



## Surface Morphological Changes in Human Enamel Following Bleaching: An *in vitro* Scanning Electron Microscopic Study

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### ABSTRACT

**Objective:** The purpose of this study was to evaluate, using scanning electron microscopy (SEM), the morphological and structural changes of the enamel induced by three bleaching agents namely old McInnes solution, modified McInnes solution and 10% carbamide peroxide gel at different time intervals.

**Materials and methods:** Fifteen freshly extracted noncarious human central incisors with intact enamel surface were selected. The teeth were sectioned at the cemento-enamel junction separating the crown portion from the root using a diamond separating disk. Following this, the samples were subjected to three different bleaching agents: Group 1: Old McInnes solution, group 2: modified McInnes solution and group 3: 10% carbamide peroxide for a period of 15, 30 and 60 minutes, 24 and 30 hours time interval. The sample stubs were subjected to scanning electron microscope and were photographed at 2000 and 10,000 magnifications.

**Conclusion:** The present study revealed no indication of either etching or significant change in surface morphology of enamel when evaluated under scanning electron microscope after 6 weeks treatment with various bleaching agents.

**Clinical significance:** Morphological alterations in bleached enamel are both concentration and time dependent.

**Keywords:** Dental enamel, McInnes solution, Peroxides.

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**Conflict of interest:** None declared

### INTRODUCTION

Esthetics, by definition is the science of beauty, it is derived from the greek word 'perception' which deals with beauty as an art.

In the modern civilized cosmetically conscious world well-contoured and well-aligned white teeth, set the standard for beauty. Such teeth are not only considered attractive,

but also indicative of nutritional health, self-esteem, hygienic pride and economic status.

Currently there are several methods available to treat discolored dentitions, such as laminates, porcelain jacket crowns and bleaching methods.

Bleaching as a treatment modality, continues to hold its century old place as the simplest, most common, least invasive and least expensive means to lighten discolored teeth.

Hydrochloric acid and hydrogen peroxide<sup>1,2</sup> have been used alone and in combination, in a variety of treatment techniques to remove stains from vital teeth. Hydrochloric acid was reportedly the first of the two chemical agents used to remove intrinsic dental stains from vital teeth.

Heat was applied on the assumption that the bleaching agents would penetrate deeper into the enamel and dentin.<sup>3</sup> But there was concern about the effect of hydrogen peroxide and heat on the pulp.<sup>4</sup>

The McInnes bleaching agent, which was introduced, did not require heat to enhance its effect, but, the hydrochloric acid had deleterious effects<sup>5,6</sup> on the enamel structure resulting in alteration of the tooth contour.<sup>7</sup>

The McInnes bleaching agent was modified by replacing the hydrochloric acid with 20% sodium hydroxide. Reports<sup>8,9</sup> suggested that the modified/new McInnes bleaching agent was much more effective and had less deleterious effects on the enamel.<sup>10</sup>

Recently, home-bleaching methods have been introduced to treat discolored teeth. This basically consists of 10% carbamide peroxide in a custom fitted mouth tray. Various studies<sup>11</sup> have suggested that 10% carbamide peroxide has less deleterious effects on teeth and surrounding tissues.

This *in vitro* study was conducted to compare enamel surface morphologic changes induced by three bleaching agents namely old McInnes, new/modified McInnes solution, and 10% carbamide peroxide gel, at different time intervals under scanning electron microscope.

## MATERIALS AND METHODS

Fifteen freshly extracted human incisors for periodontal reasons, noncarious with intact enamel surface were collected from the Department of Oral Surgery SDM Dental College, Dharwad. Teeth were stored in normal saline at room temperature till the experiments were performed.

The armamentarium used in the present study:

1. Mouth mirror and probe.
2. Saline solution.
3. Micromotor handpiece.
4. Diamond coated separating disk.
5. Polishing paste.
6. Polishing rubber cup.
7. Sterile sealed bottles for storage.
8. Teflon tape.
9. Scanning electron microscope (JOEL-JRC 1100CE).
10. Gold sputter coater (JOEL-JSM 840A).

### Bleaching Materials Tested

- |  |   |
|--|---|
| 1. Old McInnes                             | 36% hydrochloric acid<br>30% hydrogen peroxide 5:5:1<br>1% anesthetic ether |
| 2. New McInnes                             | 30% hydrogen hydroxide<br>10% carbamide peroxide gel                        |
| 3. Nupro Gold<br>Tooth whitening<br>system | 10% carbamide peroxide gel  |

### Sectioning Procedures

The labial surface of the disinfected teeth were polished using a polishing paste with the help of a rubber cup and a slow speed handpiece.

The teeth were sectioned at the cemento-enamel junction separating the crown from the root using a diamond separating disk fitted to a micromotor handpiece.

### Surface Treatment

Prior to the actual bleaching procedure, left half of the cleaned crown samples were covered with teflon tape, longitudinally, while the right half was subjected to the bleaching agents.

Fifteen samples were divided into three groups of five specimens each:

*Group I:* The five specimens were subjected to old McInnes bleaching solutions for a period of 15, 30 and 60 minutes time intervals.

*Group II:* The five specimens were subjected to new McInnes bleaching solution for a period of 15, 30, 60 minutes, 24 and 30 hours time intervals.

*Group III:* Five specimens were subjected to 10% carbamide peroxide gel for 15, 30, 60 minutes, 24 and 30 hours time interval.

After treatment the specimens were washed with normal saline and stored in sterile sealed bottles.

### Sample Preparation for Scanning Electron Microscopy

The samples were mounted on aluminum stubs with double sided scotch tape silver paste applied to make them conductive. The stubs were placed in a vacuum chamber of the ion sputter, gold coating unit (model JOEL-JFC-1100E). A thin layer (approximately 200Å) of gold under vacuum evaporated on to the surface of a tooth.

The gold layer was necessary to prevent charge build-up on the tooth surface during electron bombardment.

The sample stubs were inserted in (model JOEL-ISM-10A) to vacuum chamber of scanning electron microscope and were photographed at 1000 and 10,000 magnifications.

## RESULTS

The photomicrographs obtained from the scanning electron microscopy after treatment was examined for mild, moderate and severe alterations.

In all cases, the left side control (unbleached surface) presented a similar appearance. The surface showed no alteration or changes, indicating that the solution did not penetrate or seep under the teflon covered surface.

The surface changes were noticeable when the scanning electron microscope was moved across the border from the untouched control side of the specimen to the exposed tested surface.

## DISCUSSION

Many dental procedures are performed empirically and without knowledge of what their biological effects may be. This lack of understanding may give rise to unpredictable results, if and when subsequent treatment is required.

This *in vitro* study, evaluated the effect of three bleaching agents namely—old McInnes solution, new McInnes solution and 10% carbamide peroxide gel (Nupro Gold tooth whitening system) on enamel surface, observed under scanning electron microscope at different time intervals, showed alterations in surface morphology of enamel ranging from mild-to-moderate (Table 1) and severe in case of old McInnes bleaching solution.

