

# Atomic force microscopy and tridimensional topography analysis of human enamel after resinous infiltration and storage in water

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## ABSTRACT

**الأهداف:** تقييم تأثير التخزين في الماء على خشونة السطح لمينا أسنان الإنسان بعد معالجتها بالمادة الراتنجية و ذلك بإستخدام جهاز مجهر القوة الذرية وجهاز الطبوغرافيا الثلاثي الأبعاد .

**الطريقة:** أجريت الدراسة بعد تسجيل البحث وتوضيح الموافقة على مطابقتها للمعايير الأخلاقية بمركز البحوث في كلية طب الأسنان جامعة الملك سعود، الرياض، المملكة العربية السعودية وذلك خلال الفترة من 1 يناير 2011م حتى 15 أغسطس 2011م. تم إعداد 30 عينة من سطح مينا الأسنان مأخوذة من ضواحك آدمية خالية من التسوس. تم تقسيم العينات إلى 3 مجموعات. ولقد استخدمت المجموعة الأولى للمقارنة، أما المجموعة الثانية فقد تمت معالجتها بالمادة الراتنجية (Icon®) بينما استخدمت مادة SealRite™ على أسطح المينا للمجموعة الثالثة. تم تخزين جميع العينات في الماء المقطر لمدة 6 أشهر ومن ثم فحصت جميع العينات بواسطة مجهر القوة الذرية (AFM Veeco CP11 1.2). وبعد ذلك تم مسح بعض العينات باستخدام مجهر الأشعة السينية الشعاعي skyscan-1072. سجلت متوسط قراءات خشونة السطح وأجري التحليل الإحصائي (Package for Social Sciences Version 16) عند مستوى الدلالة الإحصائية  $p < 0.05$ .

**النتائج:** لم تسجل أية فروقات ذات دلالة إحصائية في متوسط معدل الخشونة بين المجموعات الثلاث، (المجموعة الأولى =  $0.21 \pm 0.057$ )، (المجموعة الثانية =  $0.23 \pm 0.075$ ) و (المجموعة الثالثة =  $0.20 \pm 0.039$ ) عند  $p = 0.747$ . كما أظهرت نتائج صور سطح المينا المسجلة بواسطة AFM طبقة رقيقة وغير متجانسة للمادة الراتنجية في المجموعة الثانية، وفي الوقت نفسه، أظهرت المادة الراتنجية للمجموعة الثالثة طبقة متجانسة في جميع العينات. هذا وقد دعمت صور المجهر الشعاعي نتائج جهاز AFM.

**خاتمة:** إن تواجد مادة SealRite في جميع العينات يساعد على انخفاض ذوبانها في الماء كما أن لها تأثير وافي على سطح المينا.

**Objectives:** To evaluate the effect of water storage on surface roughness (Ra) of human enamel after treatment with resin infiltrant and fissure sealant, by utilizing atomic force microscopy (AFM) and microtomography.

**Methods:** This study was conducted after registration and ethical approval clarification at the College of Dentistry Research Center, King Saud University, Riyadh, Kingdom of Saudi Arabia between January 2011 and August 2011. Thirty enamel surface specimens were prepared from caries-free human premolar teeth. Specimens were divided into 3 groups: Group I, was the control; Group II, a resin infiltrant (Icon®) was applied on the enamel surfaces; and Group III, the teeth were treated with fissure sealant (SealRite™). All specimens were stored in distilled water for 6 months and then, subjected to AFM Veeco CP11 1.2 analysis. A few specimens were scanned by skyscan-1072-x-ray microtomography. The Ra mean readings were recorded and statistical analysis was performed with the Statistical Package for Social Sciences Version 16 at the significance level of  $p < 0.05$ .

**Results:** No significant differences in the mean Ra were recorded among the 3 groups, (Group I =  $0.21 \pm 0.057$ ), (Group II =  $0.23 \pm 0.075$ ), and (Group III =  $0.20 \pm 0.039$ ) at  $p = 0.747$ . The AFM images of enamel surface show thin and inhomogeneous Icon resin in Group II, meanwhile, the SealRite in Group III showed a homogeneous layer in all specimens. The microtomography supported the findings of the AFM images.

**Conclusion:** The persistence of the SealRite in all specimens revealed its low solubility in water and its protective effect on enamel surface.

