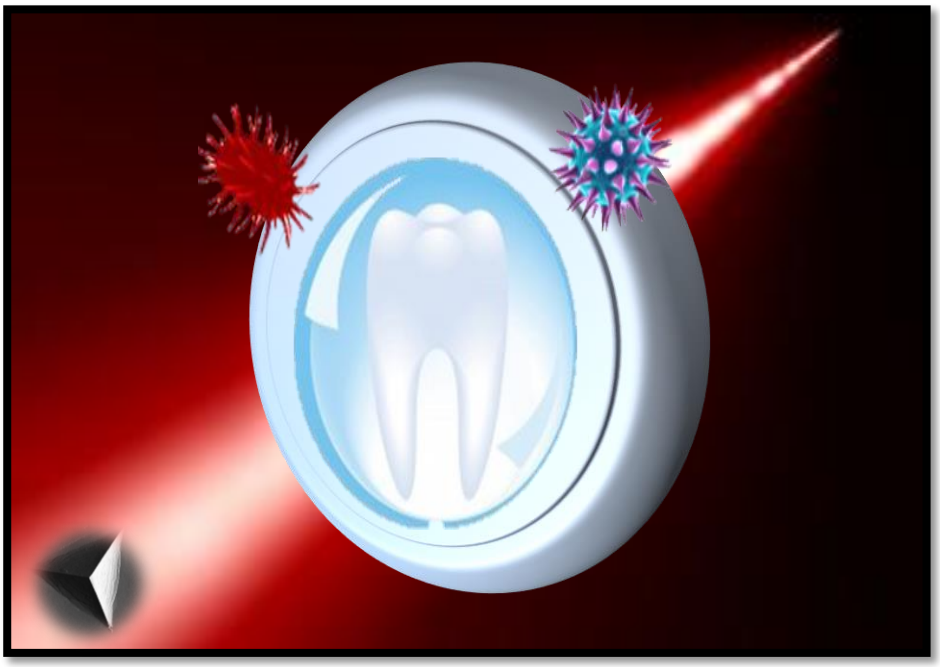
TMDU
TOKYO MEDICAL AND DENTAL UNIVERSITY

# Optical and Nano-Hardness Evaluation of Resin Coated Enamel and Adjacent Area



*Doctoral of Philosophy Thesis*

**EHAB ZAKI ALSAYED**

**2015**

A dissertation presented

By

**EHAB ZAKI ALSAYED**

to

Cariology and Operative Dentistry,
Oral Restitution Department,
Faculty of Dentistry,
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In partial fulfillment of the requirements
for the degree of

**Doctor of Philosophy in Dental Science**

Promoter: Prof. Junji TAGAMI
Advisor: Drs. Alireza SADR & Yasushi SHIMADA

**Tokyo Medical and Dental University**

**2015**
**Tokyo, Japan**

# DEDICATION

# *DEDICATION*

***This work is dedicated to the unlimited source of inspiration and motivation, my father Mr. Zaki ALSAYED, mother Rawdhah FATANI, my brother and sisters.***

***Furthermore, I dedicate this work to Dr. Ilnaz HARIRI and all my friends for their limitless support and assistance.***

***Lastly and most importantly, I dedicate this work to my little angels sons Abdulmoez, abdulmalek, Abdulhakam and my wife Hania FATANI for their love, understanding and support.***

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April 1, 2015

EHAB ALSAYED

# ABSTRACT

Promoter: Prof. Junji Tagami
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**ABSTRACT**

## Optical and Nano-Hardness Evaluation of Resin Coated Enamel and Adjacent Area

**[Background and Objective]:**

Dental caries usually occurs at earlier ages, but its risk remains throughout the entire life of natural dentition. Recently, resin coating materials have been developed that can cover enamel and dentin surfaces and potentially reinforce the tooth against intraoral challenges (biofilm attachment, erosion, abrasion) and reduce sensitivity. The aim of this study is to monitoring durability of various resin-based materials used for enamel coating using swept source optical coherence tomography (SS-OCT) in combination with nanoindentation test.

**[Materials and Methods]:**

Half of the enamel surface of resin-embedded bovine incisors was coated by one of the resin based coating materials according to manufacture instruction with other specimens served as control. The coating layer and its underlying and adjacent enamel were monitored using SS-OCT at baseline and after thermal challenge, and after demineralization (pH 4.5). The specimens were secondly resin-embedded, cross-sectioned and fine polished for nano hardness

evaluation. The findings have been confirmed with confocal laser scanning microscope and scanning electron microscopy.

**[Results]:**

Coating film-thickness differed among materials. However all the coats remained after the short thermal challenge but they showed various levels of interfacial integrity after demineralization.

Moreover, the results suggested that most of the coating resin materials effectively prevented demineralization in coated area. While, uncoated areas presented different nanohardness trends.

**[Conclusion]:**

Resin-based materials applied as a coat can protect enamel from demineralization. There was a difference among materials in their ability to protect enamel beneath and adjacent to the coating according to their properties. OCT can be used to monitor integrity of the protective coating layer and enamel changes. Also, nanohardness evaluation and mapping of nanoindentation on a wide range of locations could clarify more details regarding the effects of various coating materials on enamel structure.

**[Clinical significance]:**

Coating of enamel surface by thin resin-based materials releasing active ingredients such as Fluoride will provide an excellent approach towards caries prevention and tooth protection. These coating and enamel beneath them can be regularly monitored by OCT, as an adjunct clinical tool.

# PREFACE

# *PREFACE*

This thesis is based on the original research works by the author, to which the following articles refer:

**Article1:**

**Ehab Z ALSAYED**, Ilnaz HARIRI, Alireza SADR, Syozi NAKASHIMA, Turki A BAKHSH, Yasushi SHIMADA, Yasunori SUMI and Junji TAGAMI. **Optical coherence tomography for evaluation of enamel and protective coatings**. Dental Materials Journal 2015; 34(1): 98–107.

**Article2:**

**Ehab Z AlSAYED**, Ilnaz HARIRI, Alireza SADR, Syozi NAKASHIMA, Yasushi SHIMADA, Turki A BAKHSH and Junji TAGAMI.

**Effects of Protective Resin Coating on Nanomechanical Properties of Enamel and Adjacent Area**. Dental Materials. (Under review)

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# CHAPTER 1

# *CHAPTER 1*

# 1. BACKGROUND AND LITERATURE REVIEW

Dental enamel, the most highly calcified and hardest tissues of the human body is susceptible to be demineralized by acid from bacteria and other sources. Dental caries has a multi-causative etiology and the chance of its effective prevention still is the greatest widespread disease of many societies which affects all populations and age groups [1].

In the early stages of caries formation continuous demineralization will result in the dissolution of apatite crystallites and increase porosities. White spot lesions are the early stage of caries development characterized by intact enamel surface with subsurface demineralization. Clinically they appear as chalky white or brownish discolorations and may or may not have associated surface roughness [2]. If the process is not reversed, substantial loss of minerals lead to surface break down of the lesion (known as cavitation) [3]. One of the most susceptible areas to dental caries is proximal tooth surface. The prevention, diagnosis and therapeutic treatment of proximal caries lesions comprise a constant problem in clinical dentistry particularly after caries decline [4]. In

another hand, the recurrent caries formation has been shown to be the main reason for failure and replacement of restorations [5].

Dental erosion is defined as the chemical dissolution of dental enamel without bacterial involvement and is considered as a common problem among different ages. It acts a key role in enamel demineralization with food-induced demineralization as one of the main factors [6, 7].

Recently, resin-based coating (RBC) materials have been improved in terms of physical and chemical properties. Potentially, such coatings can cover enamel and dentin surfaces and reinforce the tooth. Some of these RBCs can release Fluoride (F) and act as reservoirs to increase F levels in tooth surface as an additional protective measure against intraoral challenges [8].

Nevertheless, there are only few studies evaluating the performance of the RBC materials to protect the smooth surfaces against demineralization partly due to the lack of materials suitable for the purpose and partly due to the unavailability of objective means to monitor the clinical efficacy of such approach.

Introduction of optical coherence tomography (OCT), as a noninvasive and nondestructive imaging method, has brought recent advancements in the field of diagnostic sciences [9]. OCT has been reported as a promising high resolution biomedical optical method to detect microstructural details of hard and soft oral tissues [10]. Swept source (SS)-OCT is one of the most recent implements of the spectral discrimination, using a wavelength-tuned laser as the light source and

providing improved imaging resolution and scanning speed [10]. The technology has recently been widely used as an experimental method for studying dental structures and diagnosis of dental disease, including caries [11]. OCT image has been previously used for in order to clearly detect such bubbles and failures in the adaptation of sealants [12]. However, few studies have to date investigated the application of OCT for assessment of dental resin coatings.

Recently, nano-indentation (NI) technique has enabled investigations of local mechanical properties of materials under various loading regimes based on load displacement data of indentations on submicron scale. Measurement of mechanical properties by NI has been suggested as advantageous over the conventional methods for its high resolution of force and accurate indent positioning [13]. Nanhardness of sound enamel has been widely investigated by NI systems [14]. This technique was also employed in assessing enamel erosion and demineralization/remineralization [15]. Moreover, a NI characterization of artificially generated white spot lesions has been reported [16]. The enamel is adapted to absorb essential mechanical and abrasive stress due to its great stiffness. This property is related to its microstructure. Understanding of nanohardness of protected enamel thus might be of importance for understanding mechanism and therapeutic strategies of enamel.

However, few studies have assessed the protective effect of enamel coating against demineralization due to the lack of proper materials and no studies have investigated the nanohardness of coated enamel and adjacent area.

In this thesis two studies based on optical and nanomechanical characterization of resin coated enamel were done. Protection efficacy of resin coated materials on enamel against demineralization was evaluated by OCT and NI in first and second studies respectively.

# CHAPTER 2:
# OPTICAL COHERENCE TOMOGRAPHY FOR EVALUATION OF ENAMEL AND PROTECTIVE COATINGS

# *CHAPTER 2*

# OPTICAL COHERENCE TOMOGRAPHY FOR EVALUATION OF ENAMEL AND PROTECTIVE COATINGS

## *2.1. INTRODUCTION AND OBJECTIVES*

Dental caries usually occurs at early ages, but its risk remains throughout the entire life of natural dentition among various populations. In simple terms, caries is the result of tooth demineralization by acids that are produced through bacterial metabolism of sugars, and diffuse into enamel and dentin. Caries is a multifactorial disease process, and the tooth surface is only one of the involving factors. Destruction of the tooth surface is initiated by loss of minerals due to a localized shift in the dynamic balance between demineralization and remineralization of the highly mineralized enamel tissue. If the process is not arrested or reversed in the early stage, substantial loss of minerals from enamel eventually leads to surface break down of the lesion (known as cavitation) [17]. Emerging concepts in caries management, which are aimed at promoting the health rather than repairing cavities, involve preventive strategies as well as new approaches to protect the teeth against caries [18].

In addition to caries, mechanical and chemical loss of dental hard tissue has been considered as a matter of concern. The prevalence and severity of dental erosion have increased significantly in the recent years [6, 19]. Tooth erosion is defined as the loss of mineral substrate by intrinsic acids from the backflow of the gastric contents through the mouth or extrinsic acids such as acidic drinks and foods where the damage is more frequently located on the cervical third of the buccal surface of anterior teeth [20]. Moreover erosive tooth wear will happen as the accelerated loss of dental hard tissue through the combined effect of erosion and mechanical wear (abrasion and attrition) on the tooth surface. Without intervention, such wear will progress as cuspal cupping with exposed dentin and eventually lead to loss of occlusal morphology [19].

Physical coverage of the tooth surface has been considered as one of the ways to protect teeth against caries and erosion. Pit and fissure sealants have been well known to decrease the incidence of caries, with a substantive amount of evidence [21, 22]. It has also been suggested that extended enamel surface sealing by appropriate coating materials can potentially provide the benefits of physical protection, especially for inter-proximal surfaces [23, 24] and smooth buccal coronal sites, and in high-risk patients such as orthodontic patients [25, 26], special-needs or handicapped patients and the elderly. A clinical study showed that up to 75 per cent of patients undergoing fixed appliance therapy were affected by decalcification of tooth surface. It was shown that gingival areas

were most affected by demineralization, particularly on anterior teeth and that these specific sites could benefit from sealing and protection by a viscous resin [27]. Nevertheless, some reports showed that the application of conventional sealants failed to provide consistent protection against white spot formation on smooth surfaces [28]. Such new lesions were usually located in areas where extensive plaque accumulations occurred.

