

TITLE

Trend-Analysis of Dental Hard-Tissue Conditions as Function of Tooth Age

SHORT TITLE

Age and Dental Hard-Tissue Conditions

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ABSTRACT

Objective: This retrospective in-vitro study investigated tooth age effect on dental hard-tissue conditions. **Methods:** Unidentified extracted premolars (n=1,500) were collected and their individual age was estimated (10-100 (± 10) years old (yo)) using established dental forensic methods. Dental caries, fluorosis and tooth wear (TW) were assessed using the International Caries Detection and Assessment System (ICDAS; 0-5 for crown and 0-2 for root), Thylstrup-Fejerskov (TFI; 0-9) and Basic Erosive Wear Examination (BEWE; 0-3) indices, respectively. Staining and color were assessed using the modified-Lobene (MLI) (0-3) and VITA shade (B1-C4) indices, respectively. Relationships between indices and age were tested using regression models. **Results:** Starting at age ~10yo, presence of caries increased from 35% to 90% at ~50yo (coronal), and from 0% to 35% at ~80yo (root). Caries severity increased from ICDAS 0.5 to 2 at ~40yo and from ICDAS 0 to 0.5 at ~60yo for coronal and root caries, respectively. Presence of TW increased from 25% (occlusal) and 15% (smooth-surfaces) to 100% at ~80yo. TW severity increased from BEWE 0.5 to 2 at ~50yo (occlusal) and ~0.3 to 1.5 at ~50yo (smooth-surfaces). Percentage and severity of fluorosis decreased from 70% to 10% at ~80yo, and from TFI 1 to 0 at ~90yo, respectively. Percentage of extrinsic staining increased from 0% to 85% at ~80yo and its severity increased from MLI 0 to 2 at ~70yo. Color changed from A3 to B3 at ~50yo (crown), and from C2 to A4 at ~85yo (root). **Conclusions:** Aging is proportionally related to the severity of caries, TW, staining, and inversely to dental fluorosis. Teeth become darker with age.

KEYWORDS

Aging, Enamel, Dentin, Caries, Tooth Wear, Fluorosis, Tooth color

CLINICAL SIGNIFICANCE

Age correlated with presence and severity of the studied dental conditions, which might require age-specific treatment protocols. Therefore, further understanding of age impact on the process of dental conditions is warranted.

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 [1]. 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 [2].

Besides improvement in dental health awareness and preventive measures, age may also impact propensity for dental diseases. 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 [3, 4]. 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 [4]. 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 change as well. Moreover, behavioral aspects 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 literature to allow deeper understandings of the age impact on dental hard-tissue conditions. Major limitations of longitudinal clinical studies, such as time and costs, prohibit conducting a comprehensive evaluation of their prevalence and severity. 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 [5, 6].

We hypothesized that 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 started exploring this hypothesis by evaluating the occurrence of clinically common dental problems in a 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.

MATERIALS AND METHODS

Teeth collection

A sample of 1500 extracted human premolars were randomly selected from an existing pool (approximately 18,000 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 teeth collection from dental practice clinics across the USA over several years. Upon receipt at OHRI, teeth were sorted, gently cleaned from tissue remnants and kept in 0.1% thymol, at 4° C. Patient metadata (e.g., age, sex, reason for extraction) were not available, rendering all samples unidentifiable. Our exclusion criteria included advanced caries lesion (i.e. International Caries Detection and Assessment System [ICDAS] index 6) [7], restored and fractured teeth. 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 a wide age range. Using approximately 300 teeth per age category, pathologies' presence could be estimated within the range 50% ($\pm 6\%$) to 3.5% ($\pm 2.3\%$), 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 [1999] [8] method was used to estimate the age of not yet fully developed teeth, which comprised 10.7% 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 $A =$

$b_0+b_1 \times$; where (A) is the estimated age, (\times) is the developing tooth length, and (b_0 , b_1) are coefficients for each tooth type (1st or 2nd premolars, in our study).

The Bang and Ramm [1970] [9] method was used to estimate the age of fully developed teeth based on root dentin translucency. The minimal and maximal translucency length values (TL 1 and 2, respectively) from the apex to the borderline of opaque root dentin coronally were recorded. The average of TL1 and TL2 (TM) was used to estimate the age (A) applying the formulae $A = B_0+B_1 \times + B_2 \times^2$ (for $TM \geq 9.0$ mm) or $A = B_0+B_1 \times$ (for $TM < 9.0$ mm); where B_0 , B_1 , B_2 are coefficients for each tooth type. A single trained examiner performed the measurements using a digital sliding caliper (Fisher Scientific, Waltham, MA, USA).

