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A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentine

M. Sulieman, M. Addy*, E. Macdonald, J.S. Rees

Division of Restorative Dentistry, Department of Oral and Dental Science, University of Bristol Dental School, Lower Maudlin St, Bristol BS1 2LY, UK

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Summary *Objective*. The aim of this study was to investigate safety concerns with bleaching procedures by studying the effects of a high concentration hydrogen peroxide (HP) in-surgery bleaching product on enamel and dentine.

Method. Flat enamel and dentine samples embedded in epoxy resin were prepared from human third molar teeth. *Erosion of enamel*: groups of enamel samples were treated with 35% HP then citric acid (CA) or brushing with toothpaste or CA alone and water alone. Enamel loss was measured using a profilometer. *Abrasion/erosion of dentine*: groups of dentine specimens were treated as follows: Group 1—brushed with water for 30 min. Group 2—brushed with 35% HP for 30 min. Group 3—power bleached for 30 min and then Group 4—brushed with toothpaste for 1 minute. Group 5—water soaked for 30 min followed by brushing with toothpaste for 1 min. Group 6—orange juice soaked for 30 min followed by brushing with toothpaste for 1 min. Treatment effects were measured using a profilometer. *Hardness tests*: enamel and dentine specimens were hardness tested using a Wallace indenter prior to and post bleaching. *Scanning Electron Microscopy*: enamel and dentine specimens were taped and the exposed tissue treated with 35% HP and then studied under scanning electron microscopy (SEM).

Results. *Enamel erosion*: bleaching enamel samples had no measurable effect on enamel. Pre-bleaching had no significant effect on subsequent CA erosion or brushing. *Abrasion/erosion of dentine*: no significant differences were found between treatments 1–5 with little change from baseline detected. Orange juice (Group 6) produced considerable and significantly more erosion than other treatments. *Hardness tests*: there were no significant changes in hardness values for enamel and dentine. *SEM*: there was no evidence of any topographical changes to either enamel or dentine.

Conclusion. Using one of the highest concentrations of HP for tooth bleaching procedures and maximum likely peroxide exposure, there was no evidence of deleterious effects on enamel or dentine. It must be assumed that studies which

* Corresponding author. Tel.: +44-117-928-4506; fax: +44-117-928-4100.
E-mail address: martin.addy@bristol.ac.uk (M. Addy).

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reported adverse effects on enamel and or dentine of bleaches reflect not the bleach itself but the pH of the formulation used.

Introduction

The desire for whiter teeth among the population has reached an all time high with many articles featured in the popular press and television on a very regular basis.

The use of a variety of bleaching techniques has attracted much interest from the profession, as they are relatively non-invasive and simple to carry out. Contemporary bleaching systems are based primarily on HP or one of its precursors, notably carbamide peroxide (CP), and these are often used in combination with an activating agent such as heat or light. Bleaching agents can be applied externally to the teeth (vital bleaching), or internally within the pulp chamber (non-vital bleaching).[1,2]

Coupled with the uncertain legal situation within the European community and especially within the UK, it has become important to investigate whether bleaching could adversely affect dental hard tissues. A number of authors have investigated the safety of bleaching procedures,[3–22] unfortunately many of the publications are abstracts,[3–8] which limits the interpretation of their findings. Most have tended to concentrate mainly on the use of CP used in 'at home' bleaching systems.[3–6,9–13,15–18,20–24] The evidence on safety published to date on the whole tends to suggest that bleaching is a relatively safe procedure[3,4,9–13,15,16,21,24] but there are some workers that have voiced concerns on structural changes to enamel that occur as a result of bleaching.[5,17,18–20,22] Relevant to this is sensitivity of teeth in some individuals during bleaching: a problem that has attracted little research attention to explain the phenomenon. Most scanning electron microscopy (SEM) studies of enamel bleached with CP show little or no change in morphology[9] although some report areas of shallow erosions[10] or more substantial changes in enamel structure.[17–19] Surface hardness and wear resistance has also been investigated with disagreements on the overall effects of bleaching. The results range from no effects[4,8,24] to significant decrease in hardness and fracture resistance of enamel.[5,19]

In summary, most studies have concentrated on the use of 10% CP, which is equivalent to only 3.35% HP. The aim of this study in vitro was to investigate the effects of one of the highest concentrations of HP used for in-office power bleaching on the integrity of human enamel and dentine. As with many models in vitro, the application would represent the maximum likely peroxide exposure.

