AGE EFFECT ON PRESENCE, SUSCEPTIBILITY AND TREATMENT OF EROSIVE TOOTH WEAR

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DEDICATION

All praise belongs to God, Who helped me to accomplish this dissertation. This thesis is dedicated to my deceased parents, who spent their lives inspiring and supporting me. To my beloved husband Aied Algarni, for his unlimited love and support. To my dearest children Mastor, Razan and Asal, for their smiles and making my life more meaningful. To my beautiful sisters Lulwa, Thana'a and Khayar, for their continuous prayers and encouragement. To my great friends Hadeel, Maryam, Afnan, Ala'a, Sara, and Layla, for their support and encouragement.

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Erosive tooth wear (ETW) is a growing dental condition often associated with aging. This in-vitro project comprised three studies aiming to investigate the impact of tooth age on ETW susceptibility and prevention. In the first study, un-identified extracted premolars were collected and had their ages estimated using validated dental forensic methods. The premolars were examined to investigate the relationship between age and presence and severity of ETW, as well as other main dental-hard tissues conditions. ETW, dental caries, fluorosis, extrinsic staining and tooth color were evaluated using established clinical indices. In the second study, the tooth age impact on ETW susceptibility and response to preventive treatments (Sn+F, NaF, and de-ionized water control) were evaluated using representative samples from the initial study. Enamel and dentin specimens were prepared and subjected to daily erosion-treatment-remineralization cycling procedure. Surface loss (SL) was determined during and after the cycling, by optical profilometry. Similar protocol was adopted in the third study with the addition of toothbrushing abrasion to the model, in order to explore the interplay between age and toothpaste abrasivity on erosion-abrasion development. SL was measured during and after the erosion-toothbrushing-remineralization cycling. The relationships between age and the investigated variables were assessed using linear regression models. In conclusion: 1. The presence and severity of ETW, dental caries, and extrinsic staining increased with age, while of enamel fluorosis decreased. Tooth also showed to be darker with age. 2. Susceptibility of enamel and dentin to demineralization increased with age. Sn+F showed the highest anti-erosive efficacy, and was not affected by age. NaF showed lower efficacy on dentin, which increased with age. 3. Enamel and dentin SL increased with toothpaste abrasivity level. Dentin SL also increased with age. Age effect on enamel SL was observed only with low abrasive toothpaste. Age-related changes on enamel and dentin affected ETW development.

Anderson T. Hara, DDS, PhD. Chair

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

The senior population (65+ years old) has increased considerably. In 2015, seniors represented 14.9% of the US population, and are expected to rise to approximately 24% by 2060 (Wan et al., 2016). Epidemiological studies revealed high prevalence of main dental pathologies among elder, including erosive tooth wear (ETW) (Van't Spijker et al., 2009).

ETW is defined as the chemical dissolution of hard dental substrate due to exposure to non-bacterial extrinsic or intrinsic acids. ETW lesion is characterized by surface softening of enamel or dentin. Increase in acid exposure makes the softened surface layer more prone to abrasive wear as a result of abrasion with harder objects (three-body abrasive wear), as in toothbrushing (Ganss, 2014). The importance of ETW comes from its potential negative impact on a person's quality of life. ETW has been related to increase in patency of dentinal tubules, and consequently, dentin hypersensitivity (West et al., 2013; Olley and Sehmi, 2017). Functional and esthetic issues have also been related to ETW (Morley, 1997; Muts et al., 2014). These undesirable outcomes are likely to be exacerbated by aging (Morley, 1997; Olley and Sehmi, 2017).

During aging, the dental hard-tissues undergo continuous structural (physical, chemical and mechanical) changes, as response to biological and environmental factors (Gustafson, 1950; Johanson, 1971). The first scientific evaluation of morphological changes due to teeth aging was done in 1950 (Gustafson, 1950). Then, forensic researchers followed up trying to identify the main age-related criteria for chronological age estimation (Bang and Ramm, 1970; Johanson, 1971). In addition to morphological changes, aging has been found to significantly impact the microstructure, as well as the mechanical and physical properties of enamel and dentin. Regarding chemical composition, there is increase in mineral, and decrease in protein contents of enamel and dentin with age (Kinney et al., 2005; He et al., 2011; Miake et al., 2016). This is accompanied by alterations in physical properties such as increase in tooth darkness, dehydration and reduction in permeability (Toto et al., 1971; Tagami et al., 1992; Mayoral et al., 2013). Generally, dental substrates' strength, fracture toughness, fatigue and crack propagation deteriorate with age (Kinney et al., 2005; Park et al., 2008). These changes can potentially affect enamel and dentin susceptibility to erosive and abrasive tooth wear.

Successful prevention of ETW should start from determination of individual risk. This can be achieved by detailed history of the patient's dietary and behavioral practices, in order to identify the etiological factors. In addition, several studies revealed positive results of fluoridated rinsing solutions in ETW prevention, with tin-containing solutions showing superior anti-erosive efficacy (Lussi and Carvalho, 2015). Fluoridated toothpastes also have been observed to reduce mineral loss after an erosive attack. Despite the benefits of fluoride, the abrasive components of toothpastes may exhibit adverse effect by mechanically abrading the softened eroded enamel and dentin (Hara et al., 2009).

Although several oral environmental factors are potentially associated to the increase in ETW prevalence, little is known about the influence of dental tissues' age on their susceptibility to demineralization. Besides, there is limited data available in the literature regarding the possible influence of tooth age-related changes on ETW-affected enamel and dentin responses to fluoride-containing rinses and toothpastes. The overall aim of this project was to explore how tooth age impacts ETW susceptibility and prevention, and whether specific age-related management protocols are needed. In order to achieve that, we evaluated extracted human teeth with different estimated ages to determine the presence of main dental hard-tissue pathologies, as well as their susceptibility to laboratory-induced ETW and responses to preventive treatments.

1.2. Project aims

This thesis presented three specific aims, individually described in Chapters:

Aim 1 (Chapter 2): to assess the presence and severity of the main dental hard-tissue problems (ETW, caries, enamel fluorosis, staining, change in tooth color) in extracted pre-molars with different estimated ages, using established indices.

Aim 2 (Chapter 3): to assess the susceptibility of enamel and root dentin of different estimated ages to erosion, and to investigate the tooth age impact on the efficacy of anti-erosive fluoride mouth rinses (Sn+F and NaF), using an in-vitro erosion-remineralization cycling model and optical profilometry.

Aim 3 (Chapter 4): to evaluate the susceptibility of enamel and root dentin of different estimated ages to erosion-abrasion, and to compare the impact of fluoridated toothpaste abrasivity (low, mid and high), using an in-vitro erosion-remineralization-toothbrushing abrasion model.

CHAPTER 2: TREND-ANALYSIS OF DENTAL HARD-TISSUE CONDITIONS AS FUNCTION OF AGE

2.1. Introduction

Population aging is a global trend, and the percentage of older individuals (65+) is expected to more than double over the next half century (United Nations, 2015). Aging is defined as the cumulative and progressive change that occurs with time, causing deterioration in structural integrity, as well as increase in disease susceptibility and debilitated function (Lopez-Otin et al., 2013).

Besides improvement in dental health awareness and preventive measures, age may also impact propensity for dental disease. Tooth aging is related to several behavioral (environmental) and biological (tooth) factors. Teeth suffer different mechanical and chemical insults throughout a person's life. The accumulation of these experiences may affect the properties and behavior of dental hard tissues. Several microstructural changes have been correlated with age, including increase in mineral content, decrease in organic bridging ligaments at enamel rods and dentin tubular occlusion (He et al., 2011; Yahyazadehfar et al., 2014). These changes are likely to impact enamel and dentin mechanical, physical and chemical properties. Increase in brittleness and decrease in fracture toughness with age cause an overall reduction on the mechanical strength of enamel and dentin (Yahyazadehfar et al., 2014). Other properties, including solubility, ion-exchange and tooth color may also alter with age. Consequently, the susceptibility to demineralization (as in dental caries and erosion), rate of remineralization, and tooth shade may all change as well. Moreover, behavioral aspect such as diet and oral hygiene may significantly impact the presence of those diseases and conditions as well as tooth appearance, including abraded fluorotic enamel and tendency to retain more extrinsic staining. Despite the importance of this topic, scarce data are available in the literature to allow deeper understanding of the age impact on dental conditions.

Major limitations of longitudinal clinical studies, such as time and costs, prohibit conducting a comprehensive evaluation of the prevalence and severity of the most common dental hard-tissue conditions and diseases. Meta-analyses from previous clinical studies are limited, due to lack of robust retrospective data of different ages. Considering these circumstances, we propose that a systematic laboratory approach using extracted teeth with estimated ages can be valuable. Tooth aging manifests a highly predictable developmental sequence of morphological and biochemical changes, which allows the forensic identification of an individual's age using mathematical models (Gustafson, 1950; Johanson, 1971).

We hypothesized that an individual's susceptibility to dental hard-tissue diseases and conditions change throughout life, suggesting a need for age-specific clinical preventive and therapeutic protocols. In the current study, we explored this hypothesis by evaluating the occurrence of clinically common dental problems in a relatively large set of extracted human premolars, with a broad age range. Our unique experimental approach consisted of using established forensic methods to estimate tooth age, and established clinical indexes to assess dental pathologies and conditions, as well as staining and color.

2.2. Materials and Methods

Tooth collection

A sample of 1500 extracted human premolars were randomly selected from an existing pool (approximately 18000 premolars) at the Oral Health Research Institute (OHRI), Indiana University School of Dentistry (Indiana University IRB # NSO 911-07). This tooth-bank was compiled through collection from numerous dental practice clinics across the USA over several years. Upon receipt at OHRI, teeth were sorted, cleaned and kept in 0.1% thymol, at 4º C. Patients metadata (e.g., age, sex, reason for extraction) were not available, rendering all samples unidentifiable. Our exclusion criteria included teeth with an advanced caries lesion (i.e. ICDAS 6) (ICDAS coordinating committee, 2009), restoration and fracture.

Sampling was performed randomly assuming similar distribution among five empiric age categories with 15 year-intervals (<25; 25-40; 41-54; 55-70; >70) to ensure proper coverage of all age stages. Using approximately 300 teeth per age category, disease presence could be estimated within the range 50% (± 6%) to 3.5% (± 2.3%), and would have 80% power to detect odds ratios for age of 1.5 or less, assuming 5% significance level and 3.5% disease presence.

Age estimation

Tooth age was estimated using one of two established forensic methods. The Liversidge and Molleson method was used to estimate the age of not yet fully developed teeth, which comprised 11.7% of our sample. After measuring the distance between the buccal cusp tip and the edge of the developing root at the midline, age was estimated by applying the formula $A = b0+b1x$; where (A) is the estimated age, (x) is the developing tooth length, and (b0, b1) are coefficients for each tooth type (1st or 2nd premolars, in our study) (Liversidge and Molleson, 1999).

The Bang and Ramm method was used to estimate the age of fully developed teeth based on root dentin translucency (Bang and Ramm, 1970). The minimal (TL1) and maximal (TL2) translucency length values from the apex to the borderline of opaque root dentin coronally were recorded. The average (TM) of TL1 and TL2 was used to estimate the age (A) applying the formulae $A = B0+B1X+B2X2$ (for $TM \geq 9.0$ mm) or $A = B0+B1X$ (for $TM<9.0$ mm); where (X) is the length of translucent dentin; B0, B1, B2 are coefficients for each tooth type. A single trained examiner performed the measurements using a sliding caliper (Fisher Scientific, Waltham, MA, USA).

Outcome measures

Coronal and root caries lesions were recorded on all surfaces according to ICDAS-II criteria (ICDAS Coordinating Committee, 2009). Enamel fluorosis was recorded on buccal, lingual and occlusal surfaces using the criteria described by Thylstrup and Fejerskov (1978) (TFI). Erosive tooth wear (ETW) was scored on occlusal, buccal and lingual surfaces, using the Basic Erosive Wear Examination (BEWE) index (Bartlett et al., 2008). For buccal and lingual surfaces, two digits were given; the first digit represented the severity, while the second digit represented the location of ETW to study the percentage of non-carious cervical lesions (NCCL) in different ages (Appendix Table 9.1). Presence of extrinsic staining was assessed on buccal and lingual surfaces, using the two-digits Modified Lobene index (MLI) (Macpherson et al., 2000). The first and second digits represent intensity and extent scores of stain, respectively (Appendix Table 9.2). The shade at the middle third of facial surfaces of crowns and roots was evaluated using a digital spectrophotometer (VITA Easyshade, Vident, USA) and recorded based on VITA classical A1-D4 shade guide (Appendix Table 9.3). For caries, fluorosis, smooth surface ETW, staining intensity, the highest score was considered in the statistical analyses.

