INTRODUCTION

Tooth bleaching has become popular among patients and dentists. Any alteration in the chroma, hue, value of tooth structure is called tooth discoloration and categorized in three categories – external tooth discoloration, internal tooth discoloration and age-related discoloration.

A dental prophylaxis performed on permanent dentition, including scaling and polishing of dentition, will only eradicate plaque, calculus and several extrinsic stains. For polishing, prophy cup is used with pumice paste. For more obstinate extrinsic and intrinsic stains, variety of methods are available such as crown, veneer and enamel micro- and macro- abrasion. Tooth bleaching technique, being one of the most conservative, esthetic and ideal solutions, has gained high patient acceptance.

Modern bleaching agents are Hydrogen Peroxide (HP), Carbamide Peroxide (CP) and Sodium Perborate (SP). During the process of bleaching, HP acts as an active oxidizing agent. It converts the carbon double bond compound, which is usually yellow in color, to colourless hydroxyl group; or simply, a long yellow colour organic chain into a short colourless chain. This oxidizing reaction diffuses through the organic matrix of enamel because of its small molecular weight and may not only oxidize the chromogens but also cause destruction of the enamel organic matrix and affects the morphology of enamel.

Bleaching agents alter the composition of enamel by decreasing its calcium, phosphate and fluoride contents thus reducing its micro-hardness and disturb, its morphology. This effect could be compensated by remineralizing effect of artificial saliva. Absorption and adsorption of calcium and phosphate from artificial saliva repairs the micro-structural defects caused by bleaching.

There are two common methods (qualitative and quantitative) for analyzing enamel and dentin surface morphology. Scanning Electron Microscopy (SEM) is a quick and suitable method for qualitative analysis of the surface morphology of enamel and dentine specimens.
after bleaching. A focused beam of electrons interacts with atoms in the sample, generates a range of signals that can be identified and have information about the sample's surface topography and composition.8

Previous investigators had reported that the bleaching procedure had caused morphological alteration with increased porosity that resulted in reduction of micro-hardness.9,10 While, others had reported no alteration or slight change on the enamel surface.11 Inspite of the fact that bleaching procedure does not cause macroscopically visible defects, microscopic alteration and surface roughness may predispose to extrinsic staining and plaque accumulation which lead to periodontal disease and carries.

The hypothesis was that different bleaching agents and methods of their application affect the enamel surface micro-morphology.

The objective of current study was to assess the effect of home-use bleaching agent containing 16% carbamide peroxide and in-office bleaching agent with 38% hydrogen peroxide on surface micro-morphology of enamel after bleaching.

METHODOLOGY

This experimental study was conducted at Material Engineering Department of NED University of Engineering and Technology, Karachi. Surface morphology was analyzed in Centralized Science Laboratory of Karachi University, Pakistan.

Forty-five sound human third molars were stored in thymol solution (Buffered 0.1%, pH 7.00) for about one week; storage and handling of extracted teeth were done according to ISO/TS 11405. Hard and soft deposits were removed with ultrasonic scaler. Only crowns of the teeth were used in this study. The transversal section was made at cementoenamel junction, dividing the root and coronal portions, with the help of digital low speed cutting saw (MTI Corp, USA) under water spray. The pulp was removed from its chamber with the help of flexible barbed broaches (XX Fine, Dentsply; USA) and longitudinal sections were made using diamond disk in digital low speed cutting saw (MTI Corp, USA) under water spray to obtain enamel slabs measuring (3 mm x 3 mm).

The slabs were embedded in polystyrene resin (Allied High Tech Product Inc, USA) by using 2.0 cm diameter PVC molds, leaving the external enamel surfaces uncovered by the resin. After 24 hours, the slabs were removed from the molds, smoothened with sand papers 1200, 2400, 4000, (Allied High Tech Product Inc, USA) and polished with 1 µm, 0.3 µm and 0.05 µm alumina polishing paste (Allied High Tech Product Inc, USA) to obtain the smooth surface. The inclusion criteria were only crown of teeth used and teeth without any carious lesion and crack with intact buccal surface and not subjected to any pre-treatment chemical agent, e.g., hydrogen peroxide. Both genders were included, irrespective of both the jaws. Exclusion criteria were teeth with fractured crowns, cracks, chipped surface and grossly carious or restored teeth.

Ninety dental enamel slabs were prepared, with a standardized area of 9 mm² (3 mm x 3 mm). The slabs were examined with stereomicroscope (Motic DMW-143-FBGC, Hong Kong) at 20x magnification in order to exclude those with cracks and stains.

The composition of artificial saliva is shown in Table I. It was made as described previously by Klimek et al.12 Each 50 ml experimental tube was filled with 20 ml artificial saliva for storing the samples. Each tube contained one sample to ensure proper immersion.

