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Hydrogen Peroxide Tooth Whitening Agent Effect on the Nanomechanical Properties of Enamel

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ABSTRACT

A pleasing smile can be considered part of an attractive appearance and having "whiter" teeth is perceived as an integral part of achieving this. Tooth whitening is a conservative, relatively quick and inexpensive option that uses bleaching agents such as hydrogen peroxide at various concentrations that have been marketed for in-office and at home use. The aim of this study was to assess the effects of 30% hydrogen peroxide on the nanomechanical properties and the protein content of enamel. The effect of bleaching with 30% hydrogen peroxide for 30 minutes and 10% Carbamide peroxide for 8 hours on the mechanical properties of enamel was investigated. This was determined by measuring the hardness and the elastic modulus of twelve sound enamel samples using nanoindentation testing with a Brecovich tip at 250 mN and 25mN force, before and after bleaching. The microhardness tests showed no statistically significant difference in the mean values of the elastic modulus and the hardness of enamel samples following exposure to the bleaching agents. The results obtained from this study showed that bleaching sound enamel with a 30% hydrogen peroxide for 30 minutes or 10% Carbamide peroxide for 8 hours does not affect the microhardness of enamel.

Key words: Tooth Whitening – Hydrogen Peroxide – Microhardness – Enamel – Mechanical properties.

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INTRODUCTION

Bleaching materials may act as either oxidizing or reducing agents. The most commonly used agents are solutions of hydrogen peroxide or carbamide peroxide in different strengths, for external bleach [1]. There are three fundamentally different bleaching approaches. These are: in-office bleaching using 25-35% hydrogen peroxide, dentist-supervised nightguard bleaching using 10% (range 5-20%) carbamide peroxide (which contains only 3% hydrogen peroxide), and over-the-counter treatments containing up to 6% hydrogen peroxide [2]. Preparations that provide lower ranges of hydrogen peroxide concentration, such as 3-7% hydrogen peroxide and 10-20% carbamide peroxide, are widely available for in-home dental bleaching [3]. Hardness (H) is broadly defined as the resistance to permanent surface indentation or penetration. It is a metric of the resistance to plastic deformation and is measured as the force required per unit area of indentation [4]. Elastic modulus (E) describes the relative stiffness or rigidity of a material [5]. The E of enamel is a function of the contact area and the effective volume of the contact stress field applied, regardless of the type of indenter tip used [6]. The effects of bleaching materials on enamel are poorly understood. There are concerns about possible side effects, including structural changes on the hard and soft dental tissues during and after bleaching procedures. Despite the aesthetic advantages of dental whitening, high concentrations of hydrogen peroxide (~25%) may have a deleterious effect on the structure of mineralized tooth tissues [7]. The literature presents conflicting views about the short-and long-term consequences of bleaching dental hard tissues, particularly enamel. with 10% carbamide peroxide solutions, including over-bleaching five-fold in excess of clinical recommendations [8, 9, 10]. In contrast, an *in vitro* study found that bleaching

for 8 hours with a 10% carbamide peroxide gel reduced the microhardness of enamel [11]. It was reported in another study that high concentrations of carbamide peroxide (35 and 37%) can promote alterations of the enamel surface. It was also noted that the resultant enamel surface roughness and stain susceptibility was bleaching concentration dependent [12]. Therefore, the aim of this study was to assess the effects of bleaching agents on the nanomechanical properties of enamel.