The surface changes caused by new McInnes solution (Table 2) and 10% carbamide peroxide gel (Table 3), consisted primarily of minor alterations, such as an apparent increase in surface porosities, grooves, deepening of fissure

Table 1: Bleaching agent—old McInnes solution			
Time interval	Mild	Moderate	Severe
15 minutes		++	
30 minutes			+
60 minutes			+

Table 2: Bleaching agent—new McInnes solution			
Time interval	Mild	Moderate	Severe
15 minutes	+		
30 minutes	+		
60 minutes	+		
24 hours		+	
30 hours		+	

Table 3: Bleaching agent—10% carbamide peroxide			
Time interval	Mild	Moderate	Severe
15 minutes	+		
30 minutes	+		
60 minutes	+		
24 hours		+	
30 hours		+	

*Mild*: Moderate alternations of aprismatic layer in the enamel surface.  
*Moderate*+: Increased porosity, deep fissures and opening of enamel prism.

*Moderate* ++: Hollowing of enamel prism centers with relatively intact prism peripheries, similar to acid etching pattern.

*Severe*: Entire enamel surface is eroded; the scalloped/ pitted dental surface with opening of dentinal tubules is seen.

and opening of enamel prisms.<sup>12,13</sup> These changes appeared to be time related, i.e. longer the exposure of the enamel to the bleaching solutions, more marked were the effect.<sup>14</sup>

Bitter NC<sup>15</sup> in a scanning electron microscope study of long-term effect of bleaching agents on the enamel surface *in vivo*, concluded that bleaching over a long time may be associated with increased enamel and altered enamel could also be vulnerable to subsequent staining.

Seghi and Denry<sup>16</sup> reported an increase of up to 40% in the fracture susceptibility of enamel, dentin and cementum after bleaching. This present study cannot support these findings because of the limitations of SEM.

McInnes, in 1966, advocated a solution of five parts 30% hydrogen peroxide, five parts 36% hydrochloric acid and one part anesthetic ether (old McInnes bleaching solution) to be applied topically on the tooth surface for 15 to 30 minutes, to treat fluorosed enamel.<sup>17</sup>

In this study, the enamel surface was exposed to old McInnes bleaching solution, at 15, 30 and 60 minutes time intervals.

In case of old McInnes bleaching agent, a 15 minutes exposure resulted in a typical acid etched surface pattern of the enamel where as a 30 and 60 minutes exposure resulted in severe changes, wherein the entire enamel surface was eroded (Figs 1 to 9).

The deleterious effect on the enamel surface pattern of the enamel, where as a 30 and 60 minutes exposure resulted in severe changes, wherein the entire enamel surface was eroded (Graph 1).

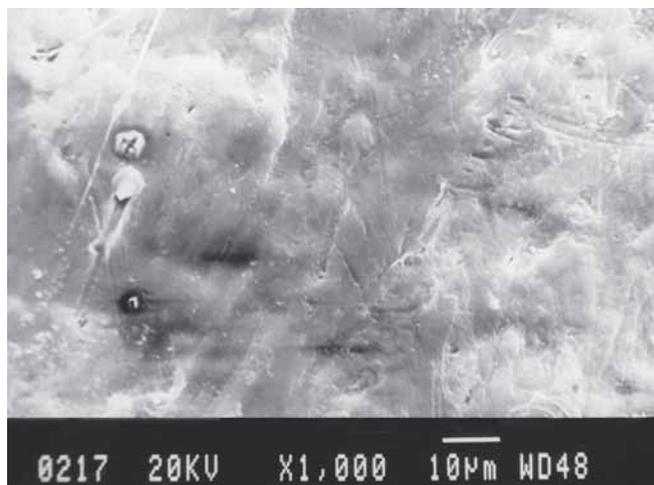


Fig. 1: Tooth surface: Control group I (x1000 magnification)

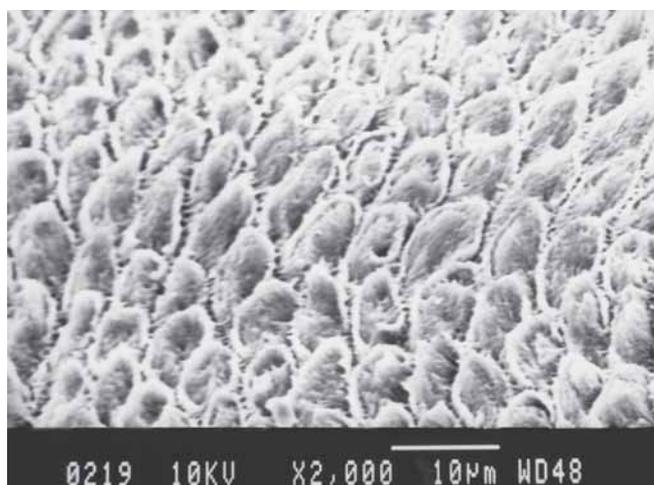


Fig. 2: Tooth surface exposed to old McInnes bleaching agent for 15 minutes. A typical acid etched pattern is seen (x2000 magnification)



Fig. 3: Tooth surface exposed to Old McInnes bleaching agent for 15 minutes (x10,000 magnification)

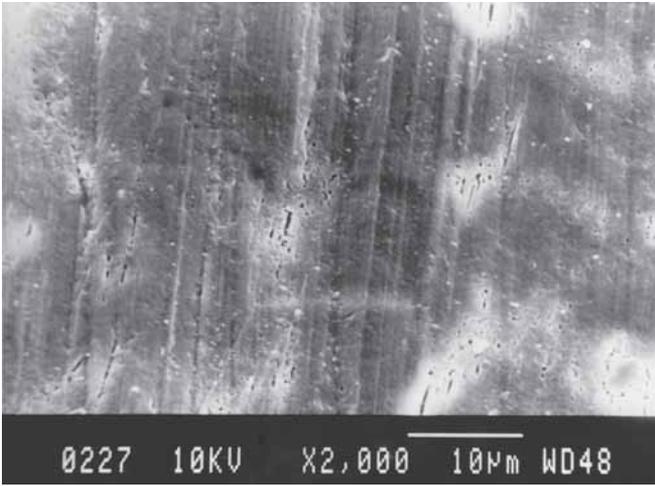


Fig. 4: Tooth surface control group I (x2000 magnification)