*Saudi Med J 2013; Vol. 34 (4): 408-414*

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*Received 10th December 2012. Accepted 2nd March 2013.*

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Enamel remineralization has been studied for approximately 100 years, and it has been suggested that the non-invasive treatment of early caries lesion by remineralization has the potential to be the major advance in the clinical management of the disease.<sup>1</sup> Minerals are dissolved out of the enamel during the development of subsurface caries lesion. These lesions appear clinically as white-spot lesions which are the result of increased porosities in the enamel surface.<sup>2</sup> White-spot lesions (WSLs) are the early stage of caries development characterized by an intact enamel surface with subsurface demineralization. Clinically, they may or may not be associated with surface roughness (Ra). White-spot lesions occur in many locations, they are most commonly found in fissure pits, proximal contact areas or cervical above the gingival margins. It is recognized that these lesions, although having visually intact enamel surfaces, are structurally weaker than sound enamel and that due care is required during examination and treatment.<sup>3</sup> Preventing iatrogenic cause of cavitations as a result of probing or ultrasonic cleaning is needed. Also, these lesions are commonly treated by enhancing remineralization for example, improving individual oral hygiene and diet.<sup>3</sup> It was stated in previous study<sup>4</sup> that enamel demineralization creates a surface layer defective and porous. This layer may permit the acid produced by bacteria to diffuse more freely along prisms and intercrystal spaces, affecting the enamel subsurface. Robinson et al<sup>5</sup> were the first to describe the infiltration of carious lesions with organic resins and demonstrate a reduction in pore volume following the application of resorcinol-formaldehyde resin. It was stated by Muller et al,<sup>6</sup> that the resin was not suitable for clinical use due to its toxic nature. Sasal et al,<sup>7</sup> recommended that sealants can be placed on teeth that are considered to be “at risk” to develop caries, including teeth that present with incipient enamel lesions. It could be assumed that only the infiltrated material influenced the progression of demineralization. Therefore, complete infiltration of a sealant should be the goal.<sup>6</sup>

In recent years, the promising approach called resin infiltration includes the application of some light-curing resins, such as dental adhesives or sealants, has

proven to hamper or even arrest demineralization. As manufacturer claimed “by means of a new virtually painless method, the caries infiltration, the product Icon<sup>®</sup> was introduced in Germany in 2009.<sup>8</sup> This product is based on a special resin with which diseased enamel can be filled and sealed. The revelatory aspect: there is no unnecessary loss of healthy hard tissue (DMG, 2009). The resin has been in the market since March 2009 and has high penetration coefficient.<sup>9</sup> This new treatment option is promising since it showed approximately the same micro hardness compared to sound enamel and a clinically acceptable surface Ra.<sup>10</sup> Also, it was concluded that the surface quality of non-processed infiltrants seemed to be perfectible.<sup>11</sup>

Hashizume et al<sup>4</sup> stated that “mature human dental enamel has a high degree of mineralization making structural studies somewhat less straight forward than for other tissues, particularly for mode of imaging involving transmission electron microscopy” as concluded by Field et al.<sup>12</sup> Despite the variety of available in vitro tests for measuring surface changes, the most common form of reporting Ra within dental studies has been the surface profile Ra average (Ra, arithmetic average) or the root mean square (Rq, geometric average). Profilometric means are usually used to evaluated surface Ra.<sup>12</sup> Roughness is a measure of surface texture. It is often quantified by the deviations of the surface from its ideal form. If the deviations are large, then the surface is considered to be rough, meanwhile, the surface is considered smooth if they are small.<sup>12</sup> Therefore, the aim of the present study was to evaluate the effect of water storage on surface Ra of healthy human enamel after application of Icon<sup>®</sup> and Seal-Rite<sup>™</sup> using atomic force microscopy (AFM) and tridimensional micro computed topography.

**Methods.** Materials tested in the present study were Icon<sup>®</sup> (Resin sealant, DMG, Hamburg, Germany) and Seal-Rite<sup>™</sup> (Pit & Fissure sealant, Pulpdent, Watertown, MA, USA). The Icon<sup>®</sup> has the following composition: Icon-Etch (HCL 15%), Icon-Dry (99% Ethanol), and Icon-Infiltrant (Methacrylate-based resin matrix, initiators, additives). Meanwhile, Seal-Rite<sup>™</sup> consists of Ultra etch: 35% Phosphoric acid, and 34.4% filled urethane dimethacrylate (UDMA).