Statistical analysis

Intra-examiner repeatability for the translucency measurement and inter-examiner agreement for ICDAS, BEWE, TFI, and MLI were evaluated using the intra-class correlation coefficient (ICC).

Presence of caries lesions, enamel fluorosis, ETW, staining and color were estimated and plotted against tooth age. A simulation-based analysis was performed to account for measurement error of the age assessments (±10 years) when evaluating the relationship between age and the outcomes. The simulated analysis used 1000 replications, wherein a normally distributed random error with mean 0 and standard deviation 10 was added to each age measurement. The nonlinear regression analysis was fitted for each simulated dataset. The point-wise median and 5th and 95th percentiles of the nonlinear regression lines were estimated and plotted in the graph.

2.3. Results

Our sample showed similar distributions for maxillary (50.6%) and mandibular (49.4%) premolars. Although the sample represented a wide age range (10-100yo), the distribution was unbalanced at the highest age categories, with few teeth ≥80yo (Appendix Figure 1). This should be considered when interpreting and comparing data obtained from those ages. ICC revealed excellent agreement for average translucency

(0.92), TFI (0.97) and staining intensity (0.90). Acceptable agreement was observed for BEWE location (0.67) and severity (0.72), staining extent (0.77), and ICDAS (0.77). Percentage and severity of coronal and root caries increased with age, with higher numbers observed for coronal caries (Figure 2.1). ETW percentage and severity for both occlusal and smooth surface, as well as occurrence of NCCL increased with age (Figure 2.2). Percentage and severity of enamel fluorosis decreased with age (Figure 2.3). Extrinsic staining presence, severity and extent increased with age, with higher extent observed in the lingual surface (Figure 2.4). Mean VITA classical shade increased for both crown and root, with more evident results for root (Figure 2.5).

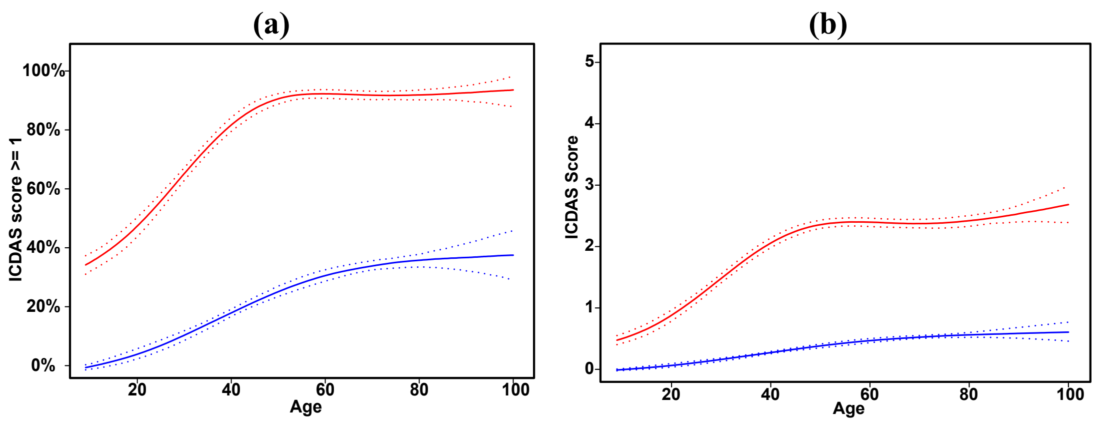


Figure 2.1. Caries lesion presence and severity increased with age.
(a) Percentage of coronal caries (Red) increased from ~35% at ~10 to reach 90% at age ~50 and above. Percentage of root caries (Blue) increased from 0% at ~10 to 20% at age ~40, and reach the highest of 35% at ~80. (b) Mean coronal ICDAS score (Red) increased steadily from 0.5 at age ~10 to 2 at ~40 and remained stable between scores 2 and 3 at older ages. Mean root ICDAS score (Blue) increased from 0 at age ~10 to 0.5 at ~60, then remained relatively stable.

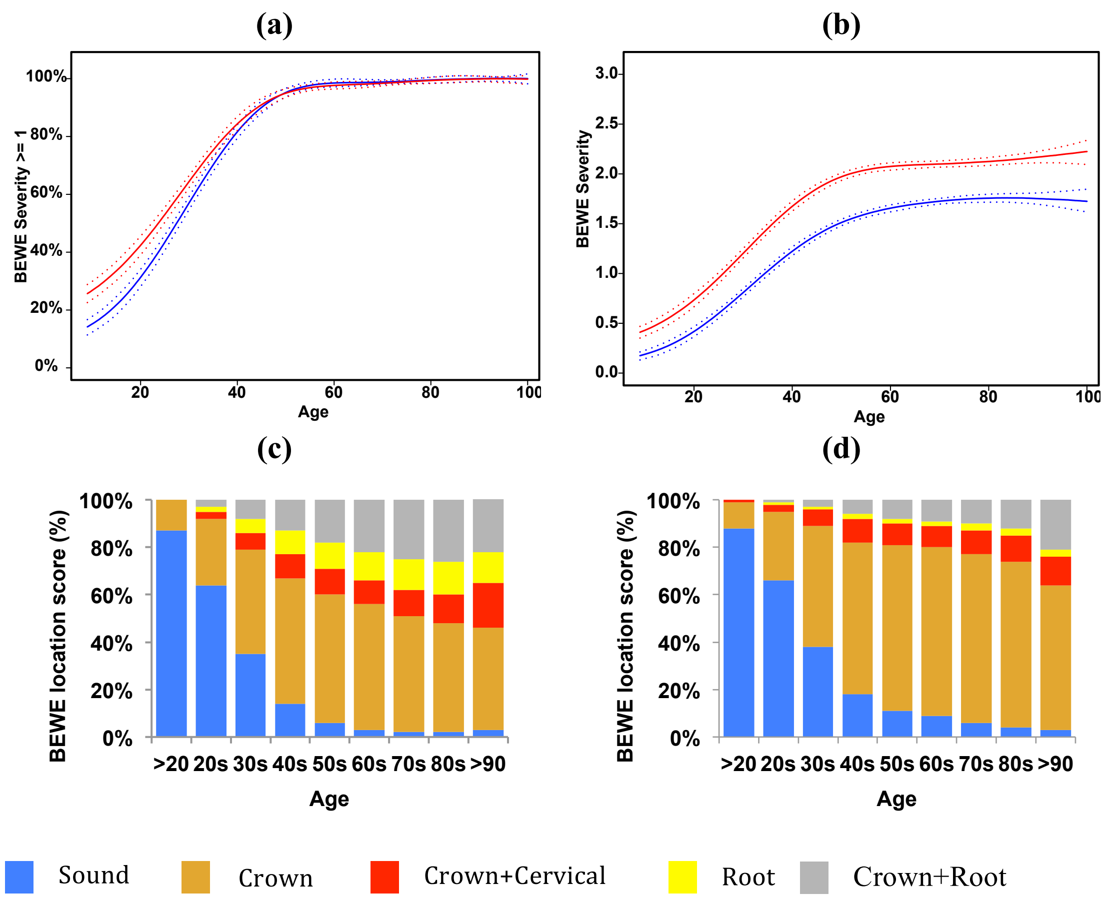


Figure 2.2. Presence and severity of tooth wear signs increased with age.
(a) Percentage of ETW increased from 25% and 15% at ~10 to reach 100% at ~80 for occlusal (Red) and smooth-surfaces (Blue), respectively. (b) Mean BEWE score increased from 0.5 and ~0.3 at age ~10 to 2 and 1.5 at ~50 remaining stable after, for occlusal (Red) and smooth surfaces (Blue), respectively. Distribution of BEWE location scores is shown for buccal (c) and lingual (d) surfaces. For both surfaces, percentage of sound teeth decreased with age until reached 0% at 90s. For all ages, higher percentage showed wear confined to crown with no involvement of cervical area (score 0). NCCL percentages (red, yellow and gray bars) start at ~30s and increasing after, with lower percentages observed in lingual surfaces.

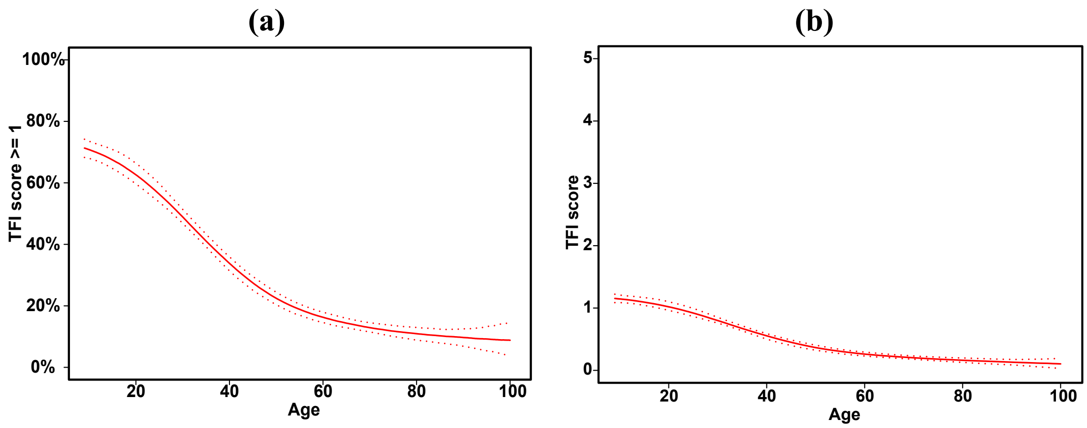


Figure 2.3. Presence and severity of enamel fluorosis decreased with age. (a) Percentage of dental fluorosis decreased from 70% at ~10 to reach approximately 10% at ~80. (b) Mean TFI stayed around 1 until age ~30 before decreasing to 0.5 at age ~40, approaching 0 at ~90 years old.

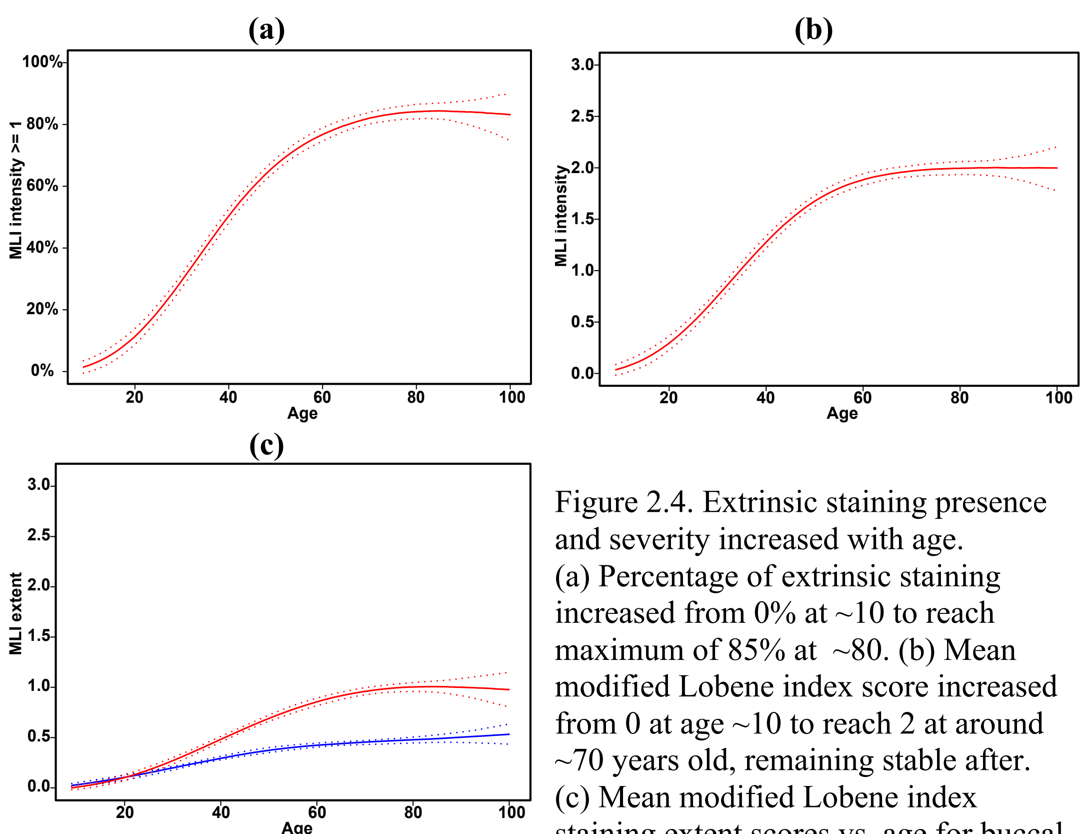


Figure 2.4. Extrinsic staining presence and severity increased with age. (a) Percentage of extrinsic staining increased from 0% at ~10 to reach maximum of 85% at ~80. (b) Mean modified Lobene index score increased from 0 at age ~10 to reach 2 at around ~70 years old, remaining stable after. (c) Mean modified Lobene index staining extent scores vs. age for buccal (Red) and lingual (Blue) surfaces. Lingual surface showed higher extent compared to buccal one.

