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Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review

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Abstract

Objective: Numerous studies investigated the impact of bleaching procedures on enamel microhardness. The outcomes of these studies reveal inconsistencies regarding the fact whether a microhardness reduction due to bleaching occurs or not. Aim of the present review was to summarize the existing literature of external bleaching therapies, which used microhardness tests for evaluation of possible effects on enamel and to weigh up different parameters of the study designs with respect to the outcome of these studies.

Sources: The data from original scientific full papers listed in PubMed or ISI Web of Science (search term: enamel AND (bleaching OR peroxide) AND (hardness OR microhardness OR Knoop OR Vickers)) and received by additional hand-search meeting the inclusion criteria were included in the review. Influences of different parameters on the outcome of the bleaching treatments were analyzed with the Fisher's-exact-test.

Data: A total of 55 studies were identified with 166 hardness measurements conducted directly after bleaching and 69 measurements performed after a post-treatment episode. Directly after bleaching, 84 (51%) treatments showed microhardness reduction compared to baseline, whereas 82 (49%) did not yield microhardness reduction. After the post treatment episode, 20 (29%) treatments showed hardness reduction and 49 (71%) did not. A significant higher number of bleaching treatments resulting in enamel microhardness reduction were observed, when artificial instead of human saliva was used for storage of the enamel samples in the intervals between the bleaching applications and when no fluoridation measures were applied during or after the bleaching phase.

Conclusion: The review shows that in those studies, which simulated the intraoral conditions as closely as possible, the risk of enamel microhardness decrease due to bleaching treatments seems to be reduced. Nevertheless more in-situ- and in-vivo-studies are needed to verify this observation.

Introduction

Numerous studies have investigated the effect of external bleaching agents on dental hard tissues. Systematic reviews could prove that bleaching therapies might have a deleterious impact on restorative materials and restorations and that external bleaching with heat might be done with caution due to unknown effects on the pulpal tissue [1]. It was shown that bleaching agents might have a negative influence on integrity of organic enamel structures, such as proteins and collagen [2]. Also, some studies found mineral loss, loss of fluoride, increased susceptibility to erosion or caries, increased surface roughness, reduced enamel micro tensile strength, reduced fracture stability or a decrease in abrasion resistance of bleached dental hard tissues [3-17]. On the other hand, there are other studies, which did not confirm these observations [18-22]. Beside this, no clinical studies or case reports in the literature have documented macroscopically or clinically visible damage due to vital bleaching or clinically relevant tissue destruction. Studies investigating external bleaching therapies often apply microhardness testing for evaluation of structural enamel defects. There is great inconsistency in the outcome of those studies. This might be due to differences in study design such as use of dental substrate (human vs. bovine), microhardness test (Knoop vs. Vickers hardness), storage conditions between bleaching intervals (no remineralizing solution vs. artificial saliva vs. human saliva), fluoridation measures (applied vs. not applied) and the question whether the study was performed in vitro or in situ/in vivo. Moreover, in some studies the microhardness is measured directly after the bleaching episodes and in other studies after a post treatment period, in which the samples had been stored in remineralizing solutions. Additionally, the concentration and kind of formulation of the bleaching agent might also influence the effect on the enamel structure.

Aim of the present review was to summarize the existing literature of external bleaching therapies, which uses microhardness tests for evaluation of possible effects on enamel and to weigh up different parameters of the study designs with respect to the outcome of these studies.

Data collection and analysis

An electronic search was performed in PubMed and ISI Web of Science on 1rst December 2007 (Search term: enamel AND (bleaching OR peroxide) AND (hardness OR microhardness OR Knoop OR Vickers)). Only original scientific full papers were included in the review, abstracts and reviews dealing with this topic were not taken into consideration. Additionally, a hand search was made in those journals, for which the electronic search had identified a study dealing with the topic. Moreover, the manual search included the reference lists of the articles found as well as of review articles concerning the topic. Furthermore the "related aticles" option on the PubMed website was used as data source.

Inclusion criteria leading to further analysis of the papers were: definition of storage conditions, clear description of bleaching conditions, description of enamel substrate (bovine or human), mentioning of parameters used for hardness determination (Knoop or Vickers, load) and performing of a statistical analysis.