Materials and methods

Production of enamel and dentine samples

Preparation of samples followed the procedure described in detail by West et al.[25] Enamel samples were prepared using un-erupted human third molar teeth removed from patients aged 18–35 years. Sections from the buccal or lingual enamel were embedded in epoxy resin in moulds measuring approximately $8 \times 5 \times 2$ mm. Once set the specimens were polished using 800 grit abrasive paper in a lapping and polishing unit (Kermet International Ltd, Sunbury-on-Thames, UK) to expose an area of enamel. The samples were then placed in a purpose machined stainless steel die with the same dimensions as the specimens and the surface profile of the enamel measured using a profilometer (Planer Products, Sunbury-on-Thames, UK). Only samples with a mean surface profile within $\pm 0.3\mu$m were accepted. These specimens were taped with PVC tape, exposing a 2 mm wide window of enamel.

Dentine taken from the roots of un-erupted third molar teeth were prepared in a similar way to enamel samples except final polishing was achieved using 1200 grit paper. Dentine specimen profiles had to be within the same tolerance as enamel. Again a 2 mm wide zone of dentine was achieved by taping.

Experimental procedure

The study was divided into three parts to determine the effects of HP on both enamel and dentine in terms of erosion, abrasion resistance and hardness. In addition specimens were prepared for SEM to observe any morphological changes to the surface of enamel and dentine specimens.

Erosion of enamel

Forty enamel samples were randomly divided into four groups of 10 and treatments were applied to the 2 mm window of exposed enamel as follows;

Group 1–35% HP application

The bleaching procedure applied to Group 1 has been previously described[26] and is essentially a power bleaching procedure using 35% HP gel (Quick White, DMDS, Lombard House, 12–17, Upper Bridge St, Canterbury, UK) and according to the manufacturers instructions. The gel, which has a pH of 7.0, was applied to the sample and activated using a plasma arc lamp (Apolite, DMDS, Lombard House, 12–17, Upper Bridge St, Canterbury, UK) for 6 s and then left in place for 10 min before being wiped off with damp gauze. The procedure was repeated three times so that the total application time of the gel was 30 min. After the third application, the samples were washed with distilled water, lightly dried and re-measured in the profilometer after removal of the taping. Two readings were taken from each specimen across the window and averaged (arithmetical mean).

Group 2—0.3% citric acid application. Specimens from Group 1 together with another 10 enamel specimens not exposed to HP were attached on optically clear acrylic blocks (ICI Ltd, Macclesfield, Cheshire, UK) using double sided adhesive tape to prevent floating and were then placed in 0.3% solution of CA (Sigma–Aldrich Ltd, Poole, Dorset, UK) adjusted to pH 3.2 with sodium hydroxide. Erosion was for a period of 30 min in 300 ml of CA in a beaker placed in a water bath at 35 °C. The CA was stirred by an overhead propeller stirrer at constant speed (270 rpm). After removal from the CA, all the samples were washed and dried before the taping was removed and re-measured on a profilometer across the 2 mm window as for the bleach specimens.

Group 3—water application under identical conditions as the CA for 30 min followed by re-measurement in a profilometer to provide the negative control to the study.

Group 4—HP application, as for Group 1, followed by brushing for 1 min using standard toothpaste (Colgate-regular, Colgate-Palmolive, Guildford, Surrey, UK).

NB. A control for Group 4 of brushing with toothpaste alone for 1 min was not used as previous in house data showed no measurable changes to enamel specimens.