Outcome measures

Coronal and root caries lesions were recorded on all surfaces according to ICDAS-II criteria [7]. Enamel fluorosis was recorded on buccal, lingual and occlusal surfaces using the Thylstrup-Fejerskov Index (TFI) [10] (TFI). Tooth wear was scored on occlusal, buccal and lingual surfaces, using the Basic Erosive Wear Examination (BEWE) index [11]. For buccal and lingual surfaces, two digits were given; the first digit represented the severity, while the second digit represented the location of tooth wear (TW) [12] to study the percentage of non-carious cervical lesions (NCCL) in different ages. Presence and severity of extrinsic staining was assessed on buccal and lingual surfaces, using the two-digits modified-Lobene index (MLI), in which the first and second digits represent staining intensity and extent, respectively [13]. 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.

Statistical analysis

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

Presence of caries lesions, enamel fluorosis, tooth wear, 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.

RESULTS

Our sample showed similar distributions for maxillary (50.6%) and mandibular (49.4%) premolars. Although the sample represented a wide age range (9-101yo), the distribution was unbalanced at the highest end, with fewer teeth ≥ 80 yo. This should be considered when interpreting and comparing data obtained from those ages. ICC revealed excellent intra-examiner agreement for average translucency (0.92), and inter-examiner agreement for TFI (0.97) and staining intensity (0.90). Acceptable inter-examiner agreement was observed for BEWE location (0.67) and severity (0.72), staining extent (0.77), and ICDAS (0.77).

Overall, percentage and severity of coronal and root caries increased with age, with higher numbers observed for coronal caries (Figure 1). TW percentage and severity for both occlusal and smooth surface, as well as occurrence of NCCL increased with age (Figure 2). Percentage and severity of enamel fluorosis decreased with age (Figure 3). Extrinsic staining presence, severity and extent increased with age, with higher extent observed in the lingual surface (Figure 4). Mean VITA classical shade increased for both crown and root, with more evident results for root (Figure 5).

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 preferred type of teeth for forensic age-estimation due to their uncomplicated and more stable root morphology [14]. While practical and convenient, premolars may not be entirely representative to 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 used are not affected by gender [8, 9], 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 [9, 15], Indians [16] and Hispanics [17], 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 evaluated for different races and ethnicities either, which could be a limitation for the younger teeth used in this current investigation.

Coronal and root caries percentages of our sample trended higher with age increase. For older age teeth (≥ 40 yo), our data showed percentages up to 90% for coronal, and 35% for root caries. This observation is in agreement with those obtained by Papas et al. [1992] [18], in which caries in premolars reached up to 89% and 35% for coronal and root caries, respectively. Overall, our results displayed comparable trend to other full mouth screening epidemiological studies, which adds justification for limiting our study to premolars [19].

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 [18]. While increased caries occurrence with age can be related to several environmental and behavioral factors, 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 [20, 21]. Therefore, our data demand further investigation of age impact on enamel and dentin susceptibility to demineralization, as well as on the efficacy of different caries preventive/therapeutic measures.

For clinical practice, our findings suggest the need for different caries management protocols for each age stage. At young adulthood, primary coronal caries prevention should be targeted, while additional root caries preventive measures may be needed for older adults (>50 yo). These results

also provide evidence that seniors should be considered a high-risk group for caries, supporting existing evidence in the literature [22].

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 was that the extensive destruction would render those teeth inappropriate for the planned subsequent phases of this project (un-published data). Nonetheless, caries presence estimates at ages older than ~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.

We found direct relationships between age and both presence and severity of TW for occlusal and smooth surfaces. More evident wear was observed on the occlusal surfaces, which may be explained by the association of erosion with mechanical forces from occlusion and food comminution. Clinically, TW is mainly a result of interaction between mechanical and chemical processes [23]. A steady increase in mean severity of occlusal TW was observed from BEWE 0 at ~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. Smooth surfaces showed slightly lower severity compared to occlusal, stabilizing at BEWE score below 2, at ~50yo. NCCL presence also increased with age, which is consistent with existing epidemiological data [24]. At ~40yo, one third of the sample started to show some extent of NCCL on the buccal surface, with increased incidence thereafter (Figure 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. Based on our findings, it is recommended that dental practitioners consider NCCL preventive measures at early ages.

Although similar trends with age were observed in a clinical study that used BEWE index [25], 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 TW visual detection. Moreover, using premolars in this study may have contributed to this difference, as different tooth types might vary in susceptibility to TW [26, 27].