Some of the RBC materials can release F and act as reservoirs to increase F levels in tooth surface, as an additional protective measure against intraoral challenges [29-31]. Many commercially available RBC materials have similar compositions to those of dental adhesives. Previous literatures have shown that some self-etching adhesives have the ability to improve the resistance of enamel and dentin against the demineralization by forming a layer of acid-base resistant zone below the interface [32, 33]. It has been also verified that coating of root dentin surface with such materials showed a remarkable reduction in caries susceptibility of the root site [34]. Nevertheless, the use of physical barrier to protect the susceptible smooth enamel surfaces against demineralization is still not fully probed; perhaps partly due to the lack of materials suitable for the purpose, and partly due to the unavailability of objective means to provide evidence on the clinical efficacy of such an approach.

Clinically, the conventional dental radiographs (X-rays) are the most widely available diagnostic method besides direct visual inspection; however,

dental X-rays are not capable of detecting early changes and demineralization of the tooth surface, and superimposition of the surrounding structures is an obstacle. In addition, the coating materials are usually radiolucent and may be thinner than the resolution of the X-rays [35]. More recently, fluorescence-based techniques have been employed for the detection of early enamel demineralization [26, 28]. It has been shown that those which provide high-resolution fluorescence pictures are likely to be more reliable than those devices that obtain a signal intensity value via a single spot [36]. However, the current fluorescence-based methods do not provide cross-sectional or in-depth images of the structure, and may be affected by the presence of resin material on the surface. Therefore, a cross-sectional imaging modality that can be used to inspect and monitor both the coating and the enamel surface is in demand.

Optical coherence tomography (OCT) is addressed as a non-invasive cross-sectional imaging of the internal biological system at the submicron scale [9, 37]. It is a promising imaging modality, which does not require cutting and processing of the specimens and allows the visualization of microstructures of tissues and biomaterials in real time [38]. Recently, clinical OCT systems equipped with hand-held probes and suitable for intra-oral imaging have been launched by some manufacturers on a trial basis and showed a potential as a detecting tool for occlusal, interproximal and secondary caries, demineralization around orthodontic brackets as well as evaluation of dental materials defects

[39-42]. The introduction of faster spectral domain systems [39] and incorporation of micro mechanical electrical systems into the probe designs [40] have facilitated three-dimensional imaging and adoption of OCT in clinical dentistry [43]. Previous studies showed that OCT imaging of sealants enabled clear detection of voids and failures in the adaptation of materials [12, 44]. It was also demonstrated that OCT could measure the inhibition of demineralization on smooth enamel surfaces peripheral to orthodontic brackets [45]. Therefore, the aim of the current study is to evaluate the efficacy of different resin materials as enamel coating against demineralization using SS-OCT. The null hypotheses of this study were that covering enamel by resin material cannot resist demineralization, and that there was no difference in enamel protection among various coating materials.

## *2.2. MATERIALS AND METHODES*

### 2.2.1 Materials used

The materials used in this study are listed in **Table 1**. The lot number and chemical compositions of each material are according to the information provided by the manufacturers. Three RBC materials: Clinpro XT Varnish (CXT; 3M ESPE, St Paul, MN, USA), PRG Barrier Coat (PBC; Shofu, Kyoto, Japan), Tokuyama Shield Force Plus (SFP; Tokuyama Dental, Tokyo, Japan), and a two-

step self-etch adhesive Clearfil SE Protect (SEP; Kuraray Noritake Dental, Tokyo, Japan) were used in this experiment.

| Material | Brand | Code | (Lot#) | Composition | Manufacturer |
|---|---|---|---|---|---|
| Resin-based coating material | Clinpro XT Varnish | CXT | N352376 | liquid: HEMA, water, camphorquinone, calcium glycerophosphate and polyalkenoic acid<br>Paste: HEMA, Bis-GMA, water, initiators and fluoroaluminosilicate glass | 3M ESPE, St. Paul, MN, USA |
| Resin-based coating material | PRG Barrier Coat | PBC | 051101 | Base: glass powder, purified water, Methacrylate monomer, S-PRG filler, phosphonic acid monomer<br>Activator: methacrylate acid monomer, Bis-MPEPP, carboxylic acid, TEGDMA, catalyzer. | Shofu, Kyoto, Japan |
| Two-step, self-etch adhesive | Clearfil SE Protect | SEP | Primer :00103A<br>Bond:00165B | Primer: MDP, MDPB, HEMA, hydrophilic dimethacrylate, water<br>Bond: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, di-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica, surface treated sodium Fluoride | Kuraray Noritake Dental, Tokyo, Japan |
| Resin-based dentin coating and desensitizer | Shield Force Plus | SFP | 009 | 3D-SR monomer, HEMA, Bis-GMA, TEGDMA, alcohol, Water, camphorquinone, fillers. | Tokuyama Dental, Tokyo, Japan |

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol-A-diglycidyl methacrylate; S-PRG, surface pre-reacted glass inomer fillers; Bis-MPEPP, 2,2-Bis[4-(2-methacryloyloxyethoxy)phenyl] propane; TEGDMA, triethyleneglycoldimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MDPB, 12-Methacryloyloxydodecyl pyridinium bromide; 3D-SR monomer, 3 dimensional self-reinforcing monomer, methacryloyloxyalkyl acid phosphate.

**Table 1: Composition of the coating and adhesive system used in this study.**

## 2.2.2 Specimens preparation

The experimental procedure of the study is schematically presented in **Fig. 1**. Forty five fresh bovine incisors were obtained from a local slaughter house (Yokohama, Japan) and stored frozen prior to the experimental procedure. Enamel blocks 6 × 3 × 3 $mm^3$ (length × width × depth) were cut from the bovine incisors using a low speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) under running water, and embedded in epoxy resin (Epoxycure resin; Buehler). The outer enamel surface was slightly polished with a 800-grit silicon carbide (SiC) paper (Sankyo, Saitama, Japan) until a flat area was obtained on the surface. This was aimed to eliminate any possible superficial enamel cracks, and create a standard flat smooth surface. Two areas, namely coated (C), and un-coated (UC), were assigned on the polished enamel surface of each block as follows; The half surface of each treated block was covered carefully by placing a tape and stayed intact as UC area, while the other half surface of each block was carefully covered by one of the four materials (as described in **Table 1**) that served as the C area (n=10/group). The specimens were then stored in water for 24 h at 37 °C. Five specimens were used as control, without any surface coating.

Following coating and water storage, the specimens were subjected to thermocycling challenge. They were placed in wire-mesh basket, aged for 5,000 thermocycles alternating between 5 °C and 55 °C water bath (Cool line CL200

and Cool Mate TE200, Yamato Scientific, Tokyo, Japan). The dwell time was 30 s in each bath with a transfer time of 5 s between baths.

### 2.2.3. Demineralization procedure

After thermal challenge, samples were subjected to a demineralization solution ($CaCl_2$ 1.5 mM, $KH_2PO_4$ 0.9 mM, $CH_3COOH$ 50.0 mM, $NaN_3$ 3.08 mM) at pH 4.5 at 37 °C for one week [46]. The volume of demineralization solution was 140 ml per each five samples in the following order of one sample in the center and four samples in the each corner of the container. On the other hand in order to clarify the ion releasing and buffering effect of each material, the solution was not refreshed till the end of treatment.

### 2.2.4. OCT system

The OCT system (Dental OCT Prototype 2, Panasonic Healthcare, Ehime, Japan) operates at 1330-nm center wavelength. Laser light is projected onto the object surface and scans across the section of interest in two dimensions using hand-held probe. The focused light-source beam is projected onto the sample and scan across the area of interest in two dimensions (x, z) using a hand-held probe. Backscattered light from the sample is returned to the system, digitized in time scale and then analyzed in the Fourier domain to reveal the depth-resolved reflectivity profile (A-scan) at each point. A cross-sectional tomogram (B-scan) may be achieved by laterally combining a series of these axial depth scans (A-

scan) from each point within the scanned area. Two-dimensional cross-sectional images can be created by converting the B-scan raw data into a grey-scale image [47]. The axial resolution of this OCT system is 12 μm in air, which corresponds to 8 μm within a biomedical structure with a refractive index (*n*) of around 1.5. The lateral resolution (spot size) of 20 μm is determined by the objective lens at the hand-held probe designed for intra-oral imaging. Detailed information of this device was previously reported [39].

### 2.2.5. OCT imaging and analysis

In-depth 2D OCT images were carried out at five stages (after 1 day of storage, after thermocycling and after 1, 4 and 7 days of demineralization) using OCT. During the scan, the OCT probe was set at 5-cm distance from the specimen surface, with the scanning beam oriented about 90 degree to the surface. In order to ensure the repeatability of the OCT scan after each treatment, the cross-sectional B-scan was performed along the same line between the two points marked by a marker pen on the specimen surface. All OCT B-scan images were taken in wet condition for the specimens to decrease the strong reflection from the surface of the specimen [9].

For image analysis, a custom code in the image analysis software (ImageJ version 1.45S; National Institutes of Health, MD, USA) was used to import the raw data of the OCT. A noise reducing median filter (size 2) was applied to the data. In order to measure the initial coating thickness, ImageJ was used to calculate the

thickness value in the thickest area. Then, a region of interest (ROI) width 1 mm × optical depth 500 μm from the surface of enamel in UC area to deeper levels was selected and lesion depth was measured using an experimental plugin which was developed for ImageJ (**Fig. 2**). Threshold function of the software allows the user to find appropriate intensity values that correspond to the visual boundary, suggesting the demineralization front or optical lesion depth [9, 37]. For the purpose of comparing OCT and CLSM measurement, all optical depth values obtained under the hydrated conditions for coating thickness and lesion depth were converted to real depth values by dividing them to the estimated refractive index of 1.5 for resin (**Fig. 2**) and demineralized enamel [46].

### 2.2.6. Cross-sectional microscopy

Direct observation of the physical cross-sections was accomplished under confocal laser scanning microscope (CLSM; 1LM21H/W, Lasertec, Yokohama, Japan). Enamel blocks were secondly embedded by polyester resin (Rigolac, Oken, Tokyo, Japan). The specimens were then cross-cut along the C and UC areas through the location that was previously imaged by OCT using the diamond saw, and fine polished to perform CLSM evaluation. Each sample was sequentially polished by SiC papers #600, #800, #1000, #1200, #1500, and #2000 in circular motion under copious cooling water, followed by diamond slurries with particle sizes of 6 μm, 3 μm, 1 μm, 0.5 μm, and 0.25 μm in a lapping machine (ML-160A;

Maruto, Tokyo, Japan).

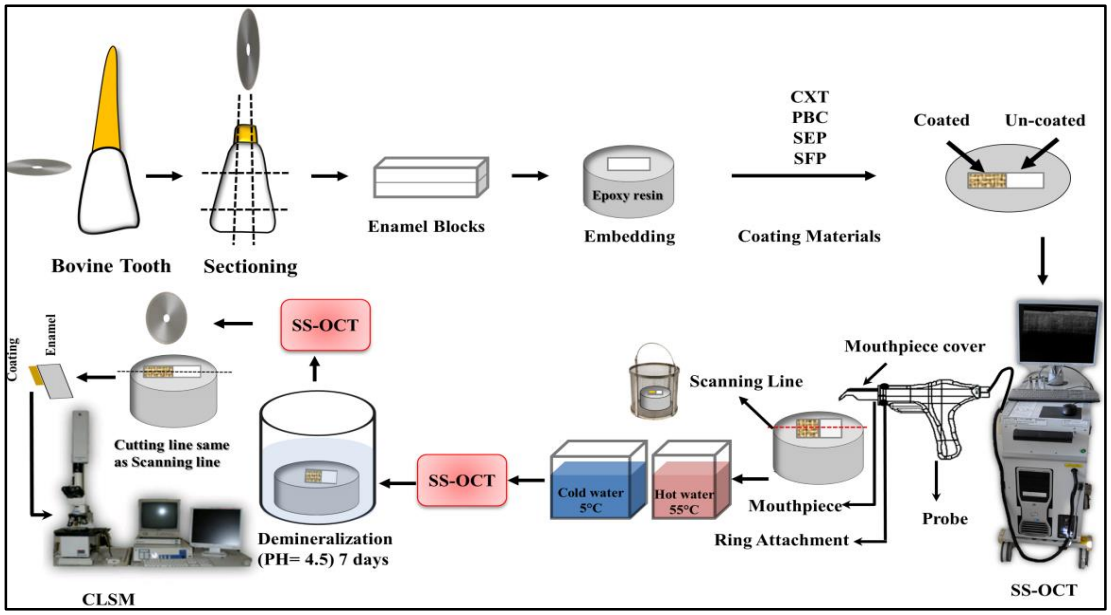


**Figure 1: Schematic drawing for the sample preparation and visualization under the OCT. Bovine enamel blocks were embedded in epoxy resin; one of the coating materials (CXT, PBC, SFP or SEP) was applied on half of the enamel surface; SS-OCT scans were obtained at the baseline, after 5,000 thermal cycles, and after 1, 4 and 7 days of demineralization; specimens were secondly embedded, cross-sectioned and polished for laser microscopy (CLSM) observation.**

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## 2.2.7. Statistical analyses

Repeated measures analysis of variance (ANOVA) was used to compare the progress of lesion depth with demineralization time among different coating materials and their interaction. This was followed by comparisons between each

two material groups with Bonferroni correction. All the statistical procedures were performed at a significance level of α=0.05 with the statistical package for social science (SPSS for windows, Version 16.0, SPSS, IL, USA).