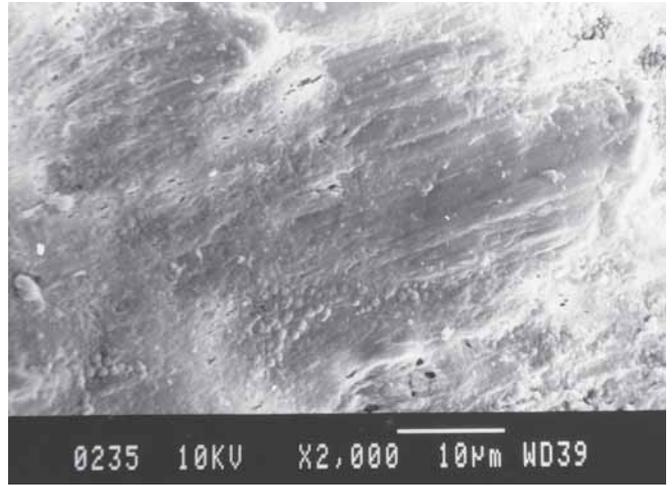


Fig. 7: Tooth surface control group I (x2000 magnification)

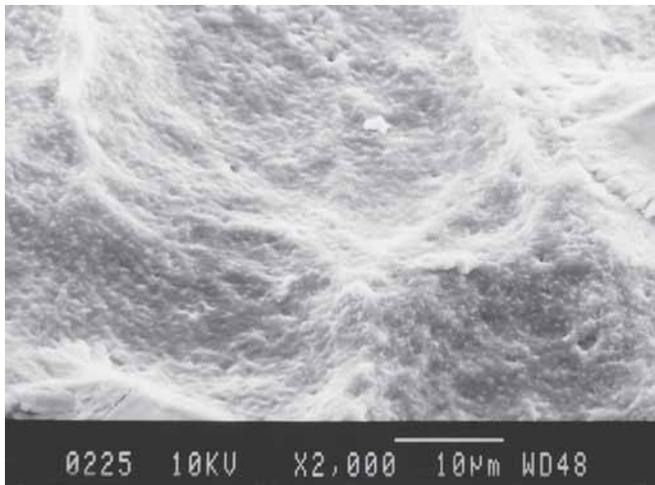


Fig. 5: Tooth surface exposed to old McInnes bleaching agent for 30 minutes. Scalloped dentinal surface is seen (x2000 magnification)

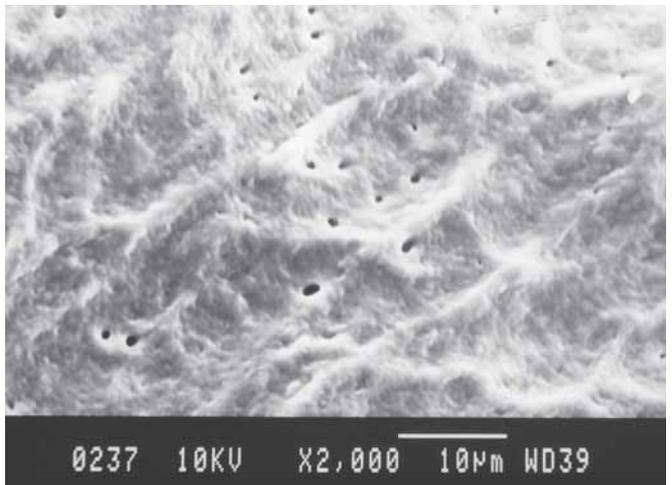


Fig. 8: Tooth surface exposed to old McInnes bleaching agent for 60 minutes. Scalloped dentinal surface with opening of dentinal tubules seen (x2000 magnification)

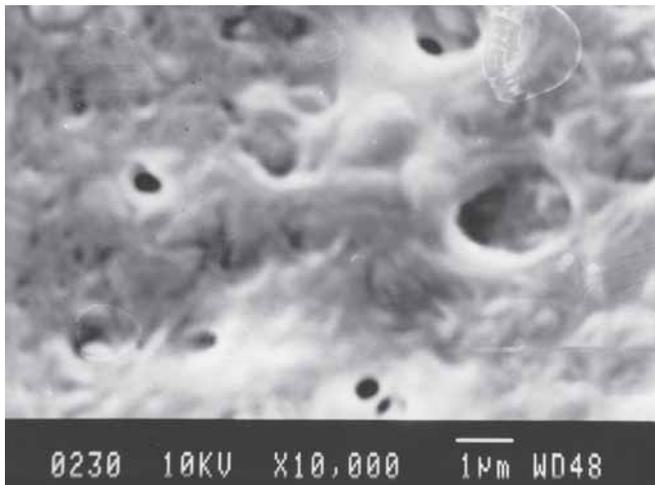


Fig. 6: Tooth surface exposed to old McInnes bleaching agent for 30 minutes. Opening of dentinal tubules seen (x10,000 magnification)

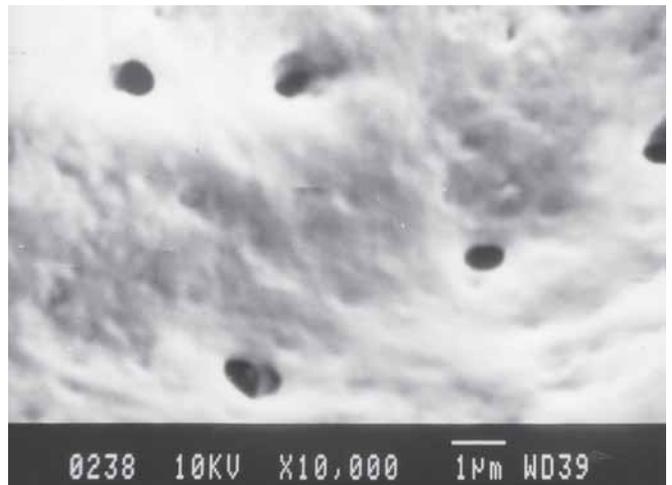
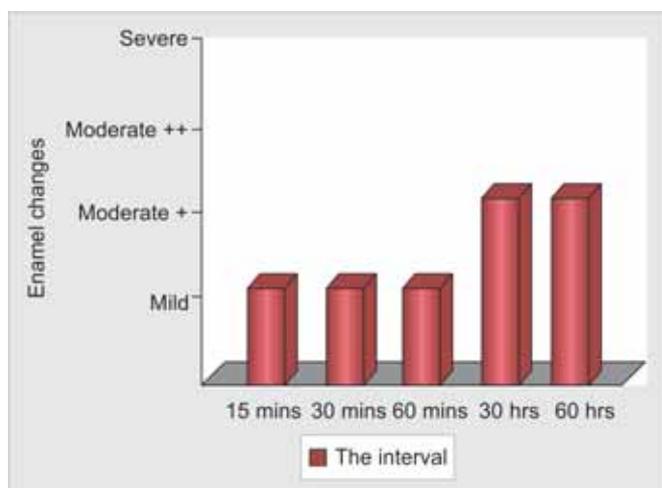