By age ~80yo almost 100% of our sample showed some extent of TW, corroborating a previous study [28]. The relationship between TW and age has been explained by physiological wear, as well as various factors, including diet and age-related microstructural changes of dental tissues [29, 30]. Previous studies have found acidic juices to be more related to erosive TW in younger subjects (15-39 years), whereas chewing hard and acidic food was more related to TW in older adults (>60) [30, 31]. Thus, effective TW 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 tooth donors. However, considering the multifactorial nature of TW, it is difficult to eliminate the possibility that chemical processes contribute to wear, unless patient's history and clinical lesions suggested otherwise. Hence, we used BEWE index as a reliable tool for measuring chemical and/or mechanical tooth wear [32].

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 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 the USA National Health and Nutrition Examination data [33]. These results may be influenced by the higher fluoride exposure of younger (more recent) generations due to increased awareness of caries prevention and oral hygiene [33]. Increase in TW 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 [34]. Moreover, premolars are considered the most permanent teeth affected by fluorosis [35], which could justify for the higher percentages of fluorosis found in this study compared to previous reports [33].

Extrinsic staining presence and intensity increased with age reaching MLI of 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 [36]. 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 stains 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 [37]. 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 [38], as does tubular occlusion and advanced glycation end-products accumulation in dentin [39]. 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 [40], 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, tooth wear, 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 immediate next steps will focus on age effect on the efficacy of preventive and therapeutic measures for dental caries and erosive tooth wear, two 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 timely and systematic fashion.

CONCLUSION

Within the limitations of our study, we concluded that the presence and severity of dental caries, tooth wear, and extrinsic staining increased with age, while of enamel fluorosis decreased. Tooth also showed to be darker with age.

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DECLARATION OF INTEREST

The authors of this paper have no interest to declare.