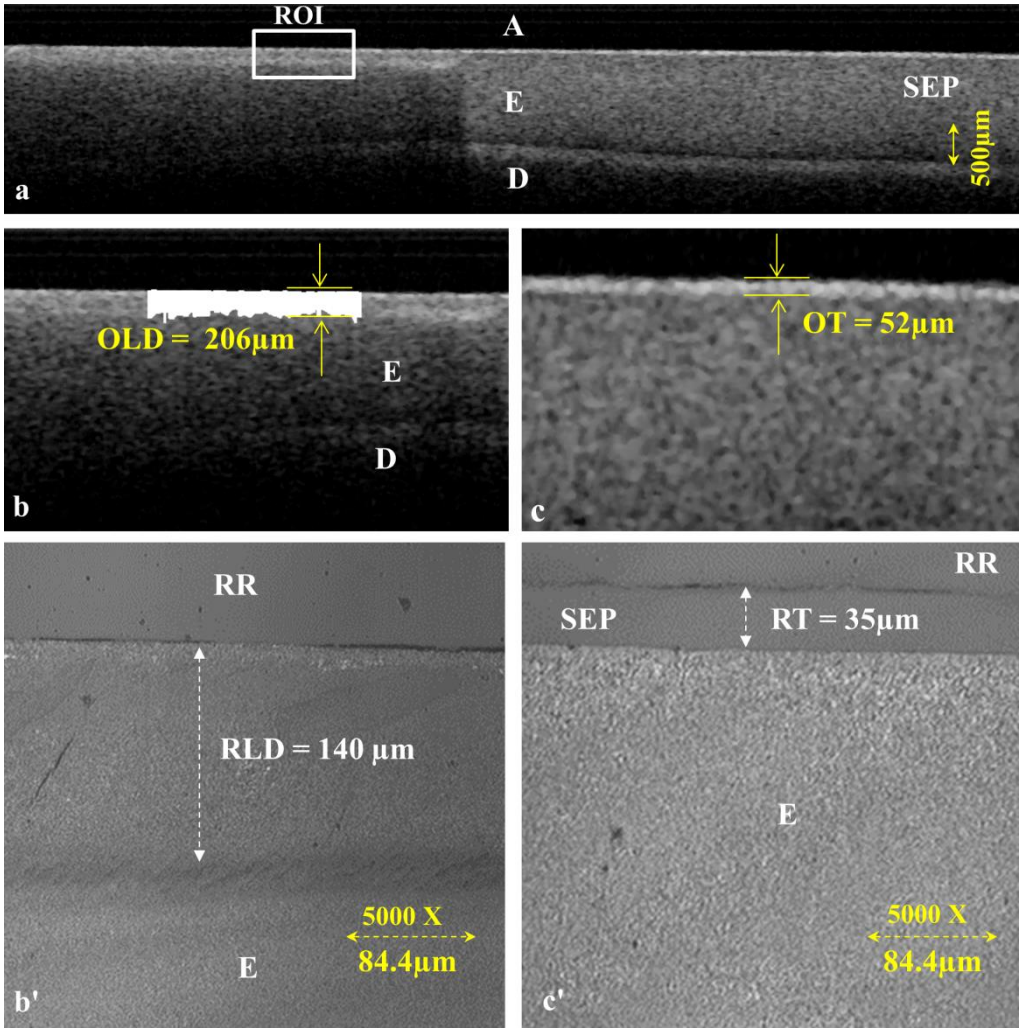


**Figure 2: Measuring the thickness of the lesion and the resin (a) Lesion depth (OLD) and coating thickness (OT) of SEP coat determination on OCT images. (b) Demineralization depth was determined through the binarization process over ROI; a visible sharp border was taken as the depth of lesion. The OLD reading from OCT image (b) was 206 µm (optical) or approximately 140 µm in real (RLD) considering *n* of 1.5 for demineralized enamel. This depth corresponds to the**

**lesion front depth marked by dashed arrow on the CLSM image of the same cross-section after cutting (b'). The OT was measured directly on the OCT image as presented in (c). The actual coating thickness (RT) was later confirmed under CLSM imaging. Note that matching the two thicknesses (OT vs. RT) suggests *n* value of (52 / 35=1.48) (c'). A=air, E=enamel, D=dentin, RR=polyester second embedding resin, ROI=Region of interest, OLD=Optical lesion depth, RLD=Real lesion depth, OT=Optical thickness and RT=Real thickness. The CLSM scale bar shows 84.4 µm distance.**

## *2.3. RESULTS*

Optical thickness of the coating and their integrity and protection against enamel demineralization are summarized in **Table 2**. The highest mean optical thickness values measured by OCT were found in CXT, followed by PBC and SEP. Representative OCT images are presented in **Figs. 3-5** and confirmatory CLSM images are presented in **Figs. 4** and **5**.

**Figure 3** a-f represented 2D OCT B-scans of sound and 4 days demineralization challenge. UC enamel area showed high reflectivity from the lesion and decreased reflectivity just beneath the lesion, revealing a lesion boundary. For CXT, a decrease in reflectivity from UC superficial areas adjacent to protected zone was noted. In most specimens, the C area appeared to be intact after 4 days of demineralization; however, localized increase in reflectivity appeared in some areas coated by SFP. While it was difficult to distinct the coating layer of SFP, its enamel protection effects could be observed.

**Figures 4** and **5** represented typical B-scan images obtained by the OCT and cross-sections as confirmed by the CLSM for each material after 7 days of demineralization. The B-scan images of the CXT sample showed bright clusters at several areas beneath the resin coating indicating interfacial gaps, which were later confirmed by CLSM imaging. A distinct area appearing similar to sound enamel was observed adjacent to the C area of CXT, which was confirmed to be a demineralization inhibition zone in corresponding CLSM image. In another hand, PBC maintained its integrity and protected enamel beneath the coating, but no defined inhibition zone was seen adjacent to the coating. Dentinoenamel junction (DEJ) was not clearly observed beneath the demineralized enamel in OCT images. All groups showed complete protection against demineralization of enamel in C areas after 7 days, except for SFP which showed patches of partial demineralization.

The values in the bar graph represent optical lesion depths (μm), which were obtained using OCT images of different groups through demineralization days (**Fig. 6**). Repeated measures ANOVA revealed that demineralization time (within subject effects; $F=175.8$, $p<0.001$) and its interaction with coating type ($F=5.2$, $p<0.001$) significantly affected the depth of lesion formed in UC area. Coating type, the between subject factor, was also significant ($F=11.3$, $p<0.001$). Pair comparisons revealed that lesion progress in UC area of CXT was

significantly different from all other groups ($p<0.05$) but there was no significant difference among other groups including the control.

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**Table 2: Optical thickness and frequency of detachment and demineralization for each coating material obtained from OCT images.**

| Material | Initial optical thickness ±SD (μm) | Detachment (>0.5 mm) | | | | Demineralization under the coating | | |
|---|---|---|---|---|---|---|---|---|
| | | After thermal cycling | 1-day DEM | 4-day DEM | 7-day DEM | 1-day DEM | 4-day DEM | 7-day DEM |
| CXT | 253.7 ± 55.26 | 0/10 | 2/10 | 3/10 | 4/10 | 0/10 | 0/10 | 0/10 |
| PBC | 123.1 ± 49.43 | 1/10 | 2/10 | 2/10 | 2/10 | 0/10 | 0/10 | 0/10 |
| SEP | 67.4 ± 29.75 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SFP | *61± 7.48 | 3/10 | 6/10 | 7/10 | 7/10 | 6/10 | 7/10 | 9/10 |
| (*)Data excludes two samples that were not measurable (thickness < 10 μm). | | | | | | | | |

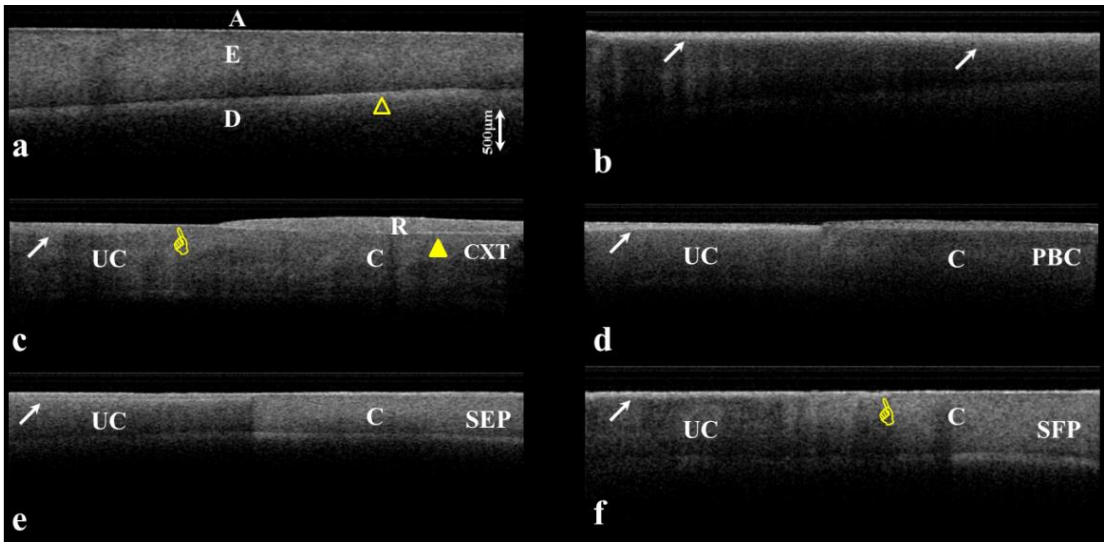


**Figure 3: 2D OCT B-scans of sound and 4 days demineralization challenge.**
**(a-f) B-scan images of sound and 4-day demineralized enamel; (a) sound enamel, (b) UC control enamel block, (c) CXT, (d) PBC, (e) SEP and (f) SFP. (a) Blank head indicates DEJ and vertical scale bar present 500 µm optical distance in axial (z) direction. (b-f) White arrows indicate the visible lesion boundaries in UC. (c) Solid head shows gap between enamel and the coating resin, and the finger pointer represents the demineralization inhibition zone in UC adjacent to C area. (f) Finger pointer represents damage of resin coating.**
**A=air, E=enamel, D=dentin, R=resin material, C=coated area, UC=un-coated area and DEJ=dentinoenamel junction.**