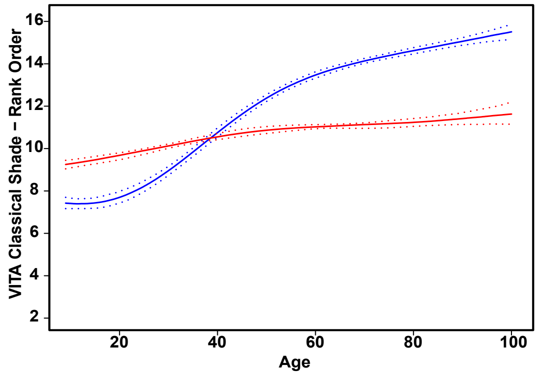


Figure 2.5. Crown and root color with age.
Mean VITA classical shade of crown (Red) increased from A3 to B3 at age ~50, then remained stable after. Mean VITA classical shade of root (Blue) starts from C2 at age ~10, to A3 at age ~30, then keep increasing to B4 at ~50, and to A4 at age ~85.

2.4. Discussion

Time, costs and ethical concerns limit the study of age's effect on dental hard-tissue pathologies and conditions in a clinical setting. Our in-vitro approach, based on the age-estimation of unidentified extracted teeth using forensic methods, allowed us to investigate a relatively large number of teeth under very controlled conditions. We selected premolars for practical reasons since they are extracted at a wide age-range due to orthodontics, prosthodontics, and periodontal disease progress. Obtaining similar numbers of other types of teeth with a comparable range of estimated ages would be more challenging. In addition, premolars are the preferred type of teeth for forensic age-estimation due to their uncomplicated and more stable root morphology (Aboshi et al., 2010). While practical and convenient, premolars may not be representative of other tooth types, and this limitation should be considered when interpreting our findings.

The forensic age estimation methods used here present advantages due to their adequate accuracy and simplicity, as they do not require tooth sectioning, special training, or any specialized tools. Besides, both methods are not affected by gender (Bang and Ramm, 1970; Liversidge and Molleson, 1999), which was appropriate since this information was not available in the studied sample. Although Bang and Ramm equations have been validated on different populations and races including European caucasians

(Bang and Ramm, 1970; Soomer et al., 2003), Indians (Acharya and Vimi, 2009) and Hispanics (Ubelaker and Parra, 2008), its accuracy and precision are still unknown for other populations, such as Asians and Africans. The accuracy and precision of the Liversidge and Molleson method has not been thoroughly evaluated for different races and ethnicities either, which could be a limitation for the current investigation.

Coronal and root caries percentages trended higher with age increase. For age group ≥40yo, our data showed percentages up to 90% for coronal, and 35% for root caries, similar to data obtained for the same age group by Papas et al. (1992), in which caries in premolars reached up to 89% and 35% for coronal and root caries, respectively. Coronal caries presence in our sample increased from 45% at ~20yo to approximately 65% at ~30yo. This is slightly lower, but still in the range of those previously reported by the NHANES survey for the same age range (82%) (Dye et al., 2015). Thus, our results were comparable to these full mouth screening epidemiological studies, which may add justification for limiting our study to premolars.

The higher prevalence of root caries among older adults might be explained by the increased frequency of root exposure with aging due to gingival recession (Papas et al., 1992). The increase of occurrence and severity of coronal and root caries are accelerated at younger ages ~10yo up to ~50yo, tapering off after that. At the first two decades of life, coronal caries lesions tend to be mostly incipient or limited to superficial enamel layers, and visibly noticeable only after air-drying (ICDAS 1). This is followed by a steady increase in depth and becoming clinically visible without drying (ICDAS 2) until ~50yo, after which it plateaus. Root caries lesions on the other hand, start to be clinically noticeable at ~20yo and increase continuously after that, stabilizing at ICDAS score below 1 (i.e. < 0.5-mm loss of anatomical contour) at ~80yo, indicating that although there is an increase in occurrence of root caries, it is not often accompanied by cavitation. It should be noted that our caries findings might be underestimated, since teeth with extensive cavitation (ICDAS 6) and/or restorations were excluded from the study. This makes the age-related findings even more remarkable. The rationale for excluding ICDAS 6 is that the extensive destruction would render those teeth inappropriate for the following phases of this project (Chapters 3 and 4). Nonetheless, caries presence estimates at ages older than ~90 and ~80 for coronal and root surfaces, respectively, are less reliable due to the large variation observed (note wide error lines in Graphs) at those ages, and thus should be interpreted with caution.

For clinical practice, our findings emphasize the need for different caries management protocols for each age stage, targeting primary coronal caries prevention up to ~20yo. After ~50yo, additional root caries preventive measures may be needed. These results also provide evidence that seniors should be considered a high-risk group for caries, and also suggest the need for future investigations of the efficacy of different caries preventive/therapeutic measures on different ages. While increased caries occurrence with age can be related to changes in water fluoridation and improved preventive methods, age-related changes may also be responsible. Reduction in fluoride and increase in carbon content of enamel may contribute to higher demineralization susceptibility with age (Kidd et al., 1984; Leventouri et al., 2009). Nevertheless, the impact of tooth aging on enamel and dentin susceptibility to demineralization remains to be investigated (see Chapter 3).

We found direct relationships between age and both presence and severity of ETW for occlusal and smooth surfaces. More evident ETW was observed on the occlusal surfaces, which may be explained by the association of erosion with mechanical forces from occlusion and food comminution. Although similar trends with age were observed in a clinical study that used BEWE index (Vered et al., 2014), our results showed overall higher percentages. We examined extracted teeth with more controlled conditions (constant light, direct visual access, dry and plaque-free surfaces), which may have improved ETW visual detection. Moreover, using premolars in this study may have contributed to this difference, as different tooth types might vary in susceptibility to ETW (Carvalho and Lussi, 2015). By ~80yo, almost 100% of our sample showed some extent of ETW corroborating a previous study (Wei et al., 2016). A steady increase in ETW severity was observed from ~10yo (no erosion) to ~30yo, when the enamel began to evince surface texture alterations (BEWE 1), advancing to involve >50% of the surface (BEWE 2) at ~50yo. No further increase in severity was observed at older ages. Smooth surfaces showed slightly lower severity compared to occlusal, stabilizing at BEWE score below 2, at ~50yo. NCCL also increased with age, which is consistent with existing epidemiological data (Smith and Robb, 1996). At ~40yo, one third of the sample started to show some extent of NCCL on the buccal surface, with increased incidence thereafter (Figure 3.2c). This may be related to root surface exposure to the oral environment starting in young adults, in addition to possible adverse effects of brushing. This is substantiated by the higher percentages of NCCL observed for the buccal surface compared to the lingual, and particularly in lesions confined to the root only. Higher occurrence of NCCL in buccal surfaces is in agreement with previous reports (Johanson et al., 2012). Besides, gingival recession showed to be higher for buccal compared to lingual surfaces (Papa et al., 1992). Based on our findings, it is recommended that dental practitioners consider NCCL preventive measures at early ages.

The relationship between ETW and age has been explained by various factors, including diet and age-related microstructural changes of dental tissues (Zheng and Zhou, 2006; Liu et al., 2014). Acidic juices were found to be more related to ETW in younger subjects (15-39 years), whereas chewing hard and acidic food was more related to ETW in older adults (>50) (Liu et al., 2014; Kitasako et al., 2015). Thus, effective ETW management should include detailed dietary history, with the understanding that food preferences may change with the patient's age. In this study, we did not differentiate between erosive and abrasive tooth wear, given challenges associated with the lack of metadata on teeth donors. However, considering the multifactorial nature of ETW, it is difficult to eliminate the possibility that chemical processes contribute to wear, unless patient history and clinical lesions suggest otherwise (Shellis and Addy, 2014). Hence, we used BEWE index as a reliable tool for measuring chemical and/or mechanical tooth wear (Bartlett, 2012).

Our sample displayed mild-moderate fluorosis severity, starting at TFI 1 (clinical visualization of white lines) from ~10yo to ~30yo, then decreasing slowly to 0.5 (between 0 and 1) at ~40yo, and reaching 0 at ~90yo. The presence of enamel fluorosis in our sample was substantially higher (70%) in adolescents compared to older adults ~50yo (~20%). This reduction in teeth affected by fluorosis with age is similar to the trend reported by NHANES data (Beltran-Aguilar et al., 2010). These results may be influenced by the higher fluoride exposure of younger generations due to increased awareness of caries prevention and oral hygiene (Beltran-Aguilar et al., 2010). Increase in tooth wear with age may also contribute to this observation. In this case, the superficial layers of fluorotic enamel would be chemically and/or mechanically removed, in a process similar to that suggested for incipient carious lesions (Nassar et al., 2014). Moreover, premolars are considered the most permanent teeth affected by fluorosis (Ramires et al., 2007), in addition to using the more sensitive TFI index, which could justify for the higher percentages of fluorosis found in this study compared to previous reports (Beltran-Aguilar et al., 2010).

Extrinsic staining presence and intensity increased with age reaching MLI close to 2 at ~50yo, indicating the presence of a clearly visible stain, varying from orange to brown. This level of staining remained consistent at older ages. The brown extrinsic staining was shown to increase in intensity with age (Eriksen and Nordbo, 1978). Higher staining extent in lingual surfaces is probably due to less accessibility to natural cleansing and brushing. The cumulative effect of age-related changes to the tooth surface on extrinsic stain adsorption and retention should be explored, as they may affect the efficacy of stain-removal treatments, requiring different clinical protocols. This may raise some esthetic issues due to staining impact on an individual’s appearance.

We observed increase in darkness of crown and root color, with more evident change in roots, consistent with the literature (Mayoral et al., 2013). The slight change in crown color may show that teeth not only become darker, but also more reddish with age, as the color scores changed from VITA shade A3 at early age, to B3 at age ~50yo. The reduction in rod sheath and crystal gaps seem to intensify the reflection of underlying dentin at younger ages (Miake et al., 2016), as does tubular occlusion and advanced glycation end-product accumulation in dentin (Miura et al., 2014). Future research should shed light on the potential impact of age-related color changes on the efficacy of various tooth-whitening approaches. Using extracted teeth is a potential limitation of this study, as specific events such as internal pulpal hemorrhage during extraction and long storage time may have influenced the outcome. However, tooth color from skeletal remains has been used for age estimation with an acceptable degree of accuracy (Ten Cate et al., 1977), which encouraged us to include it in our study. Furthermore, the meticulous treatment and preservation of the studied teeth in thymol immediately after extraction likely mitigated color change during storage.