MATERIALS AND METHODS

The study sample consisted of four permanent molars and two permanent premolars that were extracted because they were either impacted, or required as part of an orthodontic treatment plan. The selected teeth were caries-free and had no defect or crack on the enamel layer. Teeth with a history of bleaching treatment were excluded from the study. After collection of teeth, attached soft tissues were removed using scissors. The teeth were then washed and cleaned under running water with a soft toothbrush. Specimens were placed in containers encoded with the patient's identification number. Sterile Hank's balanced saline solution (HBSS, Invitrogen™), as recommended by Habelitz *et al*., 2002 was added to store the teeth, refrigerated at 4^{o}C until the time of testing [13]. The crowns were decoronated at the cemento-enamel junction and sectioned mesiodistally using a diamond bur and high-speed handpiece with distilled water for irrigation. In this way two samples from each tooth were obtained to provide a total of twelve samples that were than embedded in clear epoxy resin (Castapress, Vertex-Dental B.V. Zeist, Netherlands) with their buccal or lingual surfaces exposed. The epoxy resin was cured in a cold-water bath for 15 minutes under 2 bar pressures to dissipate heat generation from the exothermic setting reaction of the resin and to prevent development of air bubbles developing during curing. All the samples were then uniquely identified by number and stored in HBSS prior to testing. For nanoindentation testing, the samples were smoothed and polished with both surfaces of the specimen parallel (± 25 µm) using the Tegra grinding and polishing system (Struers A/S, Ballerup, Denmark) following the protocol modified from that of Mahoney [14]. All samples were polished at each step for 3 minutes in a clockwise and an anticlockwise direction. The protocol is summarised in Table 1. The Ultra Micro-Indentation System - UMIS (Fischer-Cripps Laboratories Limited, Sydney, Australia) is a nanoindentation tester that applies a loading (and unloading) force in increments, and the resulting data is plotted for a complete indentation cycle. The two mechanical properties investigated in this research are hardness and modulus of elasticity. The hardness (H) is the resistance to surface penetration, calculated by dividing the force (load) by the projected contact area. The elastic modulus (E) represents the stiffness of a material within the elastic range and is calculated by the ratio of stress to strain [14, 4]. The ultra-micro indentation was produced by a Berkovich indenter, 25mN (milli-Newton) and 250mN forces were used in this study. The Berkovich indenter is a three-sided pointed pyramid with apex angles of 65.3 degrees [15].

Table 1: Showing the steps used in enamel polishing

Step	Force (N)	Polishing abrasive pad	Polishing lubricant	Speed of rotation of polishing disk (rpm)	Cleaning time in ultrasonic bath with distilled water (min)
A	10	Waterproof silicon carbide paper, grit 320	Distilled water	150	10
B	10	Waterproof silicon carbide paper, grit 500	Distilled water	150	5
C	10	Waterproof silicon carbide paper, grit 1200	Distilled water	150	5
D	10	Waterproof silicon carbide paper, grit 2400	Distilled water	150	5
E	10	MD-Pan™ Grain size: 6µm	6µm diamond suspension	150	5
F	10	MD-Pan™ Grain size: 1µm	1µm diamond suspension	150	5

Table 2: Showing the specifications of the UMIS.

Specifications of the UMIS	
Maximum load	50mN – 500mN
Minimum contact force	2μN
Maximum depth	2μm – 20 μm
Force resolution	500nm
Depth resolution	0.03nm
Minimum sample positioning step	0.05μm
Load frame compliance	0.1nm/mN

Table 3: Showing the parameters used for nanoindentation tests.

Type of indenter	Berkovich
Number of indents per line	4
Position	Array
Direction of movement of the stage	Right
Time of hold at maximum load	10 seconds
Distance between each indentation	100μm for 250mN and 50μm for 25mN

All nanoindenters are mounted on the stable base in a chamber that provides thermal and mechanical stability to the instrument. Mechanical and thermal stability are important because of the nano-scale of the operation and the machine must be isolated to reduce both vibration and thermal variation [16, 15]. The specifications of the UMIS are summarised in Table 2 [15].

A typical set-up used with the UMIS instrument is shown in Figure 1. A microscope with 20x or 50x magnification attached to the indentation system enables precise location and section of the area to indent. A depth-sensing device records changes in the depth of indentation during the loading and unloading. This allows collection of the data needed to calculate the hardness (H) and elastic modulus (E).

