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# **Diffusion of peroxides through dentine *in vitro* with and without prior use of a desensitizing varnish**

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# Abstract

Different bleaching regimens are used in dentistry possibly penetrating the dentine and affecting the pulp. The aim of the present study was to investigate peroxide diffusion through dentine pre-treated with a desensitizing varnish (Vivasens<sup>®</sup>) in a standardized *in vitro* setup during application of different bleaching materials.

The penetration was tested using 1.3 mm thick bovine dentine slabs. The following bleaching materials were tested with and without prior application of the desensitizing varnish on the external side of the dentine slabs: Vivastyle, Whitestrips, Simply White, Opalescence (external bleaching) and sodium perborate (internal bleaching, only tested without varnish), (n=8 samples per subgroup). The penetration of peroxides was measured photometrically using 4-aminoantipyrin as a substrate, the penetration of peroxides was monitored over 240 min.

All bleaching agents yielded a diffusion of peroxides through the dentine, the kinetics of penetration were approximately linear for all materials tested. The significantly highest diffusion of peroxides was observed with Opalescence, the lowest with sodium perborate. The adoption of the desensitizing varnish reduced the diffusion of peroxides significantly for all external bleaching materials.

Peroxides penetrated the dentine during application of bleaching materials, the penetration of peroxides can be reduced by application of a desensitizing agent.

## **Introduction**

External and internal bleaching regimens have been established as relevant and accepted methods in aesthetic dentistry[1-2]. However, peroxides may induce oxidative stress for the oral hard and soft tissues and side effects of peroxides are still a point of discussion in the literature [3-4]. The antioxidative capacity in the oral cavity is limited due to the fact that peroxidase in the saliva and in the acquired pellicle as the most relevant antioxidative protein is inactivated irreversibly by its substrate [5-6]. This applies also for human peroxidase activity in the pulp chamber. Typical side effects of external bleaching are gum burning, gingival erosions and tooth hypersensitivities [1, 4, 7-8]. Furthermore, alterations of restorative materials and dental hard tissues are discussed controversially in the literature [9-12]. In this context, it has to be taken in consideration that oral soft tissues undergo a very fast turnover whereas dental hard tissues are non-regenerating structures with non-shedding surfaces. Though some of the side effects might be transient, numerous people claim hypersensitivities during application of the external bleaching agents that might lead to cessation of the regimen [1, 4, 7]. This applies especially for patients suffering from exposed root surfaces. The dentine is of much higher permeability as compared with the enamel though also the latter is permeable for hydrogen peroxide [13-14]. The considerable diffusion of peroxides through the exposed dentine into the pulp chamber recorded in several studies is regarded as main cause for this effect [1, 14-15]. Hydrogen peroxide inhibits pulpal enzymes[16]. Succinyl-Dehydrogenase is inhibited by peroxides as observed in cell cultures [13]. As a result of peroxide diffusion transient inflammation of the pulp has been observed histologically [17-18]. Also the composition of the bleaching agent and its viscosity have an impact on the release and diffusion of the hydrogen peroxide and therewith on the intensity of perceived side effects [19-22]. Due to this fact desensitizing varnishes are recommended for coating of root surfaces during application of bleaching agents [23]. However, it is not known, if these varnishes hamper penetration of peroxides through underlying dentin efficiently.

Thus, the aim of the present in vitro study was to investigate and to quantify the penetration of typical and representative bleaching materials [24] through dentine in a standardized setup. Thereby, the effect of a desensitizing varnish based on methacrylates on peroxide diffusion was tested. Sodium perborate typically adopted for internal bleaching was included in the study as a reference.

## **Methods**

### *Dentine specimens*

Dentine specimens were gained from bovine incisors. The dentine slabs were ground plane parallel till grain size 4000, the diameter of the samples was at least 8 mm, and the thickness amounted exactly to 1.3 mm. The smear layer was removed by rinsing with EDTA (5%) and aqua bidest. for 10 s each. Before the experiments, the samples were stored in aqua bidest. For 24 h. Plastic rings (polypropylene, diameter 8 mm) were fixed with wax on both sides of the dentine slabs using a template for exact positioning (fig. 1).