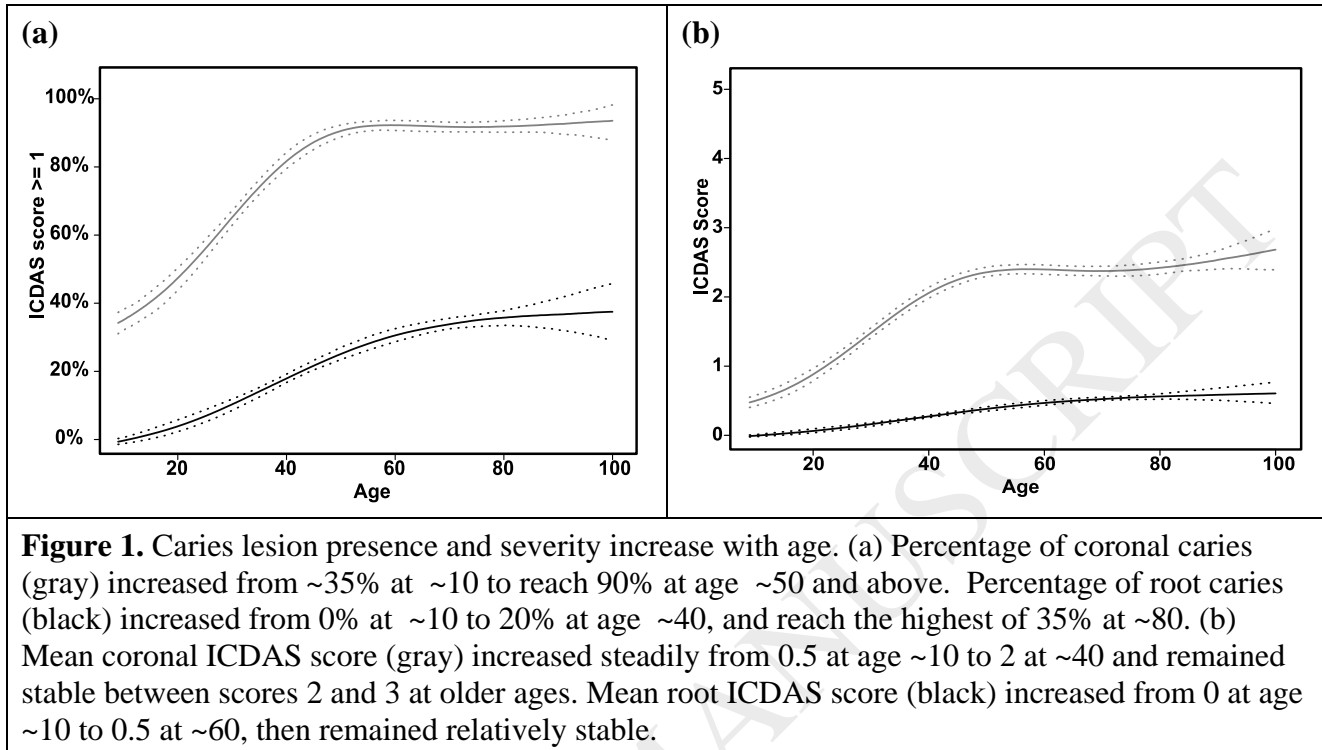
REFERENCES

- [1] World population ageing: 1950-2050: United Nations; Population Division: [accessed 2017 May 20]. http://www.aoa.acl.gov/aging_statistics/index.aspx.
- [2] C. Lopez-Otin, M. A. Blasco , L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging. *Cell* 153 (2013) 1194-1217.
- [3] B. He, S. Huang, C. Zhang, J. Jing, Y. Hao, L. Xiao, X. Zhou, Mineral densities and elemental content in different layers of healthy human enamel with varying teeth age, *Arch Oral Biol* 56 (2011) 997-1004.
- [4] M. Yahyazadehfar, J. Ivancik, H. Majd, B. An, D. Zhang, D. Arola, On the Mechanics of Fatigue and Fracture in Teeth, *Appl Mech Rev* 66(3) (2014) 0308031-3080319.
- [5] G. Gustafson, Age determination from teeth, *J. Am. Dent. Assoc.* 41(1) (1950) 45–54.
- [6] G. Johanson, Age determination from human teeth. *Odont Revg* 22(suppl. 21) (1971) 1-126.
- [7] International caries detection and assessment System, ICCMS™ Guide for Practitioners and Educators. <https://www.iccms-web.com/uploads/asset/59284654c0a6f822230100.pdf>, 2014 (accessed 13 March 2018).
- [8] H.M. Liversidge, T.I. Molleson, Developing permanent tooth length as an estimate of age, *J. Forensic Sci.* 44(5) (1999) 917-920.
- [9] G. Bang, E. Ramm, Determination of age in humans from root dentin transparency, *Acta Odontol. Scand.* 28(1) (1970) 3-35.
- [10] A. Thylstrup, O. Fejerskov, Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes, *Community Dent. Oral Epidemiol.* 6(6) (1978) 315-28.
- [11] Bartlett D, Ganss C, Lussi A: Basic erosive wear examination (bewe): A new scoring system for scientific and clinical needs. *Clin Oral Investig* 2008;12 Suppl 1:S65-68.

- [12] N.X. West, M. Sanz, A. Lussi, D. Bartlett, P. Bouchard, D. Bourgeois, Prevalence of dentine hypersensitivity and study of associated factors: a European population-based cross-sectional study, *J. Dent.* 41(10) (2013) 841-51.
- [13] L.M. Macpherson, K.W. Stephen, A. Joiner, F. Schafer, E. Huntington, Comparison of a conventional and modified tooth stain index, *J. Clin. Periodontol.* 27(11) (2000) 854-9.
- [14] H. Aboshi, T. Takahashi, T. Komuro, Age estimation using microfocus X-ray computed tomography of lower premolars, *Forensic Sci Int* 200(1-3) (2010) 35-40.
- [15] H. Soomer, H. Ranta, M.J. Lincoln, A. Penttila, E. Leibur, Reliability and validity of eight dental age estimation methods for adults, *J Forensic Sci* 48(1) (2003) 149-52.
- [16] A.B. Acharya, S. Vimi, Effectiveness of Bang and Ramm's formulae in age assessment of Indians from dentin translucency length, *Int J Legal Med* 123(6) (2009) 483-8.
- [17] D.H. Ubelaker, R.C. Parra, Application of three dental methods of adult age estimation from intact single rooted teeth to a Peruvian sample, *J Forensic Sci* 53(3) (2008) 608-11.
- [18] A. Papas, A. Joshi, J. Giunta, Prevalence and intraoral distribution of coronal and root caries in middle-aged and older adults, *Caries Res* 26(6) (1992) 459-65.
- [19] B. Dye, G. Thornton-Evans, X. Li, T. Iafolla, Dental caries and tooth loss in adults in the United States, 2011-2012, *NCHS Data Brief* (197) (2015) 197.
- [20] E.A. Kidd, A. Richards, A. Thylstrup, O. Fejerskov, The susceptibility of 'young' and 'old' human enamel to artificial caries in vitro, *Caries Res* 18(3) (1984) 226-30.
- [21] T. Leventouri, A. Antonakos, A. Kyriacou, R. Venturelli, E. Liarokapis E, V. P: Crystal structure studies of human dental apatite as a function of age. *Int J Biomater* (2009) doi: 10.1155/2009/698547
- [22] P.E. Petersen, T. Yamamoto, Improving the oral health of older people: the approach of the WHO Global Oral Health Programme, *Community Dent Oral Epidemiol* 33(2) (2005) 81-92.
- [23] R.P. Shellis, M. Addy, The Interactions between Attrition Abrasion and Erosion in Tooth Wear, *Monogr Oral Sci.* 25 (2014) 32-45.
- [24] B. Smith, N. Robb, The prevalence of toothwear in 1007 dental patients, *J. Oral Rehabil.* 23(4) (1996) 232-239.
- [25] Y. Vered, A. Lussi, A. Zini, J. Gleitman, H. Sgan-Cohen, Dental erosive wear assessment among adolescents and adults utilizing the basic erosive wear examination (BEWE) scoring system, *Clin. Oral Investig.* 18(8) (2014) 1985-1990.
- [26] T.S. Carvalho, A. Lussi, Susceptibility of enamel to initial erosion in relation to tooth type, tooth surface and enamel depth, *Caries Res.* 49(2) (2015) 109-115
- [27] Y. Kitasako, Y. Sasaki, T. Takagaki, A. Sadr, J. Tagami, Erosive Tooth Wear Among Different Tooth Types and Surfaces in Japanese Adults 15 to 89 Years Old, *Oral health & preventive dentistry* 15(4) (2017).
- [28] Z. Wei, Y. Du, J. Zhang, B. Tai, M. Du, H. Jiang, Prevalence and Indicators of Tooth Wear among Chinese Adults, *PLoS One* 11(9) (2016) e0162181.