Our findings clearly demonstrate that age impacts dental caries, ETW, fluorosis, staining and color; however, the influence of behavioral and/or biological factors in each of the considered processes still need to be elucidated. It is reasonable to suggest that clinical protocols, especially preventive ones, should consider a patient's age. In that sense, our next steps will focus on the age effect on the efficacy of preventive and therapeutic measures for ETW (Chapters 3 and 4), one of the most common clinical dental problems. The proposed experimental approach, involving the use of extracted teeth and forensic age-estimation methods, has proven to be cost-effective and will be appropriate to conduct such future investigations in a timely and systematic fashion.

CHAPTER 3: AGE IMPACT ON DENTAL EROSION SUSCEPTIBILITY AND EFFICACY OF ANTI-EROSION SOLUTIONS

3.1. Introduction

Erosive tooth wear (ETW) is an increasing dental condition that has been associated with aging (Lussi and Schaffner, 2000). Several complications have been related to ETW such as tooth sensitivity due to dentin exposure, loss of vertical dimension and alterations on the occlusal table, which may lead to poor aesthetics and functional impairment (Muts et al., 2014). Therefore, ETW may have a negative impact on the person's quality of life (Papagianni et al., 2013).

ETW is a multifactorial condition in which multiple biological, chemical and behavioral factors contribute to its dynamic process (Lussi and Jaeggi, 2008). Increases in ETW with age has been linked mainly to dietary factors such as increased consumption of acidic diets (Lussi and Schaffner, 2000). Changes in salivary flow, buffering capacity, and composition with age have also been associated with ETW (Lussi and Schaffner, 2000; Piangprach et al., 2009). In addition, the composition and structure of dental substrates (i.e. enamel and dentin) are essential biological factors that may affect the individual's risk to ETW (Hara et al., 2006). There is clear evidence for the impact of aging on the microstructure and composition of enamel and dentin. For instance, there is increase in mineralization and reduction in protein matrix for both substrates with age (Cardoso et al., 2009; Montoya et al., 2015; Miake et al., 2016). However, the impact of these changes on the susceptibility of tooth to demineralization it is still unknown.

Fluoridated mouth rinses are considered a feasible daily anti-erosion preventive measure. Several fluoride formulations with different concentrations have been investigated and demonstrated positive results in reducing the effect of erosive attacks (Lussi and Carvalho, 2015). Tin-containing fluoride solutions showed superior and more promising findings compared to fluoride only (Lussi and Carvalho, 2015). To our knowledge, there is very limited data regarding the influence of age-related changes of enamel and dentin on the efficacy of aforementioned mouth rinses. Therefore, the objective of this study was to investigate the susceptibility of enamel and root dentin of different estimated ages to simulated ETW, and their responses to fluoride-based mouth rinses.

3.2. Material and methods

Experimental design

The study followed a randomized design with mouth rinse as the main experimental factor at 3 levels: Sn+F (800 ppm Sn; 250 ppm F, pH 4.5), NaF (250 ppm F, pH 4.5), and de-ionized water (DIW) (negative control). Each group consisted of a representative sample of premolars from all ages (n=93). Tooth age was considered as a continuous variable. Enamel and dentin samples were exposed to a pH cycling procedure for 10 days. Surface profilometry was used to measure surface loss after 3, 5 and 10 days.

Sample selection and preparation

A total of 279 (93/group) premolars were selected from a previously existing collection (Chapter 2) using stratified random sampling with equal allocation among 7 age groups (<20; 20s; 30s; 50s; 60s; and ≥70). The age of each premolar was estimated using Bang and Ramm (1950) or Liversidge and Molleson dental forensic methods for developed and developing teeth, respectively. One enamel and one dentin slab (3 × 3 × 2 mm) from each premolar were cut, embedded in acrylic resin, flattened, and polished as described elsewhere (Scaramucci et al., 2015). Adhesive unplasticized polyvinyl chloride (UPVC) tapes were placed on the surface of each specimen, leaving a central area of (3×1 mm2) exposed.

Solutions preparation

Solutions were prepared as described in a previous study (Algarni et al., 2015b). Solution compositions and pH used in the study are shown in Table 3.1.

Table 3.1. Solution compositions.

Solution	**Composition (per liter)**
Demineralizing solution	(0.3%) 3.0 g citric acid anhydrous; pH 2.4
Artificial saliva	$CaCl_2 \cdot H_2O$: 0.213g, KH_2PO_4: 0.738g, KCl: 1.114g, NaCl: 0.381g, Tris buffer: 12g and 2.2 g of porcine gastric mucin; pH adjusted to 7.0
NaF	250 ppm F^-: 0.553 g NaF; pH adjusted to 4.5
Sn+F	800 ppm Sn^{+2}: 1.277 g $SnCl_2$; 2.3 g Na-gluconate; 250 ppm F^-: 0.553 g NaF; pH adjusted to 4.5

Daily cycling procedure

Samples were subjected to a cycling procedure (6 cycles/day) (Table 3.2) as described by Scaramucci et al. (2015). A complete cycle consisted of a 5-min acid challenge period in 0.3% citric acid (pH 2.4) with no agitation, and a 60-min remineralization period in artificial saliva (AS) under 150 rpm agitation. The exposure to treatment solutions (Sn+F, NaF or DIW) was sandwiched between the 1st, 3rd and 6th remineralization periods, for 2 min. Fresh erosive and treatment solutions were used every cycle; while AS was replaced twice/day. The experiment was conducted at room temperature.

Table 3.2. The Daily cycling procedure.

Steps	Procedures
Cycle 1	5-min erosive challenge; 30-min remineralization; 2-min treatment; 30-min remineralization
Cycle 2	5-min erosive challenge; 60-min remineralization
Repeat cycle 1; Repeat cycle 2 twice; Repeat cycle 1; Immersed in AS overnight	

Surface loss (SL) measurement

To assess the erosion progression and treatment effect, dentin and enamel SL was measured at the end of the 3rd, 5th, and 10thday of the cycling procedure. Optical profilometry (Proscan2000, Scantron Industrial Products Ltd., Taunton, England) was used to analyze the depth of the erosive lesions as previously described (Scaramucci et al., 2015).

Statistical analysis

SL was plotted against tooth age to visualize the relationship between tooth age and surface loss, for each treatment group. The effects of tooth age, substrate (enamel or dentin), treatment (Sn+F, NaF, DIW), and time (3, 5, 10 days) on SL were evaluated using linear mixed effects regression analysis. Substrate and time were repeated factors within each specimen; each substrate-time combination was allowed to have a different variance, and correlations between substrate-time combinations were allowed to differ. Tooth age was treated as a continuous, linear variable. A simulation-based analysis was performed to account for measurement error of the tooth age assessments. The measurement error for age was assumed to have a standard deviation of 10 years. The simulated analysis used 10000 replications, where a normally distributed random error with mean 0 and standard deviation 10 was added to each tooth age measurement. A 5% significance level was used for all tests.

3.3. Results

Relationship between tooth age and SL

The relationship between tooth age and SL was evaluated using the slope of the regression line for each time point analysis and treatment (Table 3.3). The slope indicates the change in SL for each one-year increase in tooth age. All slopes were significantly greater than zero ($p=0.0075$ for enamel- Sn+F solution -3 days, $p<0.0001$ for all other substrate-treatment-time combinations), indicating that SL increased with age for all substrate-treatment-time combinations. Regression lines are shown in Figures 3.1 (enamel) and 3.2 (dentin).

Treatment comparisons

Sn+F solution showed significantly less SL than NaF solution and DIW control regardless of substrate, time, or age ($p<0.0001$). NaF solution had significantly less SL than DIW control regardless of substrate, time, or age ($p<0.0001$). To explore the treatment efficacy with age, the slope difference between treatments and DIW control are shown in Table 3.4.

Time comparisons

Day 3 had significantly less SL than day 5 and 10; and day 5 had significantly less SL than day 10 for dentin regardless of treatment or age ($p<0.0001$). Day 3 had significantly less SL than days 5 and 10; and day 5 had significantly less SL than day 10 for enamel for NaF solution or DIW control regardless of age ($p<0.0001$). For Sn+F solution, day 3 had significantly more SL than days 5 and 10 for enamel regardless of age, and day 5 had more enamel SL than day 10 at younger ages ($p<0.0001$).

Substrate comparisons

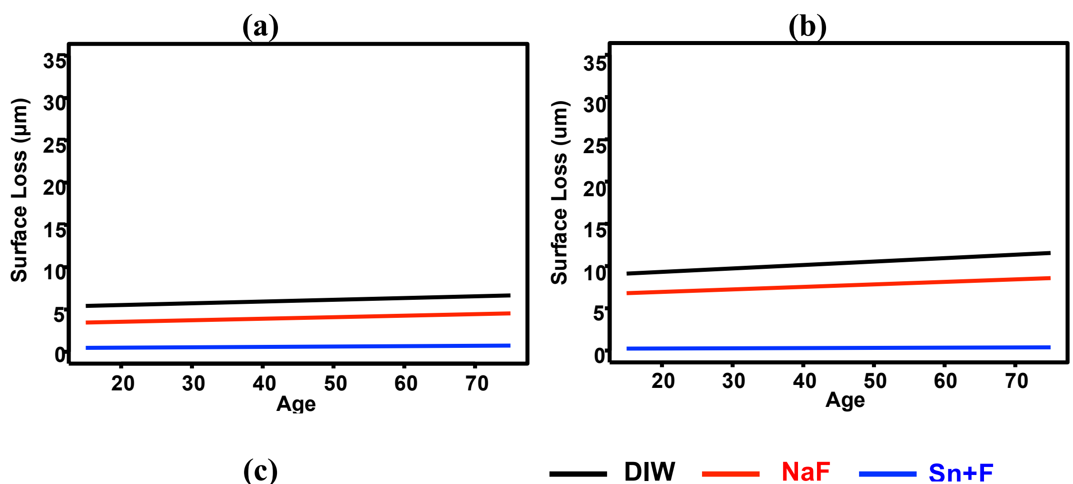
Dentin had more SL than enamel for Sn+F solution and DIW control regardless of time or age (p<0.0001). Dentin also had more SL than enamel for NaF solution, regardless of age for 3 and 5 days (p<0.0001), but for 10 days it had less SL than enamel regardless of age (p<0.0001).

Table 3.3. The regression lines' slopes for all time-substrate-treatment combinations.

Substrate	Treatment	Time point	Slope	SE	p-value
Enamel	Sn+F	3-day	0.0044	0.0010	0.0075
		5-day	0.0025	0.0009	<0.0001
		10-day	0.0039	0.0009	<0.0001
	NaF	3-day	0.0181	0.0027	<0.0001
		5-day	0.0297	0.0040	<0.0001
		10-day	0.0580	0.0066	<0.0001
	DIW	3-day	0.0207	0.0030	<0.0001
		5-day	0.0407	0.0050	<0.0001
		10-day	0.0632	0.0076	<0.0001
Dentin	Sn+F	3-day	0.0168	0.0022	<0.0001
		5-day	0.0232	0.0023	<0.0001
		10-day	0.0325	0.0029	<0.0001
	NaF	3-day	0.0366	0.0029	<0.0001
		5-day	0.0621	0.0036	<0.0001
		10-day	0.0809	0.0059	<0.0001
	DIW	3-day	0.0109	0.0023	<0.0001
		5-day	0.0619	0.0045	<0.0001
		10-day	0.1189	0.0079	<0.0001

Table 3.4. Slope difference between fluoridated solutions and DIW control.