The contents of the studies meeting these criteria were summarized (Table 1) according to selective criteria. In some studies, the outcome with respect to microhardness changes was only given in figures without giving the respective values, other authors presented microhardness reduction in percentage, other in microhardness numbers. Due to these differences in the presentation of the results, we decided to focus on the question, whether the microhardness was described as being statistically significantly lower or higher as compared to baseline ("lower", "higher"), or not significantly different ("n.s.d."). The outcome was further subdivided into the results directly after bleaching and the results obtained after a respective post treatment phase, in which the samples had continuously been stored in a remineralizing solution. In most of the studies, numerous bleaching applications on subsequent days were performed during a time period of e.g. 14 days. In the table it is additionally presented in which kind of storage media the specimens were immersed for the remaining period of the day between the bleaching applications ("human saliva", "artificial saliva" or "no remineralizing solution"). Other criteria considered in the table are, whether bovine or human enamel specimens were used ("human", "bovine"), whether the study was performed in the laboratory only ("in vitro") or "in situ/in vivo". In

the in-situ-studies the enamel samples were carried in intraoral appliances at least in the intervals between or after the bleaching episodes. In a single study, bleaching was completely performed in vivo with the microhardness test conducted after the extraction of the bleached teeth [23]. This study was also allocated to the “in situ/in vivo” category. The studies were further differentiated according to the fact, whether fluoridation measures (fluoride toothpastes/gels/rinses/varnishes, fluoridated bleaching agents) were used or not (“fluoride”, “no fluoride”). The bleaching agents were categorized according to their active bleaching substrate and the concentration (“≤10% hydrogen peroxide (HP)”, “>10% HP”, “<10% carbamide peroxide (CP)”, “≥10% CP”, “sodium chlorite and citric acid”, “19% percabonate” or “Hydroxylite”). The peroxide concentration of Hydroxylite was not given in the respective paper, so that a separate category was introduced for this product.

The impact of the different coupled parameters (“bovine” vs. “human”, “fluoride” vs. “no fluoride”, “artificial saliva” vs. “human saliva”, “in vitro” vs. “in situ/in vivo”) on the outcome of the bleaching therapy with respect to enamel hardness was statistically analyzed using the Fisher`s-exact-test. Only those parameters, which were represented by more than five bleaching treatments, were included in the analysis. Level of significance was set to $p \leq 0.05$.

Results

A total of 55 studies were identified meeting the inclusion criteria and were included in the following analysis. In the 55 studies, 166 bleaching treatments were performed in which enamel microhardness was directly measured after the treatment; 69 bleaching treatments were conducted in which microhardness was evaluated after a post-treatment phase during which the samples were in contact with either artificial or human saliva as remineralizing solution. The treatments performed in each study were allocated to the different parameters according to the fact, whether microhardness reduction was recorded or not for this treatment (Table 2). Thus, 166 treatments were included, in which hardness was directly tested after the bleaching therapy. From these 166 treatments, 84 (51%) showed microhardness reduction compared to baseline directly after bleaching and 82 (49%) did not yield microhardness reduction. From the 69

bleaching treatments, which were followed by a post-treatment microhardness test, 20 (29%) showed hardness reduction and 49 (71%) did not reveal enamel hardness reduction as compared to baseline values.

In Table 3 the results of the statistical evaluation are given. Only few parameters could be identified, which have an impact on the outcome of the microhardness changes of bleached enamel. For the measurements performed directly after bleaching, less numbers of bleaching treatments resulting in enamel microhardness reduction were seen, when human saliva was used instead of artificial saliva for simulation of the remineralizing solution and when fluoridation measures were involved in the study protocol. For the microhardness determination performed after the post-treatment episode, the use of human saliva and the application of Vickers hardness tests instead of Knoop tests were also associated with a less frequency of microhardness reductions. Both for the assessments performed directly after bleaching and for those conducted after the post-treatment phase, no impact was seen with regard to the concentration or kind of the bleaching agent and the study design.

