SURFACE TOPOGRAPHY AND THICKNESS OF HYBRID LAYER ASSESSMENT AFTER DENTIN PRETREATMENT WITH EGG SHELL POWDER AS DESENSITIZING AGENT

Ramy Abdallah*, Hadeel Farouk** and Mona E Essa***

ABSTRACT

Objectives: The aim is to investigate the effect of Eggshell powder solution (ESPS) desensitizing agents as dentin surface pretreatments on thickness of hybrid layer of a direct resin composite restoration bonded with etch and rinse adhesive systems.

Methods: A flat dentin occlusal surface will be prepared on extracted molar using a low speed diamond saw under water coolant. Teeth will be randomly divided into three main groups according to dentin surface pretreatment. First group, no dentin pretreatment (A1), second group Egg shell pretreatment applied before acid etching (A2) and third group, Egg shell pretreatment applied after acid etching (A3). A Teflon mold will be used to build up resin composite directly. Teeth will be sectioned perpendicular to adhesive joint to obtain 0.9±0.1 mm in thickness slabs. All samples will be examined under scanning electron microscope (SEM) and thickness of hybrid layer will be measured. Moreover energy dispersive analytical x-ray (EDAX) analysis will be used to count different minerals at tooth restoration interface.

Results: The SEM analysis showed that; there are established significant differences in the thickness of hybrid layers among all tested groups. The EDAX analysis showed increased quantitative amounts of Ca atomic % and P atomic % for the ESS treated groups.

Conclusion: Eggshell solution when used as desensitizing agent can significantly increase the hybrid layer thickness.

KEY WORDS: Eggshell powder, Dentin, Hybrid layer thickness, Desensitizing agent.

INTRODUCTION

Dentinal tubules play a major role in transferring stimuli and irritants to the pulp (1). One of the most common cause of postoperative dentin hypersensitivity (DH) of tooth that restored with resin-composite which pretreated with the etch-and-rinse adhesive system, the higher dentinal permeability of dentin surface; in combination with, the incomplete sealing of the opened dentinal
tubules, so; the dentinal fluid transudation with the un-polymerized adhesive may lead to formation of blisters along the adhesive-tooth interface that filled with water (2-4).

The hydrodynamic theory that considered the most accepted theory that can explain the dentin hypersensitivity (1,5) was used to illustrate the effect of water-filled blisters as follow; during mastication; these blisters may compressed causing higher rate of dentinal fluid movement within the dentinal tubules indirectly stimulating the extremities of the pulp nerves causing the postoperative dentinal hypersensitivity (4).

Dentinal hypersensitivity (DH) can be defined as “the short exaggerated, painful response elicited when exposed dentin is subjected to certain thermal, mechanical or chemical stimuli” (6).

The treatment and prevention of DH using the concept of dentin desensitization through the mechanical occlusion approach of the dentinal tubules by using chemical or physical occluding agent that can reduce the DH through reducing the dentin permeability and the movement of pulpal fluid (7,8).

The Eggshell powder (ESP) and Nano-hydroxyapatite (Nano-HA) containing dentine like substance are examples to the physically approach which plugs the open dentinal tubules with a calcium and phosphate that precipitated and prevent the fluid diffusion through the tubules into the dentin subsurface (9,10).

Eggshell is a rich source of minerals, mainly calcium carbonate and is probably the best natural source of calcium (11). The eggshell contains mainly calcium, phosphorous, magnesium, strontium, and fluoride (9,12). Demineralization resulting from loss of calcium and phosphate can be restored by using non-invasive calcium phosphate delivery system (12,13), like eggshell solution (14).

Dentinal hybrid layer (HL) is a mixture of polymerized resin infiltrated in partially demineralized dentin at a molecular-level (15). Both of smear layer and the mineralized dentin don’t allow monomer diffusion into the collagen network (16). Therefore, dentine “conditioning” must be done to remove or modify of the smear layer and to demineralize the mineralized dentin surface, thus permitting monomer diffusion into the demineralized collagen network and formation of hybrid layer (15,16).

The etch-and-rinse approach (gold standard approach) can readily be recognized by an initial etching step, the so-called “conditioning step”, followed by a compulsory rinsing phase (17). After the conditioning step, adhesion-promoting monomers are applied in one or two application steps to penetrate the exposed collagen network (17,18).

The process of dentin hybridization completely changes the physical and chemical properties of dentin and hence can help in dentin desensitization (12,15).

MATERIAL AND METHODS

Materials which are used in the present study have been illustrated in Table (1).