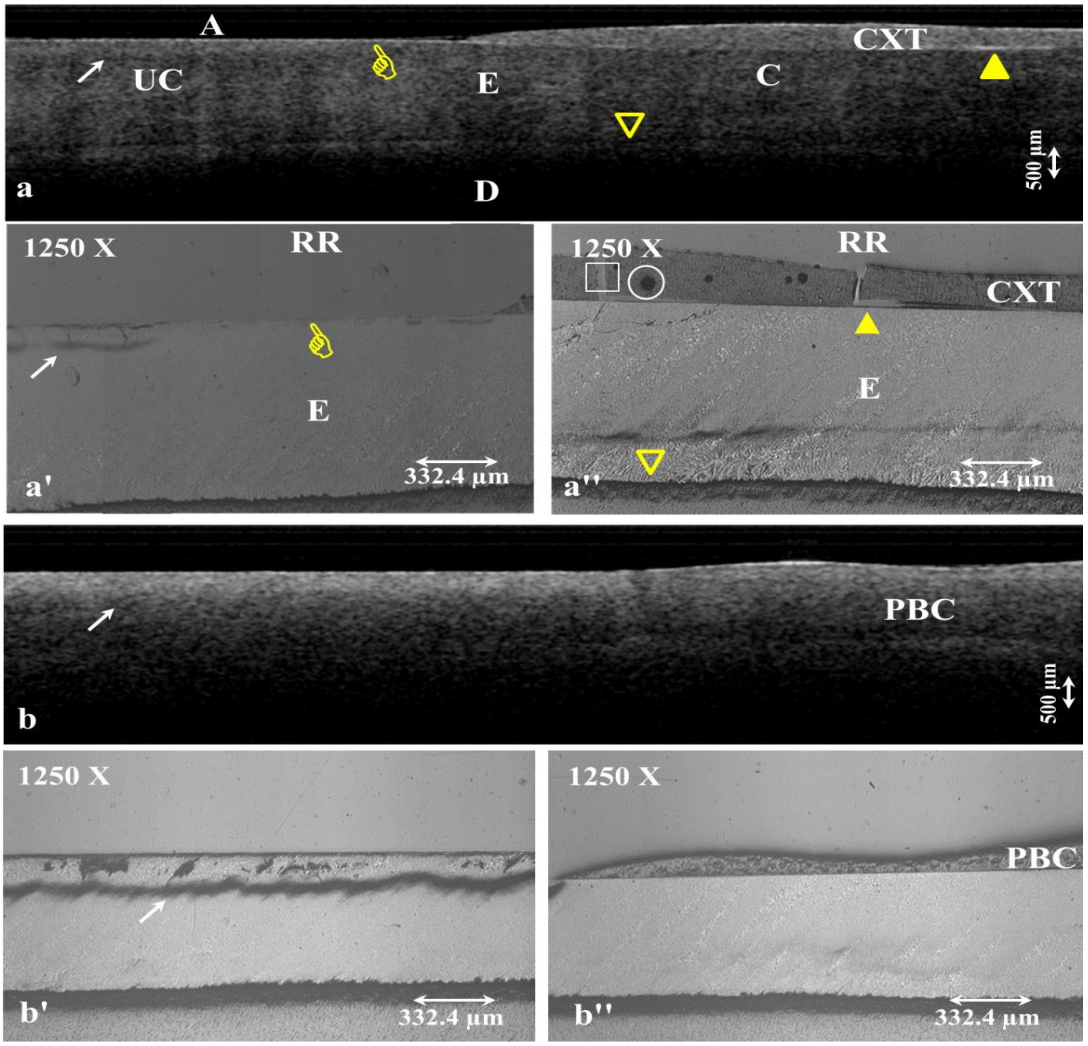


**Figure 4: B-scan images obtained by the OCT and confirmed by CLSM for CXT and PBC. (a and b) B-scan OCT images and (a', a", b' and b") CLSM images obtained for the CXT and PBC groups after 7 days. (a, a' and b, b') CXT and PBC after 7-day demineralization; white arrows indicate the lesion boundary that formed only in UC area. (a and a') Finger pointer indicates the inhibition zone in OCT with similar phenomena confirmed on CLSM image. (a and a") Arrow heads point to corresponding regions in OCT and CLSM images (solid head: gap and blank head: DEJ). (a") Circle and square indicate the void and crack, respectively.**

**A=air, E=enamel, D=dentin, C=coated area, UC=un-coated area, RR=second embedding resin and DEJ=dentinoenamel junction.**

## *2.4. DISCUSSION*

In the present study, bovine teeth were used, which are widely used in demineralization/remineralization studies and resin infiltration tests. Comparing to human enamel, it is easier to obtain bovine teeth in large numbers and good conditions with less variable composition. Bovine teeth have large flat surfaces and have not experienced prior caries challenges that might affect the test results [48]. A routine thermal cycling challenge was used to evaluate the durability of resin coats on the surface. Although thermal expansion of such thin layers on flat enamel surface may not be considered as a serious concern, previous studies have suggested that the increased number of thermal cycles accelerated degradation of resin through such mechanisms as increased water sorption and elution of ingredients [49]. Tooth brush abrasion and mechanical wear of resin coatings are also important challenges facing the application of these materials clinically [50].

The demineralization solution in this study was based on previous works, which resulted in formation of a subsurface enamel lesion [46]. It should be noted that the solution was not changed during the period of demineralization; this was done to boost the effects of any ions releasing from the materials in the in vitro study. Demineralization and remineralization processes on enamel and dentin are difficult to detect at early stages by visual inspection alone. The application of OCT for detection of enamel caries beneath composite filling and

sealants has been reported [44, 45]. The current results demonstrated the excellent potential of OCT imaging for observing coating thicknesses and changes which occurred under the coating after demineralization. Despite attenuation of OCT signal through the resin-based coats, surface of the enamel under the coats could be clearly monitored. Nevertheless, in CXT and PBC that showed higher thickness, DEJ was occasionally masked. In this regard, it was shown that the composition of resin materials affected the OCT signal pattern [38]. In addition it is difficult to detect the DEJ beneath the demineralization in some images of UC area, due to very low signal intensity from the structure. It was shown that enamel demineralization results in increased attenuation coefficient of near-infrared light and rapid loss of signal intensity [37].

There are two potential mechanisms that underlie the protective effect of the coats in this study. First and foremost, the hermetic sealing of the coating surface act as a physical barrier to impede the ingress of acid. Second, they supposedly release F and other ions that reinforce the tooth structure against acid or have buffering effects. The detected loss of interfacial integrity in this material may be related to the incomplete infiltration of CXT, which has a relatively viscous paste, into the phosphoric acid-etched enamel [22]. CXT is somewhat similar to the generic resin modified glass ionomers (RMGIs), and contains bisphenol-A-diglycidyl methacrylate (Bis-GMA) that has a high molecular weight. Moreover, in cross-sectional microscopy images (**Fig. 4**), the

coating layer appeared to be fractured. Such voids and cracks have been attributed to the continuous internal acid-base reactions known for glass ionomers [51]; and could also be due to the effect of the acidic environment on the integrity of material [52]. On the other hand, anti-caries effects of RMGIs were attributed to the presence of the loosely-bound F ions from the glass fillers, which are free to move and protect enamel surface [30]. F may reduce the rate of dissolution of enamel in the demineralization phase in acidic conditions and enhance the remineralization at the crystal surface [31, 53]. Moreover, CXT contains a compound that releases calcium into the solution [54]. The development of calcium-releasing dental restoratives is becoming a new trend [25, 55]. Calcium ions can enhance the remineralization effects of F and increase the local buffering effect at the demineralization site, which may explain significantly lower lesion progress in CXT compare to all other groups (**Fig. 6**) and the presence of the demineralization inhibition zone adjacent to the CXT coating, which is clearly observed in both OCT and confirmatory CLSM (**Fig. 4**).

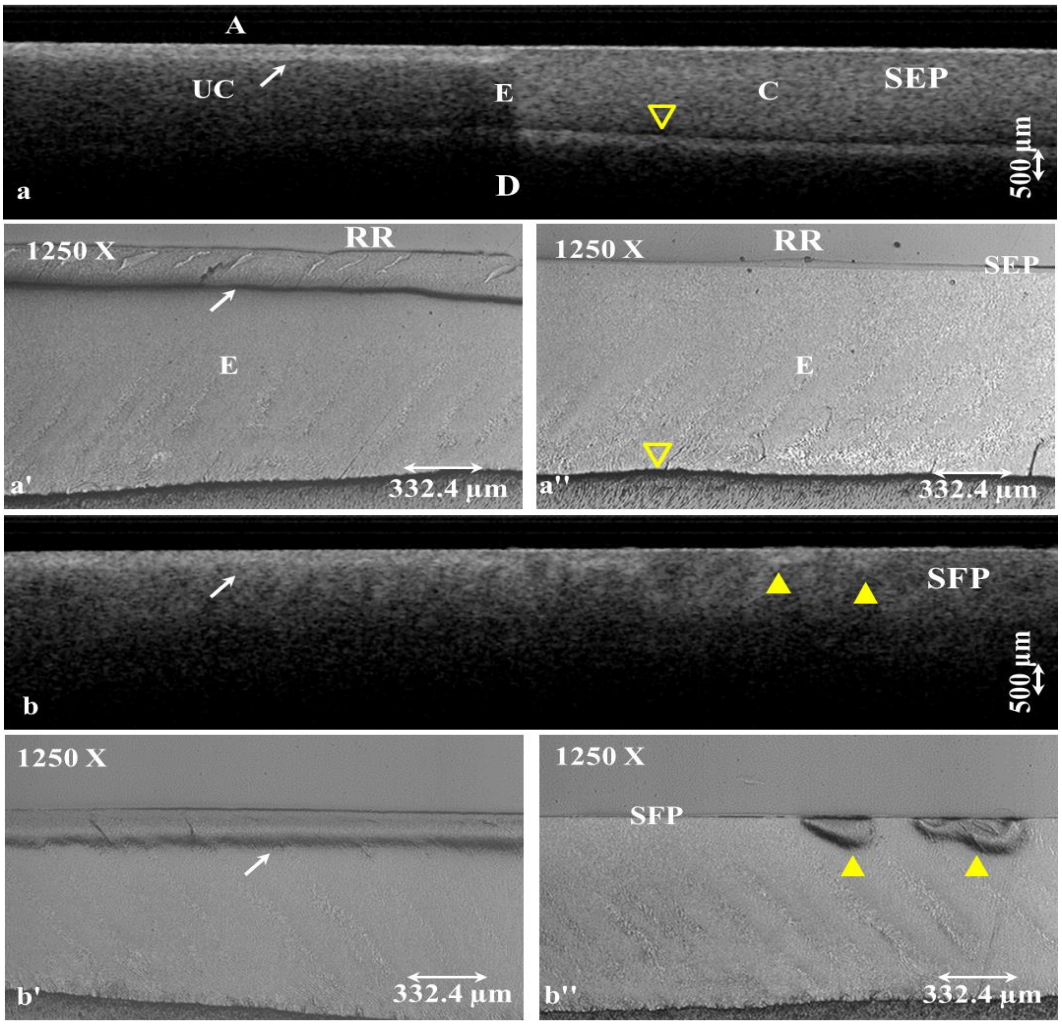


**Figure 5: B-scan images obtained by the OCT and confirmed by CLSM for SEP and SFP. (a and b) B-scan OCT images and (a', a", b' and b") CLSM images obtained for the SEP and SFP groups after 7 days. (a, a' and a") SEP after 7-day demineralization; white arrows indicate the lesion boundary that formed only in UC area. Blank heads point to DEJ in OCT and CLSM images. (b, b' and b") SFP after 7 days of demineralization; the thin coating generally protected enamel beneath it; however, patches of deep demineralized areas were seen in the C region (solid heads) in both OCT and CLSM.**

**A=air, E=enamel, D=dentin, R=resin material, C=coated area, UC=un-coated area and DEJ=dentinoenamel junction.**

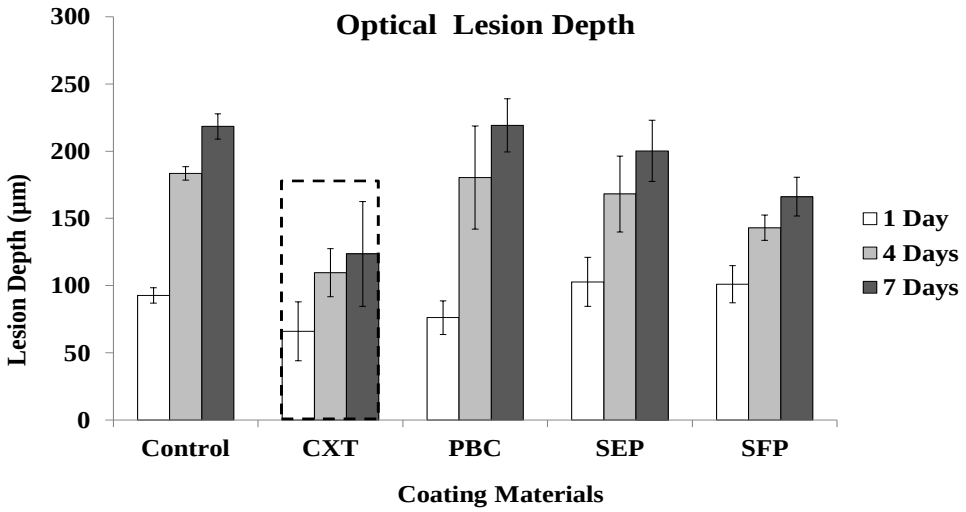


**Figure 6: The bar graph of the optical lesion depths for different groups and days. Optical lesion depth calculated by OCT of different groups through demineralization days. The dashed box indicates that lesion progress in CXT was significantly different from all other groups ($p<0.05$).**

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OCT scan in PBC group showed complete protection against demineralization despite partial detachment of the coating from enamel surface in two samples. In addition to the physical barrier effect of coating, the acid resistance of the enamel surface underlying the PBC can be explained by the release of multiple ions from the surface pre-reacted glass (S-PRG) fillers included in the composition of PBC as a giomer-based product. The release of F, silicon, boron, strontium and other ions is believed to be due to the presence of a glass ionomer phase around the glass core of the filler [56].