**Specimens' preparation.** The present study was conducted after registration and ethical approval clarification from the College of Dentistry Research Medical Center (CDRC), King Saud University from January 2011 to August 2011. Fifteen caries-free human premolar teeth that had been extracted for orthodontic reasons from subjects at the College of Dentistry,

**Disclosure.** The current research was registered at the College of Dentistry Research Center (CDRC), King Saud University (No. FR0003), dated 1st January 2011. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

King Saud University, Riyadh, Saudi Arabia. Teeth were thoroughly cleaned using slurry pumice and a prophylaxis brush in a contra-angled hand-piece. Teeth used in the current study were collected and stored in 0.025% Thymol solution (BDH Laboratory reagent, product no. 30433, BDH chemical, Ltd.: Poole, England) until the day of specimen preparation. The solution was prepared at the CDRC. Teeth with cracks, restorations or developmental lesions were excluded from the current study. The premolars with healthy enamel were included in the study. The roots of teeth were cut, and the crowns were sectioned longitudinally in a mesio-distal direction using a diamond cutting saw (Isomet 2000 Precision Saw, Buehler Ltd., Waukegan Road, Lake Bluff, IL, 664 USA) with profuse water irrigation. A total of 30 specimens were embedded in an ortho resin (Harry J. Bosworth Neocryl splint orthodontic splint resin, Bosworth Company, IL, USA). The crown was projecting to ensure that the convex smooth tooth surfaces were parallel to the scanning stage as possible.

The enamel specimens were randomly allocated into 3 groups of 10 specimens each: the first group (Group I) was considered as control. The enamel surfaces of the second group (Group II) were treated with Icon.<sup>®</sup> Seal-Rite<sup>™</sup> was utilized for the enamel surfaces of the third group (Group III). Both tested materials were used according to manufacturer's instructions as follow: for Group II, enamel surfaces were etched with Icon-Etch and allowed to set for 2 minutes. Then, it was rinsed off with water for 30 seconds and air dried. Icon-Dry was applied, and left to set for 30 seconds and air dried. Icon-Infiltrant was applied for 3 min and light-cured for 40 seconds. Again, Icon-infiltrant was used and left to set for one minute. Then, it was light-cured for 40 seconds.

Enamel surfaces of Group III were acid etched for 20 seconds with Ultra etch, rinsed off with water for 20 seconds, and air dried. Then, Seal-Rite<sup>™</sup> resin was applied and light-cured for 30 seconds. Both materials were polymerized by Elipar Highlight, ESPE, Germany; with 400-500 nm, and the intensity was measured

throughout the experiment with Optilux Radiometer (Kerr Corp. USA, 21 Commerce Drive, Dandury, CT, 09810). The specimens preparation followed was similar to previously reported investigation by Taher et al.<sup>10</sup> Specimens were stored in distilled water in an incubator at 37°C for 6 months. The specimens were measured with atomic force microscopy (AFM) Veeco CPM 1.2 nanometer (Woodbury, NY, USA). Atomic force microscopy technology represents a method of sample surface topography imaging. The topography image is observed by means of forces (nanonewtons) between sharp AFM tip and specimen surface atoms. The force sensors used are nano size needles (tip) which was placed at the end of the Si springs. The laser beam is focused at the tip edge. Photodetector was used to measure the deflection of the beam. During scanning the specimen surface, the force between surface atoms and tip are maintained constant using a feedback system. The measured tip movement gives the surface topography image of the specimen. The specimen dimensions were 50 mm (width) x 50 mm (1) x 20 mm (height) and, a scanning tunnel microscopy (STM) contact or noncontact AFM scan mode was used where the specimen was placed on an isolation table and connected to an optic microscope with a personal computer. Then, 3 specimens were scanned at an energy of 101 kV and intensity of 96  $\mu$ A with a resolution of 37.41  $\mu$ m pixel with an aluminum filter (1mm) using Skyscan-1072 x-ray micro tomograph, TomoNT (version 3-N.5, Skyscan, Belgium). Cone-Beam reconstruction (Version 2.15, Sky Scan) was performed. All scans and parameters applied were identical for the specimens and calibration rods.

The Ra mean readings were recorded and statistical analysis was performed with the SPSS program version 16 (SPSS Inc., Chicago, IL, USA) at a significance level of  $p < 0.05$ . Normality was satisfied with Shapiro-Wilk's test and the equality of the variance was determined by the Levene's test. Then, one-way analysis of variance was used to test significant differences between the 3 groups.