### *Application of the bleaching agents*

The external bleaching agents were placed on the former enamel site of the dentine specimens. If testing Whitestrips<sup>®</sup>, a piece of the strip (diameter 8 mm) was placed on the dentine slabs, for application of Opalescence<sup>®</sup> Xtra<sup>®</sup> Boost<sup>™</sup> or VivaStyle<sup>®</sup> a volume of 50 µl gel was filled in the ring. Simply White<sup>®</sup> was brushed on the dentine surface with the paint brush included in the kit. Sodium perborate was mixed with aqua bidest (1 g ad 750 µl aqua bidest.), 50 µl were adopted in the experiment. In contrast to the external bleaching materials, sodium perborate was applied on the pulpal site of the dentine samples. Detailed information on the materials is given in tab. 1. Due to the high viscosity of the bleaching agents they did not flew out of the ring. A volume of 100 µl phosphate buffer was pipetted into the ring on the

upper side (fig. 1). Peroxide diffusion was assessed 10 s after application of the bleaching agent as well as after 30, 60, 90, 120, 150, 180, and 240 min. At each time point, the buffer was completely removed, transferred into the well of a microtiter plate and replaced by fresh buffer solution. Incubation with the bleaching agents was carried out in a wet chamber at 37° C.

### *Sealant*

The following desensitizing agent was tested with the external bleaching materials:

Vivasens<sup>®</sup>, Free Stand<sup>®</sup> Single Dose (0.1 g), Ivoclar Vivadent AG, Schaan, Liechtenstein, LOT: G20436. Composition: ethanol, water, hydroxypropylcellulose, polyethyleneglycol, dimethacrylate, other methacrylates.

The desensitizing varnish was adopted according to manufacturer's instructions. One single dose stand was used for 2 specimens. Dry dentine surfaces were sealed with a thin homogenous layer of the varnish for 10 s, and then dried with oil free air for 10 s. Before application of the bleaching agents the coated samples were stored in a wet chamber for 10 min at 37° C.

### *Peroxide assay*

Peroxide diffusion was measured photometrically using 4-aminoantipyrin as a substrate as described previously [19, 25-28]. Peroxidase catalyzes the reaction of 4-aminoantipyrin and phenol with hydrogen peroxide. Inorganic peroxides are oxidised by peroxidase (oxidoreduktase EC1.11.1.7). Thereby oxygen is released subsequently oxidizing the achromatic chromogenic hydrogen donor. The product chinonimin has its maximum of extinction at a wavelength of 510 nm. For determination of peroxides a calibration curve was carried out with different peroxide concentrations. The composition of the reaction buffer was 4-aminoantipyrin (4 mmol/l), peroxidase (0,4 U/ml), and phenol (24 mmol/l) in 0.1 molar

phosphate buffer (pH 7.0)[25]. A volume of 100 µl reaction buffer was added to 100 µl sample from the dentine specimen and the absorption was read vs. 0.1 M phosphate-buffer. If necessary, samples were diluted 1:20 with phosphate buffer. Peroxide diffusion was assessed 10 s after application of the bleaching agent and after 30, 60, 90, 120, 150, 180, and 240 min.

### *Statistics*

Statistical evaluation was carried out by ANOVA followed by the Scheffé-procedure using SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA). The level of significance was  $p \leq 0.05$ . For pairwise comparison of the external bleaching agents with and without the varnish the Mann-Whitney-test was adopted.

## **Results**

The kinetics of peroxide diffusion were approximately linear for all bleaching agents tested as depicted exemplarily for Whitestrips (fig. 2).

Irrespective of the desensitizing agent, significantly different amounts of peroxides penetrated the dentine samples when adopting the different materials. The cumulated diffusion of peroxides was balanced after 60 and 240 min, respectively.

For the unsealed specimens, the bleaching material had significant impact on the amount of peroxides detected on the pulpal side (ANOVA,  $p < 0.001$ ). The Scheffé-procedure indicated that Opalescence yielded the significantly highest diffusion of peroxides as compared with the other materials ( $p < 0,001$ ). This applied for the cumulated 60 min data as well as for the 240 min values.