Discussion

Interpretation of the analysis should be done with caution, since the different parameters were mostly not directly compared in a single study. Nevertheless, the data give hints, how to interpret results in future studies and how to design future experiments analyzing enamel microhardness after bleaching. The analysis showed that the probability to decrease microhardness in bleached enamel could be significantly reduced when fluoride compounds were administered after the bleaching steps during the bleaching period. This was true for fluoride fluoride varnishes as well as for fluoride containing dentifrices. Especially those studies applying fluoridated dentifrices to the bleached enamel surface mimic the usual clinical situation closely, since the use of fluoride dentifrices is nowadays widely spread. Also, fluoridated carbamide peroxide bleaching gels were able to reduce microhardness loss and accelerated microhardness recovery in the post-treatment phase to a better extent than unfluoridated gels [24]. This might be due to the fact that fluoride containing carbamide peroxide bleaching gels induce fluoride acquisition of enamel [25].

Although this effect is less pronounced as compared to application of pure fluoride gels, it may be speculated that the fluoride component of bleaching gels might support the repair of the microstructural defects by the adsorption and precipitation of salivary components, such as calcium and phosphate. In this sense, it was shown that susceptibility of bleached enamel is lower for enamel bleached with a fluoride-containing carbamide peroxide bleaching gel [26].

The use of human saliva was less associated with microhardness reduction as compared to artificial saliva. Although artificial saliva is able to re-harden surface softened enamel [27], human saliva seems to have the better capability and mimics the natural conditions far better. Both aspects, the use of fluoride as in the everyday situation and the use of human saliva, are important for estimating the influence of bleaching agents on dental enamel under clinical conditions in the oral cavity. Moreover, it should be realized, that enamel surface microhardness is also reduced by dietary components and even by mouthrinses or saliva substitutes prescribed for patients with dry mouth symptoms [28-30]. It is supposed that these hardness reductions will resolve in the oral cavity due to the remineralizing impact of saliva. [31,32]. The same behaviour is expected to happen with bleached enamel, as observed in the studies, which re-evaluated the microhardness after a post-treatment phase, in which the samples were kept under remineralizing conditions. The majority, i.e. 71% of bleaching applications evaluating post-treatment enamel surface hardness, showed recovery of hardness to baseline values. All of the studies using human saliva in the post-treatment phase did not show difference of hardness as compared to baseline values, thus indicating complete recovery of enamel surface hardness.

The majority of studies were conducted under in-vitro-conditions. Only four studies, with a total of nine bleaching treatments, were designed as in-situ-experiments. One of those four studies, conducted by Justino [33], compared the influence of bleaching agents in an in-situ- versus an in-vitro set-up. In this study, the samples kept under in-situ-conditions did not show hardness reduction, whereas the specimens only subjected to an in-vitro-environment, revealed significant hardness reduction. This finding showed that the intraoral conditions seem to reduce the risk of creating surface microhardness due to bleaching. Unfortunately, the samples in the in-vitro-leg

of this experiment were kept in distilled water and not in human or artificial saliva, so that an estimation of in-vitro-conditions using remineralizing solutions could not be done.

The only study conducted in vivo, performing microhardness test on intraorally bleached surfaces after extraction, did not reveal microhardness reduction as compared to unbleached surfaces of the same tooth [23]. Unfortunately, there are no further in-vivo-studies dealing with this topic, so that a final conclusion on the impact of bleaching under in-vivo-conditions could not be drawn.

However, also note-worthy and important is the fact that in all studies presented in Table 1, and in all other studies and case reports in the literature, no detrimental effects on a macroscopic level (such as fracturing of teeth or visible loss of dental hard tissue) has been reported. Even in clinical studies with a long bleaching period of up to six month with using 10% carbamide peroxide gels as bleaching agent, no visible impact on the integrity of any of the treated teeth was reported [34-36].

Conclusions

The review shows that in those studies, which simulated the intraoral conditions as closely as possible (use of human saliva and fluoridation measures, and evaluation after a post-treatment phase), the risk of enamel microhardness decrease due to bleaching treatments seems to be reduced as compared to the remaining studies. Nevertheless more in-situ- and in-vivo-studies are needed to verify this observation.

Table 1: Summary of the 52 studies included in the analysis.