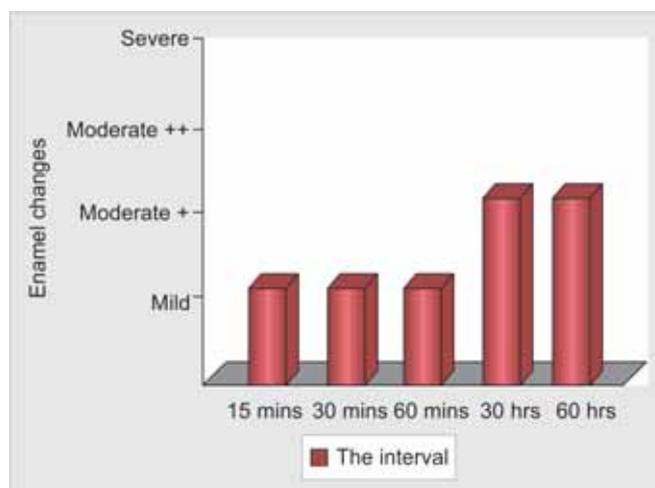
Fig. 9: Tooth surface exposed to old McInnes bleaching agent for 60 minutes. Opening of dentinal tubules seen (x10,000 magnification)

The deleterious effect on the enamel surface is mainly attributed to the 30% hydrochloric acid content of the old McInnes bleaching solution. The observations of this present study with regard to hydrochloric acid is well correlated to,

McEnvoy findings that hydrochloric acid used in concentrations ranging from 18 to 30% acts by decalcifying enamel along with the stain.<sup>18</sup> According to Griffin RE et al the decalcifying action of hydrochloric acid when used



Graph 1: Old McInnes solution



Graph 2: New McInnes solution

alone or as part of solutions containing hydrogen peroxide has been shown to cause considerable etching after 2 minutes applications *in vitro*.<sup>19</sup>

McEnvoy noted that hydrochloric acid decalcifies the superficial 16% of the enamel surface and is used to remove stains physically.

The hydrochloric acid is not selective in its mode of action and this could be the reason for the extensive surface morphologic changes when exposed to old McInnes bleaching solution.

Chen Hua ji et al<sup>20</sup> modified the McInnes solution by replacing hydrochloric acid with 20% NaOH solution to overcome the deleterious effect of hydrochloric acid. The modified new McInnes bleaching solution consisted of 30% hydrogen peroxide and 20% NaOH in a 1:1 ratio. The oxidizing ability and releasing of oxygen from hydrogen peroxide is stronger in a basic solution than acidic solution. They suggested that this mixture was effective in bleaching of stains that respond to hydrogen peroxide. Their study concluded that the decomposition of 30% hydrogen peroxide in a 20% NaOH solution was faster and more violent than in old McInnes solution.<sup>21</sup>

In the present study, the enamel surface was exposed to modified McInnes bleaching agents for a longer time intervals of 15, 30 minutes, 24 and 30 hours respectively (Graph 2).

The modified McInnes bleaching solution resulted in mild-to-moderate enamel, changes when exposed at different time intervals. Mild changes were observed up to 1 hour, where there was alteration of aprismatic layers of enamel surface, whereas exposure of up to 24 and 30 hours resulted in moderate changes consisting of increased porosities, deepening of fissures and opening of enamel prisms (Figs 10 to 21).

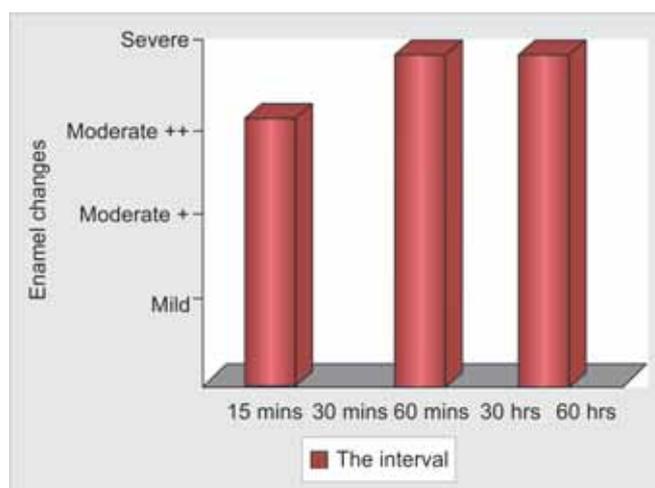
Our observations regarding the new McInnes bleaching solution are well correlated with the findings of Ji Chen et al who suggested, that new McInnes had lesser deleterious effects on the enamel surface.<sup>22</sup>

According to McEnvoy AS hydrogen peroxide being a strong oxidizing agent, the process by which it works within the enamel or dentin is most likely an oxidation process<sup>23,24</sup> which removes the stain by its oxygen releasing, mechanical cleaning action. Hydrogen peroxide is more selective than hydrochloric acid in stain removal; this mechanism of action makes hydrogen peroxide method a safe and conservative approach.

Studies have shown that teeth placed in labeled hydrogen peroxide shows increased permeability.<sup>25,26</sup> This has been attributed to the low molecular weight of hydrogen peroxide (30 g/mol) and its ability to denature proteins, thereby increasing the movement of ions through the tooth.<sup>27</sup>

Haywood BV and Heymann's clinical observations through 75 years of bleaching with 35% of hydrogen peroxide bleaching agent have never revealed any detrimental effect on enamel surface texture.<sup>28</sup>

The manufacturers of Nupro Gold tooth whitening system (10% carbamide peroxide gel) have recommended a day time schedule – exposure to the agent for at least 2 hours, and a night time schedule throughout the night, until the desired result is obtained.



Graph 3: 10% carbamide peroxide gel

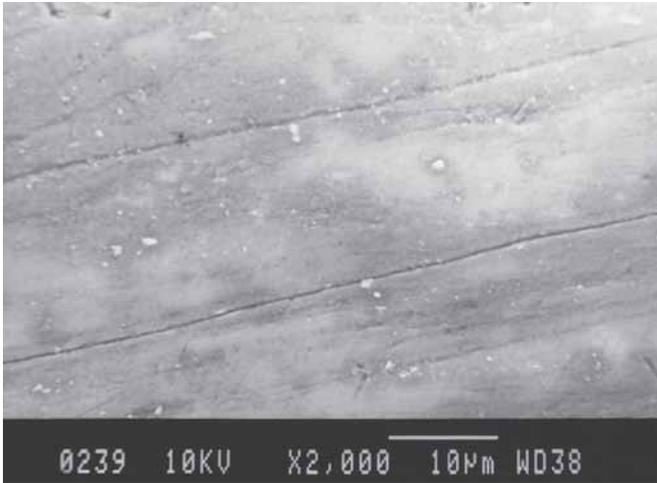


Fig. 10: Tooth surface control group II (x2000 magnification)