Substrate	**Time point**	**Compared treatments**	**Slope difference**	**SE**	**p-value**
Enamel	3-day	Sn+F vs. DIW	-0.0162	0.0032	<.0001
		NaF vs. DIW	-0.0026	0.004	0.52
	5-day	Sn+F vs. DIW	-0.0382	0.0051	<.0001
		NaF vs. DIW	-0.011	0.0064	0.09
	10-day	Sn+F vs. DIW	-0.0593	0.0077	<.0001
		NaF vs. DIW	-0.0052	0.01	0.60
Dentin	3-day	Sn+F vs. DIW	0.0059	0.0032	0.06
		NaF vs. DIW	0.0257	0.0037	<.0001
	5-day	Sn+F vs. DIW	-0.0387	0.0051	<.0001
		NaF vs. DIW	0.0002	0.0057	0.98
	10-day	Sn+F vs. DIW	-0.0864	0.0085	<.0001
		NaF vs. DIW	-0.0379	0.0098	0.00



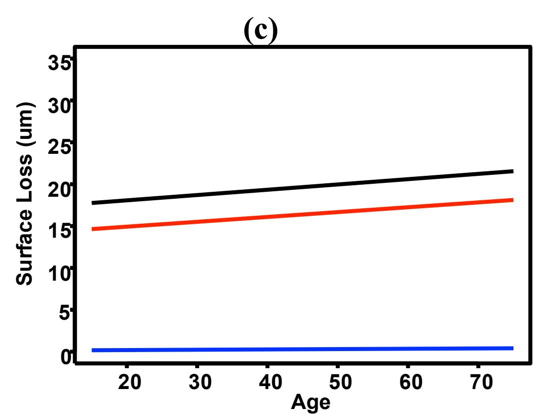


Figure 3.1. Enamel surface loss increased with age.
Enamel surface loss vs. age, after 3 (a), 5 (b) and 10 (c) days of cycling, assuming linear relationship between tooth age and SL.

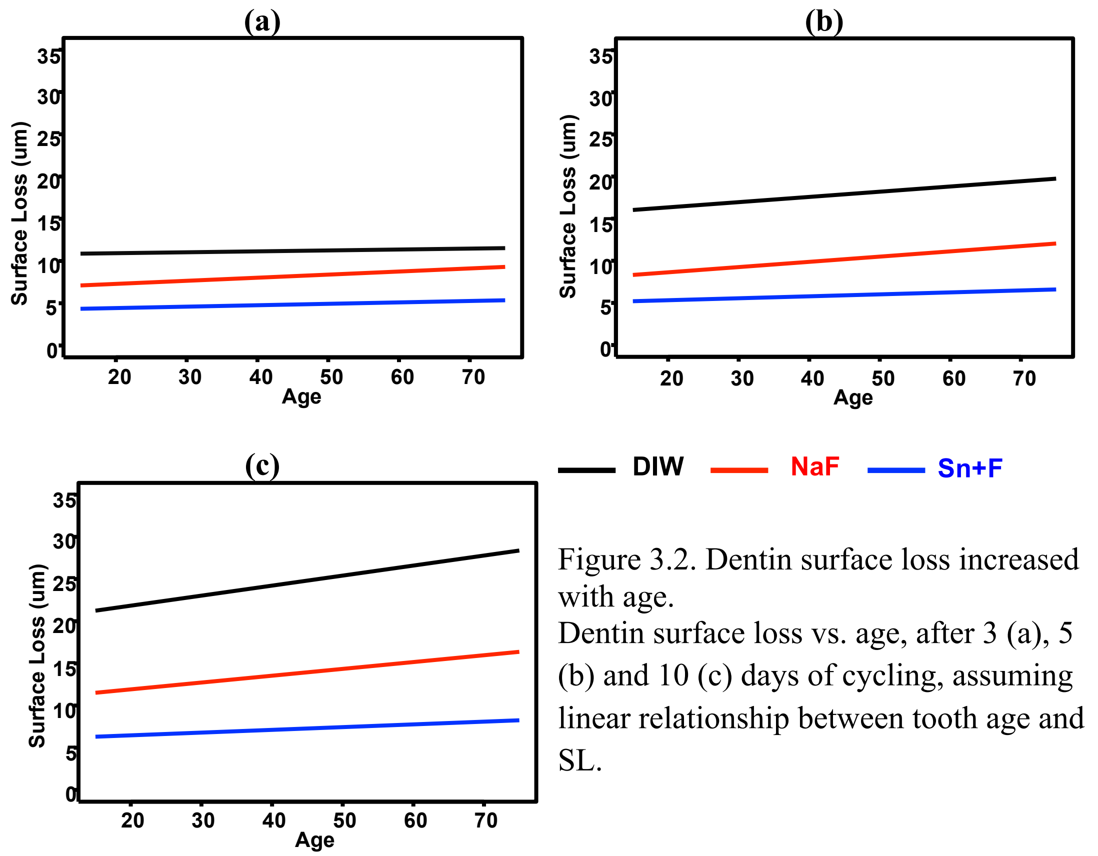


Figure 3.2. Dentin surface loss increased with age.
Dentin surface loss vs. age, after 3 (a), 5 (b) and 10 (c) days of cycling, assuming linear relationship between tooth age and SL.

3.4. Discussion

Our findings demonstrated an overall trend for increased surface loss (SL) with age, regardless of testing substrate, anti-erosive solution or time points. Increase in both enamel and dentin susceptibility to demineralization could be explained by change in their chemical composition with age. Increase in carbon (Leventouri et al., 2009), and Mg content in dental hydroxyapatites (Derise et al., 1978; Lappalaine et al., 1980) may have rendered enamel and dentin more soluble with age. Kidd et al. (1984) found that “older” premolars’ enamel (>65yo) was more susceptible to dental caries and generally thinner than younger enamel. Reduction in enamel thickness with age is most probably due to physiological wear (Bartlett and Dugmore, 2008), exposing deeper enamel layers closer to the DEJ. Based on the enamel solubility gradient, these deeper layers tend to be more soluble due to increases in Mg and C (Theuns et al., 1986; Shellis, 1996). This is consistent with studies that demonstrated an enamel mineralization gradient, in which the concentration of Ca and P tends to decrease toward DEJ, in both older and younger enamel (He et al., 2011). Moreover, the outer layer of aged enamel (~100-200 μm from the surface) exhibits an increase in mineral density compared to younger enamel, which may be assumed to be less soluble; while no significant difference was detected in middle and inner layers (He et al., 2011; Zheng et al., 2013). As a limitation of this study, we used polished samples, as usually preferred for profilometry, in which the surface layer with direct exposure to oral environment (mature enamel) was completely or partially removed. Even with that, we were able to detect a difference in enamel susceptibility to demineralization over age.

Beside its chemical composition, the crystal structure of dental apatite seems to change with age as well. Leventouri et al. (2009) revealed reduction in crystallinity of enamel and dentin with age particularly after 45 years, as a result of continuous demin-remin processes throughout one's life. The reduction in crystallite size of dental apatite could be partly responsible for the increase in enamel and dentin solubility with age. In addition to changes in the mineral phase of enamel, there is a reduction in enamel proteins with age indicated by the decrease in area and width of enamel rod-sheath in older enamel (Miake et al., 2016). It has been suggested that enamel protein matrix plays a protective role against dental erosion by acting as an electrochemical buffer against acids and alleviating H+ ions diffusion (Lubarsky et al., 2014; Baumann et al., 2015). This reduction in enamel protein may contribute to the increased enamel susceptibility to demineralization with age observed in this study. Increased solubility of enamel (for dental caries) has also been related to reduction in F content of enamel with age (Weatherell et al., 1972; Kidd et al., 1984). However, there is controversy in the literature regarding changes in enamel F content with age (Nakagak et al., 1987). Indeed, in light of our findings and due to the clinical significance of this issue, further detailed investigations are needed to understand the effect of age-related changes of enamel microstructure and composition on its solubility.

For dentin, reduction in quality and quantity of organic matrix age could be a factor that contributes to the increased SL with age. Dentin protein matrix plays an important role in prevention of demineralization progression (Kleter et al., 1994; Hara et al., 2005). It acts as a diffusion barrier that prevents further acid diffusion into mineralized dentin. It also exhibits buffering properties against acid attack by adsorbing and neutralizing H+ ions from dentin surface before it reaches inner sound dentin (Ganss et al., 2001). Non-enzymatic collagen cross-linking with age, and accumulation of advanced glycation end-products (AGEs) affect the mechanical and physical properties of dentin making it darker more prone to mechanical wear (Miura et al., 2014; Shinno et al., 2016). Consequently, this may also affect the anti-erosion properties of the matrix making dentin more vulnerable to demineralization, albeit the increase in mineral density of dentin with age. Ozdemir et al. (2012) revealed that prolonged exposure to EDTA+ NaOCl led to more excessive demineralization of older dentin, which could be due to deterioration of dentin matrix with age.

Dentin tubular occlusion and increased mineral/collagen ratio with age may also affect its susceptibility to demineralization. The process of mineral deposition and tubular occlusion was suggested to start around the third decade of life (Eldarrat et al., 2010).

Although increase in mineral deposition in dentinal tubules might enhance dentin resistance to acid diffusion, some studies revealed that the intratubular material of obliterated tubules is the most soluble portion of sclerotic dentin (Weber, 1974). Not only mineral concentration, but also dental crystallite size and composition are other factors that may affect dentin solubility and should be considered (Vogel, 2002; Leventouri et al., 2009). Porter et al. (2005) demonstrated that age-induced transparent dentin displayed smaller crystallite size when compared to normal (younger) dentin, which may help explain the increase in dentin solubility with age observed in our study.

Generally, the anti-erosive treatments in our study did not affect the increasing SL trend with age (Figures 3.1, 3.2). Utilizing regression analyses in this study allowed the estimation of enamel and dentin SL at different ages. For a 10 year difference in tooth age, the difference in SL is 10 × slope. For example, based on 10-day erosion simulation time point, if teeth from 30 and 60-years old individuals were exposed to the same erosive challenge, the latter would show more enamel SL by approximately 0.117, 1.74, and 1.89 μm for Sn+F, NaF, and DIW, respectively. Similar ranking was observed for dentin, in which a 30-year difference in age (based on 10-day simulation) would show more dentin SL by approximately 0.975, 2.427, and 3.567 um SL for Sn+F, NaF, and DIW, respectively. Therefore, Sn+F solution seems to offer enough protection against enamel and dentin ETW, regardless of their increased susceptibility with age.

In order to analyze the effect of age on treatment efficacy, we compared the difference between the slopes (regression-line inclination) of each treatment (Sn+F or NaF) with DIW control (Table 3.4). Sn+F solution provided very strong treatment effect on enamel at all time points (slope ~ 0), which limits further discussion of the age effect on treatment efficacy. No significant difference was observed between NaF and DIW at any time point (Table 3.4), indicating that the existing NaF effect on eroded enamel did not change with age. Eroded dentin, on the other hand, seems to respond differently with age at different time points. At early lesion simulation (3-day time point), Sn+F solution showed no difference in efficacy with age; while NaF showed better protection on younger ages (Table 3.4, Figure 3.2a). With lesion progression (5 and10-day time points), Sn+F solution started to demonstrate a more protective effect with age. Similarly, after prolonged erosive challenge (10-day), the effect of NaF solution started to improve with age.

In agreement with previous reports, the F and Sn combination significantly reduced enamel and dentin ETW (Algarni et al., 2015a,b). The anti-erosive mechanism of Sn+F is likely to be related to the precipitation of less soluble crystalline compounds, such as Sn2OHPO4, Sn3F3PO4 and Ca(SnF3)2 (Babcock et al., 1978). This layer acts as a protective barrier enhancing the acid resistance of dental surfaces. Our results support this hypothesis, as some enamel samples from Sn+F group showed surface “gain”, instead of loss, after 5 and 10 days of cycling, which can be explained by the formation of surface deposition. Additionally, Sn is suggested to incorporate into the surface layer of enamel, enhancing its resistance against acids attacks (Schlueter et al., 2009).

For dentin, the anti-erosive effect of Sn+F solutions is suggested to rely on tin incorporation into the mineralized phase of dentin, in the presence of organic matrix. In the case of dissolved organic matrix, the protective effect of Sn+F seems to be mainly due to the precipitation on the surface of dentin, as described for enamel (Ganss et al., 2010). The different protective effect of Sn+F solution in enamel and dentin might be due to the lower content of minerals in dentin, as Sn is believed to have higher affinity to minerals rather than protein structures (Ganss et al., 2010).

