IN BRIEF

- Concentration of carbamide peroxide does not appear to effect bleaching efficacy.
- Bleached teeth, *in vitro*, are not more susceptible to acid erosion.
- Bleached teeth, *in vitro*, are not more susceptible to caries.
- These results should prove encouraging to those individuals seeking, and providing bleaching treatments.

The effect of bleaching on enamel susceptibility to acid erosion and demineralisation

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Introduction The purpose of this *in vitro* study was to determine if enamel that had been bleached by carbamide (urea) peroxide gel (CPG) was at increased risk of either acid erosion or demineralisation (early caries) than un-bleached enamel.

Methods Human incisors were employed. The samples were randomly assigned to one of 4 groups; a) 10% CPG, b) 16% CPG, c) 22% CPG and d) 10% CPG with xylitol, fluoride and potassium. Each specimen was moistened with saliva and the appropriate formulation placed for 2 hours for a total of 40 hours of exposure. In order to ensure that bleaching had taken place, tooth shades were monitored using the Shade-Eye device. Following the bleaching process, one half of the specimen was subjected to an erosive challenge, the other to a demineralisation system with one half of each sub-sample retained as a non-bleached control. Samples were assessed longitudinally with quantitative light-induced fluorescence (QLF) and at the conclusion of the study with transverse micro-radiography (TMR)

Results Erosion was detected in all samples (ΔQ 126±23.4), in both bleached and non-bleached areas. There was no statistical difference between the bleached and non-bleached areas either within the treatment groups or between them. Caries-like lesions were detected on all samples; TMR revealed sub-surface lesions on all teeth and QLF data supported this (ΔQ 89±18.9). Following statistical analysis there were no differences detected between the bleached and non-bleached areas, nor between the different concentrations of the bleaching solution.

Conclusion These results suggest that tooth bleaching with carbamide (urea) peroxide (using commercially available concentrations) does not increase the susceptibility of enamel to acid erosion or caries.

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INTRODUCTION

With the decrease in caries among Western populations there has been an increased interest in the provision of aesthetic dentistry. One of the more commonly requested procedures provided by general dental practitioners is that of tooth bleaching, normally using hydrogen peroxide gels.[1] While in the UK there is an ongoing debate as to the legality of the procedure, a cursory glance at the dental and general press reveals that the service is widely offered and extensively marketed. The process of vital tooth bleaching is thought to operate on two main levels; the first in the removal of extrinsic stains, such as tea, coffee and tobacco, while the second is the reduction of intrinsic stains within dentine.[2]

The research in this area has been predominantly concerned with the effectiveness of various hydrogen peroxide delivery solutions, eg carbamide (urea) peroxide, gels, concentration effects and the effect of such systems on a variety of restorative materials.[1,3-5] A number of studies have examined the influence of tooth bleaching on enamel micro-hardness and fluoride uptake and have reported that while there are some numerical differences, there is no statistical difference in bleached or un-bleached samples.[6-8] SEM studies have shown that tooth bleaching causes exaggerated enamel prism peripheries and some (described as mild to moderate) prism core loss.[9] Few studies have examined the impact of tooth whitening on the susceptibility of enamel to either a carious or erosive challenge, with most concentrating on brand specific products to which anti-caries agents have been added.[9]

The purpose of this study was to examine the effect of a variety of concentrations of carbamide peroxide gels on enamel susceptibility to erosion and demineralisation and to investigate the effect of the addition of xylitol, fluoride and potassium to a CP gel and its whitening efficacy. The study was designed to ensure that the most authentic protocol for the *in vivo* situation was followed.

METHODS AND MATERIALS

A total of 24 human incisors were selected based upon their lack of caries, erosion, restoration, cracks or other enamel anomalies. Each was gently pumiced and polished with wet-and-dry paper. An initial shade reading was taken using the Shade-Eye (Shofu, Japan) colorimeter and the Vita shade noted. Transparent, non-fluorescing, acid-resistant varnish was then applied to the surface to either the gingival or incisal half (MaxFactor, Procter &

Gamble, UK). This was randomly assigned ensuring that, in each group of six teeth, 50% had their gingival half covered.

