable issues also used Sprite Light. 20,21 Likewise, Schneider and others 18 explained the use of an automatic brushing machine; in the current study, the same number of brushing strokes and bearing mass was used (100 strokes/275 g). The chronology and times for exposure in the current study are similar to those of another investigation.²² Erosion (0.3% citric acid, pH 3.2, five minutes), remineralization (artificial saliva, one hour, pH 7.0), and

abrasion (120 linear strokes with 300 g loading) varied only slightly between studies. Use of an automatic brushing machine is a common procedure; accordingly, other authors also chose comparable loadings and brushing strokes. Therefore, Yu and others²³ used 100 strokes with a load of 250g, whereas Vieira and others²⁴ performed 200 strokes with load attuned to 150g. Implementation of two brushing actions each day was among others introduced by Ganss and others.²⁵ In the current study, thermocyclic loading was additionally conducted to simulate thermal stress as thermal changes may cause defects on a dentin-adhesive surface.²⁶ Synthetic saliva pH varied between different studies with a range of 6.4 to $7.0.^{19,22,25,27}$ Use of synthetic saliva is necessary for standardization of wet surroundings of specimen's surface quality. Light microscopy was already performed in another in vitro investigation.²⁶

There was a significant difference between Gluma Desensitizer and control groups after simulation of 15 and 30 days of loading (p < 0.01). Gluma Desensitizer causes dentinal tubule occlusion by reaction of glutaraldehyde with a dentinal tubule protein, resulting in reduced diameter of dentinal tubules and dentinal tubule occlusion. 28,29 This could explain why dentin with occluded tubules might be more wear resistant than untreated dentin and why no measurable varnish layer was found. With Cervitec plus, less dentin loss was also found (p < 0.051) both after 15- and 30-day simulation (p < 0.01). It was assumed that this material might reduce the hydraulic permeability of dentin.8 This could contribute to the desensitizing effect, but it does not explain sufficiently the potential dentin protection.

After 15- and 30-day simulation of loading, Seal&Protect and Admira Protect showed a remaining varnish layer. In accordance with this, in a recent in vitro study, Seal&Protect showed more reductions in dentinal fluid flow rate than Gluma Desensitizer. 15 This also suggests a higher effectiveness and dentin protection of light-curing materials. There are no results available about remaining varnish layer thickness in vivo. However, in vitro studies have examined this topic. The investigation by Schneider and others¹⁸ reported that Seal&Protect ensured best dentin protection. Additionally, Gluma Desensitizer treatment caused lower dentin loss than untreated controls. 18 This and other investigations confirm the current study results. 30-³² One study simulated erosive impact of intrinsic and extrinsic acids using hydrochloric and citric acid and reported that Seal&Protect significantly reduced enamel mineral loss.³² In addition, Seal&Protect protected dentin from erosive wear *in situ*.³⁰ Furthermore, One Coat Bond and Optibond FL were more resistant to erosive stress from Coca Cola than Gluma Desensitizer.³¹ This confirms the conclusion that light-curing varnishes might ensure better dentin protection than self-curing materials. However, the protection appears to be for short term, because a varnish layer loss is found. This is in accordance with Zhao and others, where a short-term protection of Seal&Protect was shown, but a repetitious application is necessary to ensure long term protection of dentin.³³ To the best of the authors' knowledge, no investigation has reported on the wear resistance of Cervitec plus and Admira Protect.

Clinical effectiveness of the investigated self-curing ^{8,14,34} and light-curing varnishes ^{11,14} has already been found in several studies. In this context, light-curing materials are repeatedly discussed to ensure a better reduction of DHS compared with self-curing varnishes, especially over a period of several months. ^{11,28-30,35} Based on the results of the current study, a potential reason for this benefit is the protective varnish layer on the dentin surface, which was more stress resistant, compared with the self-curing materials, and prevented dentin loss in the simulated observation period.

In summary, neither light-curing nor self-curing materials are completely resistant to erosive/abrasive wear, but they ensure a certain degree of dentin protection. Moreover, light-curing varnishes can ensure sufficient dentin protection despite substance removal of a detectable varnish layer. Self-curing materials were at least able to reduce the dentin loss. The remaining varnish layer in light-curing materials could be a reason for their high effectiveness in available clinical investigations. However, only detected substance loss could be assessed in the current study and therefore it is impossible to draw strong conclusions on clinical effectiveness.

There were limitations in the current investigation. Based on the results of this study, the clinical effectiveness of varnishes could only be anticipated. Furthermore, different test specimens were compared post-sectioning after 15 and 30 days, and destruction of the specimen is a possible criticism. Alternative methods, such as use of an optical profilometer 36 or scanning electron microscope (SEM) and micro–computed tomography (μ CT) 6,37 might illustrate the substance loss more effectively, but they were not available for the current investigation. It must also be mentioned that the simula-

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tion of protein precipitation induced by Gluma Desenitizer in extracted teeth might differ from *in vivo* conditions as to what influences the potential to protect dentin. However, for standardization, this experimental setup was necessary, and Gluma Desensitizer as a common therapeutic option was included in the current investigation. To conclude, methods that were used are reproducible and close to the clinical situation.

CONCLUSION

Within the limitations of the study, all four varnishes protected the dentin surface compared with the untreated control. The results of the present study suggest that light-curing varnishes ensure higher dentin protection than self-curing materials and could therefore be recommended for clinical use. To verify these results *in vivo*, further clinical studies are needed.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of University medical center Goettingen, Germany. The approval code for this study is 16/6/09.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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