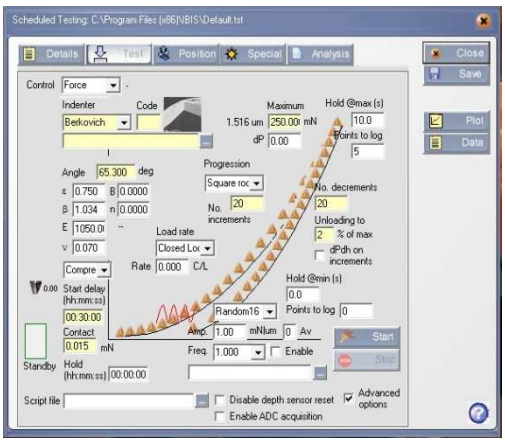

Figure 1: Showing the nanoindentation settings for testing with a 250mN force.

During UMIS testing, each sample was mounted on a metal base with sticky wax (Model Cement, Metrodent Ltd, Hudders field, United Kingdom). The sticky wax and the metal base were heated and the samples were pressed against the metal base using a paralleling machine (Leitz, Wetzlar, Germany). Prior to UMIS testing, samples were allowed to set for 30 minutes at room temperature to avoid any thermally-induced changes affecting the dimensions of the sample. A strong magnet in the mounting base ensured adequate contact with the test base in the UMIS system. The edges of the metal base were enclosed with a plastic barrier that allowed the samples to be immersed in HBSS during the initial stabilisation period and testing to ensure that the enamel was fully hydrated at all times, especially during indentation.

The UMIS is operated under Windows XP using IBIS™ software (Fischer-Cripps Laboratories Pty Ltd, Sydney, Australia). The samples were mounted on a movable stage that allowed indentations to be made at selected sites on the sample surface. A microscope (Fischer-Cripps Laboratories Limited, Kyowa lenses, Sydney, Australia) was used to set up the indentation maps for the samples. The parameters used for each test are summarised in Table 3.

After completion of the 250mN indentation test, the samples were shifted using the stage control software up 100μm and left 300μm. This point was set as the zero point for the 25mN force tests. When both tests were completed, the specimen was detached from the base and treated with 30% HP for 30 minutes and a new test was performed using the same procedure. The distance between the two sets of indentations before and after bleaching was 500 μm. There was sufficient space between the indents to avoid any residual stress influence. The procedures for testing each of the samples are shown in Figures 2 and 3.

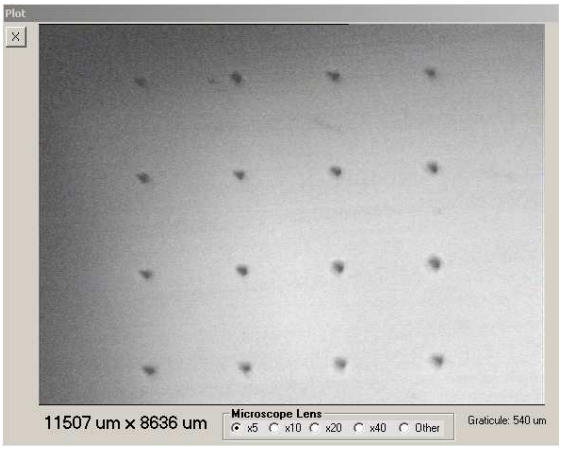

Figure 2: Showing Indentation tests on an enamel surface before application of the dental bleaching agent at a force of 250 mN. Microscopic image (5x) of one of the enamel samples showing the indentations with a distance of 100 µm between the indents.