NaF solution substantially reduced enamel and dentin SL for all ages, compared to DIW control. Fluoride anti-erosion effect is primarily through CaF2- or CaF2-like layer formation on the dental surfaces after topical application (Ganss et al., 2001). Besides CaF2 precipitation, fluoride effect on dentin has also been suggested to be due to fluoride retention in dentin porosities, as well as water content of dentin that provides a fluoride reservoir (Laufer et al., 1981; ten Cate et al., 1995). This may explain the better preventive effect of NaF on dentin, compared to enamel, after prolonged exposure (10 days) observed in this study. Moreover, the low pH of NaF solution used in our study (4.5) probably enhanced its efficacy, as fluoride agents have shown to form more stable CaF2-like precipitations at acidic pH (Yu et al., 2010).

Clinical studies would be the best approach to study the relationship between age and ETW. However, clinical trials demand extensive time, higher budget and complex documentation/ethical approval. These limitations did not apply to our in-vitro model that was based on the age-estimation of unidentified extracted teeth from a tooth bank using established dental forensic methods. This approach was successfully used in a previous trend-analysis study (Chapter 2) to identify the percentages and severity of several dental conditions (e.g. caries, ETW and dental fluorosis), which were comparable to existing epidemiological data in the literature.

The current in-vitro study design allowed us to control for most biological and environmental factors to focus on the effect of dental age-related changes on tooth susceptibility to demineralization, and response to anti-erosive treatment. A limitation of using unidentified extracted teeth is that it is not possible to understand the relationship between our results and other factors that may also change with the patient's age as dietary and salivary factors (Piangprach et al., 2009; Liu et al., 2014). One should bear in mind that only premolars were included, as they are the most available extracted teeth from all ages, due to orthodontic and periodontal reasons. Therefore, this should be considered when extrapolating our results to different types of teeth.

Our findings from this study demonstrated an increasing trend of tooth susceptibility to ETW with age. The efficacy of anti-erosive solutions on eroded enamel may not change with age. However, increasing efficacy with age was observed for eroded dentin, particularly for NaF solution. The next step would be to investigate the potential interaction between age and toothpaste abrasivity level on enamel and dentin susceptibility to erosion-abrasion lesions (Chapter 4).

CHAPTER 4: INTERPLAY BETWEEN TOOTH AGE AND TOOTHBRUSHING ON DENTAL EROSION-ABRASION SUSCEPTIBILITY

4.1. Introduction

Global aging is the most profound demographic transformation, as the number of senior individuals (>65 years) in the developed countries is predicted to increase from ~524 million in 2010 to approximately 1.5 billion in 2050 (National Institute on Aging, 2011). Therefore, deep understanding of the aging effect on dental health is required in order to ensure healthy longevity (Fukai et al., 2017).

Erosive tooth wear (ETW) is a complex, multifactorial phenomenon that involves chemical, mechanical, and biological factors. Interactions among these factors, including softening of enamel by chemically induced demineralization followed by mechanical abrasion, would potentially increase both wear rate and intensity (Shellis and Addy, 2014). The initial erosive lesion is characterized by surface softening with no enamel loss, due to exposure to non-bacterial acids (Ganss, 2006). After successive episodes of acid exposure and abrasive forces (e.g. toothbrushing), eroded surface loss starts to occur.

Prevalence and severity of ETW have demonstrated a significant association with older ages (Van't Spijker et al., 2009; Wazani et al., 2012). The age-related changes in the microstructure and mechanical and chemical properties of teeth may affect their solubility and wear resistance (Zheng et al., 2006). The importance of toothbrush abrasion and ETW comes from their possible impact on a person's quality of life. They have been related to increased dentin hypersensitivity (West et al., 2013; Sehmi and Olley, 2015) as well as functional and esthetic concerns (Ahmed et al., 2014).

Prevention of ETW occurs primarily by identifying and controlling the causative factors. In addition, behavioral changes, as toothbrushing habits, as well as increasing enamel and dentin resistance to acid attacks (e.g., through the use of fluoridated products) are also important approaches in ETW prevention. Toothbrushing with fluoridated dentifrices is one of the most common daily measures to prevent dental caries and ETW. The protective effect of fluoridated dentifrices against ETW occurs mainly by enhancing remineralization of enamel and dentin (Magalhães et al., 2014). Abrasive components of dentifrices, however, could increase the mechanical wear of the softened, partially eroded surface. In-vitro studies showed that surface loss increases with increase in dentifrice's abrasiveness (Hara et al., 2009; Magalhães et al., 2014).

Considering the wide range of abrasivity levels of commercially available fluoridated dentifrices, it is important to examine and understand the possible interplay between age-related changes and the level of fluoridated toothpaste abrasivity, and their effect on erosion-abrasion susceptibility. This laboratory study aimed to evaluate the susceptibility of enamel and root dentin of different estimated ages to simulated erosion attack, and their responses to different toothbrushing treatment protocols.

4.2. Material and methods

Experimental design

The study followed a complete randomized design, with the abrasivity of fluoridated dentifrice (1100 ppm) as the experimental factor at 4 levels; low (L), medium (M), high (H), and DIW as a negative control. Each experimental group consisted of representative enamel and dentin samples from all estimated ages (n=80/substrate). Tooth age was considered as a continuous variable. Samples were exposed to daily erosion-abrasion pH cycling procedure for 10 days. Surface loss was measured using optical profilometry after 5 and 10 days of cycling.

Sample preparation

A total of 320 (80/group) premolars with different estimated ages were selected as previously described (Chapter 2). One enamel and one dentin slab (3 × 3 × 2 mm) from each premolar were cut, embedded in acrylic resin, flattened, and polished as described elsewhere (Algarni et al., 2015). Adhesive UPVC tape was placed on the surface of each specimen, leaving a central area of (3×1 mm2) exposed to cycling procedure.

Daily cycling procedure

The cycling procedure consisted of 5-min demineralization challenge (0.3% citric acid, pH 2.6) with no agitation, 60-min remineralization period using artificial saliva (AS) under 150 rpm agitation (4×/day). Two toothbrushing episodes were sandwiched between the 1st and last remineralization periods. Fresh acid was used for each episode, while AS was replaced twice a day. At the end of each cycling day, specimens were kept in AS overnight. During tooth-brushing periods, specimens were immersed in 1100 ppm fluoridated dentifrice slurries (1 part toothpaste to 3 parts DIW), and brushed under 150 g for 15 s (45 strokes) using an automated V-8 brushing machine delivering reciprocal brushing strokes. After brushing, specimens remained in slurries for a total of 2 min. Standard toothbrushes (P40, Oral-B, Procter & Gamble, Cincinnati, Ohio, USA) were used. The cycling procedure was repeated for 10 days. Citric acid and AS were prepared as described previously (Chapter 3). The composition of toothpastes slurries is shown in Table 4.1.

Table. 4.1. Abrasive slurry compositions (120 g).

Slurry	NaF (275 ppm)	Abrasive (concentration)	CMC solution	RDA (mean ± SD)
Low (L)	0.072 g	6 g (5%) Zeodent 103	114 g	69.2 ± 2.6*
Medium (M)	0.072 g	12 g (10%) Zeodent 124	108 g	146.9 ± 10.0v
High (H)	0.072 g	18 g (15%) Zeodent 113	102 g	208.0 ± 9.4*

NaF (Sigma–Aldrich Co., St. Louis, MO, USA), Zeodent abrasives: precipitated silica particles (J.M. Huber, Etowah, TN, USA), CMC (Carboxymethylcellulose) (Blanose 7MF, Ashland Inc.). * (Nassar et al., 2014); v (Scaramucci et al., 2013).

Surface loss (SL) measurement

To assess the erosion-abrasion lesion progression and treatment effect, dentin and enamel SL was measured at the end of the 5th, and 10th day of the cycling procedure. Optical profilometry (Proscan2000, Scantron Industrial Products Ltd., Taunton, England) was used to analyze the depth of erosive-abrasive lesion as previously described (Algarni et al., 2015).

Statistical analysis

SL was plotted against tooth age to visualize the relationship between tooth age and SL. The effects of tooth age, substrate (enamel or dentin), treatment (L, M, H or DIW), and time (5 or 10 days) and their interaction with age on SL were evaluated using linear mixed effects regression analysis. The statistical analyses were performed as described in Chapter 3.

4.3. Results

Relationship between tooth age and SL

The relationship between tooth age and SL was evaluated using the slope of the regression line for tooth age (Table 5.2). All slopes for dentin were significantly greater than zero ($p<0.001$ for all treatment-time combinations), indicating that SL increased with age. For enamel, slopes for L and DIW were significantly greater than zero ($p<0.001$), while slopes for M and H were not significantly different from zero ($p=0.276$ for M-5d, $p=0.185$ for M-10d, $p=0.687$ for H-5d, $p=0.169$ for H-10d). The relationship between age and surface loss are displayed in Figures 4.1 and 4.2 for enamel and dentin, respectively.

Time comparisons

Day 5 had significantly less SL than day 10 regardless of treatment, substrate, or age ($p<0.001$).

Substrate comparisons

Dentin had more SL than enamel for DIW group, regardless of time or age ($p<0.001$). Dentin had less surface loss than enamel for L, M, and H regardless of age for 10 days ($p<0.001$). However, dentin had more SL than enamel for L regardless of age for day 5 ($p<0.001$). For M and H after 5 days, the differences between substrates varied depending on the age of the tooth – at younger ages dentin had less surface loss than enamel, while for older ages dentin had more surface loss than enamel.

Treatment comparisons

L group showed significantly less SL than M and H groups regardless of substrate, time, or age ($p<0.001$). M group had significantly less surface loss than H group regardless age for dentin after 10 days and for enamel after 5 or 10 days ($p<0.005$), but they were not significantly different for dentin after 5 days. DIW control had significantly less enamel SL compared to all tested groups (L, M and H) and more dentin SL compared to L group, regardless of age and time ($p<0.001$). Compared to DIW, M (after 5 days) and H (after 5 and 10 days) groups showed lower dentin SL at younger ages, and higher dentin SL at older ages (Figure 4.2). After 10 days, M group showed significantly lower dentin SL at all ages.

Table. 4.2. Regression lines' slopes for all time-substrate-treatment combinations.

Substrate	Treatment	Day	Slope	SE	p-value
Enamel	L	5	0.0257	0.0037	<0.001
		10	0.0410	0.0055	<0.001
	M	5	0.0027	0.0025	0.276
		10	0.0055	0.0042	0.185
	H	5	-0.0012	0.0029	0.687
		10	-0.0058	0.0042	0.169
	DIW	5	0.0337	0.0047	<0.001
		10	0.0739	0.0078	<0.001
Dentin	L	5	0.0272	0.0026	<0.001
		10	0.0664	0.0044	<0.001
	M	5	0.0380	0.0031	<0.001
		10	0.0757	0.0059	<0.001
	H	5	0.0411	0.0038	<0.001
		10	0.0922	0.0068	<0.001
	DIW	5	0.0110	0.0023	<0.001
		10	0.0445	0.0037	<0.001

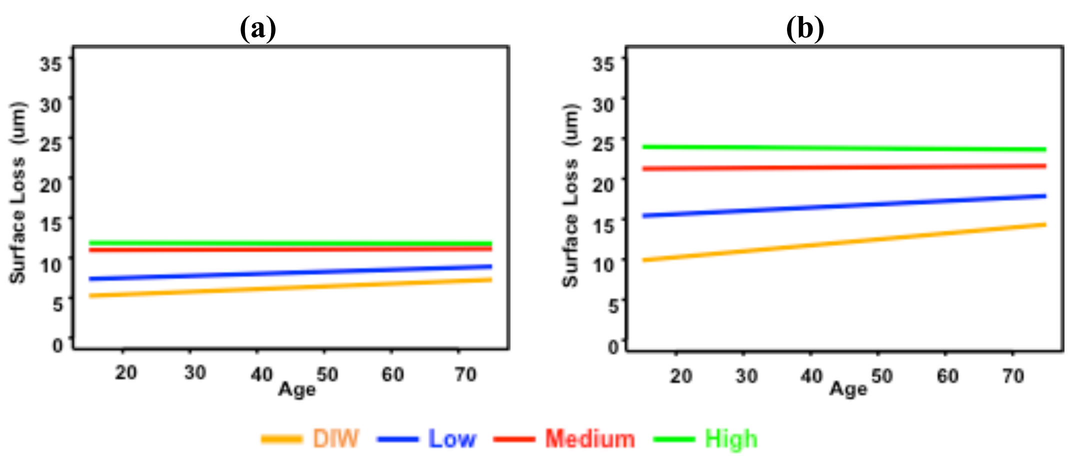


Figure 4.1. Enamel surface loss vs. age.
Surface loss of enamel after 5 days (a) and 10 days (b) of cycling, for DIW control, low, medium and high abrasivity toothpastes.