**Egg Shell Solution Preparation**

1- Calcination Process

This process used to prepare the calcium oxide from chicken egg shell (CES), the egg shell cleaned in tap water firstly, then kept in a hot water bath at 100°C for 10 minutes to facilitate removal of the internal protein membrane from the shell. After that; the egg shell was crashed using a sterile mortar and pestle and heated to in a hot air oven at 110°C for 12hrs (14) by furnace (Faculty of Dentistry, Beni-Suef University), at this temperature the shell becomes porous, fragile and very white in color. It is concluded from this fact that the egg shell CaCO$_3$ decomposes (decarbonation process) and gave CaO and CO$_2$, according to the reaction:

$$\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2$$ (14,19).
2- Preparation of Egg Shell Desensitizing Solution

To prepare the egg shell desensitizing solution; one gram of the previously prepared egg shell powder was dissolved in 20 ml of 4% acetic acid in a test tube. The clear fluid which is collected at the top was then transferred to a beaker and the pH of the solution was tested using a pH meter (JENWAY, 3505, Keison, UK) which was 11.7 (14, 20).

Sample Preparation

Fifteen molar teeth were selected for this study. All collected teeth were extracted for therapeutic reasons from patients of age group (35-45 years). The selected teeth were free of caries, cracks and showed no apparent hypoplastic defects. The selected teeth were thoroughly cleaned from calculus, tissue deposits, polished with pumice and rotating brush at conventional speed.

The occlusal enamel of teeth was removed perpendicular to the long axis of teeth to expose flat dentin surface at a standardized depth. Then a line was drawn on the external tooth surface 2 mm below DEJ using a caliper. The teeth were cut horizontally and flattened under copious water coolant. The occlusal tables were ground with a rotary grinding milling machine using #180-grit silicon carbide papers under continuous water coolant to create a uniform thickness of smear layer (4, 21).

The teeth were divided into three main groups of 5 teeth each; according to the surface pretreatment done namely; first group, no dentin pretreatment (A1), second group, Egg shell pretreatment applied before acid etching (A2) and third group, Egg shell pretreatment applied after acid etching (A3).

Etch and rinse adhesive bonding

The flat dentin occlusal surfaces were etched (conditioning) using Scotch bond universal Etchant for 15 seconds, rinsed for 10 seconds, and blotted dry with absorbent sponge pellet leaving the dentin surface visibly moist. Two consecutive coats of adhesive system (Scotch bond Universal adhesive) were applied (Group A1) using a fully saturated brush tip and gently air-thinned for 5 seconds leaving a shiny surface and then polymerized with a light-emitting diode (LED) light curing unit (Elipar LED Curing Light; 3M ESPE) for 20 seconds according to the manufacturer’s instructions (21).
Egg shell powder solution (ESPS) then passively applied on un-etched dentin using a brush for 5 minutes \(^{(9,10)}\) (Group A2). The dentin was then rinsed for 5 seconds then dried gently with absorbent paper. While (Group A3); the ESP solution applied to the etched dentin.

**Resin Composite Restorative Material Application**

A specially constructed two halves spilt Teflon round mold with a central square hole (5 mm X 5 mm in diameter and 4 mm in depth) was fabricated for resin composite build up. Nano filled visible light resin composite (Filtek Z350, 3M ESPE, USA), was built up into two increments each 2 mm in thickness on dentin wall. Each increment was packed using Teflon tipped instrument then light cured for 20 seconds using light-emitting diode Curing Light (Elipar LED Curing Light; 3M ESPE). Last increment was cured against celluloid strip matrix to avoid oxygen inhibited layer.

Each tooth was mounted on the cutting machine (Bronwill, E. McGrath Inc), and sectioned into a series of 1 mm thick slabs under water coolant. The sectioning was performed using a diamond disc of 0.3 mm thickness (IPDB40305, MTI Corporation, Richmond, USA) \(^{(10,21)}\).

Ten slabs from each group were examined using scanning electron microscope. The tooth restoration interface of each slab were air dried, mounted on aluminium stubs and sputter-coated with gold for 2 minutes and examined with a scanning electron microscope (SEM) (LEO Electron Microscopy Ltd., Cambridge, UK) operating at 10-20 kV and 4000X magnification. Thickness of hybrid layer was measured at 5 points on each slab \(^{(10,21)}\). Thickness of hybrid layer was measured from photomicrographs obtained by SEM by drawing a line form dentin side to resin composite side through hybrid layer. Six lines were drawn for each specimen.

Using the EDAX (energy dispersive analytical x-ray), the amount of Ca and P ions within the adhesive layer, hybrid layer and resin tags, in each specimen was measured in an area of (120µm X 120 µm) at 240000x magnification directly on the SEM microscope monitor \(^{(10)}\).