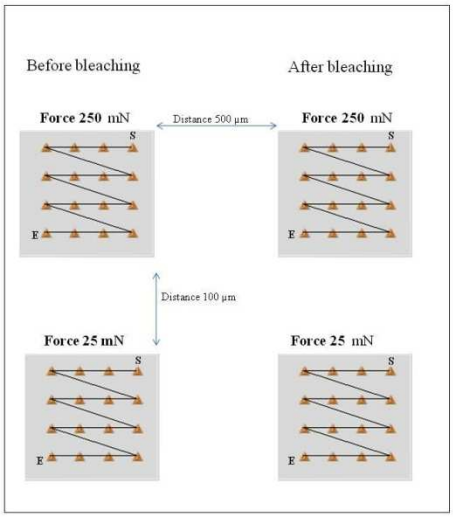

Figure 3: Showing the testing procedures for each sample. The line (S→N) indicates the path that the nanoindenter took in making the indentation (shown as triangles) in the samples. S= Starting point for the test; E=End point

All six samples were first subject to the control nanoindentation (S1 to S6). All samples were detached from the metal base and three samples (S1, S2, S3) were exposed to 30% HP solution for 30 min and the other three samples (S4, S5, S6) were exposed to 10% CP for 8 hours to simulate a course of bleaching treatment. The experimental outline is summarised in Figure 4. All samples were washed with deionised water to remove the bleaching agent and then a second series of nanoindentation test was performed on the samples in areas adjacent to previously tested sections, as outlined in Figure 4. The distance between the control (before bleaching) and study (after bleaching) groups was 500µm. The average H and E from the sixteen indentations was calculated for each sample.

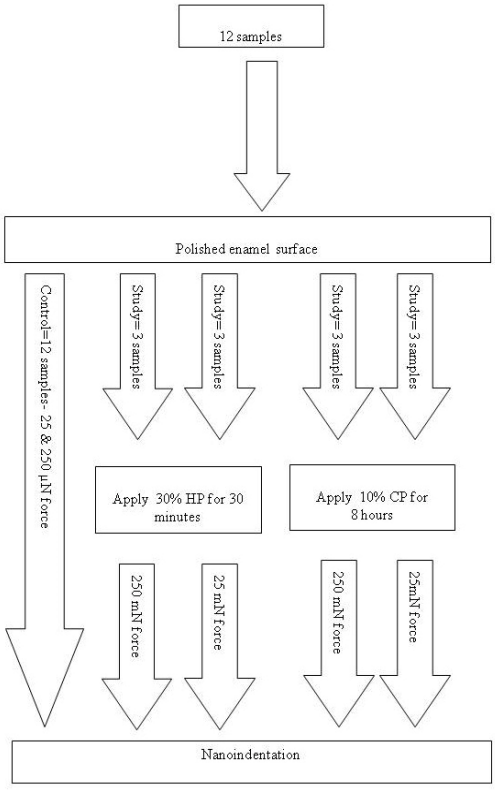

Figure 4: Showing the experimental outline for nanoindentation tests before and after dental bleaching

Statistical analysis was performed using the statistical software SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean and SD) was reported. The statistical unit was the type of bleaching agents (10% CP vs 30% HP) and differences between the two groups were assessed using an Independent t-test. The normality of the quantitative data was evaluated using a histogram, stem and leaf plot, boxplot, measures of skewness and kurtosis as well as using the Shapiro-Wilk and the Kolmogorov-Smirnov tests. An Independent t-test was used to assess the changes between the control and the test groups in terms of E and H using different bleaching agents and indentation forces. A Mann-Whitney test was used when the normality assumptions were not satisfied. The significance for statistical analysis was set at $P< 0.01$.

RESULTS

The calculation of E and H depended on the load displacement (P-h) curve. All the tests showed a similar shaped force displacement curve (Figure 3.5), in which an average indentation

penetration depth of 2.0 μm and 0.5 μm were achieved with indentation forces of 250mN and 25 mN, respectively. There was no significant difference between the mean values of E for the untreated control group and the study groups that were bleached with 30% HP or 10% CP when an indentation force of 250 mN was used (*P*> 0.01) (Tables 4 and 5). There was no significant difference between the mean H values of the untreated control group and those that were subjected to bleaching with 30% HP (for 30 min) or 10% CP (for 8 h) when an indentation force of 250 mN was used (*P*> 0.01) (Tables 6 and 7).