- [29] Q. Zheng, H. Xu, F. Song, L. Zhang, X. Zhou, Y. Shao, D. Huang, Spatial distribution of the human enamel fracture toughness with aging, *J Mech Behav Biomed Mater* 26 (2013) 148-54.
- [30] B. Liu, M. Zhang, Y. Chen, Y. Yao, Tooth wear in aging people: an investigation of the prevalence and the influential factors of incisal/occlusal tooth wear in northwest China, *BMC Oral Health* 14 (2014) 65.
- [31] Y. Kitasako, Y. Sasaki, T. Takagaki, A. Sadr, J. Tagami, Age-specific prevalence of erosive tooth wear by acidic diet and gastroesophageal reflux in Japan, *J Dent* 43(4) (2015) 418-23.
- [32] D. Bartlett, Summary of: evaluation of the basic erosive wear examination (BEWE) for use in general dental practice, *Br. Dent. J.* 213(3) (2012) 128-9. .
- [33] E.D. Beltran-Aguilar, L. Barker, B.A. Dye, Prevalence and severity of dental fluorosis in the United States, 1999-2004, *NCHS Data Brief* (53) (2010) 1-8.
- [34] H.M. Nassar, F. Lippert, G.J. Eckert, A.T. Hara, Dentifrice fluoride and abrasivity interplay on artificial caries lesions, *Caries Res* 48(6) (2014) 557-65.
- [35] I. Ramires, J.P. Pessan, F.M. Levy, M.H. Rodrigues, B.S. de Almeida, M.T. Kato, S.H. Peres, M.A. Buzalaf, Prevalence of dental fluorosis in Bauru, Sao Paulo, Brazil, *J Appl Oral Sci* 15(2) (2007) 140-3.
- [36] H.M. Eriksen, H. Nordbo, Extrinsic discoloration of teeth, *J Clin Periodontol* 5(4) (1978) 229-36.
- [37] J.R. Mayoral, M.A. Arocha, S. Dominguez, M. Roig, S. Ardu, In vivo spectrophotometric evaluation of pure enamel and enamel-dentine complex in relationship with different age groups, *J Dent* 41(12) (2013) 1245-50.
- [38] Y. Miake, S. Tsutsui, Y. Eshita, Variation in the Color of Japanese Teeth and Structural Changes in Enamel Rod Sheath Associated with Age, *Journal of Hard Tissue Biology* 25(2) (2016) 131-136.
- [39] J. Miura, K. Nishikawa, M. Kubo, S. Fukushima, M. Hashimoto, F. Takeshige, T. Araki, Accumulation of advanced glycation end-products in human dentine, *Arch Oral Biol* 59(2) (2014) 119-124.
- [40] A. Ten Cate, The estimation of age skeletal remains from the colour of roots of teeth, *Can Dent Assoc J* 43 (1977) 83-86.