SEP coat remained intact after 7 days and nor gap neither detachment occur in this group. SEP is considered as a mild self-etch system which usually has a pH of about 2 which produce a shallower enamel etching than phosphoric acid etchants. Moreover, it has been shown that 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomer forms stable salts with calcium ions, contributing to the stability of its interface with enamel [32, 57]. Moreover, SEP is a F-releasing bonding agent; previous works have shown effective release of F from this bonding agent [33], due to the presence of NaF crystals in the bonding agent. It was suggested that the total amount of F released into solution from the bonding agent over 7-day period was comparable to those of RMGI and conventional glass ionomer when applied as enamel coating [58]; however, the surface treated NaF crystals in the bonding agent may not offer the F recharging ability that has been shown for glass particles in glass ionomers and S-PRG fillers in giomers [59, 60].

In SFP group, the resin showed detachment or damage and occasional demineralization patches in C area (**Fig. 3**f and **Fig. 5**b), it is formulated to have a very thin film thickness; however, it should be noted that this resin-based dentin desensitizer has similar composition to those of all-in-one adhesive systems. Several studies have reported on the permeability of the layer formed by this type of adhesives when applied as a single coat [61]; the simultaneous presence of hydrophilic and hydrophobic domains, together with solvent within

the bonding agent might affect the curing process and the integrity of the polymer formed thereby. It has been reported that the polymerized adhesive layer was porous due to the presence of residual solvents [47].

From a conservative point of view, an ideal coating material would form very thin, durable coat that can resist chemical, mechanical and thermal challenge, with no or limited need for etching and demineralization of enamel prior to its application. The thickness and wear resistance are especially important in proximal and occlusal areas. Such a material would seal the surface completely, while actively and sustainably releasing ions that reinforce the teeth and buffer the local environment such as, but not limited to, F. Finally, the desired coat would be antibacterial, resistant against biofilm formation and have ion recharging ability. In view of these properties, none of the coating materials alone fulfilled all the requirements in the current study; nevertheless, many of these properties were cumulatively observed, confirming the possibility of developing such a protective factor for the dental practice.

OCT can be an ideal adjunct clinical tool for regular monitoring of these coatings. In this view, the coating can be repeated or repaired in required areas that can be detected by OCT. Further probing of these coating, including the mechanical evaluation of both the resin coating and the underlying and surrounding enamel is underway since one of the main concern with applying a dental resin coating is wear and tooth brush abrasion. Within the limitations of

the research, which included a narrow study design, the proposed null hypotheses were rejected as covering enamel by resin material could prevent demineralization, and there was a difference between materials in this regard.

## *2.5. CONCLUSION*

A sealed enamel surface by thin resin coatings can contribute to the protection of smooth enamel surface from acid challenge depending on the properties of the material. Coating materials that actively release ions such as F would not only protect the covered areas, but also benefit the adjacent hard tissue.

## *2.6 Acknowledgments*

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# CHAPTER 3:
# EFFECTS OF PROTECTIVE RESIN COATING ON NANOMECHANICAL PROPERTIES OF ENAMEL AND ADJACENT AREA

# *CHAPTER 3*

# EFFECTS OF PROTECTIVE RESIN COATING ON NANOMECHANICAL PROPERTIES OF ENAMEL AND ADJACENT AREA

## *3.1. INTRODUCTION AND OBJECTIVES*

Dental enamel is the hardest tissue of the human body but susceptible to dissolution of the mineral phase by acid. Enamel consists of 95 wt. % minerals, mainly an impure calcium hydroxyapatite (HAp) crystals (100–1000 nm in length, 25–90 nm in thickness) make up larger formations as prisms (3–5 μm in diameter) [62]. In the caries processes, acid produced from bacterial metabolism diffuses into enamel and then dentin, and dissolves the mineral. White spot lesions are the early stage of caries development characterized by an enamel surface zone with subsurface demineralization [63]. Once this surface is broken due to continued effect of caries development, the lesions are cavitated and generally need to be restored. On the other hand, dental erosion is different from caries; it is defined as the chemical dissolution of dental enamel without bacterial involvement due to effects of acidic foods and drinks and considered as an

increasingly common problem among different ages [6]. Subjects with gastric disorder may also suffer from sever loss of tooth enamel caused by acid reflux.

Emerging concepts in management of oral health involve preventive strategies as well as new approaches to protect the teeth against demineralization, and to provide non-surgical management options for early lesions [64]. Tooth surface coverage appears to be an immediate simple and effective way to protect enamel against acid [65]. Historically, pit and fissure sealants in newly erupted teeth have been well known to decrease caries prevalence [21]. Areas with frequently extensive plaque accumulations adjacent to bonded orthodontic brackets have also been suggested to benefit from extending proper coating materials [28].

In addition to physical protection effects, newly developed dental resins may act as a reservoir of bioactive ingredients such as F and calcium (Ca) ions or other elements [64]. F-releasing sealants have been suggested to provide additional caries inhibition effect, since the F inhibits demineralization and favors the remineralization processes [58, 66]. However, the potential advantages of newly developed thin coating materials that release F or other ions are unknown and deserve further investigation [29].

The microstructure of enamel has adapted it withstand mechanical and abrasive stress. Loss of mineral content makes enamel vulnerable to mechanical load, while enamel may be physically reinforced depending on the composition of the

surrounding environment and its degree of saturation with regard to the minerals. Traditionally, transverse microradiography

(TMR) method has been employed to estimate the mineral content of dental hard tissue based on their radio opacity, rather than their actual mechanical strength [67]. On the other hand, Nanoindentation (NI) technique has enabled investigations of local mechanical properties of materials under various loading regimes based on load displacement data of indentations on submicron scale [68]. Measurement of nanohardness by this technique has been suggested as advantageous over the conventional hardness test methods for its high resolution of force and accurate indent positioning [13]. This technique has been employed in assessing enamel erosion and demineralization/remineralization [15, 16], has shown a good sensitivity to hardness changes at different depths of enamel [69]. Nanohardness mapping of enamel beneath and adjacent to the bioactive materials can reveal information on potential benefits of the ions released. Thus, the aim of current laboratory study was to evaluate the effect of resin coating materials on nanohardness of coated enameland adjacent area after demineralization challenge. The null hypotheses proposed were that covering enamel by resin material does not influence nanohardness of the enamel and adjacent area, and that there were no difference among the materials investigated.

## *3.2. MATERIALS AND METHODS*

### 3.2.1. Specimens preparation

The experimental procedure of the study is schematically presented in **Fig 7**. Twenty extracted, sound bovine incisors obtained from a local slaughter house (Yokohama, Japan) and checked to be free from any evidence of enamel cracks were collected and used according to a protocol approved by the Institutional Review Board of Tokyo Medical and Dental University for animal studies. The teeth were cleaned with deionized water to remove any surface debris and stored at -25°C until needed. Enamel blocks 6×3×3 $mm^3$ (length × width × depth) were cut from the bovine incisors using a low speed diamond saw (Isomet; Buehler) under running water, and embedded in epoxy resin (Epoxycure resin). The outer surface was slightly polished with 800-grit silicon SiC paper (Sankyo) to remove the superficial layer and expose enamel.

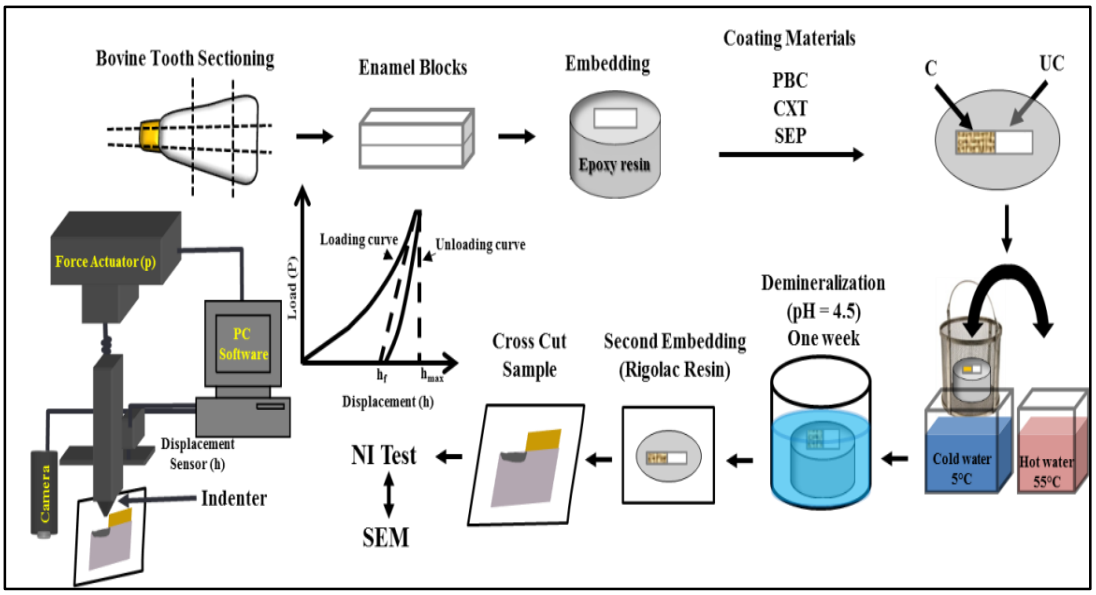


**Figure 7: Schematic drawing for the sample preparation and NI test. Bovine enamel blocks were embedded in epoxy resin; one of the coating materials (PBC, CXT and SEP) was applied on half of the enamel surface and then specimens were secondly embedded after the challenges, cross-sectioned and polished for SEM observation and NI test. Nanohardness was obtained after 5,000 thermal cycles plus one week demineralization for all groups. C= Coated, UC= Uncoated and NI= Nanoindentation.**

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## 3.2.2. Coating materials

According to the study design, the enamel blocks were divided into 4 groups (n=5/group) corresponding to the materials used and control. In the Control group, specimens received no treatment, three different resin-based materials were used to coat enamel in other groups; giomer coating PRG Barrier Coat (PBC; Shofu, Kyoto, Japan), resin-modified glass-ionomer Clinpro XT Varnish (CXT; 3M ESPE, St Paul, MN, USA); and two-step self-etch adhesive resin Clearfil SE Protect

(SEP; Kuraray Noritake Dental, Tokyo, Japan). Two areas, namely coated (C), and uncoated (UC), were assigned on the polished enamel surface of each block. Half surface of each block was carefully treated with the materials in accordance with the instructions supplied by the manufacturers as listed in **Table 3** for all groups that served as the C area while the other half stayed intact as UC area. The specimens were then stored in water for 24 h at 37°C.

### 3.2.3. Thermo cycling procedure

All specimens were placed in wire-mesh basket and subjected to 5,000 thermal cycles between 5°C and 55°C (Yamato Scientific, Tokyo, Japan), with 30 s dwell time at each temperature with an exchange time of 5 s between baths. This was aimed to allow the materials complete polymerization and water sorption under thermal aging.

### 3.2.4. Demineralization procedure

All samples were subjected to demineralization solution ($CaCl_2$ 1.5 mM, $KH_2PO_4$ 0.9 mM, $CH_3COOH$ 50.0 mM, $NaN_3$ 3.08 mM) at pH 4.5 at 37°C for one week. The pH of the solution was checked every day [33]. The volume of demineralization solution was 140 ml per each five samples. The solution was not changed to clarify the ion releasing and buffering effect of each material.

**Table 3: Composition and hardness of the materials used in this study.**

| Material | Brand | Composition | Application instruction | Hardness (MPa) |
|---|---|---|---|---|
| Giomer-based material | PRG Barrier Coat (Shofu, Kyoto, Japan) | Base: glass powder, purified water, Methacrylate monomer, S-PRG filler, phosphonic acid monomer<br>Activator: methacrylate acid monomer, Bis-MPEPP, carboxylic acid, TEGDMA, catalyzer. | Put one drop of Activator into Base and mix together.<br>Apply thin layer of the mixture,<br>Light-cure for 10 s. | 157.7±35.4 |
| Resin-modified glass-ionomer | Clinpro XT Varnish (3M ESPE, St. Paul, MN, USA) | liquid: HEMA, water, camphorquinone, calcium glycerophosphate and polyalkenoic acid<br>Paste: HEMA, Bis-GMA, water, initiators and fluoroaluminosilicate glass. | Apply acid etchant for 15 s with 35% phosphoric acid. Rinse with water.<br>Apply air for 5 s.<br>Mix paste/liquid components together rapidly for 15 s (2.5 min working time).<br>Apply thin layer to tooth surface.<br>Light cure for 20 s.<br>Wipe the coating by a moist cotton applicator. | 442.1±46.5 |
| Two-step, self-etch adhesive | Clearfil SE Protect (Kuraray Noritake Dental, Tokyo, Japan) | Primer: MDP, MDPB, HEMA, hydrophilic dimethacrylate, water<br>Bond: MDP, Bis-GMA, HEMA, dimethacrylate hydrophobic, di-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica, surface treated sodium Flouride. | Apply primer and leave for 20 s.<br>Dry with gentle air flow.<br>Apply bond.<br>Air flow gently.<br>Light-cure for 10 s. | 223.2±31.2 |

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol-A-diglycidyl methacrylate; S-PRG, surface pre-reacted glass inomer fillers; Bis-MPEPP, 2,2-Bis[4-(2-methacryloyloxyethoxy)phenyl] propane; TEGDMA, triethyleneglycoldimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MDPB, 12-Methacryloyloxydodecyl pyridinium bromide.