The teeth, in groups of six, were then allocated, randomly, to one of four treatment groups:

A 10% carbamide peroxide gel (Southern Dental Industries GmbH)
B 16% carbamide peroxide gel (Southern Dental Industries GmbH)
C 22% carbamide peroxide gel (Southern Dental Industries GmbH)
D 10% carbamide peroxide gel with Xylitol, fluoride and potassium (Biocosmetics Laboratories, Spain)

For each treatment group the appropriate gel was placed over the saliva-moistened tooth surface for a total of 2 hours, and then thoroughly removed using a water rinse. The gel was applied to cover the entire tooth surface at an even thickness, approximately 5 mm. The saliva moisture is required to ensure activation of the gel product and permit conversion of the carbamide peroxide to hydrogen peroxide. Saliva for this purpose was collected from a single individual following chewing of a paraffin wax block and was refrigerated until used. Each tooth was then mounted into an automatic toothbrushing machine and gently brushed (105 g of weight applied to brush head) for two minutes (Aquafresh brush, SmithKline Beecham, UK). Following this, the teeth were removed, the varnish inspected for any damage, a Vita shade taken using the Shade-Eye device and the process repeated. The total exposure time to the bleaching agents was 40 hours within 20 cycles. The Vita shade values, for the purposes of this study, was arranged into the following order, with each tab allocated a numerical value from 1 to 16.

B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4

The pH of each of the bleaching products was also assessed using a calibrated (buffer, pH 4) digital pH meter (Orion Instruments, UK). Following the conclusion of the bleaching cycle the nail varnish was removed using acetone and the teeth gently cleaned using pumice. The teeth were then sectioned through the midline producing a mesial and distal half. Each of the new samples was coded to ensure that the pairs could be compared at a later date. Varnish was re-applied leaving a small window of bleached and unbleached enamel. A small indicating mark was placed on the buccal surface indicating the division between the two surfaces.

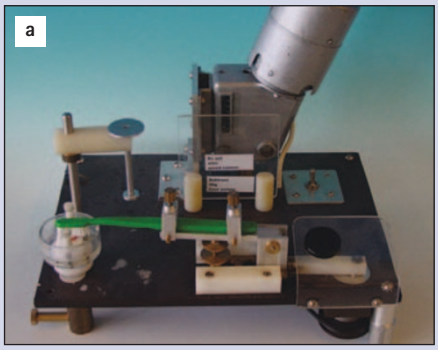
One half of the tooth was assigned to the erosion study, the other half to the demineralisation study, with a random allocation being employed to ensure that an equal number of mesial and distal halves were in each group (stratified randomisation). See Figure 1 for images from the bleaching methods, and Figure 2 for an example of the output from the ShadeEye colorimeter. See Figure 3 for an example of the tooth preparation prior to either the erosive or demineralising challenge.

Erosive challenge

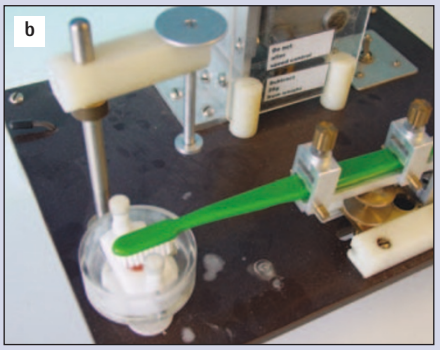
Teeth were submerged in gently agitated citric acid (0.1% citric acid (pH 2.74) at 37°C. The teeth were removed at hourly intervals, air-dried, and QLF images were taken in ASA Class I darkroom conditions and then returned to the solution. Samples were subjected to a total of 14 hours of erosive challenge. All QLF images were analysed by a blinded examiner using a set of predetermined rules. Data reported were ΔF (% fluorescence loss of eroded compared to sound enamel) and ΔQ (Δ F x lesion area measured in mm^2). Separate values were entered into SPSS for both the bleached and non-bleached areas.

Following the conclusion of the erosive study, each tooth was sectioned initially along the demarcation between bleached and non-bleached enamel. Subsequently two enamel slabs were

Fig. 1 Materials and methods from the bleaching portion of the study



View of the automatic tooth-brusher.



