

## Influence of McInnes bleaching agent on hardness of enamel and the effect of remineralizing gel GC tooth mousse on bleached enamel -An in vitro study

### Abstract

**Aim:** To evaluate the influence of bleaching agent (McInnes solution) on the micro hardness of enamel and the effect of remineralizing gel (GC Tooth Mousse) on bleached enamel surface.

**Materials & Method:** Twenty maxillary anterior teeth were selected, cut sagittally, subjected to Vickers microhardness test for baseline. McInnes solution was applied & samples were again tested for microhardness. Then GC Tooth Mousse was applied as remineralizing gel for 7 & 14 days and then again subjected for microhardness test.

**Results:** All the samples showed a decrease in the microhardness after two cycles of bleaching which was statistically significant. After application of GC Tooth Mousse the samples showed a marked increase in microhardness after seven and fourteen days.

**Conclusions:** McInnes bleaching agent does decrease the enamel microhardness by causing enamel demineralization and GC Tooth Mousse used in the study causes an increase in the microhardness of bleached enamel by maintaining a high gradient of calcium and phosphate ions at the enamel subsurface.

### Key Words

Amorphous calcium phosphate, bleaching, casein phosphopeptide, McInnes bleaching solution, Vickers hardness number.

### Introduction

The desire to have white teeth and thus a more pleasant smile has become an important esthetic need of the patient<sup>1</sup>. Tooth bleaching has been reaching great popularity as a result of the esthetic demands imposed by society; it has been indicated for teeth discoloured by aging, trauma, endodontic treatment, ingestion of colored foods and beverage, tobacco, and naturally discoloured teeth<sup>2</sup>. The successful outcome of any of the applied modalities mainly depends on the etiology, diagnosis, and proper selection of bleaching materials and the correct clinical technique<sup>3</sup>.

The technique of bleaching or whitening teeth was first described in 1877.<sup>4</sup> Hydrogen peroxide was thought to be used for the first time in 1884 and has since been established as the most effective bleaching agent because of its unique ability to penetrate the tooth structure<sup>5</sup>. Later it was McInnes who reported a technique where hydrogen peroxide, hydrochloric acid and ethyl ether were used. This technique has been found to be successful for bleaching the teeth of patients with endemic fluorosis.<sup>6</sup>

The efficacy of bleaching agents is validated by many in vitro and in vivo studies.<sup>7,8</sup> nevertheless, these bleaching agents should be used only after carefully evaluating the adverse effects on dental tissues<sup>1</sup>. In fact, the effect of bleaching procedures on enamel is still controversial and needs to be elucidated<sup>9</sup>. Increased frequency of acid exposure tends to alter the total demineralization/ remineralisation amounts resulting in greater amounts of mineral loss.

Although a reduction in enamel microhardness has been reported,<sup>10,13</sup> it must be assumed that this alteration reflects not only the bleaching procedure but also the pH of the formulation used<sup>7</sup>. In fact, some studies have found no significant differences in enamel microhardness after bleaching with hydrogen peroxide in high concentrations.<sup>14,15</sup>

Recently casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) derived from cow's milk has been reported to reduce the demineralization process of the tooth structure and enhance the remineralisation process<sup>16</sup>.

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Among laboratory studies, surface microhardness measurement is a simple method to determine the mechanical properties of enamel and dentin surface and it is not only related to the loss or gain of the mineral content of the dental structure, but also to the composition of the applied product and their pH values and the presence of other components in commercial bleaching agents<sup>1</sup>. Based on these observations the present study was undertaken to investigate the influence of McInnes bleaching agent on enamel microhardness and subsequent remineralisation by CPP-ACP on bleached enamel.