Authors	Active bleaching agent in experimental groups	Duration of bleaching	Storage conditions between bleaching episodes	Microhardness reduction directly after bleaching	Duration of post-treatment and microhardness after post-treatment	Substrate: Design, Hardness test	Remarks
Sulieman et al. [37]	1) 35% HP	3x 10 min	no remin. solution	1) n.s.d.	n.e.	H: in vitro; VH	
Maia et al. [38]	1) 10% CP 2) 7.5% HP	1 h/d: 21 d	in situ	1) n.s.d. 2) n.s.d..	n.e.	H: in situ; KH	
de Menezes et al. [39]	1-3)* 12-22% CP 4) 30% CP	1-3) 2 h/d: 21 d 4) 30 min/d: 21 d	artificial saliva	1-3) n.s.d. 4) n.s.d.	n.e.	B: in vitro; KH	
Attin et al. [26]	1-4) 10% CP with either fluoride or not and either neutral or acidic pH	1x 8h	no remin. solution	n.s.d.	n.e.	B: in vitro; KH	here: only data of the first application from a complex study

Wiegand et al. [40]	1) 10% CP 2-7) 10% CP	8 h/d: 14 d	artificial saliva 1) no F 2-7) 2x/d F-toothpaste plus either F-gel (1x/d) before or during bleaching period, or no F-Gel	1) lower 2-7) n.s.d.	14 d in saliva 1) lower 2-7) n.s.d.	B: in vitro; KH	In post treatment: 2x/d F-tothpaste plus either F-gel, or no F-Gel
Metz et al. [23]	1) 15% CP with F 2) 15% CP w.o.F	8 h/d: 4 d or 14 d	human saliva (intraoral)	1) n.s.d. 2) n.s.d.	14 d in saliva 1) n.s.d. 1) n.s.d.	H: in vivo; KH	In post treatment: continuation of dentifrice aplication
de Oliveira et al. [41]	1) 10% CP 2) 10% CP	8 h/d: 42 d	artificial saliva 1) + fluoride-free dentifrice (5 min) 2) + fluoride dentifrice (5 min)	After 42 d 1) higher 2) n.s.d.	14 d in saliva 1) higher 2) n.s.d.	H: in vitro; KH	
Rodrigues et al. [42]	1,2) 10% CP with carbopol or poloxamer	6 h/d for 7-28 d	artificial saliva	1,2) n.s.d.	14 d in saliva 1,2) n.s.d.	B: in vitro; KH	
Zhang et al. [43]	1-4) 35% HP activated with light or laser	1-4) 7.5 min	no remin. solution	1-4) n.s.d. to controls	n.e.	H: in vitro; VH	no baseline data presented
Seghi and Denry [11]	10% CP	12 h	no remin. solution (phosphate buffered saline)	n.s.d.	n.e.	H: in vitro; VH	

Araujo et al. [44]	1) 10% CP 2) 10% CP	1) 1 h/d: 21 d 2) 7 h/d: 21 d	human saliva	1) n.s.d. 2) n.s.d.	n.e.	H: in situ; KH	
Cesar et al. [45]	1-4) 35 or 37% CP plus argon laser or halogen	1-4) 2 x 40 min	no remin. solution (100% humidity)	1-4) n.s.d.	n.e.	H: in vitro; VH	
Duschner et al. [46] [46]	1) 6% HP (strips) 2) 6.5% HP (strips)	2x 30 min/d for 28 d	human saliva plus brushing with fluoridated dentifrice (twice/d)	n.e.	48-72 h in saliva 1) n.s.d. 1) harder	H: in vitro; VH	
Joiner and Thakker [47]	1) 6% HP (paint-on) 2) 6% HP (gel)	2x3 min/d for 14 d	sterile human saliva	1) n.s.d. 2) n.s.d.	n.e.	H: in vitro; VH	
Joiner et al. [48]	6% HP (gel)	2x20 min/d for 14 d	sterile human saliva	n.s.d.	n.e.	H: in vitro; KH	
Lee et al. [49]	1-3) 35-50% HP (gel/paste)	1x 1 h	no remin. solution	1-3) n.s.d.	n.e.	H: in vitro; VH	
Lopes et al. [50]	1) 10% CP 2) Hydroxylite (no oxygen) 3) 3% HP (gel)	3 h/d for 14 d	artificial saliva	1) n.s.d. 2) higher 3) lower	n.e.	H: in vitro; VH	
Murchison et al. [51]	1-3) 10 % CP	9 or 18 h/d: 5 d	artificial saliva	1-3) n.s.d.	n.e.	H: in vitro; KH	
Pugh et al. [52]	1) 10% CP 2) 7% HP (paste) 3) 12% HP (paste)	14 x 7h: 14 d	human saliva	1) n.s.d. 2) n.s.d. 3) n.s.d.	n.e.	H: in vitro; KH	