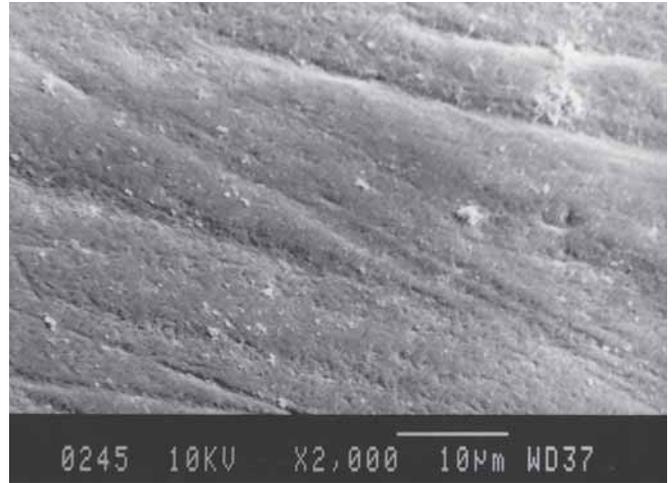


Fig. 13: Tooth surface control group II (x2000 magnification)

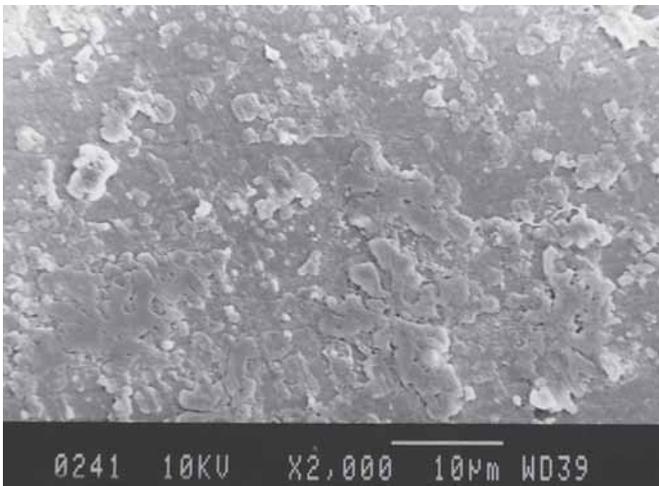


Fig. 11: Tooth surface exposed to new McInnes bleaching agent for 15 minutes (x2000 magnification)

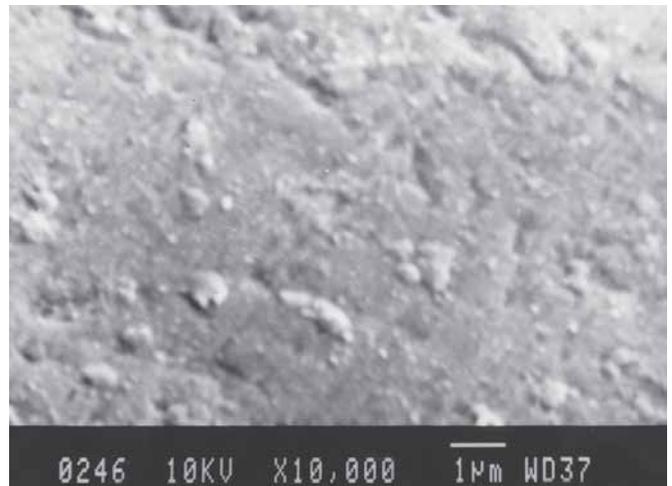


Fig. 14: Tooth surface subjected to new McInnes bleaching agent for 30 minutes (x10,000 magnification).

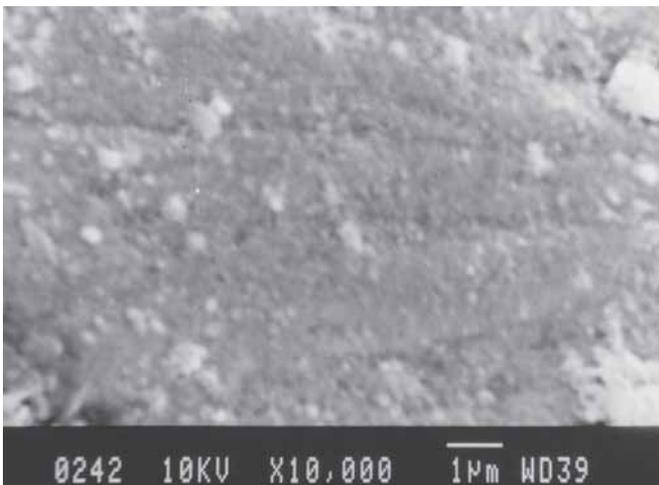


Fig. 12: Tooth surface exposed to new McInnes bleaching agent for 15 minutes (x10,000 magnification)

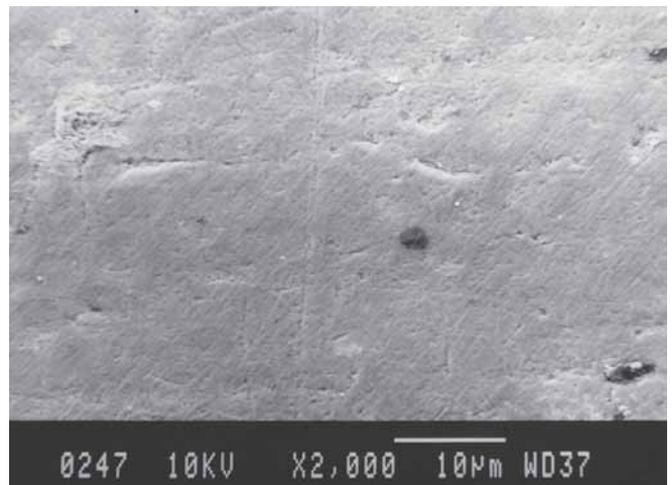


Fig. 15: Tooth surface control group II (x2000 magnification)

In this present study the enamel surface was exposed to 10% carbamide peroxide gel for time of 15, 30, 60 minutes, 24 and 30 hours (Graph 3).

The carbamide peroxide bleaching gel cause mild-to-moderate changes, which was more marked as the duration of exposure increased (Figs 22 to 31).

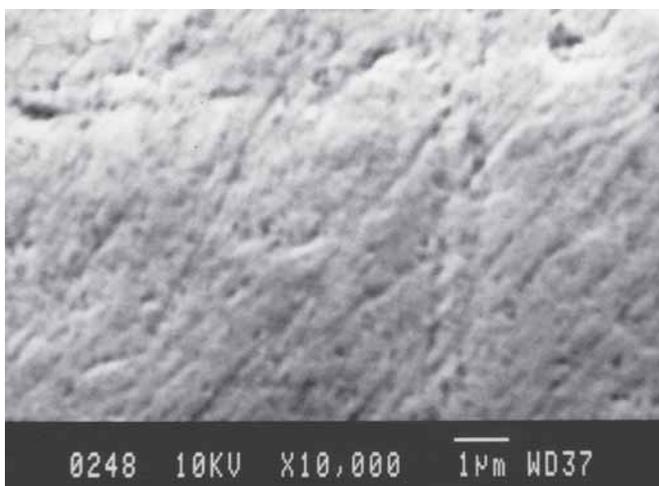


Fig. 16: Tooth surface subjected to new McInnes bleaching agent for 60 minutes (x10,000 magnification)