### Materials And Methods:

A total of 20 freshly extracted maxillary anterior teeth were selected. The teeth were then cut sagittally using diamond disc and the buccal surface were

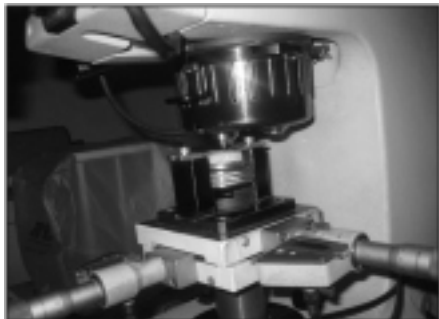
impregnated in the cold cure acrylic resin (DPI) facing upwards (Fig 1). Using the plastic moulds the resin were made in to pellets. These specimens were then kept in artificial saliva to prevent dehydration. The samples were then rinsed in water and dabbed dry with absorbent paper and then subjected to baseline microhardness test using Vickers Micro Hardness Tester (AKASHI-MVK-H2).

Fig 1: Showing sectioned specimen with buccal surface impregnated in the cold cure acrylic resin facing upwards.



The test specimens were placed on the stage of tester and stabilized (Fig 2). Then area to indent was selected by focusing with 10x objective lens. After this the load of 100 g was applied on the surface of specimen for 30 seconds, never close to any edge of the specimen. The indentation formed was viewed and measured with 10x objective lens. The average microhardness of the specimen was determined from three indentations to avoid any discrepancy, since the enamel surface has a curvature. The procedure was repeated for all the twenty specimens.

Fig 2: Showing the test specimen was placed on the stage of Vickers Micro Hardness Tester (AKASHI-MVK-H2).



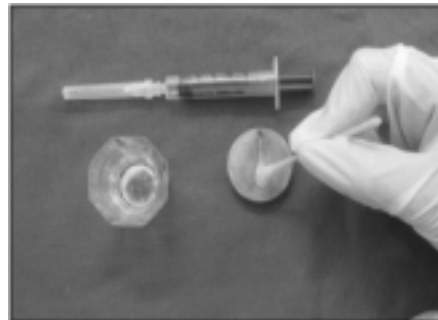
### Bleaching process

After recording the baseline hardness values McInnes bleaching was prepared using mixture of 1 ml of 36% hydrochloric acid (S D Fine Chem. Limited, Mumbai), 1ml of 30% hydrogen peroxide (S D Fine Chem. Limited, Mumbai) and 0.2 ml of diethyl ether (S D Fine Chem. Limited, Mumbai) which is mixed in the ratio of 5:5:1. The mixture was prepared freshly in a dappen dish before each application.

1) First cycle of bleaching: The bleaching agent thus prepared was applied to the enamel surface using a cotton applicator for five minutes (Fig 3). It was then washed under deionized water, damped dry with absorbent paper and then subjected for microhardness. After this, the samples were stored in artificial saliva for 24 hours to prevent dehydration.

2) Second cycle of bleaching: Again after 24 hours, the second application of bleaching agent was carried out as described earlier and the microhardness values were recorded.

Fig 3: Showing the application of McInnes Bleaching agent.



### Remineralisation process

1) First cycle of Remineralisation: GC Tooth Mousse (Recaldent) was applied with cotton applicator tips on the post bleached samples, every day for seven days with minimum application time of three minutes (Fig 4). The samples were then washed under deionized water, stored in artificial saliva for next seven days and then subjected for microhardness test as described earlier.

2) Second cycle of remineralisation: Following this, GC Tooth Mousse was applied for seven more days and at the end of fourteen days the samples were again subjected for microhardness testing using the same procedure as described earlier.

Fig 4: Showing the application of Remineralising gel (GC Tooth Mousse)



### Results:

The data obtained from the following test were subjected for statistical analysis. A "P" value of 0.05 or less was considered

for statistical significance. The changes in microhardness at different times of assessment were analysed using the "ONE WAY ANOVA".