**Table 1** - The mean and standard deviation (SD) of surface roughness (Ra) of the tested materials.

Groups	N	Mean	SD	95% Confidence interval for mean		P-value
				Lower Bound	Upper Bound	
Control group (Group I)	10	0.21	0.06	0.17	0.25	0.747
Icon group (Group II)	10	0.23	0.08	0.17	0.28	
Seal-Rite group (Group III)	10	0.20	0.04	0.18	0.23	

\* $p < 0.05$



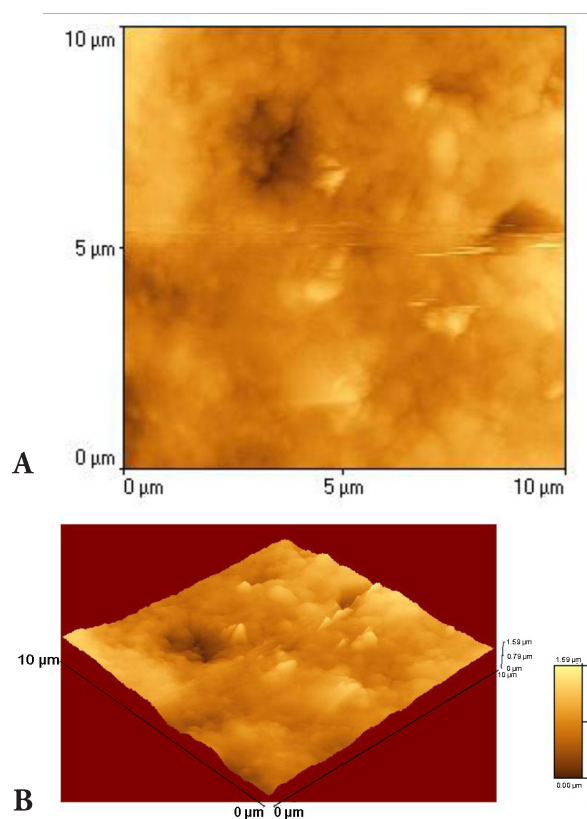
**Results.** The mean values Ra and the standard deviation (SD) of all groups are presented in Table 1. The Ra values of control group (Group I) was  $0.21 \pm 0.06$  and the recorded values for Group II were higher ( $0.23 \pm 0.08$ ) than the Group III and Group I. Meanwhile, the Group III was recorded the lowest ( $0.20 \pm 0.04$ ) values among the other tested groups. However, no significant differences in the mean Ra were found among the 3 groups ( $p=0.747$ ).

The AFM images of the Group I show the topography at the same location of the tooth surface, but one represents a 2-dimensional image (Figure 1A) and the other represents a 3-dimensional (Figure 1B) image.

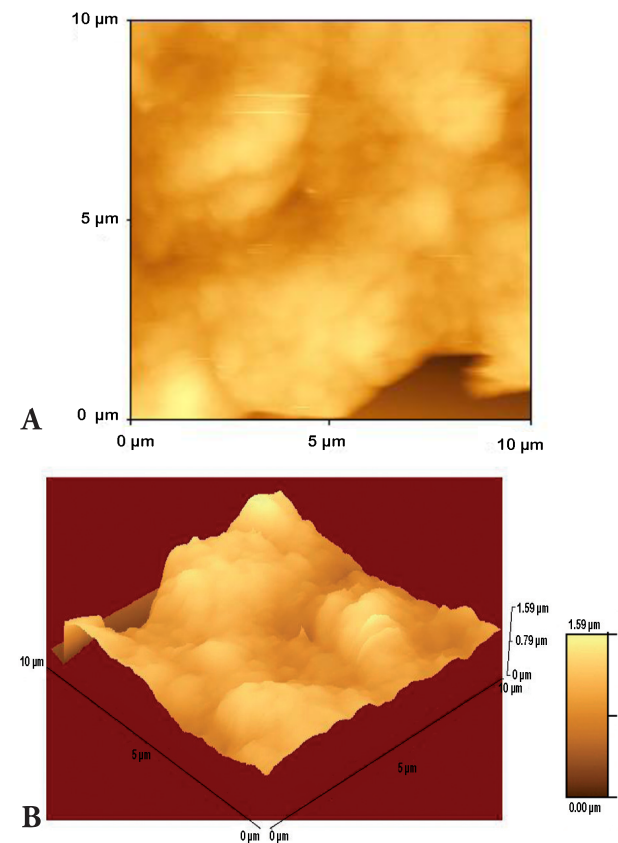
Figures 2A & 2B summarized the intact and homogenous layer of Seal-Rite resin on enamel surfaces. Meanwhile, demonstrates presence of a thin inhomogeneous Icon resin layer (Group II) (Figures 3). Also, very few small enamel grains are