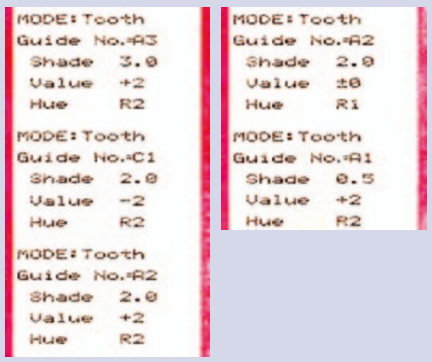
Picture of a tooth specimen in the tooth-brusher.

Example of sample undergoing bleaching, in this case the incisal half.

The Shade-Eye device.

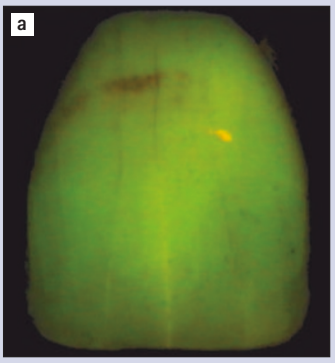
Non-branded Carbamide Peroxide whitening gels available to UK dentists.

Biocosmetics Laboratories product with additional xylitol, potassium and fluoride added.

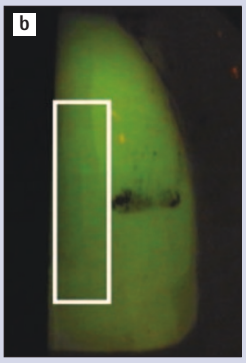


These results were obtained from a single tooth undergoing bleaching with the 22% carbamide peroxide gel. The tooth began at shade A3, value of 9, and ended at A1, value of 2, thus underwent a total of 7 shade changes.

Fig. 2 Example of output from the Shade-Eye system. The Shade-Eye is a colorimeter with an integral flash that can be used to determine Vita shades or porcelain recipes from either natural or porcelain teeth.



Full tooth sample undergoing bleaching



Sectioned half of tooth, boxed area indicates the exposed enamel and the mark to the right hand side indicates the division between bleached and non-bleached enamel.

Fig. 3 Example of sample preparation prior to demineralisation or erosion

sectioned (approximately 250 µm) using a water-cooled diamond saw (Well, Walter Ebner, Switzerland) from each of the sections giving a total of four per eroded sample. These were mounted onto custom brass anvils with varnish and polished using a diamond disc to give planoparallel specimens of 100 µm thickness. The fine sections were then mounted onto a microradiographic plate-holder with an aluminium stepwedge (25 µm steps). Kodak high-resolution plates (type 1A) were employed with a 15 minute exposure using a Cu(Kα) X-ray source (Philips B.V., The Netherlands) operating at 25 kV and 10 mA at a focus-specimen distance of 30 cm. Plates were developed using Kodak brand materials following manufacturers' instructions.

Following developing, the microradiographs were analysed using a Leica DMRB microscope (Leica, Germany) with image capture via a CCD video camera (Sony, Japan) connected to a PC. TMRW v.1.22 (Inspektor Research Systems BV, The Netherlands) was used to determine the integrated mineral loss (vol%µm), lesion depth (µm) and width (µm) using a two-stage analysis procedure (ΔZ). Values of ΔZ for bleached and non-bleached samples were recorded and entered in SPSS.

Demineralisation challenge

The teeth were submerged in a gently agitated demineralising solution (2.2 m*M* KH_2PO_4, 50 m*M* acetic acid, 2.2 m*M* of 1 *M* $CaCl_2$, 0.5 ppm fluoride at pH 4.5) at 37ºC. At 24 hourly intervals the teeth were removed, allowed to air-dry and then QLF images were taken as previously described. Following a total of 10 days of demineralisation each tooth was sectioned as per the erosive samples and subjected to transverse micro-radiography (TMR) examination following a standard analysis system. Values of ΔZ for bleached and non-bleached samples were recorded and entered in SPSS.