Abrasion/erosion of dentine

Thirty dentine samples were randomly divided into five groups of six specimens each, and treated as follows, with pre- and post-treatment profiles recorded as for enamel;

Group 1—brushed for 30 min using distilled water (negative control).

Group 2—brushed for 30 min using the 35% HP gel.

Group 3—power bleached for 30 min using 35% HP gel and activated with a plasma arc lamp as described above.

Group 4—Group 3 post-bleaching specimens brushed for 1 min using standard toothpaste.

Group 5—water soak for 30 min followed by brushing using standard toothpaste for a further 1 min as for Group 3.

Group 6—orange juice soak for 30 min followed by brushing using standard toothpaste for a further 1 min (positive control).

Brushing of enamel and dentine specimens was performed by a reciprocal action electric motor driven brushing machine carrying two brush heads (Oral B 35, Gillette Group UK, London, UK) under a 200 g load and at 50 cycles per minute. The travel of the brushes allowed up to four specimens to be placed in the brushing machine reservoir. The well was filled to a depth of 4 mm of the respective liquids. The toothpaste was a 3 g in 10 ml water slurry, to simulate dilution during brushing.[27]

Hardness testing

Ten enamel and 10 dentine specimens prepared as before were glued on to circular acrylic discs designed to fit onto the suction cup of a hardness indenter (Wallace and Co Ltd, Unit 4 St Georges Industrial Estate, Richmond Road, Kingston, Surrey UK).

The loadings of the indenter were 1 g contacting and 300 g indenting and the instrument tip was a Vickers diamond. The depth of indentation was recorded in microns.

The samples were taped as before to expose a 2 mm window for treatment. Baseline hardness measurements were carried out to all the samples using a Wallace indenter within the treatment window. All samples were then power bleached using 35% HP gel as before for 30 min before being re-measured for hardness in the Wallace indenter. A total of five indentations for each specimen, before and after bleaching, were made and arithmetical pre- and post- means calculated.

Scanning electron microscopy

Ten enamel and dentine specimens prepared as before were taped so that only half the samples was exposed to power bleaching using 35% HP gel and light activated using a plasma arc lamp.

Total treatment time was three passes of 10 min each. Taping was then removed and samples washed using distilled water and lightly dried before being prepared for the SEM. Thus specimens were sputter coated with gold and then viewed in the SEM with working conditions of 20 kv frequency and a 10.3 mm working distance. Photomicrographs were obtained from randomly chosen areas on the treated and un-treated sides of specimens at a magnification of $\times 4850$.

Statistical methods

For the enamel erosion experiments, the data showed gross heterogeneity of spread and were analysed using non-parametric statistics. Thus paired Wilcoxon tests were used to analyse for within group changes and un-paired Mann–Whitney tests for between treatment differences. For the dentine abrasion/erosion experiments, because of the same reason of data distribution non-parametric statistics were used for analyses. The distributional form of the hardness data was Gaussian and before and after bleaching was compared using paired t-tests together with the construction of 95% confidence intervals.

Results

Enamel erosion

The pre- and post-treatment results for enamel samples bleached and then eroded using CA (Group 1), eroded only with CA (Group 2) and the water treatment (Group 3) are shown in Table 1. Observationally, it is clearly apparent that bleaching alone, bleaching and brushing and water soaking had no measurable effect on enamel and specimens remained within baseline acceptance values: within and between these groups analyses showed no significant differences ($p \gg 0.05$). CA exposure with and without prior bleaching resulted in statistically significant within group changes ($p \gg 0.002$) but no significant difference between groups ($p \gg 0.05$). The acid groups were highly significantly different from the non-acid groups ($p < 0.001$).

Dentine erosion/abrasion

The baseline (pre-treatment) and post-treatment means and standard deviations for the six treatment groups are shown in Table 2. It is apparent in mean terms that treatments 1–5 had little effect on dentine specimens and post treatment profiles were either minutely increased (Groups 1, 2, 4, 5) or the same (Group 3) as baseline: all specimens remained within baseline acceptance profiles. Within and between treatment group analyses for Groups 1–5 showed no significant differences ($p \gg 0.05$). For Group 6, orange juice followed by toothpaste brushing, produced comparably marked dentine loss, which was significantly increased from baseline ($p < 0.001$) and significantly different from treatments 1–5 ($p < 0.0022$).