### 3.2.5. Nanoindentation test

The epoxy resin embedded enamel specimens were secondly embedded in polyester resin (Rigolac) to prevent breaking the surface or chipping of the specimen edges, and cross-sectioned along the longer edge of specimen using the low speed diamond saw into two halves to perform NI evaluation. To produce a smooth surface, the cross-section was sequentially polished by SiC papers #600, #800, #1000, #1200, #1500, and #2000, followed by diamond slurries with particle sizes of 6 μm, 3 μm, 1 μm, 0.5 μm, and 0.25 μm, with a lapping machine (Maruto, Tokyo, Japan). Every specimen was observed by CLSM after polishing to ensure there was no scratch on the surface.

On one cross-section obtained from each enamel block, 3 areas of interest, each 100 μm (lateral dimension) by 200 μm (axial dimension or depth) were defined to map nanohardness and draw nanohardness versus depth profiles at the center of C and UC area as well as the intermediate (INT) areas at the edge of coating (INT-C and INT-UC) using a NI device (ENT-1100a; Elionix, Tokyo, Japan). The maximum load was 2 mN at a loading rate of 0.2 mN/sec and 1 s holding segment with a Berkovich diamond tip at chamber temperature of 27.5°C. For this purpose, indentations were performed along 22 rows in each area, where each row included 10 points, with an axial spacing of 2.5 μm between each 2 neighboring points for the first 50 μm depth, then 50 μm and 100 μm spacing for the last two lines up to 200 μm from the surface. The lateral spacing between

points on each row was 10 µm, and the rows were programmed to avoid overlapping of neighboring points. This NI mapping strategy is schematically presented in **Fig. 8**. Nanohardness was calculated by dividing the maximum load over the area projected under the load, as described previously [58].

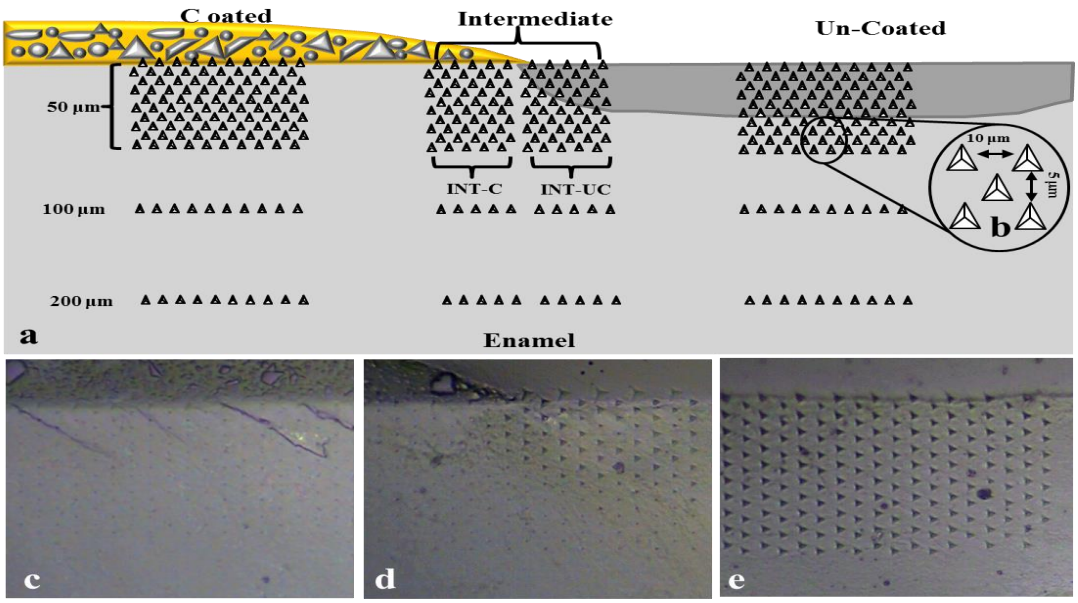


**Figure 8: (a-e) Schematic representations of the indentation points on cross-sectioned enamel. (a) Two matrices programmed on 20 lines with axial pitch distances of 2.5 µm (first 50 µm), 50 and 100 µm among rows for enamel (C, INT-C, INT-UC and UC). The total distance of the last raw from the surface was 200 µm. (b) The magnified area represents indentations programmed for nanohardness measurement. (c-e) A real optical microscopic image of polished of enamel surface after 50-µm depth NI of C, INT and UC enamel respectively. C= Coated, UC= Uncoated, INT= Intermediate and NI= Nanoindentation.**

### 3.2.6. Statistical analysis

Two variables were defined and subjected to statistical analysis in this study; surface zone nanohardness (SNH) that considered the average enamel nanohardness at the first 10 μm, and integrated nanohardness (INH), which was defined to compare the overall hardness values by calculating the area under the hardness profile curve at the first 50 μm, corresponding to the demineralized lesion body. Statistical analyses were performed using 2-way ANOVAs with experimental groups (PBC, CXT, SEP and control) and different areas of enamel (C, INT-C, INT-UC and UC) as factors, followed by multiple comparisons between each two pairs with Bonferroni correction. All the statistical procedures were performed at a significance level of $\alpha=0.05$ with the statistical package for social science (SPSS for windows, Version 16.0, SPSS, IL, USA).

### 3.2.7. Scanning electron microscope (SEM) examination

The observation was performed on the cross-sectioned surfaces either after polishing or after NI test. The specimens were cleaned ultrasonically in distilled water for 3 min, dehydrated at room temperature for 24 h, gold sputter-coated (300 Å), and finally observed in SEM (JSM-5310LV, JEOL, Tokyo, Japan).

### 3.2.8. Fluoride ion release

After one week of demineralization challenge, 10 ml of the demineralization solution in each container (PBC, CXT, SEP and control) was mixed with 1 ml of

buffer solution (sodium acetate trihydrate 0.1 M, pH 5.5). The test solution was placed in a beaker over a non-heating magnetic stirrer, and readings were taken after a five-minute immersion period using F ion selective electrode (8010-10C, 2060A-10T, HORIBA, Kyoto, Japan) connected to an ion analyzer (F-53, HORIBA). Standards were also prepared from sodium fluoride solution with concentrations of 0.05, 0.1, 0.5, 1, 10 and 100 ppm to which a buffer solution was added in order to obtain a constant background ionic strength. These standard solutions were used to plot the calibration graphs. The temperature of the solution was maintained at 25°C. F ion concentration in each solution was measured 3 times, and the mean value was reported in ppm.

## *3.3. RESULTS*

Nanohardness profiles in each group were presented in **Fig. 9** (a-d). **In Fig 9** (a) in C area, PBC and SEP continuously showed high values, while in CXT, the hardness value was lower for a few micrometers immediately beneath the surface but rapidly increased to reach the sound level and then remained constant.

The nanohardness trend of the control surface is presented together with those of UC area, uncovered zone away the coating, of different groups in **Fig 9** (b). Different nanohardness trends were found in each group; while SEP almost

followed same behavior as control with nanohardness trend representing a typical demineralized lesion, PBC showed a remarkable peak at the surface zone reaching a value of around 1600 MPa before reaching values as low as the control group, and CXT showed relatively high hardness values at all depths, which never fell below 1000 MPa.

Hardness profiles in INT-C area, at the edge of the coating beneath the material were presented in **Fig 9** (c). All materials showed a level of hardness drop when compared to C area, with lesion patterns forming in PBC and SEP despite superficial protection. In INT-UC area (**Fig 9** (d)), PBC showed a reinforced surface zone, typical of this material in all areas, while CXT and SEP showed low superficial hardness. Nevertheless, CXT exhibited rapid recovery with depth after 20 micron, resembling a trend similar to that of UC area.

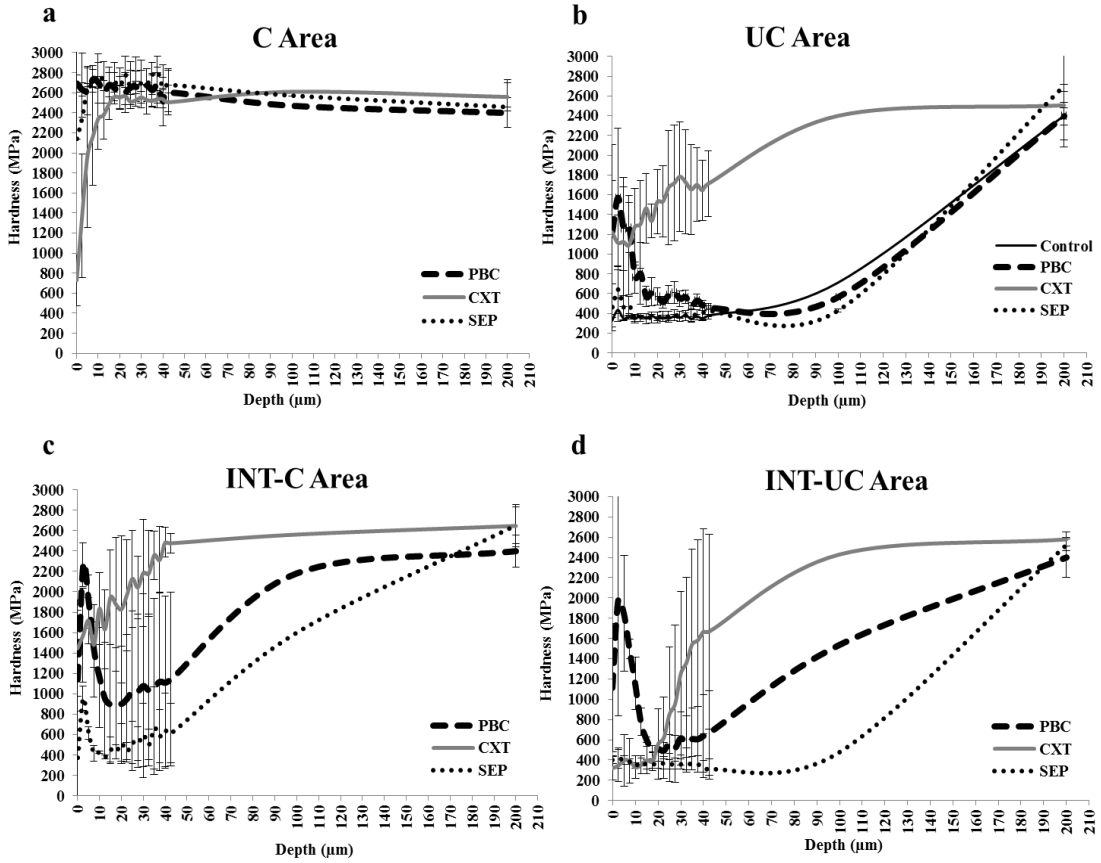


**Figure 9: (a -d) Mean overall nanohardness profiles with standard deviation covering an axial area of 200 µm for enamel samples coated by PBC, CXT and SEP for C, UC, INT-C and INT-UC areas respectively. C= Coated, UC= Uncoated and INT= Intermediate.**

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The values in the bar graphs in **Fig 10** (a and b) represent SNH at 10 micron depth and INH at 50 micron depth respectively, which were obtained using NI device of different groups through different areas (C, INT-C, INT-UC and UC). Two-way ANOVAs revealed that the coating material (PBC, CXT, SEP and control) and the indentation areas (C, INT-C, INT-UC and UC) and their

interactions were significant ($p<0.05$). Pair comparisons revealed that SNH values in C area were significantly lower in CXT compared to either PBC or SEP. While in INT-C, PBC and CXT showed higher SNH than SEP. In INT-UC significantly higher SNH values were found for PBC only. As for the UC area, PBC and CXT showed higher SNH than SEP and Control.

When INH was considered, there was no difference among groups in C area. On the other hand, the INH was significantly different among all groups ($p<0.05$) in INT-C, but not different in INT-UC. INH value in UC area of CXT was significantly different from all other groups ($p<0.05$), but there was no significant difference among other groups including the control.