FIGURES



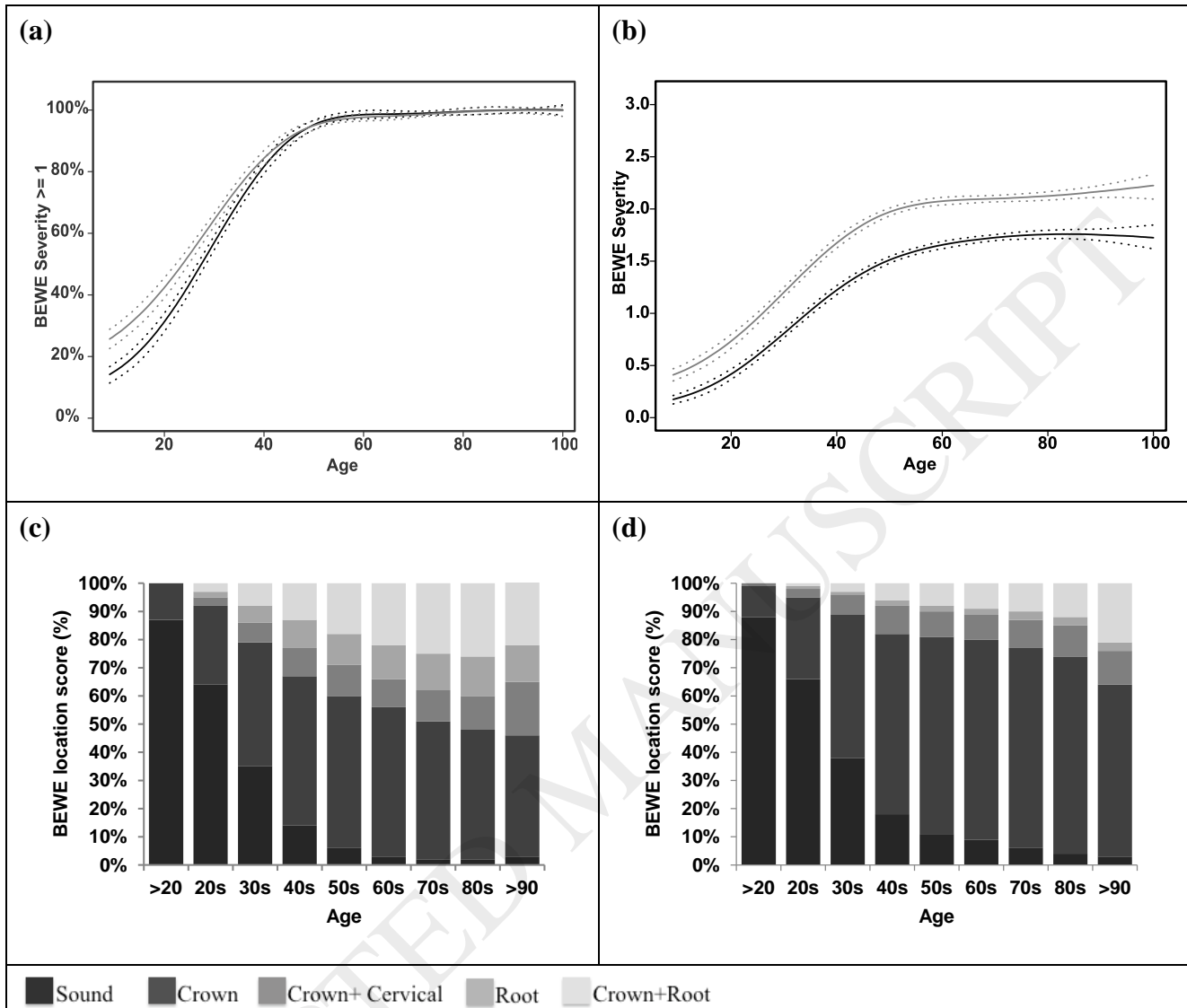


Figure 2. Presence and severity of tooth wear signs increase with age. (a) Percentage of tooth wear increased from 25% and 15% at ~10 to reach 100% at ~80 for occlusal (gray) and smooth-surfaces (black), respectively. (b) Mean BEWE score increased from 0.5 and ~0.3 at age ~10 to 2 and 1.5 at ~50 remaining stable after, for occlusal (gray) and smooth surfaces (black), 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 (Crown+Cervical, Root and Crown+Root) start at ~30s and increasing after, with lower percentages observed in lingual surfaces.