There was no significant difference between the mean values of the elastic moduli for the control group and the study groups that were bleached with 30% HP (for 30 minutes) or 10% CP (for 8 hours) when an indentation force of 25 mN was used (*P*> 0.01) (Tables 8 and 9).

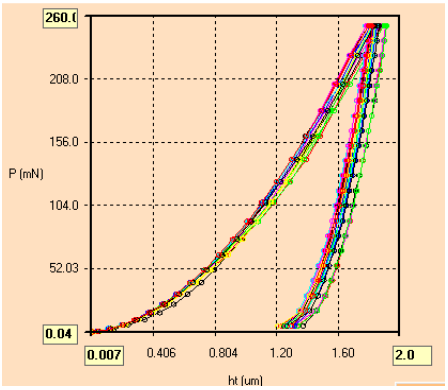

Figure 5: Showing Examples of a load displacement (P-h) curve with a nanoindentation test using 250 mN and control enamel. The x-axis represents the displacement, and the y-axis represents the load. The results of 16 indentations are shown on the graph.

Table 1 Elastic moduli of enamel samples in the control and study groups treated with 30% hydrogen peroxide for 30 min and tested with an indentation force of 250mN

Sample	Control Before bleaching	Study After bleaching
	Mean ± SD (GPa)	Mean ± SD (GPa)
S1	88.69 ± 3	91.27 ± 2.78
S2	106.68 ± 5.44	106.04 ± 4.54
S3	109.18 ± 2.12	109.99 ± 2.85
Average of 3 samples (S1, S2, S3)	**101.51 ± 11.17***	**102.43 ± 9.86***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 2 Elastic moduli of enamel samples in the control and study groups treated with 10% carbamide peroxide for 8 h and tested with an indentation force of 250mN

Sample	Control Before bleaching	Study After bleaching
	Mean ± SD (GPa)	Mean ± SD (GPa)
S4	100.79 ± 1.90	94.61 ± 2.18
S5	100.58 ± 2.08	103.23 ± 2.40
S6	102.10 ± 2.31	97.47 ± 3.03
Average of 3 samples (S4, S5, S6)	**101.06 ± 1***	**98.44 ± 4.39***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 3The hardness of enamel samples in the control and study groups after beaching with 30% HP for 30 min and tested with an indentation force of 250mN

Samples	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S1	3.88 ± 0.20	3.87 ± 0.14
S2	3.98 ± 0.11	4.40 ± 0.38
S3	4.40 ± 0.13	4.46 ± 0.09
Average of 3 samples (S1, S2, S3)	**4.09 ± 0.27***	**4.24 ± 0.32***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 4The hardness of enamel samples in the control and study groups after beaching with 10% carbamide peroxide for 8 h and tested with an indentation force of 250mN

Sample	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S4	4.30 ± 0.17	3.63 ± 0.13
S5	4.42 ± 0.14	4.40 ± 0.14
S6	3.96 ± 0.28	3.20 ± 0.16
Average of 3 samples (S4, S5, S6)	**4.22 ± 0.23***	**3.74 ± 0.60***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 5 Elastic moduli of enamel samples in the control and study groups treated with 30% hydrogen peroxide and tested with an indentation force of 25mN

Sample	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S1	91.03 ± 6.26	90.02 ± 4.35
S2	104.67 ± 8.61	112.84 ± 10.13
S3	104.53 ± 4.74	109.17 ± 2.12
Average of 3 samples (S1, S2, S3)	**100.08 ± 7.83**	**104.01 ± 12.25***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 6 Elastic moduli of enamel samples in the control and study groups treated with 10% carbamide peroxide for 8 hours and tested with an indentation force of 25mN