Potocnik et al. [53]	10% CP	42 x 8h	no remin. solution	n.s.d. compared to untreated surface	n.e.	H:in vitro; VH	Only subsurface hardness evaluated
Teixeira et al. [54]	1-4) 6-9.5% HP 5) 10% CP	1-4) 30 min/d: 14 d 5) 6 h/d: 14 d	artificial saliva	1-4) n.s.d. 5) n.s.d.	n.e.	H: in vitro; KH	
Ünlü et al. [55]	1-2) 10% CP 3-4) 15% CP	1-2) 1x 4 h or 7x 4h 3-4) 1x 4 h or 7x 4h	no remin. solution	n.e.	24 h in artificial saliva: 1-4) n.s.d.	H: in vitro; VH	
White et al. [56]	1) 5.3% HP (strips) 2) 6.5% HP (strips) 3) 10% CP 4) 20% CP	1) 2x 2h/d -> 14 h 2) 2x 2h/d -> 70 h 3) 2x 2h/d -> 70 h 4) 2x 2h/d -> 70 h	human saliva	n.e.	48-72 h in saliva 1) higher 2) n.s.d. 3) n.s.d. 4) n.s.d.	H: in vitro; VH	
White et al. [57]	1) 5.3% HP (strips) 2) 6.5% HP (strips) 3) 10% CP 4) 20% CP	1) 7x 2 h 2) 7x 2 h 3) 35 x 2 h 4) 35x 2 h	human saliva	n.e.	48-72 h in saliva 1) higher 2) n.s.d. 3) n.s.d. 4) n.s.d.	H: in vitro; VH	
White et al. [58]	19% sodium percarbonate (5.3% HP)	8 h/d for 14 d	human saliva	n.s.d.	n.e.	H: in vitro; VH	

Götz et al. [59]	1) 13% HP (strip) 2) 16% HP (strip)	2x 30 min/d: 28 d	human saliva plus 2x 60 min/d brushing with fluoridated dentifrice	n.e.	48-72 h in saliva 1) n.s.d. 2) n.s.d.	H: in vitro; VH	
White et al. [19]	1) 5.3% HP (strips) 2) 6.5% HP (strips) 3) 10% CP 4) 20% CP	1) 2x 2h/d -> 14 h 2) 2x 2h/d -> 70 h 3) 2x 2h/d -> 70 h 4) 2x 2h/d -> 70 h	human saliva		48-72 h in saliva 1) n.s.d. 2) n.s.d. 3) n.s.d. 4) n.s.d.	H: in vitro; VH	Only subsurface microhardness
Ulukapi [60]	1) 10% CP 2) 35% HP (heat)	1) 14x for 8 h 2) n. described	no remin. solution	1) n.s.d. 2) lower	1-7 d in art. sal. 1) n.s.d. 2) n.s.d. after day 3	H: in vitro; VH	
da Costa & Mazur [61]	1) 10% CP 2) 10% CP (F, PN) 3) 10% CP (APF) 4) 10% CP 5) 10% CP (F, PN) 6) 10% CP (APF)	8 h/d for 21 d	artificial saliva	1) lower 2) lower 3) lower 4) lower 5) lower 6) lower	14 d in saliva 1) lower 2) lower 3) lower 4) lower 5) n.s.d. 6) n.s.d.	H: in vitro; KH	1.23% F-gel applied once after bleachig period on day 21 in groups 4, 5 and 6
Lewinstein et al. [62]	1) 35% HP 2) 35% CP 3) 15% CP 4) 10% CP	1) 3x in 35 min 2) 3x in 35 min 3) 14x 1 h 1) 14x 1 h	no remin. solution (100% humidity)	1) lower 2) lower 3) lower 4) lower	after 0.05% SnF (5 min bath) 1) n.s.d. 2) n.s.d. 3) n.s.d 4) n.s.d	H: in vitro; KH	