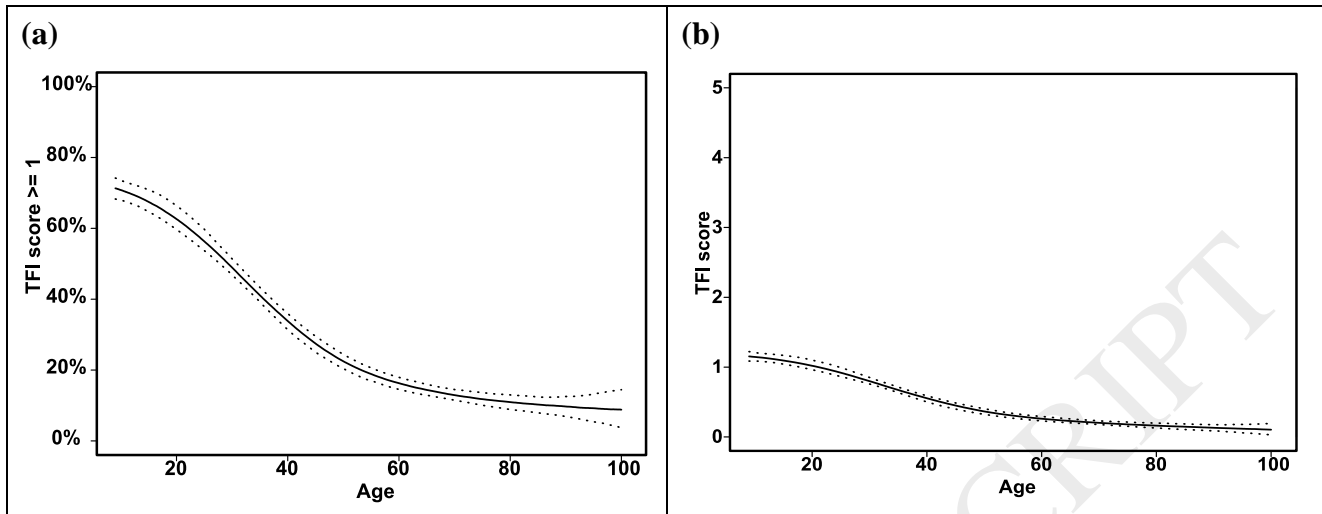


Figure 3. Presence and severity of enamel fluorosis decrease with age. (a) Percentage of dental fluorosis decreased from 70% at ~10 to reach approximately 10% at ~80. (b) Mean TFI was around 1 until age ~30 before decreasing to 0.5 at age ~40, approaching 0 at ~90 years old.