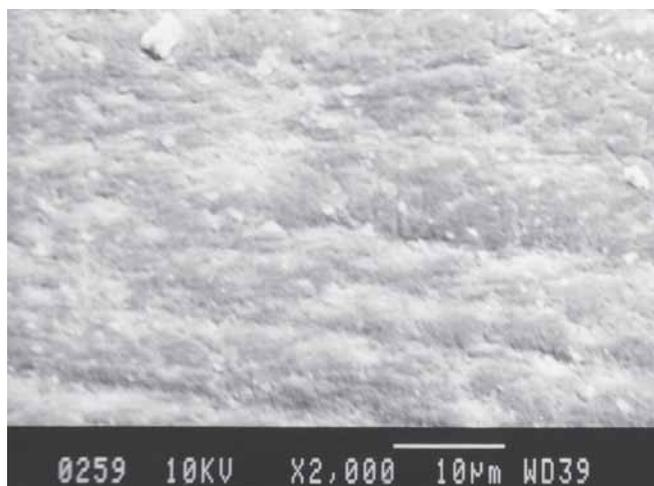


Fig. 19: Tooth surface control group II (x2000 magnification)

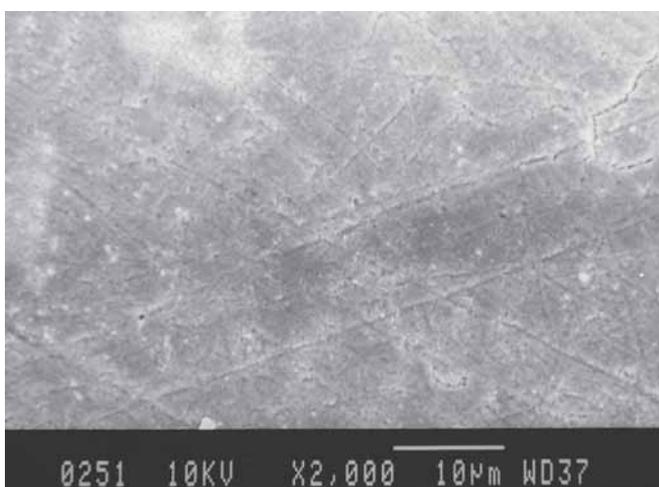


Fig. 17: Tooth surface control group II (x2000 magnification)

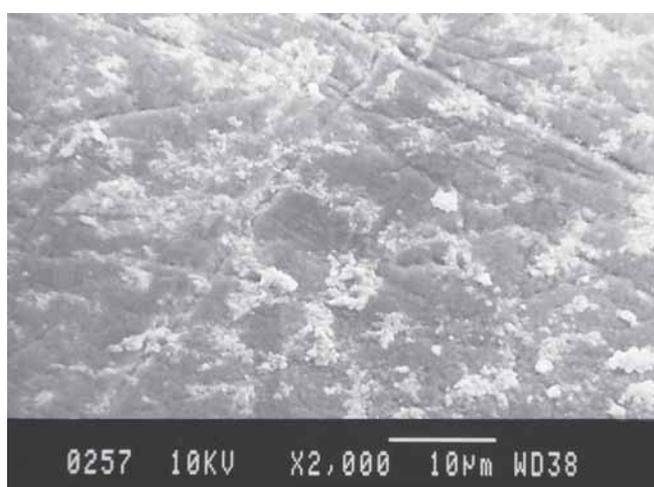


Fig. 20: Tooth surface exposed to new McInnes bleaching agent for 30 hours (x2000 magnification)

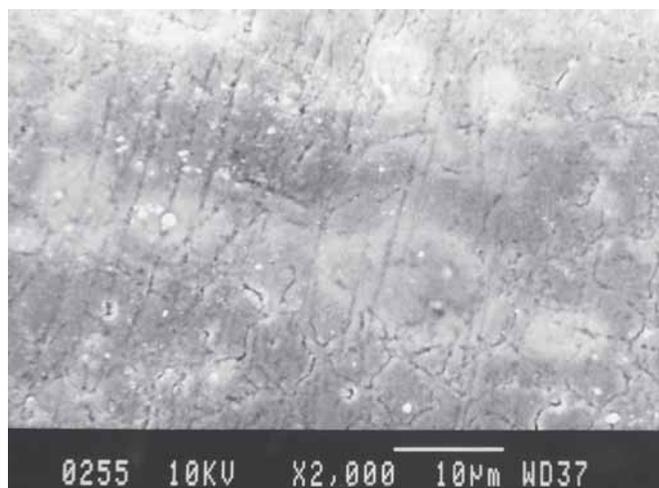


Fig. 18: Tooth surface subjected to new McInnes bleaching agent for 24 hours (x100,000 magnification)

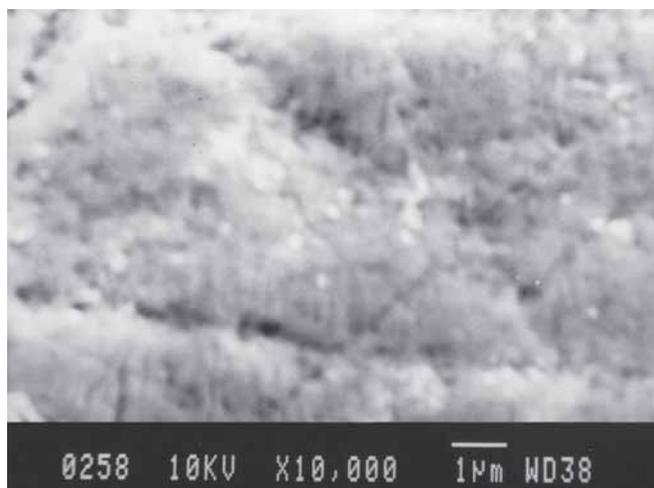


Fig. 21: Tooth surface exposed to new McInnes bleaching agent for 30 hours (x10,000 magnification)

Researchers have studied the effect of carbamide peroxide on human enamel, though their conclusions varied.