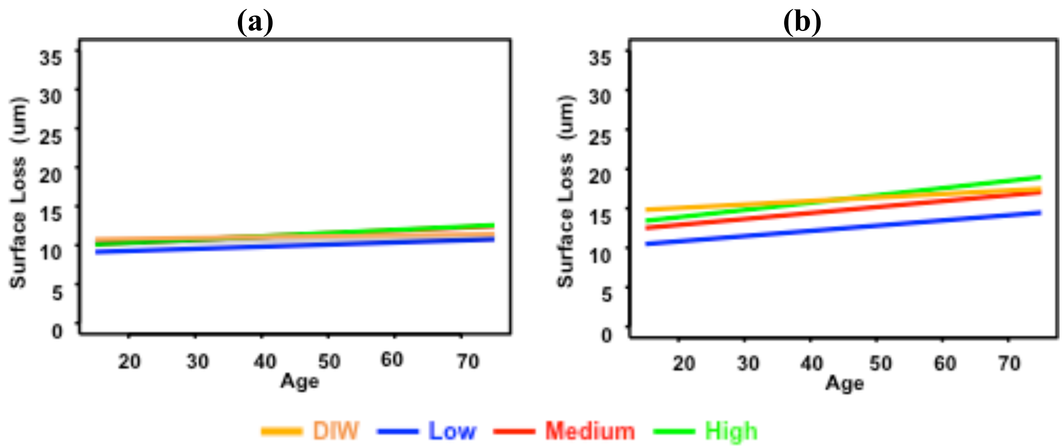


Figure 4.2. Dentin surface loss vs. age.
Surface loss of dentin after 5 days (a) and 10 days (b) of cycling, for DIW control, low, medium and high abrasivity toothpastes.

4.4. Discussion

This study aimed to investigate the susceptibility of enamel and dentin from different estimated ages to erosive tooth wear (ETW) after exposure to an established erosion-toothbrushing abrasion cycling model (Hara et al., 2013). Three different levels of toothpaste abrasivity were tested in attempt to represent the wide range of commercially available dentifrices. The negative control (DIW) shows the effect of toothbrushing with no toothpaste or fluorides. Higher variability was observed in the DIW group, as individual variations in demineralization susceptibility between teeth become more apparent due to the absence of an abrasive effect. The presence of abrasives in the other test groups overcame the individual variations, and this was more evident in enamel compared to dentin. Moreover, DIW showed an increase in SL susceptibility and progression with age for both enamel and dentin. This is in agreement with previous observations without toothbrushing (Chapter 3). However, the SL from this study was approximately 40% lower than in the previous study (Chapter 3) for both substrates, indicating that wear was less severe due to reduced acid exposure time (20 vs. 30 min), despite the inclusion of toothbrushing abrasion. Therefore, the effect of the erosive component on SL seems to predominate over the abrasive effect of dentifrices in the cycling model used in this study.

The DIW control showed the least enamel SL compared to all tested fluoridated toothpastes. The effect of abrasivity level on eroded enamel was clearly demonstrated by the increase in enamel SL with increase in toothpaste abrasivity (L, M and H), regardless of age or time. This observation is in agreement with previous reports that suggested a direct relationship between enamel SL and abrasivity of fluoridated toothpastes (Hara et al., 2009). Toothbrushing is predominantly a 3-body abrasion in which tissue loss is mainly related to toothpaste abrasives rather than toothbrush itself (Ganss et al., 2014). Besides, the eroded enamel surface is believed to be more prone to abrasion, which may explain our observations (Carvalho et al., 2015).

In our model, L group showed lower dentin SL compared to DIW, which is probably due to the protective effect of fluoride (Hara et al., 2009). However, the increase in abrasivity level seems to overcome the protective effect of fluoride, as indicated by the higher SL observed by M and H groups compared to L and DIW control. This observation is in agreement with previous findings (Hooper et al., 2003; Hara et al., 2009; Lippert et al., 2017). Moreover, after simulated prolonged exposure to acid (10 days), the difference between the three abrasivity levels was accentuated. Generally, dentin is considered more prone to demineralization than enamel, and less responsive to remineralization. Therefore, the higher SL observed by dentin compared to enamel in lower abrasive systems (i.e. L and DIW) was expected. However, with higher abrasive toothpastes (M and H), less dentin SL was observed compared to enamel, particularly after prolonged exposure (10 days). Although the exact reason is unclear, this could be explained by the fewer brushing strokes (45 strokes twice a day), which may not be enough to completely remove the dentin matrix. Preserved collagen matrix may act as a buffering agent and a physical barrier preventing further acid diffusion into deeper layers of eroded dentin (Hara et al., 2009). Moreover, the use of optical profilometer may detect only the outer surface of the demineralized dentine including the collagen matrix without measuring the underline dentin with mineral loss (Shellis and Addy, 2014). Besides, the demineralized dentin layer has found to be resistant to abrasion (Shellis and Addy, 2014).

The use of linear regression model in our study allowed estimating the difference in SL between different ages. For a 10-year difference in tooth age, the difference in SL is 10 × slope. Applying this formula and comparing the difference in enamel and dentin SL among the three toothpastes helps in visualizing the effect of the interaction between age and abrasivity level on SL. For example, if teeth from 30 and 60-year old individuals were exposed to the same erosive-abrasive challenge using low abrasive toothpaste, the latter would show more SL by approximately 1.23 and 2 μm, for enamel and dentin respectively (Table 4.3). This illustrates the higher susceptibility of older teeth to erosion-abrasion wear. Moreover, this difference increases as the abrasivity level increases for dentin, but not for enamel (Table 4.3).

Table. 4.3. 30-year difference in SL based on 10-day slopes.

Abrasivity level	**Difference in enamel SL (μm)**	**Difference in dentin SL (μm)**
L	1.23	2.00
M	~0	2.27
H	~0	2.77

For enamel, an increasing trend in SL with age was observed in L and DIW groups, which became more accentuated after prolonged acid exposure (10 days). However, the measureable impact of age on enamel ETW almost disappeared at higher abrasivity levels (M and H), indicating that the mechanical abrasion may have overcome the age effect. Therefore, age might play a role when using less abrasive toothpaste systems. The increase in enamel susceptibility to demineralization has been shown previously (Chapter 3). Several age-related changes in the mineral and organic phases of enamel may explain the increase in its susceptibility to erosion and abrasion. The increase in carbon content, as well as decrease in crystallinity with age may explain the increase in enamel solubility (Leventouri et al., 2009). Reduction in enamel protein concentration with age, as indicated by decrease in inter-prismatic sheath width, was shown to increase susceptibility to dental erosion. Although enamel proteins only comprise 1% of enamel, they provide bridging elements between enamel prisms as well as hindering mineral dissolution after acid attack (Lubarsky et al., 2014; Baumann et al., 2015).

Age-related changes are also reflected in the mechanical properties of enamel. The increase in enamel stiffness, surface micro-hardness and brittleness, have been related to increased mineral density and reduction in enamel protein content with age (Park et al., 2008a; Park et al., 2008b;Yahyazadehfar and Arola, 2015). Enamel organic matrix acts as unbroken bridging ligaments that play an essential role in reducing crack propagation. This may also explain the reduction in enamel fracture toughness with age (Zheng et al., 2013;Yahyazadehfar et al., 2016). Moreover, Zheng and Zhou (2006) revealed that the wear resistance of older (55 years) teeth is significantly lower than of younger ones. Those changes in mechanical properties may increase enamel susceptibility to abrasive wear and contribute to the increasing trend in enamel SL with age in L group observed in our study.

For dentin, all test groups showed increase in SL with age. This could be either due to the increase in susceptibility of dentin to erosion and abrasion with age, or reduction in fluoride response with age. The latter is not supported by our previous study (Chapter 3), where we also observed a clear increase in dentin susceptibility to demineralization with age. Conjointly, comparing SL from three tested toothpastes may suggest that the increase in SL with age is mainly due to increased susceptibility of dentin to abrasive wear, as all groups differ only in their abrasivity level.

Changes in dentin microstructure may help us understand the increase in dentin susceptibility to erosion and abrasion with age. There is increase in mineral density, tubular occlusion, accumulation of advanced glycation end-product in the collagen matrix, and dehydration with age (Toto et al., 1971; Montoya et al., 2015; Shinno et al., 2016). This deterioration in dentin collagen matrix quantity and quality with age may impact its acid buffering and acid diffusion prevention role (Ganss et al., 2001; Hara et al., 2005); and consequently increase susceptibility to demineralization (Chapter 3). In addition, there is evidence that dentin fracture toughness and resistance significantly decrease with age (Montoya et al., 2015; Shinno et al., 2016). This might explain the increase in dentin susceptibility to abrasive wear observed in our study. Our observations also suggest that age impact may be more evident on dentin than enamel. For higher abrasive groups (M and H at 5-day), older dentin showed more SL compared to enamel substrate; with opposite observation was found at younger ages. As discussed earlier, this could be related to the considerable changes on dentin microstructure with age.

Using un-identified extracted premolars is a limitation of this study, as demographic metadata of donors are unknown. Therefore, other factors essential for ETW diagnosis and assessment, such as salivary factors, dietary habits and fluoride regime, are also unknown. Measuring the proportions of fluoro- and hydroxyapatites is suggested for future research to help further understanding the difference in tooth susceptibility to demineralization. Moreover, since only premolars were included, generalizing our data to other tooth types should be done with caution. Even so, the proposed in-vitro approach enabled the study of aging impact on tooth susceptibility to demineralization, and response to anti-erosive treatment by controlling all other biological and environmental factors. Indeed, we were able to observe a trend of SL with age, which indicates the appropriateness of this approach in conducting such studies in a short timeframe with minimal budget.

CHAPTER 5: GENERAL DISCUSSION

As global life expectancy is increasing, maintaining oral health becomes essential to ensure healthy longevity of aging societies, as advocated by the Tokyo Declaration on Dental Care and Oral Health for Healthy Longevity (2015). Erosive tooth wear (ETW) is one of the most common dental issues associated with age (Van't Spijker et al., 2009), and may have a potential negative effect on individual's daily life (Al-Omiri et al., 2006). In that context, this project aimed to provide a better understanding of the tooth age impact on ETW susceptibility and treatment.

In Chapter 2, the percentage and severity of ETW and dental caries showed growing trends with age, corroborating existing epidemiological studies (Van't Spijker et al., 2009; Dye et al., 2015). This could be related to several changes in biological and behavioral factors associated with age, including microstructure and composition of enamel and dentin. Only few studies explored tooth susceptibility to demineralization among different ages. Most of these studies investigated simulated carious lesions, and they demonstrated controversial results. For instance, Kotsanose and Darling (1991) revealed that caries lesion depth is inversely related to age, due to increase in enamel mineralization and reduction in permeability with age. In contrast, other studies reported increase in susceptibility of older enamel to demineralization (Kidd et al., 1984; Gangler et al., 1993).

Chapter 3 of this project was conducted in order to examine the potential influence of enamel and dentin age-related changes on their susceptibility to demineralization, simulating erosive lesions. For this purpose, we used an in vitro pH-cycling model, which controlled several factors relevant to ETW development, focusing solely on the impact of age. A proportional relationship between tooth age and its susceptibility to demineralization was clearly observed. The anti-erosion efficacy of fluoridated solutions reduced the effect of age on the susceptibility of enamel and dentin to ETW. Sn+F showed superior anti-erosive effect on enamel and dentin compared to F alone, which is in agreement with the literature (Lussi and Carvalho, 2015). Overall, age did not seem to impact the efficacy of tested solutions on enamel. For dentin, however, efficacy of both solutions increased with age, after prolonged erosion simulation (10 days).