Basting et al. [12]	1) 10% CP (commercial gel) 2) 10% CP (pure)	8 h/d: 42 d	artificial saliva	After 42 d: 1) lower 2) lower	14 d in saliva 1) lower 2) n.s.d.	H: in vitro; KH	carbopol and glycerin also decreased KH
Basting et al. [63]	1-2) 10% CP 3-6) 16-22% CP 7) 20 % CP	8 h/d: 42 d	artificial saliva	1-3) lower 3-6) lower 7) lower	14 d in saliva 1-2) lower 3-6) lower 7) n.s.d.	Human: in vitro; KH	
de Oliveira et al. [64]	1) 10% CP 2) 10% CP+0.05% Ca 3) 10% CP+0.1% Ca 4) 10% CP+0.2% Ca 5) 10% CP+0.2% F 6) 10% CP+0.5% F	6 h/d: 14 d	artificial saliva	1) lower 2) lower 3) lower 4) lower 5) lower 6) lower	7 d in saliva 1) lower 2) lower 3) lower 4) lower 5) lower 6) lower	H: in vitro; KH	
Hairul Nizam et al. [17]	30% HP (solution)	24 h	no remin. solution	lower	n.e.	H: in vitro; nano-indentaion	
Smidt et al. [65]	1-3) 10% CP	6 h/d: 16 d	no remin. solution (not specified)	1-3) lower	n.e.	H: in vitro; VH	controls were placed in saline

Pinto et al. [66]	1) 10% CP 2) 10% CP 3) 7.5% HP 4) 37% CP 5) 35% CP 6) 35% HP	1) 6 h/d (14 d) 2) 6 h/d (5 d) 3) 30 min/d (14 d) 4) 4 x 30 min (5d) 5) 4 x 30 min (5 d) 6) 4 x 15 min (7 d)	artificial saliva	1) lower 2) lower 3) lower 4) lower 5) lower 6) lower	n.e.	H: in vitro; KH	
Al-Salehi et al. [67]	1) 3% HP (solution) 2) 10% HP (solution) 3) 30% HP (solution)	24 h	-	1) lower 2) lower 3) lower	n.e.	B: in vitro; VH	
Attin et al. [68]	1) 35% HP 2) 35% CP 3) sodium chlorite + c. a. 4) 5.3% HP 5) 10% CP 6) 15% CP	During 10 d: 1) 2x 30 min 2) 2x 1 h 3) 2x/d: 20 min 4) 2x/d: 30 min 5) 8 h/d 6) 4 h/d	artificial saliva	1) lower 2) lower 3) lower 4) lower 5) lower 6) lower	n.e.	B: in vitro, KH	only subsurface microhardness was determined
Park et al. [69]	1-3) 30% HP (solution)	1) 24 h 2) 72 h 3) 120 h	no remin. solution	1) lower 2) lower 3) lower	n.e.	B: in vitro; VH	no difference compared to controls (distilled water)
Basting et al. [70]	10% CP	8 h/d: 21 d	human saliva (in situ)	lower: compared to placebo	n.e.	Human: in situ; KH	