Table 1 Pre- and post-treatment groups: enamel specimen profiles in microns (minus sign indicates enamel loss).

Treatment	Mean	Standard deviation
Group 1 Pre-bleach	+0.09	0.07
Group 1 Post-bleaching	+0.07	0.07
Group 1 Post-bleaching and post-citric acid	−4.60	0.74
Group 2 Pre-citric acid	+0.11	0.07
Group 2 Post-citric acid	−4.56	1.13
Group 3 Pre-water	+0.09	0.08
Group 3 Post-water	+0.08	0.06
Group 4 Pre-bleach/brush	+0.07	±0.07
Group 4 Post-bleach/brush	+0.08	±0.06

Enamel and dentine hardness

Table 3 shows the results of hardness testing using a Wallace indenter for both enamel and dentine pre- and post-bleaching with 35% HP gel. The mean and standard deviation of the differences from baseline, together with 95% confidence intervals and paired sample test, for enamel and dentine are shown in Table 4. The mean data clearly demonstrate little if any evidence of a softening effect of bleach on enamel or dentine. Thus baseline hardness values for enamel resulted in an average penetration of 6.52 μm by the Vickers diamond and that for dentine was

Table 2 Dentine loss (microns) following erosion/abrasion treatments.

Post treatment	Baseline mean (SD)	Post mean (SD)
Group 1	−0.06 (0.11)	−0.16 (0.44)
Group 2	−0.06 (0.06)	−0.12 (0.10)
Group 3	−0.04 (0.18)	−0.03 (0.12)
Group 4	−0.03 (0.12)	−0.17 (0.06)
Group 5	−0.03 (0.08)	−0.08 (0.23)
Group 6	−0.14 (0.09)	−2.38 (2.16)

SD, standard deviation.

Table 3 Summary statistics for enamel and dentine hardness pre and post bleaching (microns).

	Baseline mean enamel hardness	Post bleaching mean enamel hardness	Baseline mean dentine hardness	Post bleaching mean dentine hardness
Mean	6.52	6.34	14.43	14.44
Median	6.56	6.32	14.40	14.48
Std. deviation	0.37	0.18	0.47	0.46
Minimum	5.96	6.07	13.81	13.71
Maximum	7.06	6.65	15.34	15.18

14.43 μm. Post bleaching with 35% HP, these values were 6.34 μm and 14.44 μm for the enamel and dentine, respectively. Paired *t*-tests revealed no significant changes in hardness values for enamel and dentine after treatment with HP.

Scanning electron microscopy

Figs. 1 and 2 show a typical SEM view of an enamel and dentine sample respectively from areas treated (top) and untreated (bottom) with HP. There was no evidence of any topographical changes to either enamel or dentine. The smear layer derived from sectioning and polishing the samples is evident on both the treated and un-treated sides of both the enamel and dentine samples. The dentine samples showed no exposed tubules indicating the bleaching treatment applied did not remove the smear layer.

Discussion

The method used measures of erosion, resistance to erosion and abrasion, hardness and visual change as outcome measures. The HP gel is one of the highest concentrations of peroxide used for in-office bleaching and therefore must represent a product with the most likelihood of causing damage to enamel or dentine if these tissues are indeed susceptible to HP. Clearly, it is important to establish first whether such a formulation does bleach teeth whether applied directly to

Table 4 Paired samples test to compare change in hardness of enamel and dentine.