The data were analyzed by SPSS version 20 using One-ANOVA. The test was performed to determine a statistically significant difference in hybrid layer thickness, and Tukey’s post hoc multiple comparisons test was used to compare between more than two groups in non-related samples, test and \( P \leq 0.05 \) was considered statistically significant.

**RESULTS**

**SEM Analysis:** Results of the SEM analysis showed that; there are established significant differences in the thickness of hybrid layers among all tested groups.

The SEM analysis showed that the highest mean value of hybrid layer thickness was found in (Group A3) (35.86 µm) followed by (Group A2) (22.26 µm), and the lowest mean value of hybrid layer thickness was found in (Group A1) (14.04 µm) (Table 2). Multiple group comparison showed that there was significantly different among the all tested groups.

SEM image of the tested groups (Group A1 and Group A2) showed a continuous and homogenous hybrid layer thickness distribution without any gap formation at the dentin–adhesive interface at the scanned area. In addition, SEM image of resin/ dentin interface for (Group A3) showed a gap formation and the dentinal tubules blocked with egg-shell minerals (Figure 1).

**Atomic Analysis by EDX**

The results of atomic analysis of calcium and phosphorus in all samples by energy dispersive X-ray spectrometry showed that; although there is no significant difference in Ca/P ratio among the (Group A1) and (Group A2), but the quantitative amounts of Ca atomic % and P atomic % is greater for the ES treated groups (Group A2 and A3), while the untreated group, demineralized group, (Group A1) shows the lowest Ca and P atomic % (Table 3).
Dentin is the core hard substance of the tooth which is covered by enamel on the crown and cementum on the root. Dentin is the calcified product of the odontoblasts which line the inner surface of the dentin within the periphery of the external pulp tissue. Therefore, the dentin and pulp are morphologically and embryologically a single unit (22).

Hybridized dentin reduces the risk of microleakage, the incidence of secondary caries and post-operative sensitivity that can be caused by such leakage (23). Also, hybrid layer and resin tags formation have been reported to play a major role in resin retention (23).

Coronal dentin discs were used in this study to evaluate tubule occlusion and depth of penetration of the desensitizing agents into the tubules. Important variables such as dentin surface area, thickness, and surface characteristics can be controlled in coronal dentin discs compared to cervical dentin discs. This model was found appropriate for application of the experimental agents on a flat dentin surface and also enabled standardized comparisons of different treatment protocols with ease. This remains a time-tested screening model to study tubule occlusion by potential desensitizing agents (24).

TABLE (2): The mean, standard deviation (SD) of thickness of hybrid layer thickness in all tested groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Thickness of hybrid layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Group (A1)</td>
<td>14.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (A2)</td>
<td>22.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (A3)</td>
<td>35.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*p-value* <0.001<sup>*</sup>

*Means with different letters in the same column indicate statistically significance difference.*

TABLE (3): Ca / P atomic ratio of all tested groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ca%</th>
<th>P %</th>
<th>Ca/P atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A1)</td>
<td>5.95</td>
<td>5.66</td>
<td>1.05</td>
</tr>
<tr>
<td>Group (A2)</td>
<td>8.84</td>
<td>8.4</td>
<td>1.05</td>
</tr>
<tr>
<td>Group (A3)</td>
<td>18.93</td>
<td>11.47</td>
<td>1.65</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Dentin is the core hard substance of the tooth which is covered by enamel on the crown and cementum on the root. Dentin is the calcified product of the odontoblasts which line the inner surface of the dentin within the periphery of the external pulp tissue. Therefore, the dentin and pulp are morphologically and embryologically a single unit (22).

Hybridized dentin reduces the risk of microleakage, the incidence of secondary caries and post-operative sensitivity that can be caused by such leakage (23). Also, hybrid layer and resin tags formation have been reported to play a major role in resin retention (23).

Coronal dentin discs were used in this study to evaluate tubule occlusion and depth of penetration of the desensitizing agents into the tubules. Important variables such as dentin surface area, thickness, and surface characteristics can be controlled in coronal dentin discs compared to cervical dentin discs. This model was found appropriate for application of the experimental agents on a flat dentin surface and also enabled standardized comparisons of different treatment protocols with ease. This remains a time-tested screening model to study tubule occlusion by potential desensitizing agents (24).
Since HAp exhibits excellent bioactive properties, this may be considered the material of choice for treating hypersensitivity in the near future (25). Although various sources are available for the synthesis of HAp, chicken eggshells were chosen in this study owing to its high calcium content and cost effectiveness. These eggshell waste help in reducing the cost of high-quality calcium source and at the same time promote recycle of material (26,27).