Only the materials for external bleaching were tested after sealing with desensitizing varnish. Also with this coating, the bleaching material still had significant impact on the amount of peroxides detected on the pulpal side after 60 and 240 min (ANOVA,  $p < 0.001$ ). Opalescence

yielded the significantly highest diffusion of peroxides as compared with the other materials (Scheffé-procedure,  $p < 0,001$ ).

The application of Viavsens led to a significant reduction of peroxide diffusion for all external bleaching materials after 60 as well as after 240 min ( $p < 0.05$ , Mann-Whitney-test). The most pronounced effects were observed for Simply white.

## **Discussion**

Peroxide diffusion through dentine has been observed in the present study. As in previous studies on bioadhesion, bovine dentine specimens were used for purpose of standardization. [29-33]. The physico-chemical characteristics do not differ considerably from human dentine [34]. Typically, the diameter of the tubules in bovine dentine is 3-5  $\mu\text{m}$  with 20,000 tubules/ $\text{mm}^2$  as measured microscopically in our laboratory [35], in human dentine in the outer layers near the enamel 0.5-1.2  $\mu\text{m}$  have been recorded for the mean diameter with 10,000-25,000 tubules/ $\text{mm}^2$  [36]. This has to be considered when interpreting the observed peroxide diffusion. The adopted assay for quantification of the diffused peroxides has been used previously for precise measurement of peroxides in the oral cavity during application of bleaching regimen. It is not impaired by salivary components [25, 37].

For direct comparison all bleaching materials were adopted for the same time though different periods of application are common. This has to be taken into account when estimating the peroxide diffusion occurring clinically. A pure in vitro setup has been chosen. In the oral cavity, peroxide catabolism and peroxide diffusion would be modulated by the saliva and the acquired pellicle [5-6]. The antioxidative potential in the oral cavity is limited due to the fact that peroxidase in the saliva and in the acquired pellicle is inactivated by its substrate hydrogen peroxide [5-6]. Accordingly, the protective properties of the pellicle are limited during application of bleaching agents [6]. The observed penetration of peroxides through the



dentine is in good accordance with several previous studies [13, 17, 21-22]. The distinctly highest peroxide diffusion was recorded for Opalescence with a peroxide concentration of 38% and a very high viscosity of the bleaching gel. Free H<sub>2</sub>O<sub>2</sub> penetrates dentine to a significantly greater extent as compared with peroxides released from carbamide peroxide [21]. Opalescence and Simply White contain pure H<sub>2</sub>O<sub>2</sub>. Furthermore, bleaching agents of high viscosity yield a more pronounced peroxide diffusion than low viscous ones [20]. This corresponds well with the observed peroxide diffusion which was most pronounced for the high viscous Opalescence and the likewise viscous Simply White. Significantly lower diffusion was measured for Vivastyle and Whitestrips. However, the extent of peroxide diffusion was described to be not correlated with the concentration of the adopted bleaching agent [21]. In contrast to this previous finding, the highest peroxide release was observed in the present study with Opalescence featuring also the highest peroxide concentration.

Sodium perborate typically adopted for internal bleaching was also included in the study and yielded the significantly lowest peroxide diffusion. Sodium perborate mixed with tap water can be regarded as a low drug release compound [38]. Due to this fact, peroxide contamination of the periodontal structures seems to be rather low and antioxidative enzymes are assumed to be able to compensate for the oxidative stress. If sodium perborate was prepared with hydrogen peroxide instead of water a more pronounced diffusion of peroxide would have been expected. Adverse effects of internal bleaching have been recorded especially if sodium perborate was used together with H<sub>2</sub>O<sub>2</sub> for internal bleaching [39-41].

Anyhow, if balancing the peroxide release into the periodontal structures during internal bleaching, the application over up to one week has to be taken into consideration.