	Paired differences							
				95% Confidence interval of the difference				
	Mean	Std. deviation	Std. error mean	Lower	Upper	*t*	df	Sig. (2-tailed)
Post bleaching mean enamel hardness minus Baseline mean enamel hardness	−0.18	0.45	0.14	−0.50	0.14	0.13	9	0.23
Post bleaching mean dentine hardness minus Baseline mean dentine hardness	0.02	0.31	0.10	−0.21	0.24	0.16	9	0.88

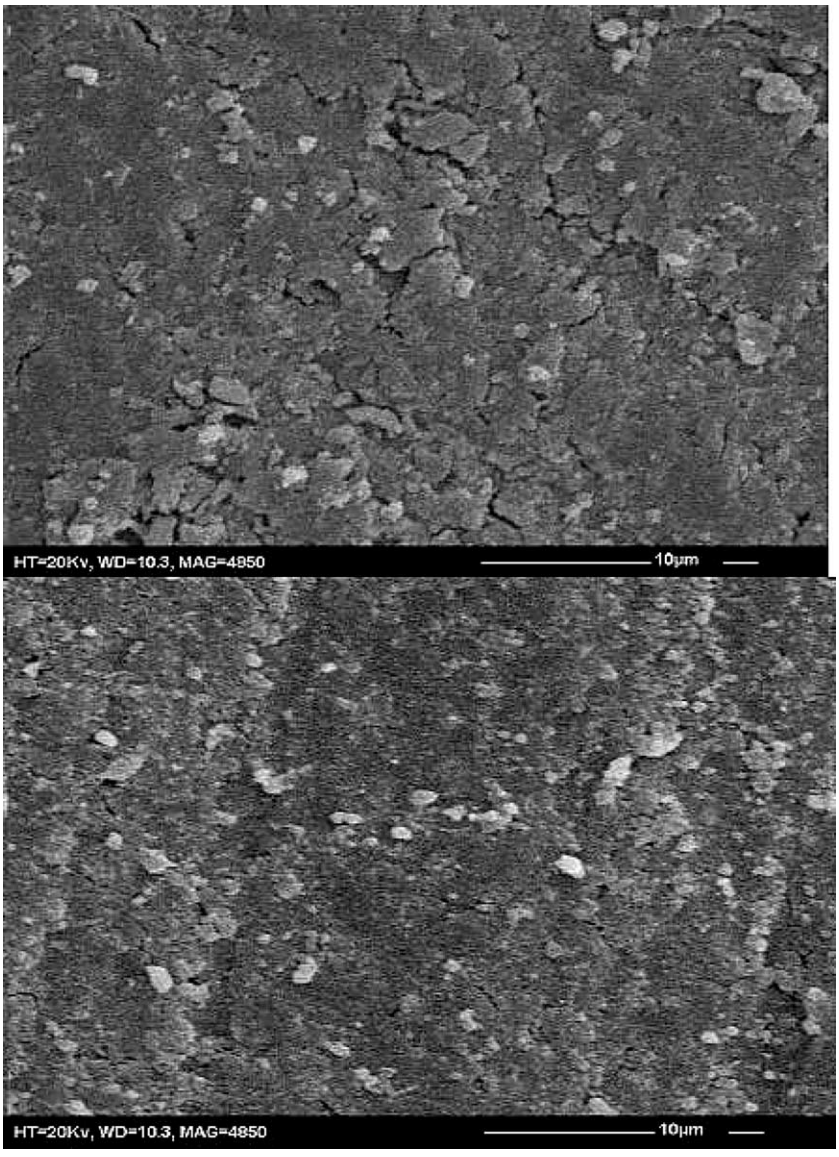

Figure 1 SEM view of a typical enamel sample showing bleached (top) and un-bleached section (bottom).

the enamel or the dentine. This was shown in vitro by the same authors prior to conducting these experiments.[26] Also, the 35% HP produced the greatest and most rapid bleaching compared to lower concentrations.[28] Un-erupted human third molars from a relatively narrow age range of subjects were used in an attempt to reduce biological variation to erosion in specimens, which from other studies was still probably quite large.[25,29] The potential disadvantage is that these teeth would probably be hypo-mineralised compared to erupted teeth. This apparent limitation may be seen as an advantage to this model, since where bleach harmful it would be more readily seen in such specimens: a conclusion drawn for the same model used to study low erosive soft drinks.[29] In the event, the model revealed overall no evidence of detrimental changes to the dental hard tissues. It would seem pertinent to discuss each experiment under their respective headings.