presented uncovered with Icon resin. The representative 3-dimensional topography image (sky scan) of the enamel surface of the control group is presented in Figure 4A. The enamel surface infiltrated with Icon resin is shown in Figure 4A and Figure 5, where a thin and inhomogeneous layer of resin is demonstrated in the image. Meanwhile, the surface texture of enamel of the third group (persistence of homogenous and intact SealRite resin) is shown in Figures 4C.

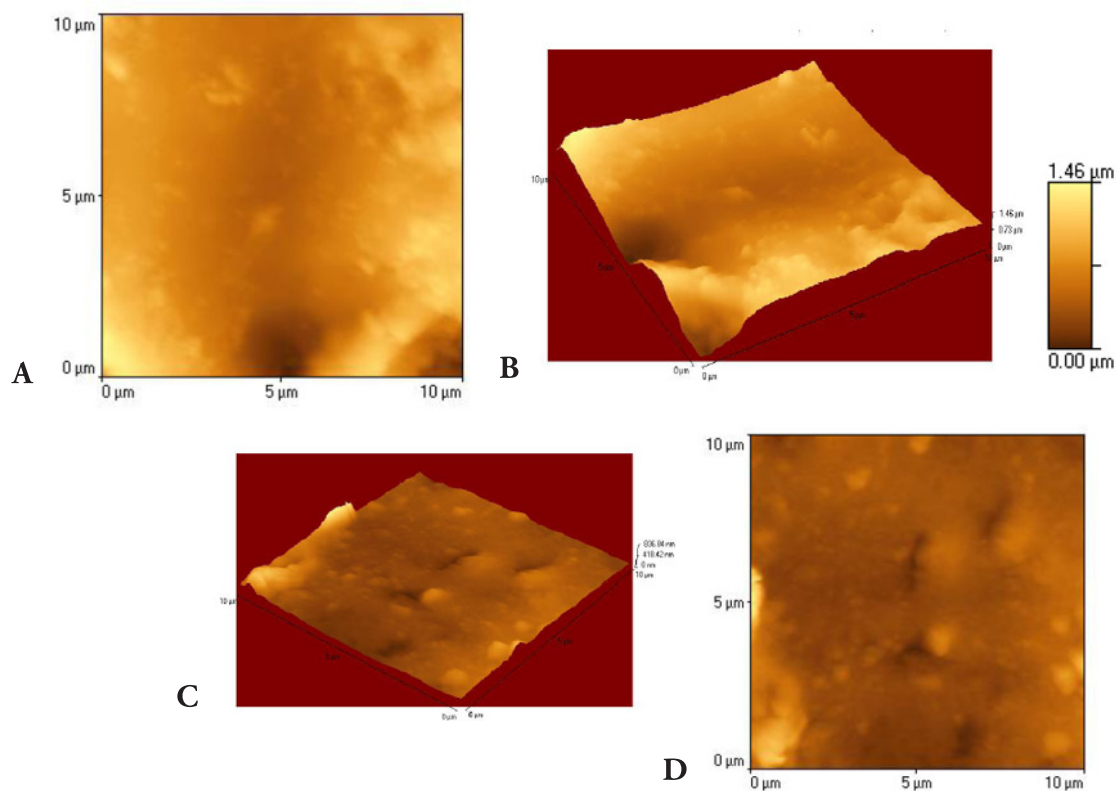
**Discussion.** The atomic force microscopy is a useful tool to study site-specific structural topography of enamel and the changes in enamel after application of materials.<sup>13</sup> Its main advantage over the other technologies is that it provides a detailed qualitative and quantitative characterization data about Ra.<sup>13</sup> Also, it allows determination of enamel pore depth which reveals details of the ultra-structure of the enamel surface.<sup>14</sup> Meanwhile, one disadvantage of the AFM is