Peroxides are considered to be the key factor inducing tooth hypersensitivity. Apparently, the adopted sealant prevented peroxide diffusion significantly. Probably, the organic and apolar components of the material formed a layer and barrier against the extensive penetration of peroxides. It has been shown in a clinical study that Vivasens and other desensitizing agents

are effective in alleviating dentine hypersensitivity. However, peroxides as a stimulus were not tested [42]. In a previous clinical study, the degree of hypersensitivities and the number of subjects with hypersensitivities were lower if Vivasens was adopted, though the difference was not significant as compared with controls [23]. The reduced peroxide diffusion through the dentine as recorded in the present study might reduce the efficacy of the bleaching agent. However, a previous clinical study indicated that Vivasens had no negative effect on the whitening effect of bleaching agents [23]. This was also shown in an in-vitro-simulation [43]. Additionally, Vivasens is able to reduce dentin dehydration during bleaching, which is also supposed to contribute to bleaching induced hypersensitivities [44].

From a toxicological point of view and for prevention of side effects, Vivasens in combination with low dose home bleaching systems adopted with individual splints or polyethylene foils seemed recommendable.

## **Conclusions**

- Peroxide diffusion through dentine depends on composition and concentration of the bleaching agent used.
- Application of a desensitizing agent significantly reduces peroxide penetration through dentine.

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

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<b>Bleaching agent</b>					
	<b>Clinical application</b>	<b>composition</b>	<b>pH</b>	<b>Lot No. #</b>	<b>manufacturer</b>
VivaStyle®	individual tray (home-bleaching)	carbamide peroxide 10%, glycerine, buffered polycarbonic acid	6.6	GL1029	Ivoclar Vivadent AG, Schaan, Liechtenstein
Whitestrips®	polyethylene-strips (home-bleaching)	water, glycerine, H <sub>2</sub> O <sub>2</sub> 6,5%, saccharin, Carbopol 956, sodium hydroxide, acidic sodium pyrophosphate, sodium stannate	6.4	5146BT2C	Procter & Gamble Technical Centres Ltd., Egham, UK
Opalescence® Xtra® Boost™	in-office	H <sub>2</sub> O <sub>2</sub> 38%, carotene, water, glycerine, thickening agent	7.3	BØBYV	UP Dental GmbH, Köln, Germany
Simply White®	paint-on (home-bleaching)	ethanol, water, PEG-2M, PEG-12, 5.9% H <sub>2</sub> O <sub>2</sub> , glycerine, carbomere, sodium phosphate, phosphoric acid, BHT	8.9	3344AM 5068AM	Colgate-Palmolive, Hamburg, Germany
Sodium perborate	internal bleaching	sodium perborate-(trihydrate), chloride, sulfate, iron	10.2	22349512	Caesar & Lorentz, Hilden, Germany

Tab. 1: Bleaching agents tested in the study.

<b>a: Cumulated Peroxide diffusion over 60 min [nmol]</b>					
	<i>Whitestrips</i> <sup>®</sup>	<i>VivaStyle</i> <sup>®</sup>	<i>Simply White</i> <sup>®</sup>	<i>Opalescence</i> <sup>®</sup>	<i>sodium perborate</i>
without desensitizing agent	<b>5.3 ± 5.8</b>	<b>1.1 ± 1.9</b>	<b>26.4 ± 35.4</b>	<b>1603 ± 1022</b>	<b>0.0 ± 0.0</b>
with desensitizing varnish (Vivasens)	<b>1.4 ± 2.1</b>	<b>0.3 ± 0.3</b>	<b>0.6 ± 0.7</b>	<b>41.1 ± 28.3</b>	
<i>% reduction</i>	-74%	-73%	-98%	-97%	
<b>b: Cumulated Peroxide diffusion over 240 min [nmol]</b>					
	<i>Whitestrips</i> <sup>®</sup>	<i>VivaStyle</i> <sup>®</sup>	<i>Simply White</i> <sup>®</sup>	<i>Opalescence</i> <sup>®</sup>	<i>sodium perborate</i>
without desensitizing agent	<b>103 ± 62.3</b>	<b>158 ± 179</b>	<b>491 ± 314</b>	<b>8021 ± 3007</b>	<b>3.7 ± 2.9</b>
with desensitizing varnish (Vivasens)	<b>22.8 ± 20.8</b>	<b>5.2 ± 2.8</b>	<b>5.3 ± 1.8</b>	<b>221.7 ± 89.2</b>	
<i>% reduction</i>	-78%	-97%	-99%	-97%	

Tab. 2 a, b: Cumulated peroxide diffusion through the dentine slabs during application of the different bleaching agents with and without desensitizing varnish (Vivasens) after 60 and 240 min. MV±SD, n=8 samples/ subgroup.