Erosion of enamel

Bleaching of enamel samples with an in office power bleaching product of 35% HP actuated by a plasma arc lamp produced no evidence of erosion of enamel both by comparison of within treatment measurements (Group 1 pre- and post-bleaching) and between bleaching and control treatments (Group 1 vs. Group 3). Moreover, pre-bleaching enamel samples did not affect the lesion that resulted following erosion for 30 min with 0.3% CA. The 30-min exposure to CA was chosen as this approximates to the total

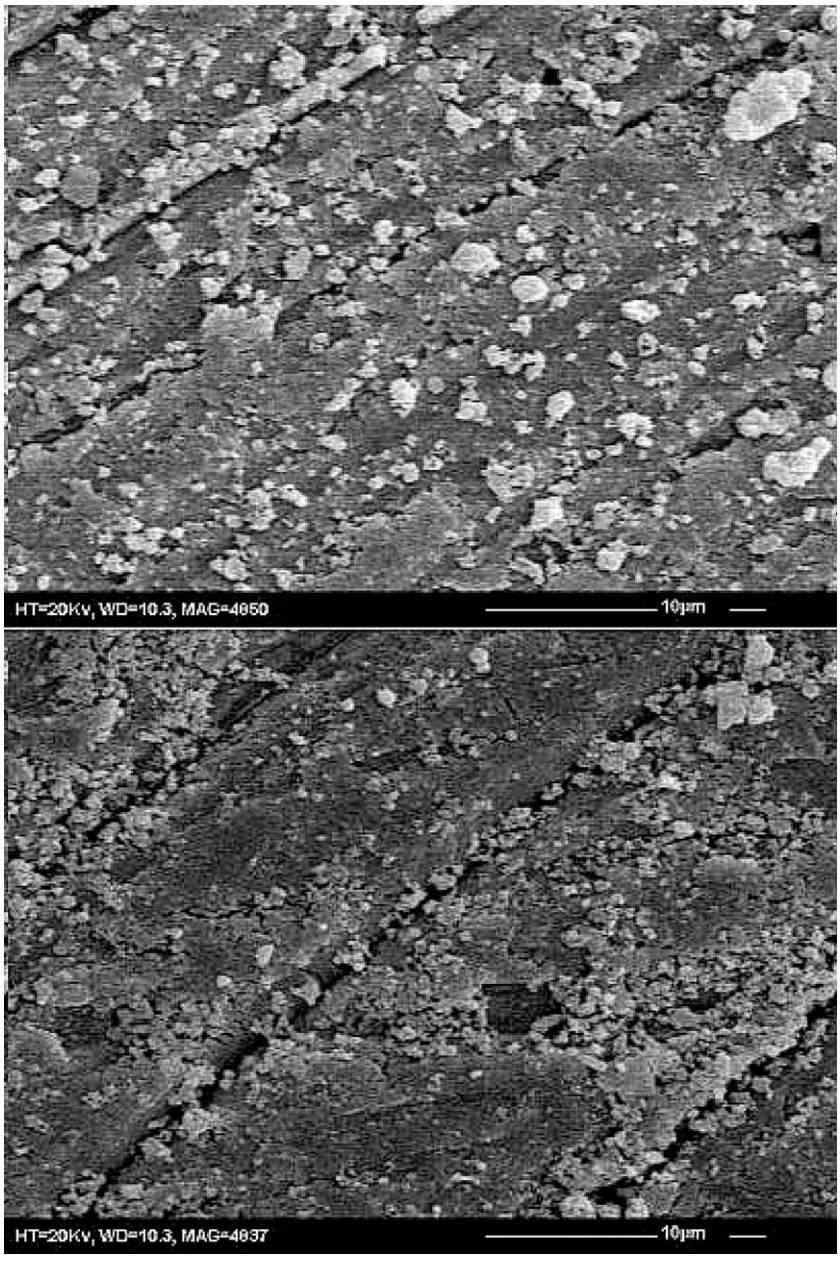

Figure 2 SEM view of a typical dentine sample showing bleached (top) and un bleached sections (bottom). Note absence of exposed tubuli and presence of the smear layer and striations across sample made by production and polishing of the sample.