Statistical tests

Analysis of variance was conducted between the bleached and non-bleached samples when all treatments were summed, and also between each of the treatment modalities. ANOVA was also employed to detect any differences between each of the time intervals examined during the study and of the Vita shade data provided by the Shade-Eye device. Statistical differences were further examined using post-hoc *t*-tests and α was set at 95%.

RESULTS

The results from the pH analysis of each of the bleaching products is shown in Table 1. One of the criticisms of previous bleaching studies is that there is often no evidence presented that bleaching actually occurred. Figure 4 illustrates the data obtained from the Shade-Eye device following 20 and 40 hours of bleaching exposure.

Table 1 Results of the pH testing on each bleaching product (3 measurements per sample)

Sample	% hydrogen peroxide	pH	SD
10% Carbamide peroxide	3.651	6.52	0.12
16% Carbamide peroxide	5.842	6.51	0.21
22% Carbamide peroxide	8.033	6.50	0.11
10% Carbamide peroxide with xylitol	3.651	6.97	0.32

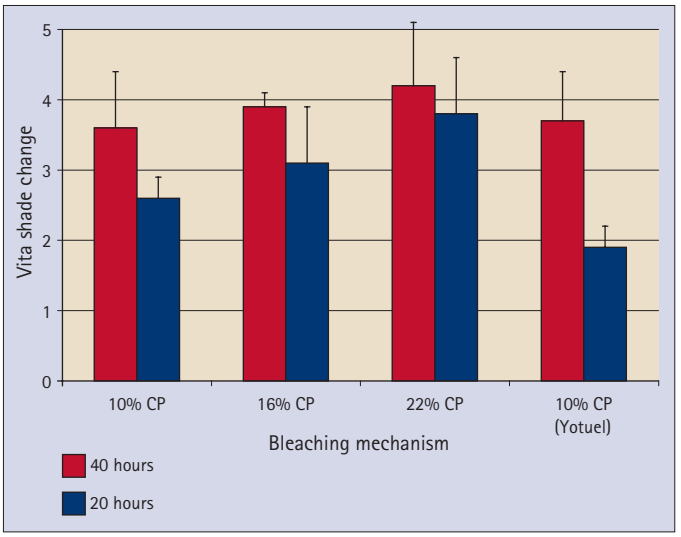


Fig. 4 Results from the Shade-Eye assessment of bleaching

Erosion data

QLF detected erosion in all of the samples analysed represented by a mean ΔF of 12.15 (±1.03) and a ΔQ of 153.2 (±23.2) following 14 hours of exposure on bleached enamel and ΔF of 11.7 (±1.4) and a ΔQ of 183.2 (±19.4) on non-bleached samples. The device detected erosion on all of the samples by 2 hours of exposure and longitudinally monitored the increase over time. Figure 5 demonstrates the data obtained from the eroded samples. In the 16% CPG group, two of the enamel slabs were lost during the grinding process and three from the 10% carbamide peroxide with xylitol group. The mean mineral loss (Vol%µm, ΔZ) from the bleached samples was 5,452.75 (±297.3) and from the non-bleached samples 5,366 (±467.2) statistical analysis both within

Fig. 5 (a)

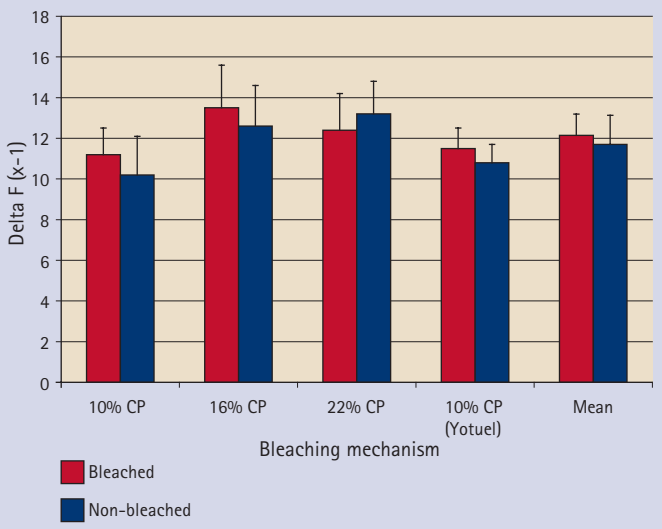


Fig. 5 (b)