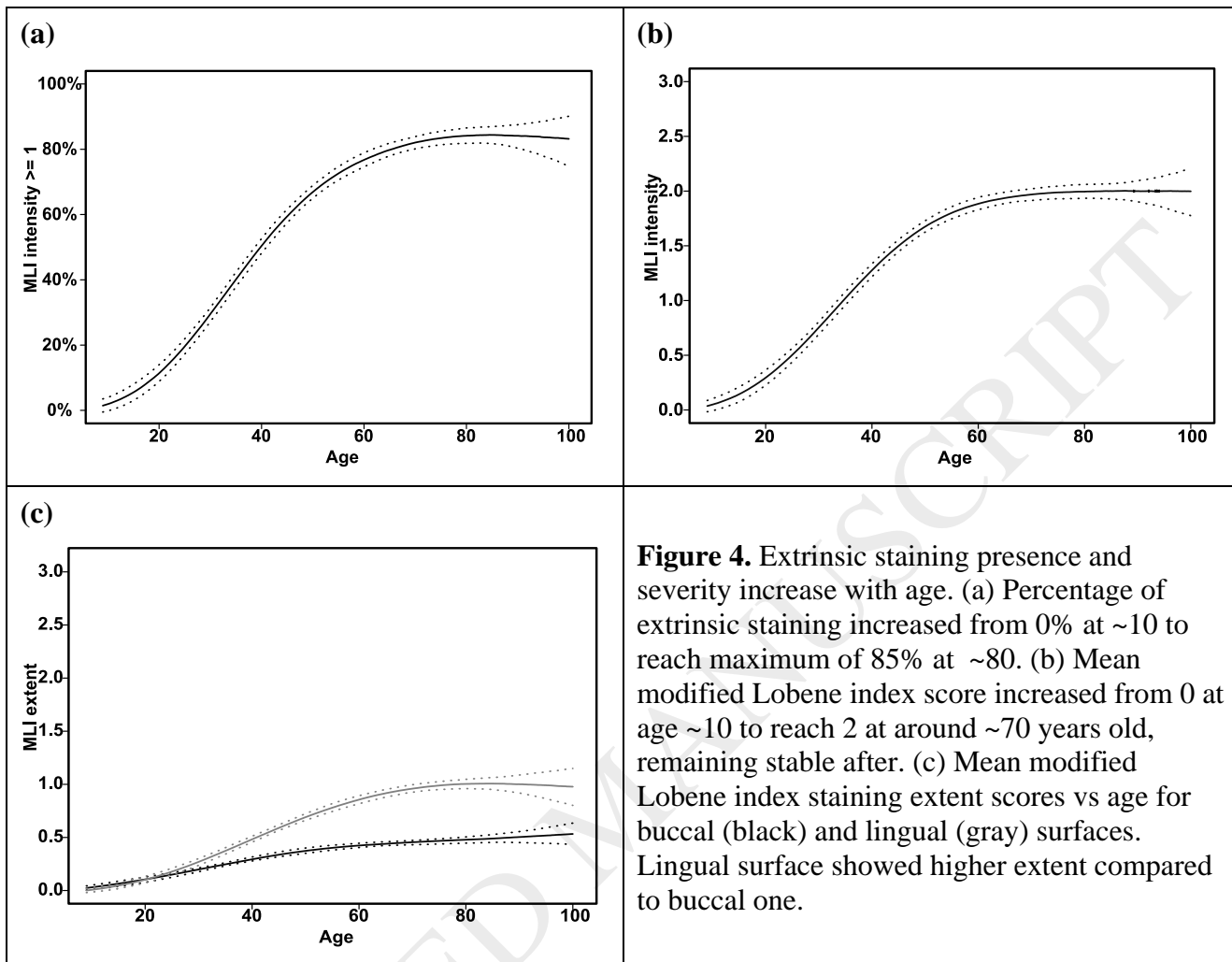


Figure 4. Extrinsic staining presence and severity increase with age. (a) Percentage of extrinsic staining increased from 0% at ~10 to reach maximum of 85% at ~80. (b) Mean modified Lobene index score increased from 0 at age ~10 to reach 2 at around ~70 years old, remaining stable after. (c) Mean modified Lobene index staining extent scores vs age for buccal (black) and lingual (gray) surfaces. Lingual surface showed higher extent compared to buccal one.

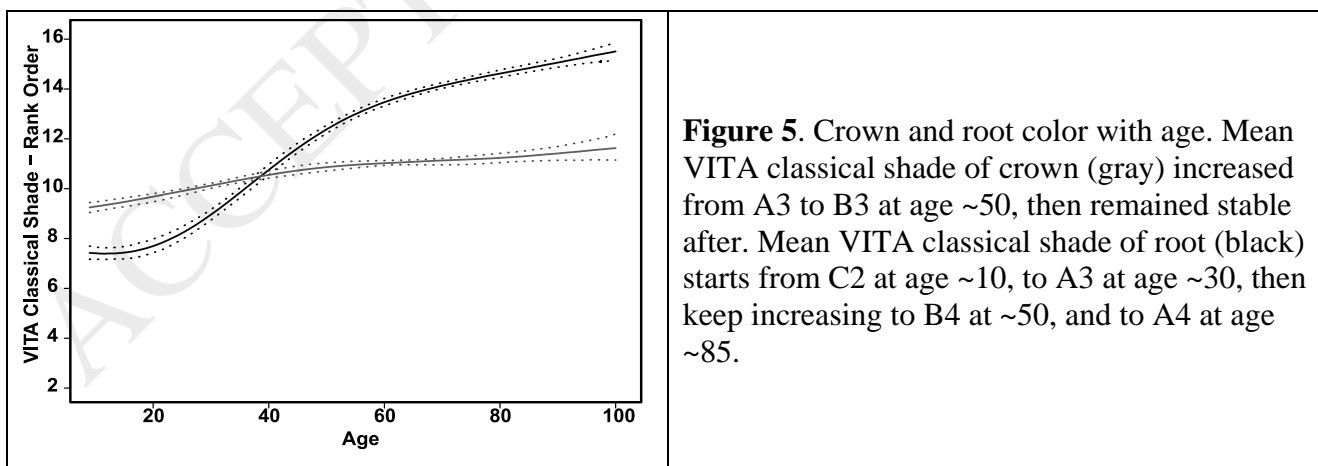


Figure 5. Crown and root color with age. Mean VITA classical shade of crown (gray) increased from A3 to B3 at age ~50, then remained stable after. Mean VITA classical shade of root (black) starts from C2 at age ~10, to A3 at age ~30, then keep increasing to B4 at ~50, and to A4 at age ~85.