time in 1 day that teeth are commonly contacted by acidic soft drinks. A similar 4 by 10 min exposure time has been used in studies in situ.[25,29] Clearly the erosion in vitro would be greater than in situ and has been proven in in situ/ in vitro studies.[29-31] This single, phase experiment was chosen to simulate the possible intake of soft drinks following a professional bleaching visit. It was assumed that if HP did demineralise the enamel surface the effects of acid intake would only occur on the same day as remineralisation would likely reharden the enamel by the next day.

This would suggest that even using HP at this very high level, there is neither actual loss of enamel or any effect on its surface structure, which might predispose to more marked erosion. Various workers have investigated the structural changes that can occur following bleaching of enamel and dentine.[3-22] However, the majority of the work concentrated on the use of various concentrations of CP used predominantly in home or night guard vital bleaching techniques and not on HP directly. Although, CP does breakdown to produce HP and urea, the resultant concentration from a 10% CP is

only 3.35% HP (HP). The present study used a 35% HP gel, which is only used for in-surgery power bleaching techniques with full soft tissue and gingival protection due to its irritant nature. Many workers who used weaker concentrations of 10% CP (3.35% HP) at neutral or alkaline pH reported no deleterious effects on enamel.[9–14] Other studies however, that also used 10% CP but in acidic products did, perhaps not surprisingly, report surface erosions of enamel.[3,17] Concerns regarding bleaching solutions has mainly centred around lower pH based materials and followed reports that the demineralisation process of enamel begins when the pH falls below the 'critical' pH of 5.5.[32,33] Haywood[10] points out that the absence of perceptible damage to enamel appears to be related to the fact that the pH of the bleaching solution rises rapidly upon exposure to oral fluids. Thus CP breaks down to HP and urea, which then further breaks down to ammonia and carbon dioxide.

The present formulation had a pH of 7.0, which is well above the critical pH for enamel dissolution, and supports our conclusion that it is the pH of formulations and not the bleach itself, which produce the changes.

Abrasion/erosion of dentine

All groups receiving brushing (Groups 1, 2, 4, 5) showed a minute change in profile, which although not significant suggests, as would be expected, that brushing with or without toothpaste can abrade dentine. Importantly however, Group 2 (brushing with HP) or Group 4 (brushing with toothpaste after bleaching) were not significantly different from their respective controls (Groups 1 and 5). Thus, bleaching did not render dentine more susceptible to toothpaste abrasion and brushing with 35% HP was no different to brushing with water. Brushing with HP clearly is not normal clinical practice but it does represent a further enhanced bleach challenge to dentine. Also if regulations change it is not out with the realms of possibility that 'brush on' products containing moderate to high levels of HP could become available.

Pre-bleaching followed by brushing with a standard toothpaste also showed mean values for erosion lesions well within baseline values and similar to water soaking and brushing in standard toothpaste (negative control). The only significant erosion, for dentine samples, followed exposure to orange juice followed by brushing. The result was expected from other studies in vitro and in situ for effects of soft drinks on dental tissues.[25,29–31] The 30 min soak was used for the same reasons alluded to for CA and enamel.[25] Again the challenge in vitro would of course be greater than in vivo or in situ and is a limitation of all such models in vitro.

Kalili et al.[6] reported tooth brush abrasion was more significant in the presence of bleaching agents while in a different study by Scherer et al.[14] who used the home bleaching agent (CP) to brush with, there was no significant effect found on enamel. The findings of this study are contrary to those of Hunsaker et al.[4] who reported the removal of the dentine smear layer following bleaching. Jay[34] also reported that over the counter bleaching products caused erosion of the enamel and the toothpaste provided within the kit was abrasive to tooth surfaces. Both products used in these studies[4,34] however, had low pH values and given that the critical pH for dentine dissolution is higher than enamel this may explain their findings. Although, the critical pH for dentine is higher than for enamel (6.0–6.5) a pH of 7.0 is again above this critical level and demineralisation should not be expected unless another mechanism for structural damage by bleach was involved.