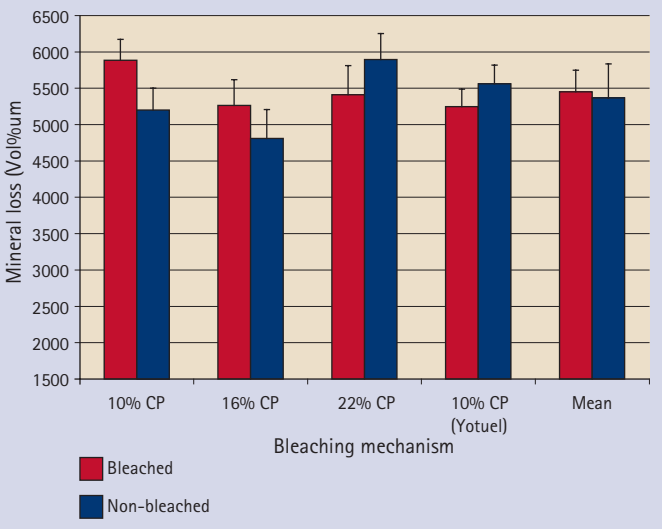


Fig. 5 QLF (a) and TMR (b) data from the erosive challenge

Fig. 6 (a)

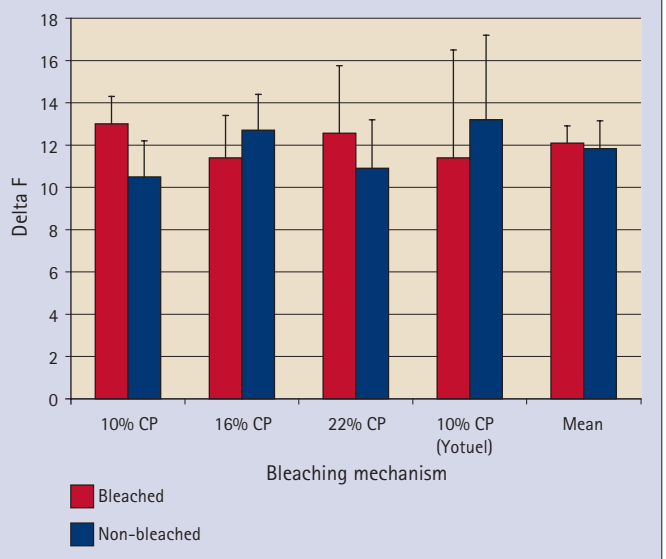


Fig. 6 (b)

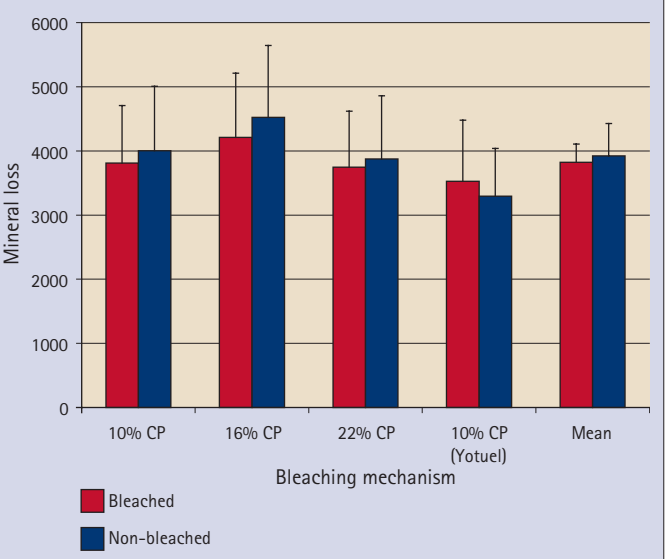


Fig. 6 QLF (a) and TMR (b) data from the demineralisation challenge

and between the groups failed to detect any significant differences either from the QLF data over each time interval or from the TMR data at the conclusion of the study.