In this study these comparisons were done between the groups:

- 1) In the first comparison baseline was compared with bleaching and remineralisation cycles (Table 1).
- 2) The next comparison was done between the 1st cycle and 2nd cycle of bleaching (Table 2).
- 3) The third comparison was done between the 2nd cycle of bleaching and cycles of remineralisation (Table 3).
- 4) The next comparison was done between the 1st and 2nd cycle of remineralisation (Table 4).

The recorded values were then subjected to statistical analysis.

### Discussion:

The lightening of the colour of a tooth through the application of a chemical agent to oxidize the organic pigmentation in the tooth is referred to as 'Bleaching'. Bleaching or tooth whitening dates back to the 18<sup>th</sup> century. In early nineteenth century, hydrogen peroxide alone and in combination with other materials, was used as bleaching agent<sup>17</sup>.

It was in the year 1966, that McInnes reported a technique that combined hydrochloric acid and hydrogen peroxide to remove fluorosis stains. He used a solution of five parts of 30% hydrogen peroxide, five parts 36% hydrochloric acid and one part diethyl ether and applied the solution with a cotton wrapped toothpick to the areas of the teeth affected by the stain. After 10 to 15 minutes the teeth were washed with water and neutralized with a sodium bicarbonate paste.

It has been reported<sup>18</sup> that McInnes bleaching technique were specifically recommended for the treatment of teeth exhibiting endemic dental fluorosis because of its superficial nature, easy manipulation and its quality of being less expensive when compared to other commercially available agents like carbamide peroxide<sup>19</sup>. In the present study McInnes bleaching agent was selected as it was commonly used in clinical set up for treating dental fluorosis.

The effects of bleaching on enamel microhardness are probably related to their pH, as well as alteration of the organic matrix of enamel under the chemical action of hydrogen peroxide. The strong oxidising effect of hydrogen

Table 1 show that baseline microhardness values when compared with first cycle of bleaching decreased by a small margin of 0.4% which was not significant ( $P=0.034$ ) and a decrease of 4% which was highly significant ( $P<0.001$ ) when compared with second cycle of bleaching. But no significant difference was found when baselines microhardness values were compared with remineralisation values in hardness.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BASILINE MICROHARDNESS	20	319.09	27.983	6.257	305.99	332.18	274	355
1ST CYCLE OF BLEACHING	20	317.88	27.897	6.238	304.82	330.93	273	354
2ND CYCLE OF BLEACHING	20	293.61	22.616	5.057	283.02	304.19	267	345
1ST CYCLE OF REMINERALISATION	20	314.99	27.820	6.221	301.97	328.01	271	353
2ND CYCLE OF REMINERALISATION	20	319.46	28.046	6.271	306.33	332.58	275	356

Table 2 shows the comparison in the microhardness values between the first and second cycle of bleaching which showed a 3% decrease and was significant ( $P<0.05$ ).

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1ST CYCLE OF BLEACHING	20	317.88	27.597	6.238	304.82	330.93	273	354
2ND CYCLE OF BLEACHING	20	293.61	24.616	5.057	283.02	304.19	267	345

Table 3 shows 2.8% & 3.5 % increase in Vicker's hardness number and was significant ( $P<0.01$ ) when the 2nd cycle of bleaching was compared with 1st and 2nd cycle of remineralisation respectively

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
2ND CYCLE OF BLEACHING	20	293.61	22.616	5.057	283.02	304.19	267	345
1ST CYCLE OF REMINERALISATION	20	314.99	27.820	6.221	301.97	328.01	271	353
2ND CYCLE OF REMINERALISATION	20	319.46	28.046	6.271	306.33	332.58	275	356

Table 4 showed an increase in the VHN by 1% and was significant ( $P<0.05$ ) when comparison was done between first and second cycle of remineralisation

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1ST CYCLE OF REMINERALISATION	20	314.99	28.520	6.221	301.97	328.01	271	353
2ND CYCLE OF REMINERALISATION	20	319.46	27.416	6.271	306.33	332.58	275	356

peroxide on the organic matrix of teeth plays a predominant role in the alterations observed after bleaching<sup>10,12</sup>. Hydrogen peroxide as diffuses through the enamel and dentin releases free radicals, reactive oxygen molecules and hydrogen peroxide ions that converts long chained dark colored chromophores in to light colored chromophores. This effect can probably be increased by low pH of the bleaching agent, causing subsequent alterations in the mineral composition, decreasing enamel and dentin microhardness<sup>20</sup>.