Ninety enamel slabs were then randomly divided into 3 groups. Each group contained 30 specimens (n=30). It was kept in a humid environment for 1 day until the beginning of the bleaching regimens. Control group was kept in artificial saliva at 37°C in incubator (Memart, Germany) during whole experiment. Group 2 was treated with power whitening gel (White Smile 2011, Germany). The gel was applied with the help of given syringe on the enamel slabs of group 2, left for 15 minutes then the gel was removed with the help of deionized water and the procedure was repeated for four times according to manufacturer’s instruction. Group 3 was treated with tooth whitening pen (White Smile 2011, Germany). Enamel slabs were brushed with tooth whitening pen for 2 minutes, left for 20 minutes then washed with deionized water. After bleaching session, specimens were thoroughly rinsed with deionized water again for 10 seconds and stored in artificial saliva at 37°C in incubator. Artificial saliva was changed after every two days. The specimen was kept in artificial saliva to evaluate possible re-mineralizing effect of

**Table I: Chemical composition of the artificial saliva used as storage medium.**

<table>
<thead>
<tr>
<th>Chemicals used</th>
<th>In gm/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.580</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.170</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.160</td>
</tr>
<tr>
<td>KCl</td>
<td>1.270</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.160</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.330</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.030</td>
</tr>
<tr>
<td>Urea</td>
<td>0.200</td>
</tr>
<tr>
<td>Na₃HPO₄</td>
<td>0.340</td>
</tr>
<tr>
<td>Aqua desta</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Table II: Manufacturer, concentration and bleaching regimen of the whitening products used in this study.**

<table>
<thead>
<tr>
<th>Bleaching agent</th>
<th>Concentration</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power whitening gel (White Smile 2011, Germany)</td>
<td>38% Hydrogen peroxide</td>
<td>4 applications (15 minutes each) per session</td>
</tr>
<tr>
<td>Tooth whitening pen (White Smile 2011, Germany)</td>
<td>16% Carbamide peroxide</td>
<td>1 application (20 minutes)</td>
</tr>
</tbody>
</table>
The enamel surface after bleaching with 16% CP showed minimal surface alteration compared to the control. However, some random depression and spots were noticeable in SEM photos after application of 16% CP in comparison to 38% HP. However, no surface alteration and prism exposure were seen.

Frequency and percentage of all groups are shown in Table IV.

## DISCUSSION

In the present study, all tests were done on extracted (non-vital) teeth due to the impossibility of performing SEM on teeth in patient’s mouth. Comparison the results attained from non-vital to vital teeth could be a problem. Although, previous researches have stated that there are no large differences between shades of teeth before and after extraction. Therefore, we also considered that teeth in mouth would not show large differences in terms of their mechanical, chemical and physical properties after extraction. Thus, future researches will have to be executed in order to confirm this hypothesis. For in-vitro studies, demineralized water and saliva are considered to be ideal storing solutions.

In present study, neutral pH artificial saliva was used because it is considered to create no surface changes on enamel. Furthermore, pH and the mineral content of saliva can protect the enamel surface from possible negative effect during storage time in vitro. The remineralizing capability of saliva is actually in its calcium and phosphorus-saturated contents.

Duschner et al. in 2006 and Nucci and colleagues in 2004 had reported that lower levels of bleaching agent like 6.5% HP and 6.0% HP; 25% CP, 15% CP, and 10% CP were also shown to have no significant effects on enamel surface morphology after simulated product usage. The results of current study were similar to Duschner and Nucci study results as human whole saliva was used as a key part for mimicking oral environment.

Yeh and colleagues have reported some changes that described as mild or slight localized area of pitting in morphology of enamel after treatment with HP or CP. The results of current study were in disagreement with Yeh et al. results for bleached samples and showed minimal changes without any pitting. The difference may be attributed due to storage of their samples between bleaching sessions in distilled water and low pH products.

Ben-Amar and colleagues in 1995 and Hegedus and colleagues in 1999 have concluded slight or mild changes on enamel morphology after bleaching with HP or CP. Hence, results of present study were in agreement with Ben-Amar and Hegedus findings and reported negligible changes on enamel surface and these changes were considered to be within the normal difference existing in natural teeth.

Turkun et al. has suggested slight raise in enamel porosity after 14 days of treatment with 10% CP,
immediately. However, findings of present study were not similar to Turkun as there was no increase in enamel porosity. The difference in results were possibly due to variation in methodology related to exposure, pH of solution, teeth type, and storage medium.\(^{22}\)

Shannon and colleagues and Zalkind and colleagues have reported most severe changes in enamel topography after 7 days’ bleaching with 30% HP. The findings of current study were in disagreement with the results of Shannon and Zalkind, as the discrepancy might be attributed due to use of low pH (4.3) bleaching solution in their studies.\(^{16,23}\)

In the current study, enamel specimens have shown a negligible difference in between control and bleached groups. The results of current study were in agreement with Akal and colleagues and Spalding and colleagues, as they had also reported minor changes after bleaching with 30% HP (20 min) and 10% CP (12 hours/day for 1 week).\(^{24,25}\)

The results of different studies are contradictory concerning the effects that bleaching agents exert on the enamel surface. The reason for the lack of uniformity may be due to a variety of factors such as the use of non-standardized methodologies in different studies. In addition, alteration in the morphology and physical properties of the enamel can occur due to the use of enamel samples at different age groups (teeth erupted or not), immediate re-mineralizing effect of saliva after removal of the bleaching agent, and the pHs of the product as well as consumption of some erosive food products.

**CONCLUSION**

Bleaching caused a mild morphological change on molar crowns surface, which was not significant.

**REFERENCES**