Similar to fluoride containing solutions, fluoridated toothpastes may enhance eroded tooth surface remineralization and resistance to further erosive attacks (Magalhães et al., 2014). However, toothpastes contain abrasives that may negatively affect ETW, by promoting abrasive wear (Ganss et al., 2017). In Chapter 4, we investigated how age and toothpaste abrasivity can interact and modulate tooth susceptibility to ETW. We found that surface loss of eroded enamel and dentin increases as toothpaste abrasivity increases, particularly after extended erosive challenge (10 days). For enamel, only low abrasivity level showed increasing trend with age, and this effect disappeared with medium and high abrasivity levels. While for eroded dentin, surface loss increased with age for all toothpaste abrasivity levels tested.

Changes in enamel and dentin compositions and properties may explain the results of Chapters 3 and 4. Reduction in protein matrix quantity and quality of enamel and dentin, and increase in carbon contents of hydroxyapatite may increase tooth solubility with age (Leventouri et al., 2009; Lubarsky et al., 2014; Shinno et al., 2016). Moreover, there is evidence of increase in enamel and dentin brittleness and reduction in their fracture toughness, which may explain the reduction in tooth resistance to mechanical wear with age (Zheng et al., 2006; Park et al., 2008; Nazari et al., 2009).

In this thesis project, we observed the increased presence and severity of main dental hard-tissue pathologies, including ETW and caries, as well as increased susceptibility of tooth to demineralization with age. Accordingly, individual’s age should be considered when designing dental preventive and therapeutic management plans, in addition to other biological and behavioral factors. Moreover, the daily use of Sn+F mouth rinse and low abrasivity toothpastes may be recommended for individuals with high-risk to ETW.

GENERAL CONCLUSIONS

In Chapter 2, we concluded that the presence and severity of dental caries, ETW, and extrinsic staining increased with age, while of enamel fluorosis decreased. Tooth also showed to be darker with age.

In Chapter 3, the susceptibility of enamel and root dentin to demineralization increased with age. Fluoride solutions (Sn+F and NaF) could reduce this susceptibility to demineralization, with Sn+F showing the best results. NaF treatment efficacy on dentin increased with age at simulated advanced lesions. While Sn+F treatment seemed to effectively prevent erosive wear, regardless of tooth age, especially for enamel substrate.

In Chapter 4, the susceptibility of enamel and dentin to erosion-abrasion lesions increased with age. The effect of age on eroded enamel wear was more prominent when using lower abrasive systems. Surface wear of eroded root dentin increased with age as well as toothpaste abrasive level.

APPENDIX

Table A-1. BEWE index.

Score	Severity (1st digit)	Location (2nd digit)*
0	No erosive tooth wear	Crown with no involvement of the cervical area
1	Initial loss of surface texture	Crown with involvement of the cervical area
2	Distinct defect, hard tissue loss <50% of the surface area (dentin often is involved)	Root only
3	Hard tissue loss ≥50% of the surface area, (dentin often is involved)	Involvement of both crown and root

* Applies only to Severity scores 1, 2 and 3. Location scores 1, 2 and 3 indicate non caries cervical lesions (NCCL).

Table A-2. Modified Lobene index.

Score	Intensity (1st digit)	Extent (2nd digit)*
0	No stain present, natural tooth coloration	---
1	Faint stain	Up to 1/3rd of the surface
2	Clearly visible stain, orange to brown	Between 1/3 and 2/3rd of the surface
3	Dark stain, deep brown to black	> 2/3 of the surface

* Applies only to Intensity scores 2 and 3.

Table A-3. Vita classical shade guide and their given scores, in descending order based on their color value.

B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

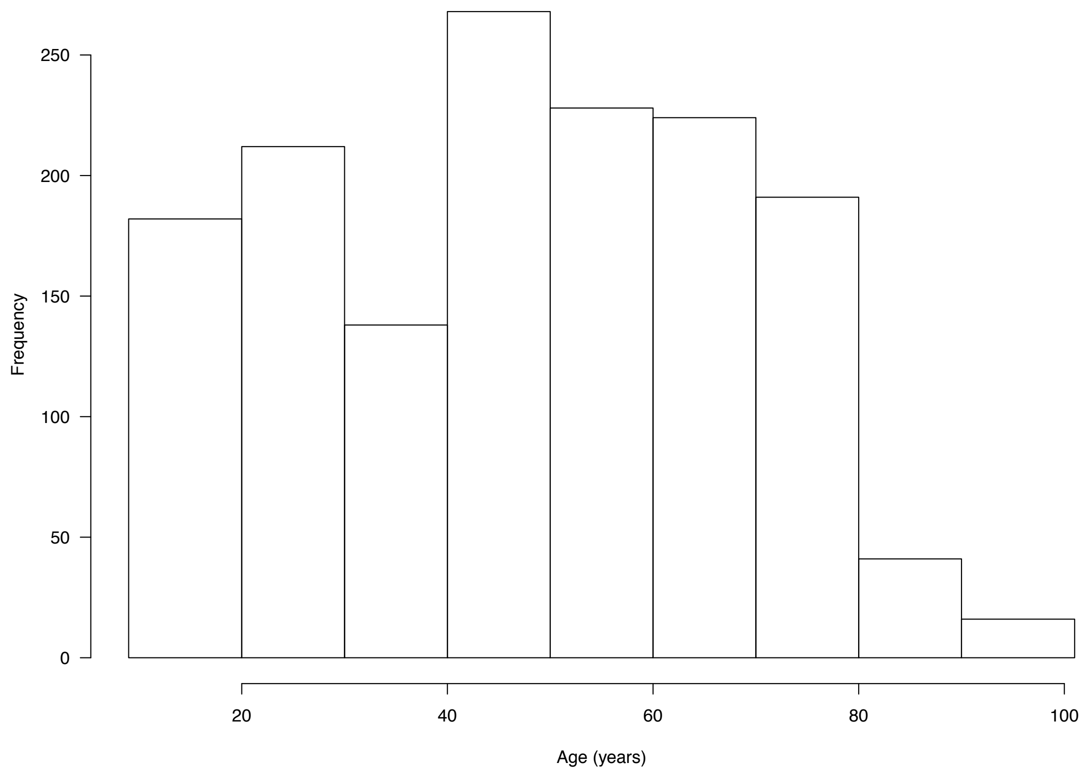


Figure A-1. Distribution of our sample based on age (10-year intervals).

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CURRICULUM VITAE

Amnah Abdullah Algarni

Education

2013-2018	PhD in Dental Sciences. Indiana University, Indianapolis, IN, USA.
2010-2013	Master of Science in Dental (MSD). Major in Preventive Dentistry and Operative Dentistry, Minor in Dental Materials. Certificate in Operative Dentistry.
2001-2007	Bachelor of Dental Medicine & Surgery (BDS) from King AbdulAziz University, Jeddah, KSA.

Professional Experience

2010-2013	Residency in Operative/Preventive dentistry, and undergraduate students clinical supervision at Indiana University school of dentistry.
2008-2010	Demonstrator (Teacher Assistant) at Taiba University, Department of Restorative Dentistry, College of Dentistry, Taibah University, Al Madina Al Munawarah, Saudi Arabia.
2007-2008	Internship rotation program: Rotation among different specialties clinics.

Research Experience and publications

- Liu WC, Ballenger B, Algarni A, Velez M, G Chu TM. FTIR characterization and release of bovine serum albumin from bioactive glasses. J Appl Biomater Funct Mater. 2017;15(4):e347-e355.
- Algarni AA, Kang H, Fried D, Eckert GJ, Hara AT. Enamel Thickness Determination by Optical Coherence Tomography: In Vitro Validation. Caries Res.

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- Algarni AA, Lippert F, Hara AT. Efficacy of Stannous, Fluoride and Their Combination on Dentin Erosion Prevention In vitro. Braz Oral Res. 2015;29 DOI: 10.1590/1807-3107BOR-2015.vol29.0081.
- Algarni AA, Mussi MCM, Moffa EB, Lippert F, Zero DT, Siqueira WL, Hara AT. The Impact of Stannous, Fluoride Ions and its Combination on Enamel Pellicle Proteome and Dental Erosion Prevention. PLoS One. 2015;10(6):e0128196.
- Algarni AA, Yassen GH, Gregory RL. Inhibitory effect of gels loaded with a low concentration of antibiotics against biofilm formation by Enterococcus faecalis and Porphyromonas gingivalis. J Oral Sci. 2015;57(3):213-8
- Abu Alenain DA, Amin HE, Al-Zahrani NA, Al Harbi NA, Al-Qadi SF, Algarni AA. Bleaching Efficiency and Side Effects of Three Home Bleaching Systems. JKAU: Med. Sci. 2009;16(3):43-58.
- Participation in a survey and conducting study: Prevalence of plaque and gingivitis among intermediate School pupils in Jeddah. 2007

<u>Presentations & Posters</u>

- Algarni AA, Lippert F, Ungar P, Eckert GJ, González-Cabezas C, Platt JA, Hara AT. Tooth Age Impact on Dental Erosion Susceptibility and Prevention. AADR conference 2018 in Fort Lauderdale, FL, USA and IUSD Research Day 2018 in Indianapolis, IN, USA.
- Algarni AA, Ungar P, Lippert F, Martinez-Mier EA, Eckert GJ, Hara AT. Effect of tooth age on the presence and severity of dental hard-tissue conditions. ORCA Congress 2017, Oslo, Norway and IUSD Research Day 2017, Indianapolis, IN,

USA

- Algarni AA, Kang H, Fried D, Ecker GJ, Hara AT. Enamel thickness determination by optical coherence tomography: in vitro validation. 63th ORCA Congress. 2016, Athens, Greece.
- Algarni AA, Yassen G, Gregory R. Biofilm inhibition by antibiotic gels against endodontic pathogens. IADR conference 2015 in Boston, MS, USA and IUSD Research Day 2015. Indianapolis, IN, USA.
- Algarni AA, Siqueira W, Lippert F, Zero D, Hara AT. Interaction between enamel acquired pellicle and tin/fluoride-containing solutions on erosion prevention in-vitro. AADR conference 2014 in Charlotte, NC, USA and IUSD Research Day 2014, Indianapolis, IN, USA.
- Several presentations at IUSD: Dental composites; CAD CAM; Fiber reinforced composite; Chlorhexidine varnish and caries prevention; Caries assessment and management.
- Al-zahrani N, Algarni A, Abouzenada S, Alyamani A. Midface distraction osteogenesis to treat sever orbital exorbitism. The 5th Pan Arab Association of Oral & Maxillofacial Surgeons Conference 2008, Jeddah, Saudi Arabia.
- Educational presentation (lecture & pamphlet) on oral health and plaque control. KAU, Jeddah, Saudi Arabia, 2006.
- Comprehensive care clinic case presentation; Orthodontics case presentation. KAU, Jeddah, Saudi Arabia, 2005.
- The effect of polyethylene fibers in endodontically treated teeth; oral medicine case Presentation. KAU, Jeddah, Saudi Arabia, 2005.

Conferences, Meetings & Workshops

2016	Writing effective multiple-choice exam questions workshop by Center for the Integration of Research, Teaching and Learning
2016	Several meetings by National Center for Faculty Development & Diversity (NCFDD)
2015	Molecular Biology Workshop
2015	IADR conference 2015 in Boston, MS, USA.
2014	AADR/CADR conference 2014 in Charlotte, NC, USA
2011-2018	Research day at IUSD
2008	Implant workshop (KAU).
2008	Intensive course in Infection Control in King Fahad General Hospital (KFGH).
2007-2008	Several international and local conferences by KAU and SDS

Awards

2016	Golden Key International Honor Society nominee, as one of the top 15% of top-performing graduate students at IUPUI
2015	Received Travel Fellowship Award from the IUPUI Graduate Office
2014-2015	Received Certificate of Appreciation for participating as a speaker in IUSD research day
2015	Received Graduate Professional Educational Grant
2008	Received Certificate of Appreciation for participating as a speaker in The 2nd international KAAU and the 19th Saudi dental society conference

2007 Received Plaque of Academic Honor for Achieving GPA with a second honor degree.

Professional Affiliations

- Member of IADR / AADR
- Member of Saudi Dental Society.
- Professional Registration as a General Dentist