Pinheiro et al. [71]	1, 2) 10% 3) 16% CP	8 h/d: 7 d	artificial saliva	1, 2) lower 3) lower	n.e.	H: in vitro; KH	
Cimilli & Pameijer [72]	1-4) 10% CP 5-8) 15% or 16% CP	6 h/d: 5d and 10 d	no remin. solution (distilled water)	1-4) lower 5-8) lower	n.e.	H: in vitro; VH	comparison to controls, no baseline data
Rodrigues et al. [73]	1) 37% CP plus 10% CP 2) 37% CP plus placebo 3) placebo plus 10% CP	37% CP: 2 x 30 min 10% CP: at night for 21 d	human saliva (in situ)	1) lower 2) lower 3) lower	n.e.	H: in situ; KH	
Lewinstein et al. [74]	1) 30% HP at 37° (solution) 2) 30% HP at 50° (solution) 3) SP+30% HP at 37° (sol.) 4) SP+30% HP at 50° (sol.)	5-30 min	no remin. solution	1) lower 2) lower 3) n.s.d. 4) n.s.d.	n.e.	H: in vitro; VH	1-2) Lower after 15 min
Akal et al. [75]	1) 10% CP 2) 12% CP+PF	1) 6 h/d: 28 d 2) 3 h/d: 28 d	artificial saliva	1) lower 2) higher	n.e.	H: in vitro; VH	
Faraoni-Romano et al. [76]	1) 10% CP 2) 15% CP 3) 22% CP	2 h/d: 21 d	artificial saliva	1) lower 2) lower 3) n.s.d.	n.e.	B: in vitro; KH	comparison to controls; no baseline data
Leonard et al. [77]	1) 19% sodium percarbonate 2) 8.75% HP 3) 10% CP	8 h/d: 14 d	artificial saliva	1) n.s.d. 2) lower 3) n.s.d.	7 d in saliva 1) n.s.d. 2) lower 3) n.s.d.	H: in vitro; KH	

Zantner et al. [78]	1) 8% CP 2) 8% CP 3) 5.9% HP 4) 8% CP 5) 10% CP 6) 5.9% HP (strips) 7) sodium chlorite + c. a.	1) 20 min/d: 14 d 2) 2x 20 min/d: 14 d 3) 30 min/d: 14 d 4) 2x 5 min/d: 14 d 5) 1 h/d: 14 d 6) 2x 30 min/d: 14 d 7) 2x 10 min/d: 14 d	artificial saliva	1) n.s.d. 2) n.s.d. 3) lower 4) n.s.d.. 5) n.s.d. 6) lower 7) lower	42 d in saliva 1) n.s.d. 2) n.s.d. 3) n.s.d. 4) n.s.d.. 5) n.s.d. 6) n.s.d.. 7) lower	H: in vitro; KH	
Justino et al. [79]	10% CP	8 h/d: 14 d	1) distilled water 2) human saliva (in situ)	1) lower 2) n.s.d.	n.e.	H: in vitro, in situ; VH	
Attin et al. [80]	1) 10% CP 2) 10% CP 3) 10% CP	12 h/d for 4 d	artificial saliva + 1) 2.23% F-varnish (1 h/d) 2) 0.2% F-solution (1 min/d) 3) artificial saliva	1) n.s.d. 2) n.s.d. 3) lower	n.e.	B: in vitro; VH	
Attin et al. [10]	1) 35% HP 2) 35% CP 3) sodium chlorie + c. a. 4) 5.3% HP 5) 10% CP 6) 15% CP	during 10 d: 1) 2x 30 min 2) 2x 1 h 3) 2x/d: 20 min 4) 2x/d: 30 min 5) 8 h/d 6) 4 h/d	artificial saliva	1) n.s.d. (VH) 2) n.s.d. (VH) 3) lower (VH) 4) lower (VH) 5) n.s.d. (VH) 6) n.s.d. (VH)	n.e.	B: in vitro, KH, VH	1-6): lower, when KH was applied

Ferreira et al. [81]	1) 10% CP 2) 7.5% HP 3) 5.5% HP 4) 3.5% HP (strips) 5) 4.5% HP	2x30 min/d: 14 d	artificial saliva	1) n.s.d. 2) n.s.d. 3) higher 4) n.s.d. 5) n.s.d.	n.e.	H: in vitro, VH	
Nathoo et al. [82]	1) 10% CP	2x30 min/d: 14 d	human saliva	1) n.s.d.	n.e.	H: in vitro, KH	only subsurface hardness after polishing was tested; comparison made to controls
McCracken and Haywood [83]	1) 10% CP 2) 10% CP	24x1 h	-	1) n.s.d. 2) lower	n.e.	H: in vitro, KH	only subsurface hardness
Rodrigues et al. [84]	1) 10% CP 2) 10% CP	8 h/d: 42 d	artificial saliva	1) n.s.d. 2) lower	n.e.	H: in vitro; KH	