Demineralisation data

QLF detected demineralisation in all samples analysed, represented by a mean ΔF of 12.09 (±2.4) and a ΔQ of 78.3 (±33.4) following 10 days of demineralisation on bleached enamel and ΔF of 11.8 (±2.5) and a ΔQ of 112.4 (±28.6) on non-bleached samples. QLF detected demineralisation on all samples by 6 days' exposure and longitudinally monitored the increase over time. A linear relationship of demineralisation with time was observed. For TMR data ΔZ was 3,823 (±285.2) for bleached and ΔZ was 3,923 (±504.7) for unbleached sites. Statistical analysis both within and between the groups failed to detect any significant differences when using either data sourced from QLF (at any time point) or TMR (at the single data collection point). See Figure 6. TMR imaging demonstrated the presence of early, subsurface lesions for each tooth.

DISCUSSION

Bleaching efficacy

A criticism that has been made of previous studies investigating tooth whitening has been the lack of data demonstrating that tooth bleaching, as measured by colour change, actually took place.[3] Without these data, it is difficult to justify any conclusions regarding the effects, positive or negative, of tooth bleaching as one cannot be sure that it occurred. Recently, models have been developed that examine tooth whitening using colorimeters, spectrophotometers and QLF to detect colour changes. Such changes can be reported as the ubiquitous Vita values,[10] or, more properly, using the international standard, Commission Internationale de l'Eclairage color spaces (CIELAB), where L* a* b* values and their differences, ΔE, can be described.[11]

This trial determined that each of the peroxide containing gels, (carbamide peroxide contains approximately 34% hydrogen peroxide), caused tooth whitening and that, after 40 hours of bulk exposure, there were no significant differences between them, ie one gel was not more effective than another. The addition of xylitol, fluoride and potassium did not decrease the bleaching ability of the proprietary gel. Matis and co-workers[12] described an experiment in which individuals were provided with varying levels of carbamide peroxide gels (10, 15 and 20%) for at-home bleaching of tetracycline affected teeth. The study obtained similar results to the current work, while the bleaching effect was obtained more quickly with the higher concentrations, the end result was similar for each. Indeed, they also reported a reduced incidence of side-effects from the lower concentration gels, and this formed the basis of their recommendation that the 10% carbamide peroxide gel should be employed.[12]

A different result was obtained by Gerlach *et al*[13] who compared a whitening strip containing 6% hydrogen peroxide to a tray system containing 5% carbamide peroxide (approximately 2% hydrogen peroxide). They found that both systems, when assessed using L*a*b*, achieved significant shade changes, but that the 6% hydrogen peroxide was statistically superior to the 5% carbamide peroxide. However, the delivery systems employed here are quite different, ie a closely opposed cellulose strip compared to a customised tray, and therefore it could be the delivery method, rather than the concentration that causes the difference.[13] Further studies on whitening strips have, however, shown that when the concentration of hydrogen peroxide is increased from 5.3% to 6.5% a significantly improved whitening effect is seen.[14] The researchers at Procter & Gamble also investigated the effect of tooth brushing on the efficacy of the bleaching treatment and found a significant improvement if the teeth were pre-brushed prior to the application of the whitening strip.[14]

The current study employed 'bulk' bleaching applications, ie the gel was placed upon the tooth surface in bulk and did not undergo dilution or degradation from factors within the oral environment. Even using close fitting custom trays, studies have found that the active agent can be reduced by as much as 54% within two hours.[15-17] It is likely that the bleaching mechanism employed within the current work represents extensive bleaching, perhaps obtaining optimum results for each tooth irrespective of the preparation. We can conclude that, in short-term use, higher concentrations of active bleaching agent will cause more rapid colour change, but following long-term use this difference is not significant. Given that there is compelling evidence to suggest that high concentrations of either hydrogen peroxide or carbamide peroxide can lead to adverse effects, such as gingival irritation or tooth sensitivity, it is perhaps wise to prescribe lower concentrations of bleaching agents over longer time periods.[18]

The addition of additional ingredients to the proprietary product assessed in this study, namely Xylitol and fluoride (for anti-caries effect) and potassium (to reduce sensitivity) appear to have had no adverse effect on bleaching efficacy. While this study has not tested the efficacy of these additions when added to a CP gel, the data suggest that their addition has no impact on the product's ability to alter tooth shade.