Hardness

The hardness of both enamel and dentine were not affected by bleaching with 35% HP in this study with baseline and post bleaching readings very similar and ranging between 23.5–27.8 and 54–60.4 units for enamel and dentine, respectively.

The effect of bleaching on enamel and dentine hardness is probably the most widely researched area of bleaching, with the majority of workers concluding that hardness is not affected.[7,8,16,20,24] These workers have used both CP and HP in various concentrations and forms in their studies. Shannon et al.[17] performed an in situ study to correlate the effects of saliva and three different commercially available 10% CP agents on enamel slabs embedded in mandibular acrylic appliances and found no statistically significant changes in enamel micro hardness. In addition to different concentrations of CP, polyethylene strips, impregnated with 5.3 and 6.5% HP, have been investigated by Kozak et al.[7] and White et al.[8]. Both workers support the findings of the present study in that no statistically significant difference in hardness of enamel and dentine was found as a result of bleaching.

Seghi and Denry[15] reported on a bleaching/re-mineralisation cycle, which showed that 10% CP applied for 12 h significantly decreased enamel hardness and that the application of fluoride improved the re-mineralisation of the enamel.

Similarly Lewinstein and Hirschfield[35] examined the effect of 30% HP at various temperatures and found that a significant reduction in micro hardness of enamel and dentine occurred after 15 min, concluding that the solubility and possibly permeability increased with prolonged bleaching. In reviewing this study, Kelleher and Roe[36] flawed its findings because it used HP at pH 3, which would in effect erode the enamel surface and produce surface softening.

SEM

Generally it is thought that bleaching does not etch the enamel surface or alter its surface topography as reported by various workers.[3,7,8,10,24] Haywood et al.[11] who examined extracted premolars exposed to a commercially available 10% CP gel for 245 h found no effect on surface morphology on tooth surface replicas viewed under SEM. Similarly, Covington et al.[3] examined enamel and dentine surfaces by electron spectroscopy and found no surface composition changes. The effect of HP and CP on tooth surface changes was investigated by White et al.[8] using confocal laser scanning microscopy (CLSM) in reflection mode, which provides a useful means for non-destructive microscopic examination of ultra structural characteristics of enamel and dentine. The effects of in vitro bleaching on enamel and dentine were compared at the enamel surface, the enamel dentine junction (EDJ) and at dentine 5 μm subsurface to the polished surface under an oil immersion objective. In addition, environmental variable pressure scanning electron microscope (VP-SEM) was used to provide a second investigation device. Whitened teeth exposed to 30 h of bleaching revealed no significant micro-morphological changes associated with the whitening process in subsurface enamel, EDJ and dentine areas using both the CLSM and the VP-SEM.

The present study made similar observations to White et al.[8] in that the bleaching process did not cause the removal of the smear layer to expose tubules in dentine.

A few researchers have reported contrary findings to the present study in that the enamel surface topography and underlying structure was greatly affected by bleaching.[2,19,23] Bitter[19] reported on a study, which after 14 days exposure of teeth to 10% CP produced alteration of the enamel surface with exposure of enamel prisms. Further, a 21–90 day post-exposure SEM evaluation demonstrated alteration of enamel indicating exposure of the enamel prismatic layer, frequently to the depth of the enamel rods and possibly dentine. The study fails to mention the product used and may well have had the changes due to a very low pH that is used in some products especially the OTC agents, which merely etch the tooth.

Conclusion

The present study in vitro using one of the highest concentrations of HP for tooth bleaching and under maximum likely peroxide exposure failed to show any evidence of deleterious effects on enamel or dentine. It must be assumed that studies which reported adverse effects on enamel and or dentine of bleaches reflect not the bleach itself but the pH of the formulation used.

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