Sample	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S4	87.43 ± 10.04	84.73 ± 5.99
S5	100.94 ± 4.95	101.69 ± 6.11
S6	92.71 ± 3.69	82.48 ± 5.52
Average of 3 samples (S4, S5, S6)	**93.69 ± 6.80***	**89.63 ± 10.50***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 7The hardness of enamel samples in the control and study groups after bleaching with 30% hydrogen peroxide and tested with an indentation force of 25mN

Sample	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S1	4.17 ± 0.22	4.00 ± 0.19
S2	4.34 ± 0.21	5.51 ± 0.79
S3	4.82 ± 0.15	4.40 ± 0.13
Average of 3 samples (S1, S2, S3)	**4.44 ± 0.33***	**4.64 ± 0.78***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

Table 8The hardness of enamel samples in the control and study groups after bleaching with 10% carbamide peroxide and tested with an indentation force of 25mN

Sample	Control Before bleaching	Study After bleaching
	Mean (GPa) ± SD	Mean (GPa) ± SD
S4	3.77 ± 0.76	2.58 ± 0.34
S5	4.03 ± 0.26	4.13 ± 0.34
S6	2.95 ± 0.42	2.08 ± 0.18
Average of 3 samples (S4, S5, S6)	**3.58 ± 0.56***	**2.93 ± 1.02***

SD = Standard deviation , S = sample
**Independent t-test (P > 0.01)*

There was no significant difference between the mean H values of the untreated control group and the samples subjected to dental bleaching with 30% HP (for 30 min) or 10% CP (for 8 h) when an indentation force of 25 mN was used (*P*> 0.01) (Tables 10 and 11). The results obtained from all nanoindentation tests performed in this study are summarized in figures 6 and 7.

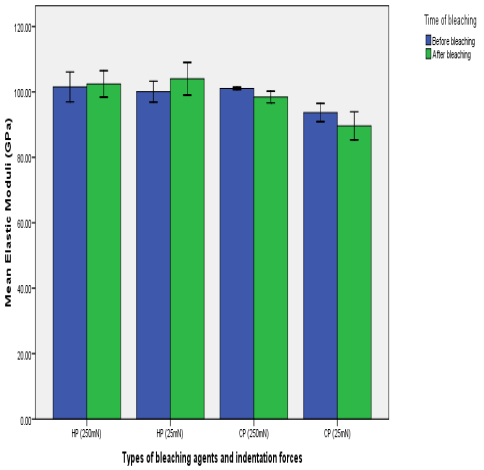

Figure 1: Showing elastic moduli of enamel samples in the control and study groups with 30% hydrogen peroxide (HP) and 10% carbamide peroxide (CP) tested with indentation forces of 25 and 250 mN.

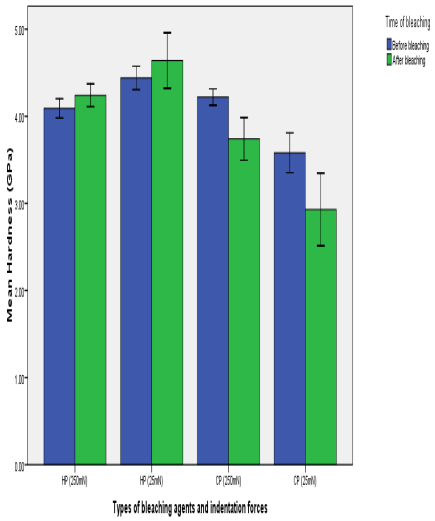

Figure 2: Showing hardness of enamel samples in the control and study groups with 30% hydrogen peroxide (HP) and 10% carbamide peroxide (CP) tested with indentation forces of 25 and 250 mN.