Erosion

The results suggest that, after extensive bleaching with carbamide peroxide gels, *in vitro*, there is no increased risk of enamel dissolution by citric acid, ie no increased susceptibility to erosion. This experiment is in agreement with previous works, although they each differ from the current study in important ways. Shannon *et al*[19] described an experiment in which enamel slabs were subjected to three different 10% carbamide peroxide (CP) solutions over a protracted period (15 hours per day, for either 2 or 4 weeks) after which microhardness values were measured. They determined that there was no difference in microhardness between any of the control or CP enamel slabs. Using CP at 10% and hydrogen peroxide (HP) at 3%, with human enamel slabs, Lopes *et al*[8] found that there was no effect on microhardness following bleaching with the CP formulation, but HP gel caused a significant decrease in surface hardness. In neither of these studies was evidence shown that the teeth had actually undergone bleaching.

Microhardness was again the measure used by Burgmaier[6] who studied the effect of 10% CP on bovine enamel. Again, there were no differences in microhardness between bleached and non-bleached samples either before, or after submersion in 1% citric acid. Significant differences between all samples after 1% citric acid challenge were noted. Again, no evidence was provided within this report that bleaching, or activation of the CP, took place. Similar results were reported by Potocnik and colleagues,[20] again using 10% CP. One study, by researchers at Procter and Gamble, used a colorimeter to prove bleaching effects and then undertook surface hardness measurements.[21] They found that using 10% and 20% CP, or 5.3% and 6.5% HP caused no detectable decrease in microhardness measures. Again, this study did not relate directly to erosion, but the results are confirmatory of the findings of the current work.

Only the study by Burgmaier *et al*[6] claimed to measure the effect of bleaching on erosion, the other studies claimed, correctly, only to investigate surface softening. The use of microhardness to quantify erosion is problematic, measuring only the softened portion of the lesion. The method is unable to quantify the crater loss. Studies using microhardness, reporting either Vickers or Knoop values, can be supplemented with profilometry to quantify the lost tissue from the eroded crater. In this study, the use of TMR enables the measurement of both crater and demineralised areas combined with the proof of bleaching, lends more weight to the conclusions drawn, namely that bleaching with commercially available concentrations of CP does not lead to greater enamel erosion following a citric acid challenge.

In this study all the gels employed had a neutral pH, and therefore the erosive results obtained are confirmatory in nature. However, not all whitening gels are pH neutral and it is important to note that the erosive findings in this study are therefore limited to those gels with such a pH. Prescribing dentists should read the product information and safety data sheets carefully before recommending individual products.

Demineralisation

The data obtained from the current work suggest that, following bleaching with CP, teeth are not at a higher risk of demineralisation than teeth which have not undergone bleaching.[20] No other study was found within the literature that assessed the demineralisation effects of bleached enamel. A study undertaken by Amaechi *et al*[22] examined the effects of differing methods of enamel sterilisation prior to employment *in situ* models. One of the methods was application of sodium hypochlorite. It was noted that application of this bleaching agent did not significantly affect the demineralisation severity compared to controls of distilled water. This is the closest study to the current work. It is important to note that, in the current work, the TMR method is a 'slice' system; ie sections from the lesion are taken. Carious lesions are rarely homogenous and therefore there can be a selection-bias. However, the QLF system analyses the lesion in its entirety and therefore the conclusions drawn are valid.

Due to the lack of either confirmatory or contrary evidence, the conclusion of this study stands somewhat isolated. It will therefore be necessary to repeat this experiment, possibly employing an *in situ* model for further validation.

CONCLUSION

Within an *in vitro* model, the application of hydrogen peroxide, via carbamide peroxide, to enamel to obtain bleaching, does not increase the susceptibility of the tissue to either acid erosion or caries-like demineralisation. The concentration of peroxide does not appear to affect bleaching efficacy following long-term use, given this it would appear sensible to recommend lower concentration gels, as individuals using such products report lower incidences of post-bleaching sensitivity. The addition of xylitol, fluoride and potassium to a CP gel does not have an adverse effect bleaching efficacy when compared to standard CP gels.

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