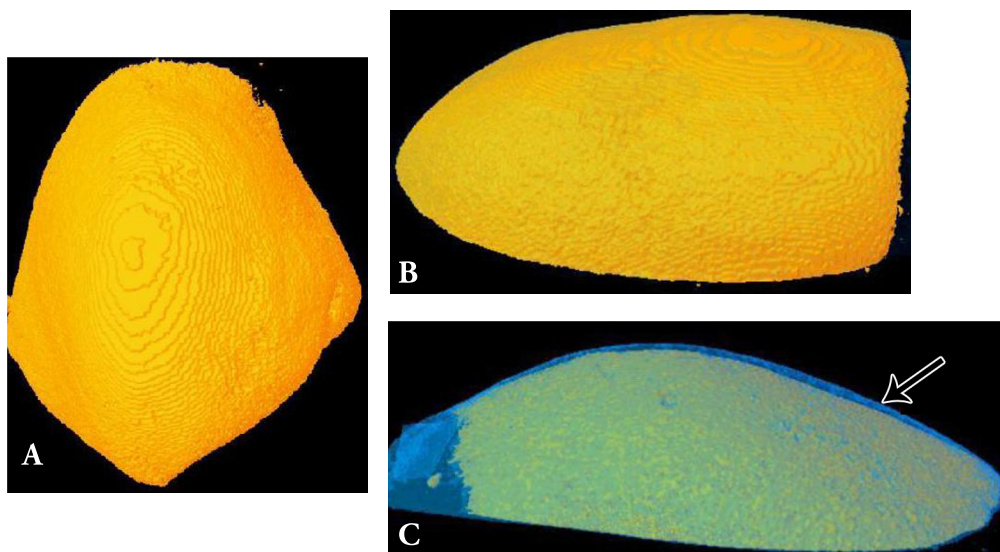
**Figure 1** - Atomic force microscopy showing A) Top view of tapping mode images of the tooth (control group) recorded in height (surface morphology) mode. The image reveals the distribution, size, and shape of numerous closely packed grains at the outer enamel surface. B) Three-dimensional representative image of the enamel surface (control group) recorded in height (surface morphology) mode.



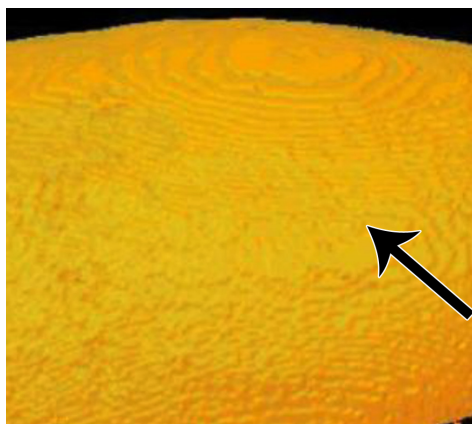
**Figure 2** - Presentation of the atomic force microscopy images of enamel treated with SealRite showing the persistence of the resin homogenous layer. A) Top view of the surface morphology. B) Three-dimensional representative image of the enamel surface.



**Figure 3** - A-D) Top-view atomic force microscopy tapping mode images of the enamel reveal the presence of the resin (thin layer) in the icon group and existence of few very small enamel grains.



**Figure 4** - Micro computed topography of the control group A) where the images clearly reveal the enamel surface, B) the surface morphology of enamel is demonstrated and some resin still covering the enamel surface in the Icon group, and C) the SealRite resin (as demonstrated by the arrow) presents a homogenous and intact layer covering the enamel surface.



**Figure 5** - Close view of the thin layer of Icon resin covering the enamel surface (arrow).

the size of the scan area; commonly it cannot be greater than 100 mm x 100 mm.<sup>13</sup> The AFM images in the current research were collected using the “tapping” technique and presented in height. Images recorded in the “height” mode, most frequently used in AFM microscopy, show surface morphology of the imaged area. In the 3-dimensional image (Figure 1B), the upper part of the sample is colored brighter (light) than in the lower parts of the surface. Accordingly, one could qualitatively and quantitatively interpret data and characterize such image features (as particle size, shape, height of the observed features).<sup>14</sup> The images of the control group (Figures 1A & 1B) recorded in the present study are similar to what Batina et al<sup>14</sup> reported, were closely packed, grains of small (0.5 μm) and large size (1.09 μm) can be observed on the enamel surface. Since the introduction of the first dental sealant Nuva-Seal in 1971, sealants have gradually become a critical component to contemporary dental practice, having demonstrated great success in the prevention of dental caries.<sup>15</sup> The fissure sealant establish a diffusion barrier on the enamel surface.<sup>16</sup> The AFM images of the surface texture evaluation (Figures 2A & 2B) revealed that treating the enamel surface with Seal-Rite resulted in intact and homogenous surface after storage in water. These findings in the present study are supported with the previous investigations where it was concluded that in general the effectiveness of sealants depends on long-term retention and depth of penetration of these materials.<sup>7</sup> Meyer-Lueckel et al<sup>17</sup> concluded that the tested fissure sealant in their study showed good penetration into initial subsurface, lesion and homogenous resin layer. Also, the low recorded Ra value of Group III in the current study might have positive interpretation, as stated by Mueller et al<sup>11</sup> a