DISCUSSION

Enamel is a heterogeneous material. It varies in mineral content, amount of organic matrix and chemical constitution [17]. Mature human enamel has inorganic and organic phases. The inorganic phase is approximately 95% mineral by weight (~87% by volume) and primarily involves calcium and phosphate in the form of hydroxyapatite. The organic phase contributes ~1% by volume of the overall enamel mass while the remaining 4% is water [18, 19]. The organic component of mature enamel includes peptide fragments, which are mainly breakdown products of the enamel matrix proteins amelogenin and enamelin that developed during tooth maturation prior to eruption but also includes material contributed by a number of other proteins. The mechanical properties of enamel depend on the location in the tooth [20]. Location affects both chemical composition and prism orientation [21, 22, 6].

The reason for the wide variation in SD was likely to be due to variation in the different enamel samples that were extracted from the different sample teeth. It has been suggested that enamel varies in mineral content, amount of organic matrix and its chemical constituency [17]. The mechanical properties of enamel also change from one location to another on the same tooth, and the chemical composition and prism orientation alters at different sites on the enamel surface [21, 22, 6]. Variations in the physical and chemical properties of enamel within the same tooth and between different teeth may explain the variation in our measurements of H and E.

Microhardness changes have been related to a loss (demineralisation) or gain (remineralisation) of the mineral/organic content of the dental structure, the direction of hydroxyapatite crystals and the anisotropic nature of enamel [23, 24, 6]. The nanoindentation microhardness test is suitable for determining small changes in surface microhardness and elastic modulus of dental hard tissue [25]. Nanoindentation testing was performed with a Berkovich diamond tip using the Ultra-Micro-Indentation System (supplied by Fischer-Cripps Laboratories Limited, Sydney, Australia) before and after application of bleaching treatment (10% CP and 30% HP).

Traditionally, microindentation methods such as the Vickers and Knoop tests haven't been used to measure microhardness. However, the large-scale indentations used by the microindentation methods to measure the microhardness have important limitations. In particular, these methods are not sufficiently sensitive to detect changes in the superficial fraction (< 2 μm) of enamel because relatively high force usually applied at the penetrating point of the indenter

(~ 0.24N - 0.98N) causes relatively deep indentations. The penetration or displacement used by the nanoindentation test is measured in nanometers with indentation depths of around 1 μm [26 , 4, 27]. The nanoindentation method is used to determine the mechanical properties of enamel, measured at a depth of 0.5 – 2 μm. However, the need to prepare samples with polished parallel surfaces is an important limitation of the nanoindentation method because the normal surface of the enamel is removed [14].

A total of sixty-four Berkovich indentations were evaluated before and after the bleaching procedure on each specimen, with measurements after bleaching made in an area in close proximity to baseline indentations measurements (500 μm intervals between control and test sites). Indentations were conducted using loads of 25mN and 250 mN at room temperature, with specimens submerged in the sterile HBSS (Invitrogen™). The time of the loading was 10 secs and gave mean indentation penetration depths of 2 μm for 250 mN and 0.5 μm for 25 mN, with similar shaped force displacement curves generated for both forces. The use of two different forces was undertaken to investigate whether the different loads altered the enamel surface in different ways. As expected, the heavy load penetrated more deeply into the enamel than the lighter load [28].

The study found that before the application of the bleaching agent the average values for elastic modulus (E) detected by applying 250 mN or 25 mN nanoindentation forces with the Brekovich tip were 102 ± 11 GPa and 100 ± 8 GPa, respectively. The corresponding hardness (H) measurements were 4.09 ± 0.27 GPa and 4.44 ± 0.33 GPa. These measurements are within the range reported by other investigators and showed that the E and H values were not significantly changed between the application of 250 mN and 25 mN forces [6]. This indicates that the physical properties of enamel samples were constant from the surface to a depth of ~ 2 μm.

Microhardness evaluation in our study showed no significant softening of the enamel caused by exposure to 30% HP for 30 minutes or 10 CP for eight hours. This is in agreement with several previous reports [29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44]. In contrast, a few studies have reported changes in the mechanical properties of enamel [45, 46, 47]. These differences are possibly related to different methodologies, including choice of samples, *in vitro* media used to store teeth and enamel samples, concentration of bleaching agent applied and treatment time.