Haywood et al showed no changes in surface morphology of human enamel outside that of normal variations using SEM evaluations in two studies.<sup>29</sup>

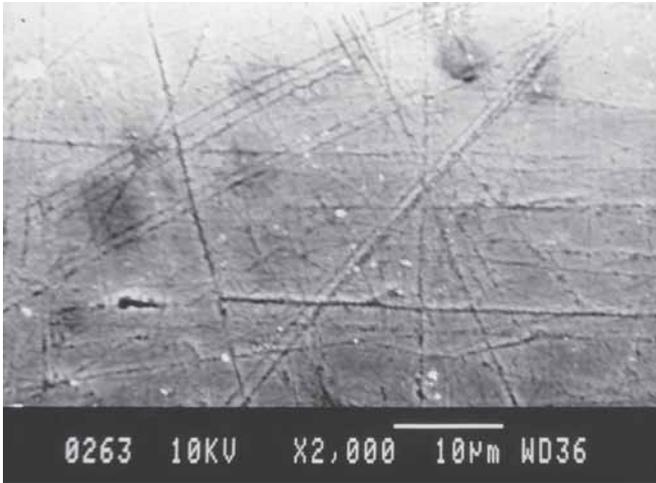


Fig. 22: Tooth surface control group III (x2000 magnification )

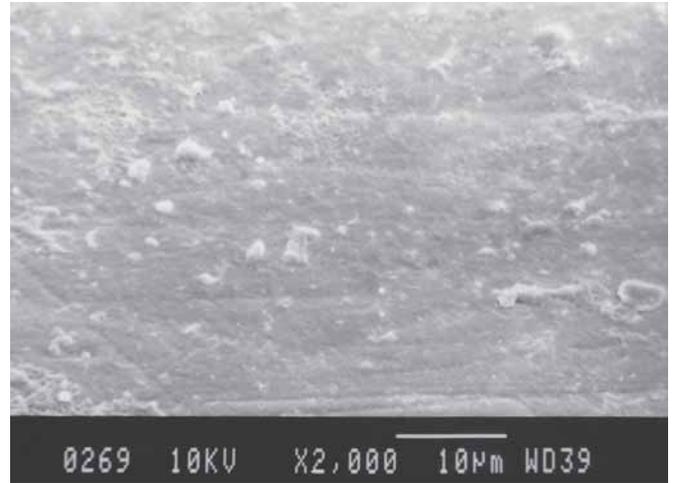


Fig. 25: Tooth surface subjected to 10% carbamide peroxide gel for 30 minutes (x2000 magnification)

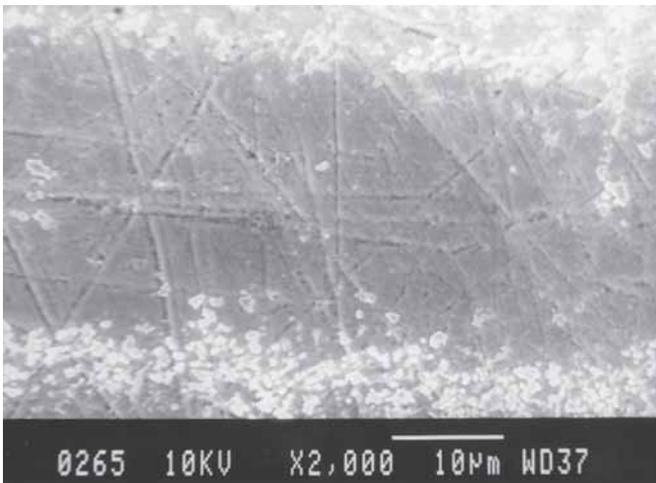


Fig. 23: Tooth surface subjected to 10% carbamide peroxide gel for 15 minutes (x2000 magnification)

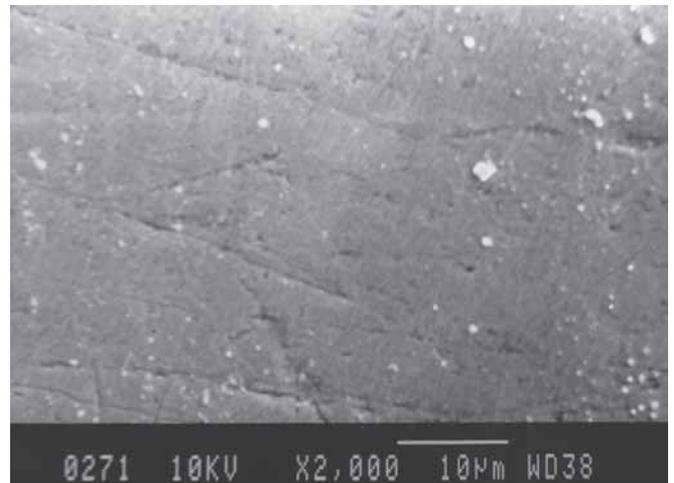


Fig. 26: Tooth surface control group III (x2000 magnification)

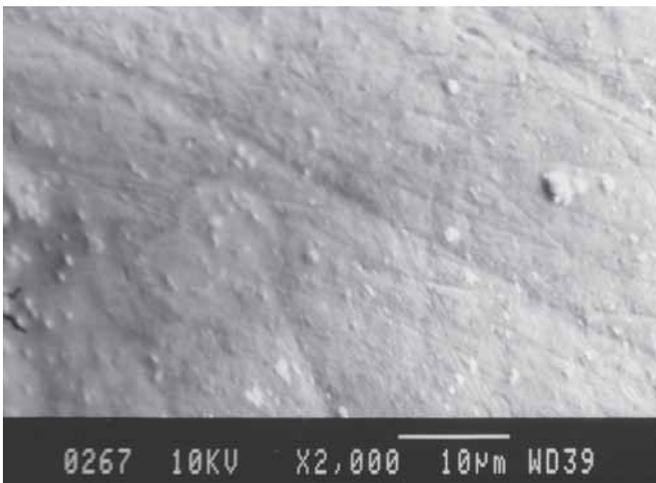


Fig. 24: Tooth surface control group III (x2000 magnification)

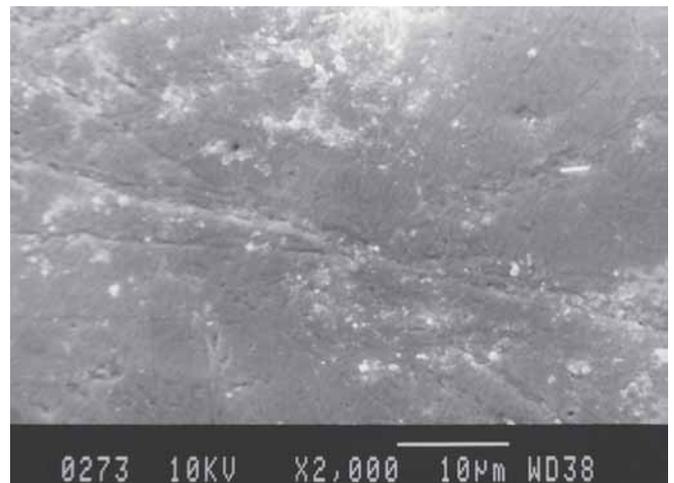
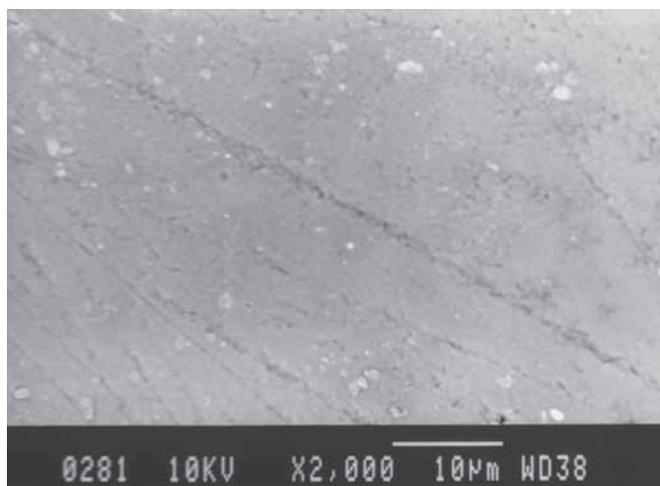