The nanohardness results obtained in INT-C and INT-UC in **Fig 9** (c and d) were in line with SEM images of these areas for PBC, CXT and SEP respectively (**Fig 11**a-c). In INT-UC of PBC (**Fig 11**(a)), a clear honeycomb pattern below the superficial zone indicated subsurface demineralization, while that of CXT (**Fig 11**(b)) showed superficial demineralization but intact deeper enamel. **Figure 11**(c) suggested demineralization at INT areas of SEP, extending a few micrometers into the INT-C area at the edge and severely affected surface at INT-UC area.

**Figure 11**(d) is a SEM micrograph at higher magnification showing the arrangement of triangular NI marks of SEP in INT area.

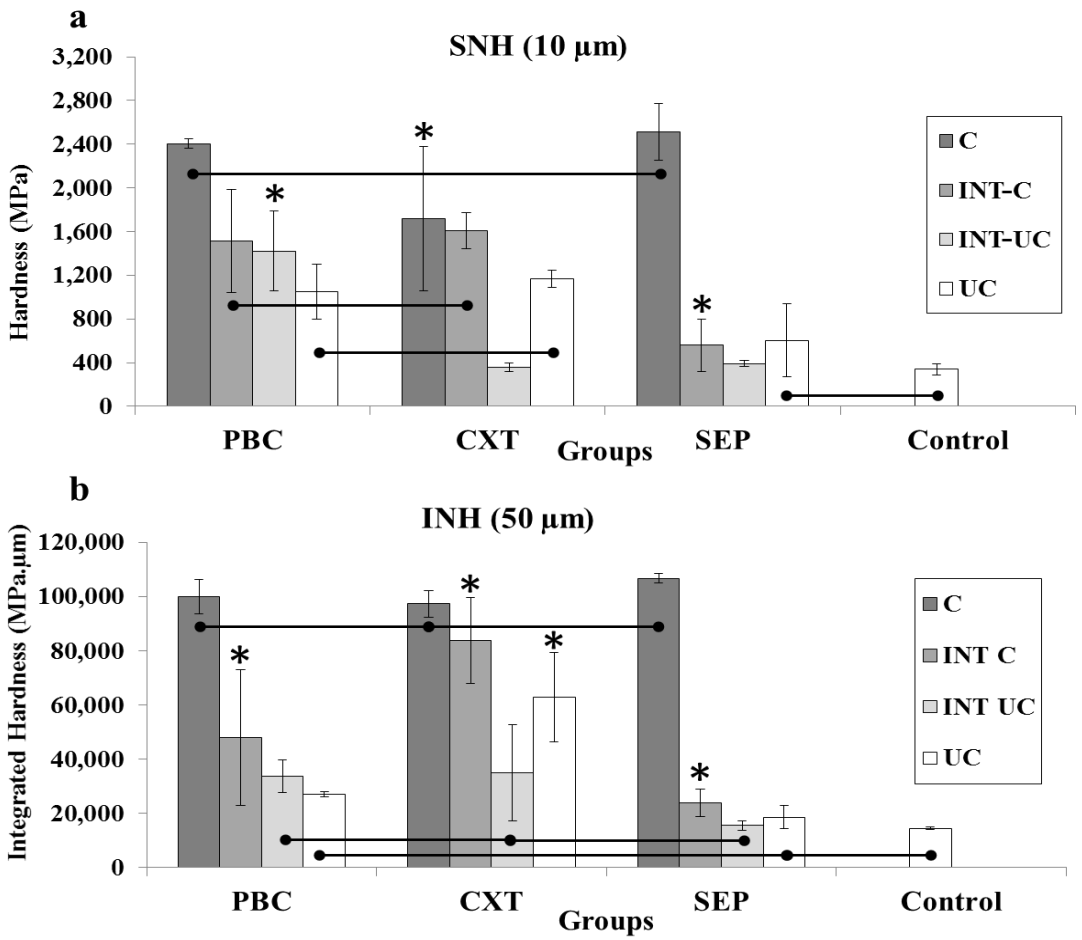


**Figure 10: (a) A bar graph showing SNH of all groups of C, INT-C, INT-UC and UC, till 10 µm; asterisk (*) shows significant difference ($p<0.05$). Horizontal bar indicates no significant difference among area subgroups ($p>0.05$). (b) A bar graph showing INH of all groups of C, INT-C, INT-UC and UC till 50 µm; asterisk (*) shows significant difference ($p<0.05$). Horizontal bar indicates no significant difference among area subgroups ($p>0.05$). C= Coated, UC= Uncoated, INT= Intermediate, SNH= Surface zone nanohardness and INH= Integrated Hardness.**

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Hardness values of the coating layers measured by NI are shown in **Table 3**. The highest mean hardness values were found in CXT. The greatest amount of

F release was also obtained in CXT solution (0.528 ppm) followed by PBC (0.149 ppm) were recorded by the ion selective electrode. In SEP and control groups, voltage values corresponding to <0.1 ppm were recorded that were converted to 0.010 and 0.002 ppm, respectively.

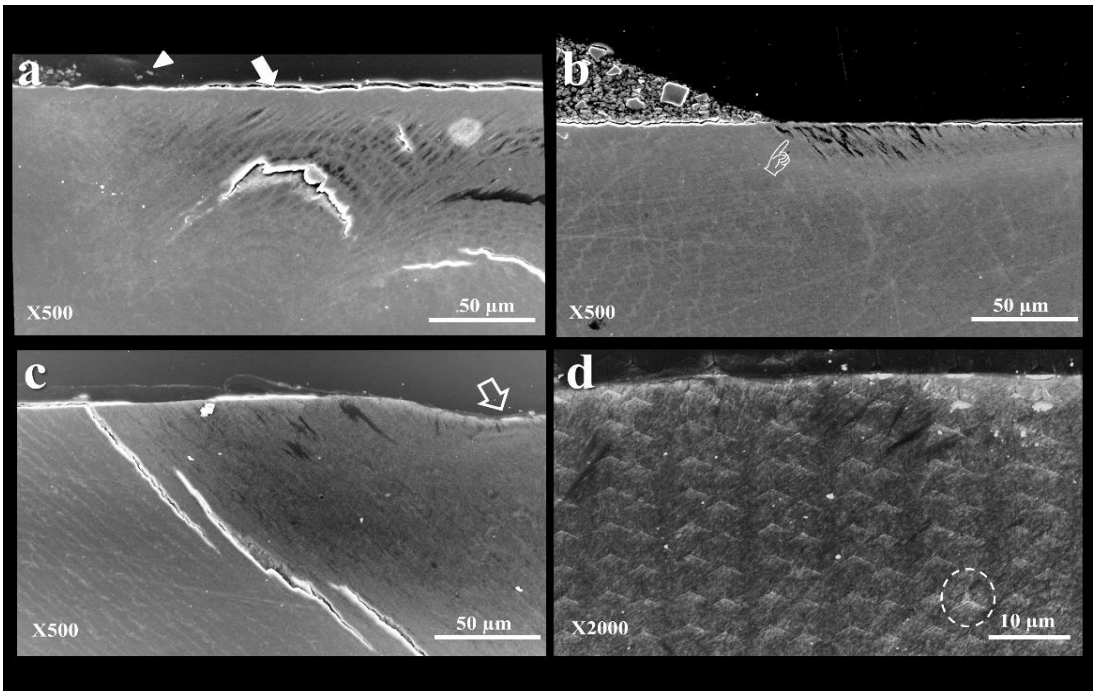


**Figure 11: (a-d) Representative SEM observations of INT-C and INT-UC enamel areas. (a) White arrow points to intact enamel surface in INT-UC area for PBC. White arrow head shows no filler area at edge of PBC coat. (b) Finger pointer indicates the lesion at INT-UC area. (c) Blank arrow shows the severely affected surface at INT-UC area. (d) SEM image of INT-C and INT-UC of SEP at 2000 x after NI test. Indentation marks are clearly observed on enamel (dashed circle). C= Coated, UC= Uncoated, INT= Intermediate and NI= Nanoindentation.**

## *3.4. DISCUSSION*

The coating of enamel could effectively protect underneath enamel against demineralization; however, hardness trends were remarkably changed at different areas and among groups. The small-force NI results in indentation depth in the order of a few hundred nanometers that can be termed nanohardness, and reflects the mechanical resilience of substrate that cannot be determined from other test such as TMR [67]. It should be noted that nanohardness mapping with micrometer spacing between adjacent points increases data resolution; however, given the time required to perform each indentation, sensitivity of the test at a small load, and the large amount of data produced, such a strategy is technically difficult for a large number of specimens [69]. The large amount of produced data in this study was analyzed by introducing two parameters; a depth-integrated variable (INH) to compare overall nanohardness and a local average (SNH) to compare only surface zones.

This in vitro study was performed bovine enamel, which consider to be suitable substrate for de/remineralization studies [37, 46, 70], even though lower hardness values have been consistently reported for bovine enamel compared to human enamel [70]. Under the 2 mN load, the NI with sharp Berkovich tip resulted in indentation maximum depth range 100 nm to 700 nm. Considering the tip geometry, such depth will result in dimensions of 0.75 μm to 5.3 μm for the indentation size on the cross-sectioned surface of enamel. This size is

confirmed by SEM image (**Fig 11**d), showing that the indentation mark was around 3 μm in lateral dimension. In general, a reduction in nanohardness of demineralized enamel was attributed to a less dense prism structure in the contact area projected under NI load, due to mineral loss after acid challenge [71].

Overall, all coating materials showed effective prevention of demineralization in C area, which was not unexpected due to the physical protection effects[72] . However, there were notable differences among groups, rejecting the second hypothesis. One interesting finding was the high SNH values of superficial INT-UC area in PBC, which may be due to multiple ions release from the glass-ionomer phase around the glass core of the filler of this material. The surface prereacted glass-ionomer (S-PRG) releases aluminum, sodium, silicon, boron, Strontium (Sr) and F ions; silicon and aluminum are elements that form the structure of glass, while Sr and F are added into glass as a modifier [73]. A recent study investigating an adhesive based on S-PRG fillers demonstrated that the adjacent dentin and enamel tissue took up F from the material, resulting in an acid-resistant zone [56, 74]. The release of F from the coating was confirmed in the current study, but considering the pattern observed in INT-UC of strong resistance of the surface zone and demineralization development at deeper areas (**Figs 9**(d) and **11**(a)), the role of other ions should be considered. This finding could be also explained by Sr release, which may further enhance acid resistance of enamel through formation of strontium-apatite complex [75]. Sr is a

homologous element of Ca and is thought to promote mineralization as a substitute and also has the capacity to enhance enamel remineralization in conjunction with F [56, 74-77]. It has also been suggested S-PRG filler had antibacterial and modulation effect on acidic solutions through releasing ions, bringing pH values closer to neutral, similar to the conventional glass ionomer cements [74, 78-80]. Nevertheless, the decrease in nanohardness value after 10 micron depth was remarkable, and can be due to disrupted ion transport into deeper layer by decreased pore volume of the surface layer [81], resulting in INH values not different from the control.

In UC area, the highest INH values were obtained in CXT compared to other groups. The phenomenon may be explained by high F release from CXT which contains fluoroaluminosilicate glass [31], as confirmed by the F measurement test. It has been shown that low concentrations (up to 1 ppm) of F in a solution can reduce and even inhibit enamel demineralization [82]. The formation of intraoral reservoirs capable of supplying ions for a prolonged period is crucial for the success of topical treatments [30]. Furthermore, CXT contains a calcium-phosphate compound (calcium glycerophosphate) that may release Ca into the solution, Ca may also play role in preventing demineralization or re-hardening of enamel surface [54].

On the other hand, CXT presented the lowest SNH value in C area compare to other groups, probably due to destructive etching effect of 35% phosphoric

acid [83]. Similarly, CXT showed a distinct lower SNH in INT-UC area. As confirmed by SEM image (**Fig 5**b), this zone continuously extended a few hundred micrometers from the coating edge. This finding may be attributed to phosphoric acid etching effect during application of CXT. Phosphoric acid is commonly used in dentistry to etch enamel surface and prepare it for resin infiltration, resulting in micromechanical retention. The phosphoric acid increases the selective solubility of the enamel and exposes the prism structure and roughens the surface [84]. This finding has a clinical implication as well; acid etching of enamel that will not be fully covered by the resin material (whether for preventive or restorative purposes) should be avoided as this may facilitate penetration of acids and increase the risk of caries development at the resin edge.