Legend: CP = carbamide peroxide, HP = hydrogen peroxide, SP = sodium perborate, d = days, w = weeks, h = hours, KH: Knoop hardness, VH: Vickers hardness, n.s.d. = not significantly different from baseline, n.e. = not evaluated, lower/higher = lower/higher as compared to baseline, the agents are in gel formulations unless differently specified, F: fluoride, PN: potassium nitrate; PF = potassium fluoride, APF = acidulated phosphate fluoride, c.a. = citric acid, no remin. solution = no remineralizing solution* experimental groups

Table 2: Number of treatments (percentages) in the 52 studies with/without microhardness reduction directly after bleaching or after the post-treatment interval subdivided according to the different categories.

	directly after bleaching				after post-treatment			
	reduction		no reduction		reduction		no reduction	
bleaching agent								
<10% HP	9	(41%)	13	(59%)	0	(0%)	11	(100%)
≥10% HP	12	(50%)	12	(50%)	1	(0%)	3	(100%)
≤10% CP	38	(49)	40	(51%)	13	(48%)	24	(52%)
>10% CP	21	(60%)	14	(40%)	5	(33%)	10	(67%)
sodium chlorite & citric acid	4	(100%)	0	(0%)	1	(100%)	0	(0%)
Hydroxylite	0	(0%)	1	(100%)	0	(0%)	0	(0%)
19% percarbonate	0	(0%)	2	(100%)	0	(0%)	1	(100%)
total	84	(51%)	82	(49%)	20	(29%)	49	(71%)
remineralizing solution								
no remin. solution	26	(53%)	23	(47%)	1	(20%)	4	(80%)
artificial saliva	54	(54%)	45	(46%)	19	(41%)	27	(59%)
human saliva	4	(22%)	14	(78%)	0	(0%)	18	(100%)
fluoridation measures								
no fluoride	82	(54%)	70	(46%)	14	(29%)	38	(71%)
fluoride	2	(14%)	12	(86%)	6	(35%)	11	(65%)
enamel substrate								
bovine	23	(49%)	24	(51%)	1	(11%)	8	(89%)
human	61	(51%)	58	(46%)	19	(32%)	41	(68%)
study design								
in vitro	80	(52%)	75	(48%)	20	(30%)	47	(79%)
in situ / in vivo	4	(36%)	7	(64%)	0	(0%)	2	(100%)
hardness test								
Knoop hardness	58	(55%)	47	(45%)	19	(40%)	29	(60%)
Vickers hardness	35	(50%)	35	(50%)	1	(0.5%)	20	(99.5%)
nanoindentation	1	(100%)	0	(0%)	0	(0%)	0	(0%)

Table 3: P-values of the comparative analysis (Fisher`s-exact-test) for different parameters regarding enamel microhardness recorded directly after bleaching or after a post-treatment episode. P-values are only given for comparisons with significant difference, others are designated as not significant (n.s.) The parameter with the statistically less frequency of microhardness reduction is additionally named.

	directly after bleaching		after post-treatment	
	p-value	less frequency of reduction	p-value	less frequency of reduction
bleaching agent				
≥10% HP vs. >10% CP	n.s.	-	-*	-
≤10% CP vs. >10% CP	n.s.	-	n.s.	-
<10% HP vs. ≥10% HP	n.s.	-	-*	-
remineralizing solution				
human saliva vs. artificial saliva	0.0193	human saliva	0.006	human saliva
fluoridation measures				
fluoride vs. no fluoride	0.0049	fluoride	n.s.	-
enamel substrate				
bovine vs. human	n.s.	-	n.s.	-
study design				
in vitro vs. in situ/in vivo	n.s.	-	-*	-
hardness test				
Knoop vs. Vickers	n.s.	-	0.0033	Vickers

* not statistically analyzed due to the low number (<5) of bleaching treatments recorded for one of the parameters. HP = hydrogen peroxide, CP = carbamide peroxide

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