The process of egg shell calcination is responsible for pathogens removal and an increase in its alkalinity (28,20). Also, the calcination process is responsible for calcium carbonate formation which acts as a calcium rich layer that can binds to the negatively charged dentine surface resulting in the plugging of dentine tubules and blocking diffusion of fluids and acts as desensitizing agent (10).

In the current study, the thickness of the hybrid layer formed was studied using an SEM. However, SEM has certain benefits for analyzing surfaces and interfaces (29) and has become the most popular and easiest tool to morphologically examine the bonding mechanism and bonding interfaces. Also, a few SEM studies indicating the effective thickness of hybrid layer have been published (16).

In etch-and-rinse systems, phosphoric acid treatment not only removes possible superficial debris, the so-called smear layer, but also exposes a net of collagen fibers besides opening the dentin tubules. By penetrating this collagen mesh and dentin tubules, the infiltrating resin will form two well-defined structures which are known as hybrid layer and resin tags, respectively (30,17). Collagen fiber network should be in a fully expanded state to facilitate resin penetration “wet bonding” (30,17).

The effective bonding relies mostly on the ability of the bonding agent to completely infiltrate the exposed collagen mesh, ideally sealing and protecting it from all sorts of degradation pathways; i.e. quality of hybrid layer. In the same way, it has been suggested that the bonding effectiveness does not depend on the number nor on the length of resin tags (31,32).

In (Group A1); when using etch-and-rinse systems, proper hybrid layer is classically achieved through the infiltration of resin monomers into the exposed collagen mesh by using the so-called wet-bonding technique. In this protocol, water remaining from the rinsing step maintains the collagen network expanded, allowing resin monomers to properly infiltrate it and hybrid layer formation (30,33).

The hybrid layer of (Group A2) was thicker when compared to (Group A1); may depend on effective penetration of bonding adhesive (MDP) to partially demineralized dentin (hybrid layer formation) (34), as the application of eggshell solution on dentin surface before acid etching may lead to decreasing the demineralizing action of acid etch due to the buffering capacity of the eggshell minerals and its high alkalinity. So, after rinsing, collagen fibrils are not completely deprived from hydroxyapatite, leaving residual hydroxyapatite still attached to collagen, which may serve as a receptor for additional chemical bonding (35), that emphasis by our EDX results which showed in (Table 3).

The application of carboxylic acid-based monomers “functional monomer”, 10-methacryloxydecyl dihydrogen phosphate (MDP), which included in the bonding agent (36), have a chemical bonding potential to calcium of residual hydroxyapatite (35). So, the increased thickness of hybrid layer may be due to the possibility of incorporating the residual minerals into the hybrid layer and formation of “hybridized complex” which comprises two portions: the zone of authentic hybrid layer and the zone of hybridized minerals (37,38). So, the addition of eggshell solution before etching have benefit of formation of thick hybrid layer in addition to desensitization of dentin by residual hydroxyapatite (39,40).

The thickness of hybrid layer of (Group A3) showed the maximum value when compared to (Group A1) and (Group A2), which were
statistically significant. Possibly could be due to that in case of (Group A3), the specimens were etched with phosphoric acid and the reaction products were rinsed off. This results in complete removal of smear layer and smear plugs from the dentin, so that the dentin permeability increases (2) then, after the application of eggshell solution on etched dentin surface, calcium plays an active role due to a very high percentage of bio-available calcium in ES solution (14,41).

Also, the pH of a ESP solution was 11.7, which is favorable, as it increases the ion activity of anions such as hydroxyl ions in the solution (14,41). The water which retained in collagen fibrils and in dentinal tubules act as a vehicle and facilitate the movement of calcium ion through it (3), then 10-MDP (10-methacryloxydecyl dihydrogen phosphate) (36) present in universal adhesives have a chemical bonding potential to calcium ions in both collagen fibrils and in dentinal tubules forming a thicker hybrid layer.

In (group A3); when the water inside the collagen network is not completely displaced, the polymerization of resin inside the hybrid layer may be affected or least, the remaining water will compete for space with resin inside the demineralized dentin (42). That results in gap formation shown in (Figure 1).

CONCLUSION

Under the limitations of this in vitro study, it can be concluded that all the ES solution as experimental agents can occluded the dental tubules to varying degrees and also penetrated to varying depths into the dentinal tubules forming different thickness of hybrid layer depending on its application strategy (before or after dentin etching).

Although Eggshell powder solution was beneficial regarding thickness of hybrid layer and Ca/P atomic ratio, further clinical studies are needed to determine its effectiveness over time.

REFERENCES