All the samples used in the study showed no change in the microhardness immediately after first cycle of bleaching. Around 0.4% reduction in the microhardness was seen which was not

significant.

When the bleaching cycle was repeated after 24 hours (the second cycle of bleaching) there was significant reduction in the VHN, which shows 4% decrease in the microhardness, which was significant.

Artificial saliva was used for storing the specimens in between the bleaching cycles, because it is believed that artificial saliva contributed to a slight increase in the microhardness, after demineralisation<sup>21</sup>.

The amount of demineralisation of enamel by bleaching was assessed by microhardness tester. Microhardness test was selected mainly because it was economical compared to other tests and was easily available<sup>22</sup>.

Microhardness measurement of tooth material can be done in three different ways like; Knoop's hardness number(KHN), Vicker's hardness number (VHN), and Brinell's hardness number(BHN). In the present study Vicker hardness number was chosen over Knoop's hardness number because a square shape of indent obtained in VHN was easy and accurate to measure. Even the minute changes in the square shape indent after the test can be easily detected, whereas the Knoop hardness test gave a rhomboid shape indentation with opposing surfaces parallel to each other and detecting the error was difficult.

The average hardness value for enamel is in the range from 270 to 350 KHN range or from 250 to 360 VHN range. In this study the microhardness values were in the range from 266.7 to 360 VHN which was within standard range.

One of the factors that affect the hardness measurement was the specimen's preparation, because any tilt or not flat surface would yield a too large an indentation and thus a smaller Vickers's hardness measurement. Hence three indentations were made to avoid any operational bias, then average of these indentations were taken for statistical analysis.

Various techniques have been followed to neutralize the effect of bleaching; the use of baking soda, prophylactic paste containing fluoride, APF gel and use of copious amount of water<sup>23</sup>. In the present study GC Tooth Mousse has been used, which is commercially available Casein Phosphopeptide (CPP), stabilized amorphous calcium phosphate (ACP) product<sup>24</sup>.

GC Tooth Mousse was developed by Prof Reynolds at the University of Melbourne in 1998<sup>25</sup>. CPP stabilizes ACP and forms Nano complexes with ACP at the tooth surface thereby providing a reservoir of calcium and phosphate ions which favours mineralization. CPP also buffers the pH of plaque, depresses demineralisation and enhances remineralisation which also results in the anticariogenic property of CPP-ACP<sup>26</sup>.

The values obtained after the application of remineralizing solution (second cycle of remineralisation) showed an increase in VHN by 1%.

Although most of the remineralizing solutions were supersaturated with respect to amorphous and crystalline calcium phosphate phases, the solutions

which were stabilised by the CPP-ACP, such as in GC Tooth Mousse, the spontaneous precipitation of calcium phosphate did not occur and thus the longer the duration of solution in contact with the teeth, the better was the remineralisation<sup>27</sup>.

According to manufacturer's instructions for the maximum benefit the solutions of GC Tooth Mousse should be left on the tooth surface as long as possible. Hence in the present study the excess GC Tooth Mousse was wiped off after three minutes leaving the residues on the samples and then stored in artificial saliva.

### Conclusions:

From the above study it can be concluded that

- McInnes bleaching agent does decrease the microhardness of enamel by causing enamel demineralisation.
- GC Tooth Mousse used in the study causes an increase in the microhardness of bleached enamel by maintaining high gradient of calcium and phosphate ions at the enamel subsurface.

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