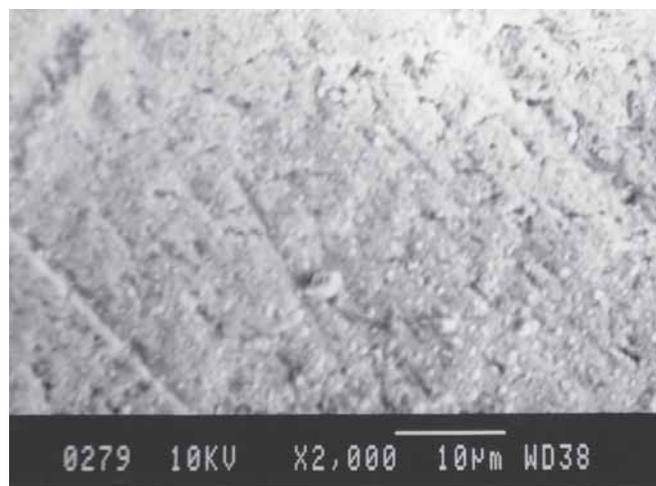
Fig. 27: Tooth surface subjected to 10% carbamide peroxide gel for 60 minutes (x2000 magnification)

Shannon et al demonstrated slight morphological changes using SEM analysis. Covington et al reported changes in surface morphology utilizing SEM analysis, but did not find major changes in the composition of human analysis.<sup>30</sup>

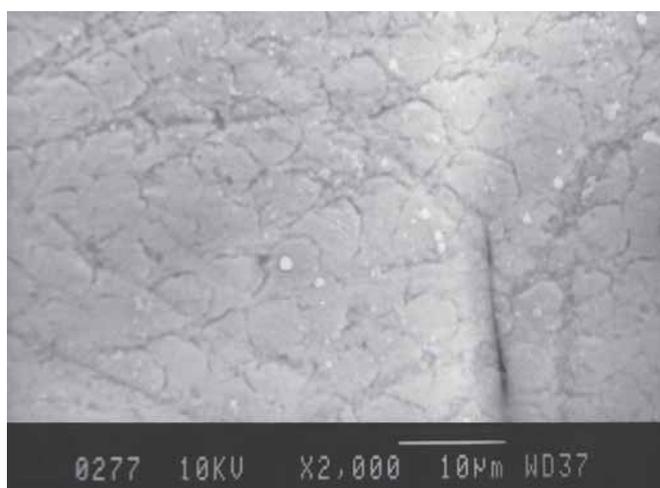
The observations in this present study is in agreement with the study of Bitter CN who evaluated the effect of carbamide peroxide bleaching agent on the enamel surface and observed alterations of direct relation to length of time



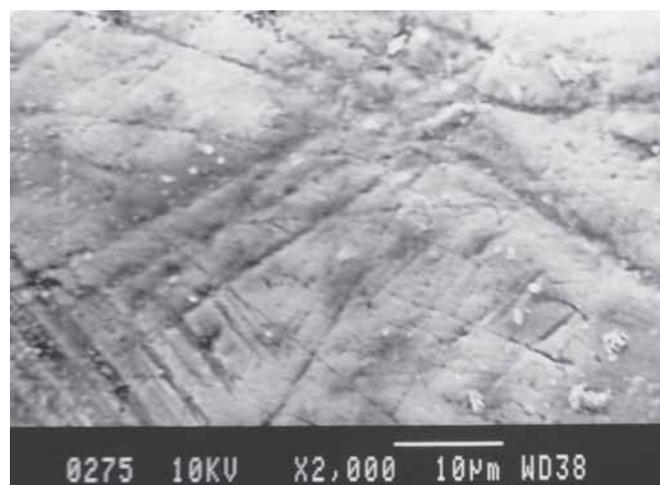
**Fig. 28:** Tooth surface control group III (x2000 magnification)



**Fig. 31:** Tooth surface subjected to 10% carbamide peroxide gel for 30 hours (x2000 magnification)



**Fig. 29:** Tooth surface subjected to 10% carbamide peroxide gel for 24 hours (x2000 magnification)



**Fig. 30:** Tooth surface control group III (x2000 magnification)

that the enamel surface was exposed to the bleaching agent. Ten percent carbamide peroxide bleaching agent ultimately breaks down into 3% hydrogen peroxide and 7% urea, it

has been shown that urea also moves freely through both enamel and dentin. Urea also has a low molecular weight (64 g/mol).

Haywood and Haymann reported concern regarding the bleaching solution with a lower pH, based on reports that the demineralization process of enamel begins when the pH falls below the critical pH of 5.2 to 5.8. However, no evidence of this process has been noted to date in any clinical trails or laboratory tests.

One explanation for this finding may be in the breakdown products of urea from carbamide peroxide. The ammonia and carbon dioxide released on degradation of the urea appears to have the effect of elevating the pH.

Stephan reported that the application of urea raises the pH of plaque material, even in presence of carbohydrate to as high as 9.0. The absence of perceptible damage on the enamel surface when exposed to carbamide peroxide may be due to the oral conditions of the patient.

Tencate JM and Duijsters have shown that fluoride concentration of 2 ppm at pH 4.5 is sufficient to effectively inhibit demineralization.

Therefore the absence of perceptible damage to the enamel may be to be related to the fact that:

1. The pH of the carbamide peroxide bleaching solutions rises rapidly upon exposure to oral fluids and is related to the concentration of urea that is present.
2. Demineralization is inhibited by the fluoride concentration in the tooth.

More recent studies conducted at the University of North Carolina have shown no indication of either etching or significant change in surface morphology of enamel when evaluated under scanning electron microscope at magnification of x100, x200, x1000 and x4000 after 6 weeks treatment with various bleaching agents. These included

the 10% carbamide peroxide preparations and hydrogen peroxide containing preparations.

The present study is in agreement with the above observations, in relation to new McInnes bleaching agent and carbamide peroxide gel, which did not produce any significant damage to the enamel.

## CONCLUSION

The following conclusions can be drawn from the present study:

1. The dissolution and increased porosity of the enamel structure should be seriously considered when recommending the use of bleaching agents. The added effect of brushing in conjunction with the bleaching agents should also be weighed when considering their use.
2. The enamel surface subjected to remineralization by oral fluids or whether its surface is renewed to form a new base of enamel prism structure is open to question and a worthy subject of further investigation.

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