The selection of teeth may influence the response of teeth to different bleaching agents. Tooth morphology, along with age-related factors such as the presence of erosion and demineralization defects, may reduce the depth and strength of the hypermineralised surface layer of enamel [48, 49]. This in turn may contribute to disparities in the literature. Another important factor that should not be overlooked when interpreting the results is the use of storage media. Several studies highlighted the importance of using a suitable storage medium that would maintain the chemical stability of enamel samples, since the loss of calcium, phosphate and organic components during storage or bleaching might significantly reduce microhardness [39, 6]. In contrast, Mahoney *et al*. suggested that the choice of storage medium should not have a significant effect on measurements as long as the control and treated samples are stored in the same medium [14]. Hank's balanced salt solution was used in the present study because it should reduce or, prevent surface demineralization and hence preserve the mechanical properties of enamel [50, 47]. A remineralizing agent was not added to the bleaching agents used in this study despite the possible benefit of increased microhardness [34, 37]. This allowed us to evaluate more directly the effects of the concentrations of bleaching agents used in the present study.

Although the present study was unable to detect any effect of bleaching on the mechanical properties of enamel, there are some important caveats. It is not known if any potentially deleterious surface effects of bleaching extend past the surface into deeper enamel or if only surface changes are of clinical significance [51]. Thus, the need to prepare flat, highly polished surfaces which do not include the enamel surface and their storage may compromise results. Although we used Hanks' solution to minimize demineralization, other studies have stored samples in distilled or deionised water, used artificial saliva or Hanks balanced medium. This makes comparison between studies and even the ability to draw significant conclusions from some studies difficult. Some reports on the mineral and organic contents of enamel after bleaching contradict the conventional view that

bleaching does not affect mechanical properties of enamel. All studies of bleaching effects on the mechanical properties of enamel need to be assessed carefully before conclusions are drawn. It seems likely that some of *in vitro* methodologies used to detect change are either flawed or insufficiently sensitive to detect changes that reflect the *in vivo* situation. For example, nanoindentation hardness testing can only be performed on flat and highly polished surfaces that do not necessarily reflect the normal tooth surface. As the roughness of a surface increases, nanoindentation measurements can give ranges of values that deviate increasingly from the actual hardness [45]. In the present study, we prepared enamel samples that had the outer surface removed to facilitate nanoindentation measurements. This may have compromised our ability to detect surface localised modification of enamel by HP that happens in clinical situation.

CONCLUSION

There are no significant deleterious consequences on the mechanical properties of sound enamel after bleaching with 30% HP for 30 minutes and 10% CP for 8 hours. Although most studies have suggested that chemical bleaching does not deleteriously affect the mechanical properties of enamel, some other studies have indicated that adverse effects can occur. Some of the disparate conclusions reached may be affected by factors such as sample selection and storage, properties of the bleaching agents applied, testing methodologies, the concentration and duration of application of the whitening agent, the pH of the reaction, and the use of *in vitro* methodologies that do not reflect *in vivo* contexts. More sensitive and well-controlled studies that test appropriate materials under standardized conditions are needed to determine the effects of bleaching agents on the mechanical behaviour of the tooth surface.

Future research and recommendations

The effects of occlusal forces on the attrition and wear behaviour of bleached enamel have yet to be evaluated.

Acknowledgements

Ethical approval (category A, approval number 10/112) from the University of Otago Ethics Committee have been granted. Informed consent was obtained from participants who voluntarily donated their extracted teeth.

Conflicts of interest*:* The authors declare that there is no conflict of interest regarding the publication of this article.

Author's Contributions:

Reza Shah Mansouri: Performed all the procedures, conducted a literature review of similar articles and contribute significantly to the discussion.

Nabil Khzam: Contributed significantly to the drafting and revision of the manuscript.

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