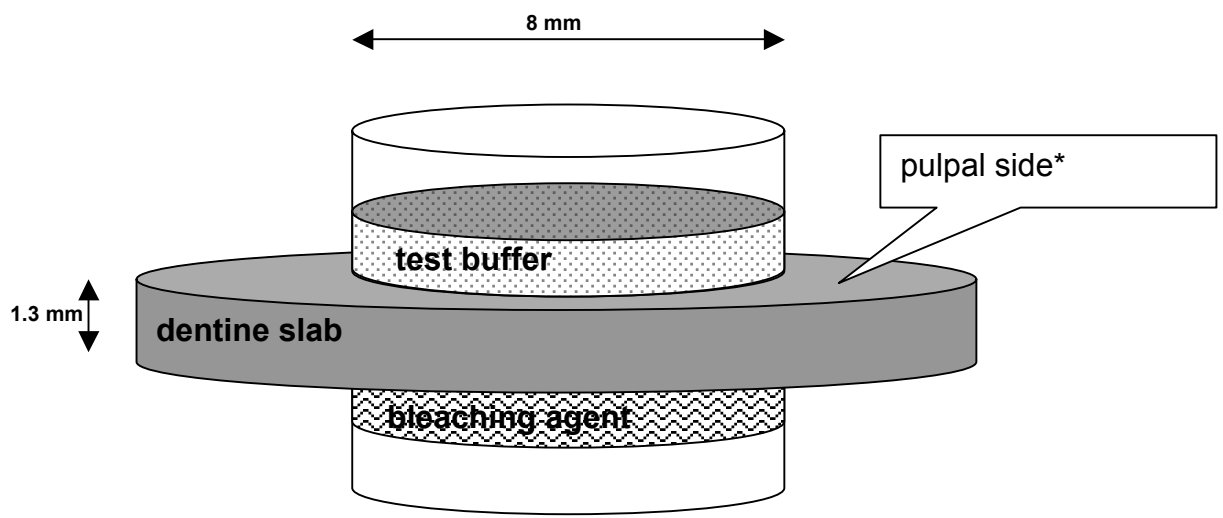


Fig. 1: Setup of the in vitro experiments, \* sodium perborate was applied on the pulpal side.



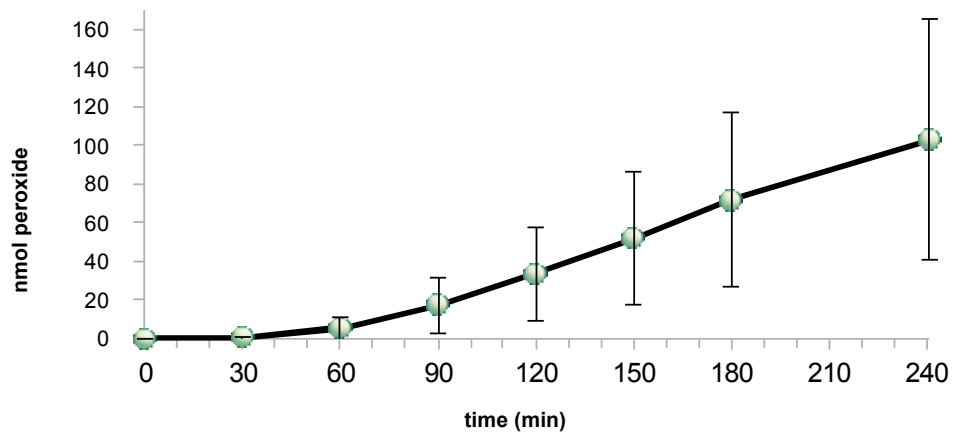


Fig. 2: Kinetics of peroxide diffusion, application of Whitestrips over 4 h, the amount of peroxides was cumulated.  $MV \pm SD$ ,  $n=8$  samples, no desensitizing agent was adopted.