high quality treated surface (meaning a surface with minimal Ra), would seem mandatory to lower any risk of plaque accumulation, thereby further decreasing the onset of caries.<sup>11</sup> The result of the current study seemed to be promising regarding this issue. It was reported that experimental infiltrants containing high amounts of triethylene glycol dimethacrylate (TEGDMA) and ethanol showed the fastest penetration, whereas a high content of bisphenylglycidyl dimethacrylate (BisGMA) affected penetration capability negatively.<sup>18</sup> Our study was supported by the previous studies<sup>17,19</sup> wherein it was assumed that a solvent such as ethanol might affect the penetration behavior positively. In the current investigation, the enamel surface that was infiltrated with Icon presented thin and inhomogeneous surfaces in some specimens, where, resin can be seen clear on enamel grains (Figures 3A & 3B). These observations are similar to what was reported by Mueller et al.<sup>11</sup> On the other hand, it was reported that the inhomogeneities might be caused by oxygen inhibition, polymerization shrinkage, or incomplete evaporation of the solvent.<sup>16</sup> Meyer-Lueckel et al<sup>17</sup> stated that an evaporation of the solvents during the penetration period might lead to unfilled pores within the resin layer. The loss of the oxygen-inhibited layer should be expected after clinical performance; in particular with Icon, it seems that this layer could be washed away or worn-off (by salivary flow or mechanical action, respectively) which might lead to re-exposure of the enamel substance.<sup>11</sup> Although, the methodology and the materials are differing, the previous investigations<sup>11,17</sup> the results support the findings of the current research. In order to have more quantities' manner for the topography image of the tested specimens in the current research, the average surface Ra factor RMS [Rq] was estimated for few specimens from the 3 groups.

In the present study, the skyscan was also utilized, which is a micro computed tomography or “micro-CT”. It really represents a form of 3-dimensional microscopy, where very fine scale internal structure of objects is imaged non-destructively. No sample preparation, no staining, no thin sectioning is required. It has a nano-CT scanner with submicron spatial resolution. Skyscan images (Figures 4A-4C & Figure 5) in the present study support the findings of the AFM images and are in agreement with the results of previously reported investigations.<sup>11,17,19</sup> The presence of Seal-Rite resin on all tested specimens after storage in water (as shown in all the images, where the enamel granules are not clearly shown) probably denotes the low solubility of the material by water. This behavior of Seal-Rite resin could protect the enamel prism clinically. However,



long-term clinical behavior and further investigations are needed.

In conclusions, within the limitation of an in vitro study, it can be concluded that, although the fissure sealant group revealed the lowest Ra values, there were no statistically significant differences of the surface Ra for enamel surfaces among the treated and non treated surfaces. The images of the enamel tooth surface revealed infiltration with Seal-Rite and Icon materials. The persistence of Seal-Rite resin in all tested enamel surfaces might reveal that the Seal-Rite material was less affected by water storage than Icon resin, denoting its protective effect on the enamel surface. Long-term clinical behavior for the both tested materials warrants further investigation

**Acknowledgment.** *The author would like to thank Dr. Khalid Y. Al-Yamani, Director of the Nano-Technology, National Center for Utilizing the Atomic Force Microscopy (AFM) device and sincere appreciation to Eng. Waiel Al-Abbad, King Abdulaziz City for Science and Technology for his great assistance in scanning and examining the samples using AFM device. Also, special thanks to Dr. Khaled Al-Hazimi for his help in utilizing the sky scan. I am also grateful to Prof. Ahmad AlHejazi for his kind consultation and advice.*

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