Enamel Mineral Content in Patients with Severe Tooth Wear

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Purpose: The amounts of calcium (Ca), magnesium (Mg), zinc (Zn), and copper (Cu) in enamel may be crucial for maintaining its integrity and to attenuate potential environmental effects on teeth. The aim of this study was to examine whether the mineral composition of enamel could influence tooth wear. ***Materials and Methods:*** A total of 50 patients with severe tooth wear were compared with 20 healthy volunteers. Tooth wear was assessed using clinical examination according to the protocol of Smith and Knight. Subsequently, the maxillary central incisors of each subject were subjected to acid biopsies to assess the mineral composition in the enamel. Atomic absorption spectroscopy with an air/acetylene flame was used to analyze for Ca, Zn, and Mg. Graphite furnace atomic absorption spectroscopy was used to analyze for Cu. ***Results:*** The concentrations of Ca and Mg in tooth enamel were comparable in the study and control groups. Zn enamel content was higher in patients with tooth wear, and Cu enamel content was lower in these patients compared with the control group. ***Conclusion:*** The differing Zn and Cu contents in tooth enamel might offer a reason for excessive tooth wear in these patients. However, the results require further, more detailed study. *Int J Prosthodont 2013;26:423–428. doi: 10.11607/ijp.3209*

Tooth enamel is the hardest tissue in the human body. Its unique structure consists of highly organized and tightly packed crystals that comprise 87% of the enamel volume and 95% of its weight.[1,2] In its mature form, enamel consists solely of intercellular substances. The enamel structure is formed during the long-term process of odontogenesis. Sufficient availability of organic nutrients and building minerals, eg, calcium (Ca) and magnesium (Mg), is needed for the proper functioning, structure, and resistance of these hard tissues. Ameloblasts secrete amelogenin, enamelin, and enamelisine proteins, which play a vital role in controlling the enamel calcification processes.[1] In the final stage of maturation, enamel is composed of 90% minerals and 3% residual proteins and lipids. The proteins in enamel are primarily present as a very thin covering on individual crystals and comprise approximately half of the organic matter. The water content in enamel is sufficient to enable diffusion of acids and other components through interprismatic enamel pores into the tooth and of minerals (calcium and phosphate) out of the tooth during the demineralization and remineralization processes.[3,4]

Correctly formed enamel is the most resistant to wear, and alterations in the enamel composition may considerably influence its resistance to conditions in the oral cavity.[1] The presence of different trace elements in the tooth enamel can influence the enamel composition before and after tooth eruption. The hypothesis is that the main enamel components might influence the susceptibility of the enamel to erosive conditions in the oral cavity environment. Ca and Mg are well-known building minerals (hydroxyapatite and magnesium phosphate) that are essential for structure and resistance, but trace elements, ie, zinc (Zn) and copper (Cu), are very important for integrity and elasticity.[5] The effect of Zn and Cu on the process of tooth demineralization and remineralization is quite well known.[6,7] Results presented by Brookes et al[8] show that Cu appears to have a direct protective effect on the dissolution of enamel in an acidic environment that is fundamental in driving both caries and erosion. Zn has been reported to reduce enamel solubility.[7,9] It has also been suggested that Zn was incorporated into enamel during remineralization in situ, but the increase in Zn concentration was modest.[10]

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Tooth wear is a normal, age-dependent, physiologic process that leads to the loss of enamel and dentin. According to the Smith and Knight definition, tooth wear can be regarded as pathologic when the teeth become so worn that they do not function effectively and/or their appearance is significantly damaged before they are lost.[11] The distinction between acceptable and pathologic tooth wear at a given age is based on the prediction of whether the tooth will survive the current rate of wear.[11–14]

The reasons why enamel is prone to excessive wear in some individuals remain under discussion. Only limited information is available about the impact of trace elements on the process of wear. Thus, the aim of this study was to evaluate the association between mineral composition of enamel and tooth wear severity.

Materials and Methods

The outcomes for 50 patients with advanced tooth wear were included in the research. Sixteen women and 34 men with a mean age of 49.5 ± 9 years were included in the study. The patients were referred to the Department of Prosthetic Dentistry at the Medical University of Białystok, Poland, due to clinically apparent decrease of occlusal vertical dimension and a consequent reduced self-esteem of facial esthetics. On average, these subjects possessed 22 teeth contacting in 9 occluding pairs (5 to 6 occluding pairs of anterior teeth with a mean of 6 occluding pairs and 1 to 8 occluding pairs of posterior teeth with a mean 3 occluding pairs; on average, 4 teeth were out of occlusion). The control group consisted of 20 healthy subjects aged 48.5 ± 6 years, including 8 women and 12 men, without signs of pathologic tooth wear. They were asked to volunteer for the study as they presented for prosthetic procedures relating only to a single tooth (eg, crown or inlay). On average, these subjects possessed 27 teeth contacting in 13 occluding pairs.

The study participants presented with a considerable decrease in the occlusal vertical dimension (more than 4 mm). They possessed more than 50% of their own teeth that were free of clinical signs of dental caries or periodontal disease. They underwent no prosthetic rehabilitation prior to study recruitment. They were healthy adults who were not taking any medication and had not undergone any preventive professional application of fluoride in a dental clinic. They were also available during the study period.

Study Design

The data were collected in the Department of Prosthodontics at the Medical University of Białystok, Poland, and conformed to the criteria of The Helsinki Declaration, ICH Guideline for Good Clinical Practice. All participants were clinically examined to evaluate tooth wear. Tooth wear was assessed according to the protocol of Smith and Knight.[11] Their tooth wear index (TWI) was selected because it allowed the authors to visually evaluate the level of wear. The maxillary central incisors of each subject underwent acid biopsies to assess the mineral composition of the enamel.[15]

Ethical Approval

This protocol was approved by the Local Ethical Committee of the Medical University of Bialystok, Poland, with an approval number of R-I-003/6/2006. Informed consent was obtained from each participant at the beginning of the study prior to confirmation of their eligibility. The participants were able to withdraw from the study at any time and for any reason without prejudice.