SEP is a self-etching adhesive material that does not require a separate acid etching step as it performs mild etching and resin infiltration simultaneously. Moreover, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomer can penetrate into the demineralized enamel and envelop the HAp crystals upon curing and forms stable salts with Ca ions, making hermetic sealing act as a physical barrier and protect underneath enamel from acid attack [32, 85, 86]. In another hand, SEP presented low INH and SNH values among other areas (INT-C, INT-UC and UC), suggesting acid diffusion through enamel at the uncoated and intermediate zones, as confirmed by SEM image. Unlike PBC and CXT, the demineralization resulted in a severely affected surface zone in UC

area of SEP (**Fig 5**c), which was attributed to the level of F release. Although SEP is considered to be F-releasing material, in this study very small amount of F (<0.1 ppm) was detected in this group. Two possibilities may explain this finding; first, F ions were quickly lost during the thermal cycle challenge from the surface pretreated Sodium Fluoride (NaF) filler in SEP, due to the high solubility of NaF in water [87]. Second, smaller amount of F released by SEP could be attributed to its lower layer thickness compared to CXT and PBC [88], which meant smaller volume of material for each sample.

The current in vitro study highlights the ability of coating materials to cover tooth in order to withstand the demineralization challenge. However, due to anatomical structure of the tooth and accessibility difficulties, such a goal is hard to be achieved in all areas. The results emphasize on the ability of coating materials to protect adjacent area as well as coated area. Effective hermetic sealing without degrading of enamel surface upon application, and compositions capable of releasing ions (e.g., F, Sr and Ca) to protect and remineralize enamel, both superficially and in-depth, are the most desired characteristics of coating materials. Another important characteristic of coating materials is their ability to protect edge of coating, which is a critical site, susceptible to plaque accumulation.

The short-term effects on adjacent enamel were favorable for glass-filler containing coatings. However, other requirements such as durability and wear

resistance can make coating materials even more desirable clinically. Relatively low nanohardness values of materials presented (**Table 3**) indicate that these coating are likely to be worn out under intraoral conditions. Clearly, the oral cavity is a mechanically dynamic environment due to occlusion, mastication and tooth brushing abrasion. Future studies should be undertaken to examine wear resistance characteristics in relation to film thickness and composition of materials.

## *3.5. CONCLUSION*

Within limitations of the current in vitro study, evaluation of nanohardness on a wide range of locations could clarify the effects of various coating materials on enamel structure. The coating material releasing Sr and F (Giomer) protected enamel against demineralization superficially adjacent to coated areas. A resin-modified glass-ionomer with Ca release improved in-depth protection. Phosphoric acid-etched enamel without effective resin coverage at the edge of coating was highly susceptible to demineralization.

## *3.6. Acknowledgments*

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# CHAPTER 4

# *CHAPTER 4*

# GENERAL CONCLUSIONS

In both two studies, experiments were done in the laboratory. However, the potential for OCT-based diagnostics in the oral cavity is excellent.

A number of factors influencing the effectiveness of resin coating materials to protect enamel against demineralization were identified using OCT and nanoindentation and discussed in the presented studies

- **In chapter 2,** A sealed enamel surface by thin resin-based bioactive coatings can contribute to the protection of smooth enamel surface from acid challenge in high/moderate risk patients depending on the properties of the material. There was a difference among materials in their ability to protect enamel beneath and adjacent to the coating. Coating materials that actively release ions such as F would not only protect the covered areas, but also benefit the adjacent hard tissue. While very thin layers (<10 μm) are not clearly detected under OCT, the modality can be used to non-destructively monitor enamel

changes beneath these protective coatings and adjacent to them. In addition SS-OCT is a modality that can be clinically utilized to monitor these coatings as well as their underlying enamel, provided that appropriate image analysis and interpretation methods are established.

- **In Chapter 3,** concluded that within limitations of the current in vitro study, evaluation and mapping of nanohardness on a wide range of locations could clarify more details regarding the effects of various coating materials on enamel structure. Resin-based materials applied as a coat with a few micrometer thicknesses to cover the enamel surface will be protected from erosive acid challenges. Coatings with F-releasing glass fillers (PBC and CXT) contributed to reinforcement of adjacent enamel. However Sr and F released from PBC can protect superficially neighboring enamel against demineralization. While Ca released from CXT will improve in-depth protection. Phosphoric acid-etched enamel without resin coverage at the edges was susceptible to demineralization.

This work will have an important role in the laboratory and clinical evaluation of the success of achievement of a sealed tooth which will contribute

to the whole concept of "super tooth" and practice of restorative dentistry, as well as patient satisfaction. This will be existing on the full enamel coating by resin material and that will lead to resist the demineralization. In addition strengthens the enamel with F ions.

Finally, this non-invasive imaging technique (OCT) is very promising technology for laboratory research on the durability and effectiveness of new dental protective polymer thin films, and potentially for clinical monitoring of this new class of materials placed on the patients' teeth.

Nanomechanical evaluation would more clarify the relationships between the various coating materials and enamel structures. Such investigations would offer possibly new ideas for novel materials design and synthesis to protect enamel surface against intraoral challenges.

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# BIOGRAPHY

# ***BIOGRAPHY***

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**Date of Birth:** October 28th 1981

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# MY MISSION, VISION AND VALUES:

## MISSION:

Providing a high quality of the dental service to all patients by excellent work.

## VISION:

The ability to save the teeth from demineralization, attrition, abrasion and erosion by covering with Bio-active Coating Materials. Moreover, make the Kingdom of Saudi Arabia in the ranks of developed countries, which can completely eliminate teeth decay.

## VALUES:

-Perfection in the work.

-Increasing my knowledge and experience in Dentistry.

-The working team to achieve my goals.

## EDUCATION:

Bachelor Degree in Dental Medicine and Surgery (B.D.S spring semester 2005-2006) in King Abdul-Aziz University (KAU), Jeddah, Saudi Arabia (SA).

## PROFESSIONAL EXPERIENCE:

• Operative dentistry. TMDU Hospital, Tokyo, Japan (November 11, 2013 – till date).

• General Dentist. Ministry of Health, Yanbu, SA (November 1, 2007 – January 30, 2012).

- Two years and 6 months (restorative and root canal treatments). Dental Center, Yanbu General Hospital.
- 6 months (fillings, root canal treatments and extractions). Yanbu Primary Health Care.

• Dentist / Intern. KAU (September 2, 2006 – September 31, 2007).

- 5 months (emergency and oral surgery department). KAU, Jeddah, SA.
- 3 months (restorative department). King Fahd General Hospital (KFGH), Jeddah, SA.
- 3 months (endodontic and pedodontic departments). King Khalid General Hospital (KKGH), Najran, SA.

## RESEARCH INTEREST:

- Advanced Bio-Medical imaging systems.

- Nano-technology in the restorative dental sciences

- Adhesion of biomaterials to dental tissues.

- Caries research.

- Physic-chemical and manipulative properties of restorative materials.

- Bio-active coatings.

- Enamel structure.

## MEMBERSHIP:

- International Association of Dental Research (IADR).

- Super-student Member of Global Center Of excellence (AISS - GCOE).

- Optical Society of America (OSA).

- Member of Saudi Dental Society (SDS).

## COMMUNITY ACTIVITIES:

- Member in dental education program (Clean your teeth all your days) for primary schools. Yanbu, SA (2010).

- Member in dental education program for primary schools. Yanbu, SA (2009).

## PUBLICATIONS:

1- Effects of Protective Resin Coating on Nanomechanical Properties of Enamel and Adjacent Area. Dental Materials, (Under review 2015).

**Ehab Z Alsayed**, Ilnaz Hariri, Alireza Sadr, Syozi Nakashima, Yasushi Shimada, Turki A. Bakhsh, Junji Tagami.

2- Monitoring of enamel protection against demineralization by resin coating using optical coherence tomography. Dental Materials Journal, (published), Vol. 34, No 1, pp. 98-107, Feb, 2015.

**Ehab Z Alsayed**, Ilnaz Hariri, Alireza Sadr, Syozi Nakashima, Turki A Bakhsh, Yasushi Shimada, Yasunori Sumi and Junji Tagami.

3- Concurrent evaluation of composite internal adaptation and bond strength in a class-I cavity. Journal of dentistry (published), Vol. 41, No 1, pp. 60-70, Oct, 2013.

Turki A Bakhsh, Alireza Sadr, Yasushi Shimada, Mona. M Mandurah, Ilnaz Hariri, **Ehab Z Alsayed**, Junji Tagami and Yasunori Sumi.

## RESEARCH EXPERIENCE:

- Prevalence of dental caries among premature schoolchildren. KAU, Jeddah, Saudi Arabia (2006).

## CONFERENCES:

## Conferences & Continuous Education:

1- AADR/CADR Annual Meeting and Exhibition at the Charlotte Convention Center. Charlotte, N.C., USA (March 19 - 22, 2014).

2- Molecular mechanism in bone and tooth, its clinical implication. TMDU, Tokyo, Japan (February 17 - 18, 2014).

3- 5th TMDU International Summer Program. Tokyo, Japan (August 26 - 30, 2013).

4- Optical Coherence Tomography in Dentistry. TMDU, Tokyo, Japan (June 20 - 21, 2013).

5- IADR/AADR/CADR General Session & Exhibition. Seattle, Washington, USA (March 20 – 23, 2013).

6- Molecular Science in Oral-Systemic Medicine. TMDU, Tokyo, Japan (February 3 – 4, 2013).

7- International symposium adhesive dentistry. Tokyo, Japan (May 19, 2012).

8 - 5th Jeddah Dental Esthetic Conference 2011. Jeddah, SA (May 9 - 11, 2011).

9- Art of dealing with people - lecture. Yanbu General Hospital, Yanbu, SA (Jan 19, 2010).

10- The 4th annual meeting of Saudi orthodontic society. Jeddah, SA (November 2009).

11- 2nd International KAU and 19th Saudi Dental Society Conference for Dental Research and Technology. Jeddah, S.A (2008).

12- Tempromandibular joint problems and how to treat - lecture. KKGH, Najran, SA (2007).

13- The UAE International Dental Conference and Arab Dental exhibition ADEEC. DUBAI, United Arab Emirates (2004).

## ORAL PRESENTATION:

1- Protection of Demineralized Enamel by Resin coating: Nanoindentation evaluation. OSA Chapter, TMDU, Tokyo, Japan (February 28, 2014).

2- Demineralization Prevention by Enamel Resin Coating: Optical Coherence Tomography Evaluation. IADR, Seattle, Washington, USA (March 23, 2013).

3- Demineralization Prevention by Enamel Resin Coating: Optical Coherence Tomography Evaluation. TMDU, Tokyo, Japan (March 6, 2013).

4- Long-term Evaluation of Enamel Coated by Resin Thin Film using SS-OCT. International student research GCOE, TMDU, Tokyo, Japan (31st October, 2012).

5- Bone histology and embryology. Maxillofacial Department, KFGH, Jeddah, SA (2007).

6- Mineral trioxide aggregate (MTA).KAU, Jeddah, SA (2006).

7- Single tooth movement (case presentation). Orthodontic Division, KAU, Jeddah, SA (2006).

8- Adult comprehensive care clinic (CCC case presentation). Dental Faculty, KAU, Jeddah, SA (2006).

## POSTER PRESENTATIONS:

1- Protection of Demineralized Enamel by Resin Coating: Nano-indentation Evaluation. AADR/CADR Annual Meeting and Exhibition, Charlotte Convention Center, Charlotte, N.C., USA (March 19 – 22, 2014).

2- Monitoring and Measuring of the Enamel Lesion Depth using Optical Coherence Tomography. Molecular mechanism in bone and tooth, its clinical implication, TMDU, Tokyo, Japan (February 17 – 18, 2014).

3- Evaluation of Enamel Coated by Resin Thin Film using SS-OCT and Nano-indentation. 5th TMDU International Summer Program, Tokyo, Japan (August 26 – 30, 2013)

4- Optical and Nano-indentation Mechanical Properties Evaluation of Enamel Coated by Resin-thin-film. Optical Coherence Tomography in Dentistry, TMDU, Tokyo, Japan (June 20 – 21, 2013).

5- Long-term Evaluation of enamel Coated by Resin Thin Film using SS-OCT. Molecular Science in Oral-Systemic Medicine, TMDU, Tokyo, Japan (February 3 – 4, 2013).

## AWARD/PRIZE:

1- Award of Excellence Research from Saudi Cultural Office. Tokyo, Japan (March 3, 2015).

2- Award of Excellence Research from Saudi Cultural Office. Tokyo, Japan (March 13, 2014).

3- Award of Excellence Research from Crown Prince Salman Abdul-Aziz. Tokyo, Japan (February 22, 2014).

4- GCOE award as super student in research. TMDU, Tokyo, Japan (2014).

5- Prize from Japanese Society for Adhesive Dentistry was introduced during the 32nd annual meeting of the society for the work on "Nanoindentation of bonding resins at the cavity floor dentin" as 2nd author. Fokuka, Japan (December 1, 2013).

6- GCOE award as super student in research. TMDU, Tokyo, Japan (2013).

7- Award for joining the Pre-Clinical Supervision Program for the Japanese dental student. Cariology and Operative Department, TMDU, Tokyo, Japan (2013).


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