Clinical Procedure

The bioptates were taken between 10 and 11 am, ie, approximately 3 hours after using tooth paste. All study participants maintained their customary habits regarding oral hygiene. The enamel of the labial surface of the maxillary central incisors was cleaned with pumice and then rinsed and dried. Three analytic grade filter paper disks were placed in the middle part of the prepared surface. Next, 1 µl of 0.1 mol/L perchloric acid solution ($HClO_4$) was pipetted directly onto the middle of each of these disks. The acid was transferred using a micropipette (Eppendorf Varipipette 4710, Eppendorf-Nethler-Hinz) and allowed to work on the enamel for 60 seconds. Immediately after removing the filter paper disks, the biopsy area was rinsed with distilled water and dried. Fluormex gel containing 1.25% amino-fluorides (Chema) was applied to promote remineralization. The bioptates were transferred to 1.5-mL sterilized, capped tubes (Safe-Lock, Eppendorf), and 1.5 mL of concentrated nitric acid and 0.5 mL of distilled water were added to the samples. Bioptates were mineralized using microwave mineralization (Uni Clever II, Plazmatronika). This method was used to completely degrade any organic matter and convert it into inorganic substances.

One well-qualified individual performed all biopsies. No clinical procedures affecting the enamel surface (ie, preventive or conservative therapy, scaling and/or polishing, impression-taking procedures) were performed on the day of the examination before collection of the bioptates.

Laboratory Procedure

The amounts of Ca, Mg, and Zn in the enamel bioptates were established using atomic absorption (AA) spectroscopy with an air/acetylene flame (Hitachi Model Z-500, Spectro). The concentration of each element was calculated using a calibration curve, and the curve for each element was constructed using the instrument. The concentration of Cu was established using an electrothermic method with argon gas on the AA spectrometer, as calculated from the appropriate calibration curve.

Reproducibility and Reliability

Ca, Mg, Zn, and Cu concentration values are reported as the mean value from three tests. Twenty measurements were retested by the study investigator, who was familiar with the employed methods. The reproducibility agreement was found to be 90%.

Another investigator, who was also familiar with the methods used, repeated the enamel bioptate analyses for 10% of the samples. Ninety percent agreement was found.

The cut-off points for the methods used were: 0.31 mg/L for Ca, 0.017 mg/L for Mg, 0.42 μg/L for Cu, and 0.011 mg/L for Zn. The cut-off point is the lowest concentration of the parameter analyzed that could be measured by the apparatus used. If the concentration is lower than the cut-off point, the apparatus registered it as 0.00.

Statistical Analysis

A statistical analysis of all of the studied attributes was carried out. In the case of quantitative attributes, mean and dispersion measures were used, ie, arithmetic mean and standard deviation. The levels of studied attributes between the groups were compared using the *t* test. The strength of relationships between pairs of measurable parameters was determined using Pearson correlation coefficient, and its significance was assessed using the *t* test for the correlation coefficient. The influence of potential factors on a measurable dependent variable, eg, tooth wear indices, was assessed using analysis of variance (ANOVA). Differences and relationships were considered to be statistically significant at $P < .05$.

Results

In total, 1,017 teeth from the study group and 523 teeth from the control group were evaluated for tooth wear based on four surfaces of each tooth. The TWI values were determined for buccal, lingual, and cervical surfaces, and these values were observed to be ≤ 1 in both the control and study groups; however, the TWI values for occlusal and incisal surfaces were considerably different. Table 1 presents the TWI values for occlusal and incisal surfaces in both the study and control groups.

Table 1 Comparison* of Tooth Wear Index (TWI) for Occlusal and Incisal Surfaces

	Group		
TWI	Study (n = 50) Mean ± SD	Control (n = 20) Mean ± SD	*P*
Anterior teeth in the maxilla	2.86 ± 0.56	0.74 ± 0.36	< .001
Posterior teeth in the maxilla	1.95 ± 0.82	0.74 ± 0.39	< .001
Anterior teeth in the mandible	2.22 ± 0.70	0.89 ± 0.50	< .001
Posterior teeth in the mandible	1.95 ± 0.63	0.90 ± 0.46	< .001
Teeth in the maxilla	2.44 ± 0.71	0.74 ± 0.34	< .001
Teeth in the mandible	2.11 ± 0.56	0.94 ± 0.46	< .001
Anterior teeth	2.53 ± 0.63	0.82 ± 0.38	< .001
Posterior teeth	1.92 ± 0.60	0.82 ± 0.41	< .001
Total teeth	2.27 ± 0.52	0.83 ± 0.36	< .001

SD = standard deviation.
*Student *t* test.

Enamel bioptates were obtained from the labial surface of maxillary central incisors. No statistically significant differences were noted in the Ca and Mg contents based on different degrees of tooth wear. In the case of enamel Zn content, a significant difference between teeth with first-degree and fourth-degree wear was observed ($P < .05$) (Fig 1). Moreover, a significant difference in enamel Cu content between teeth with first-degree and second-degree wear was also found ($P < .05$) (Fig 2).

Spectroscopic evaluation indicated that the Zn and Cu contents in the enamel bioptates were significantly different between the study and control groups. Zn was found in higher concentrations in the study group bioptates than in the control group bioptates, whereas Cu was found in lower concentrations in the study group bioptates than in the control group bioptates. Table 2 summarizes the concentrations of the studied elements as determined in the bioptates of the study and control groups.

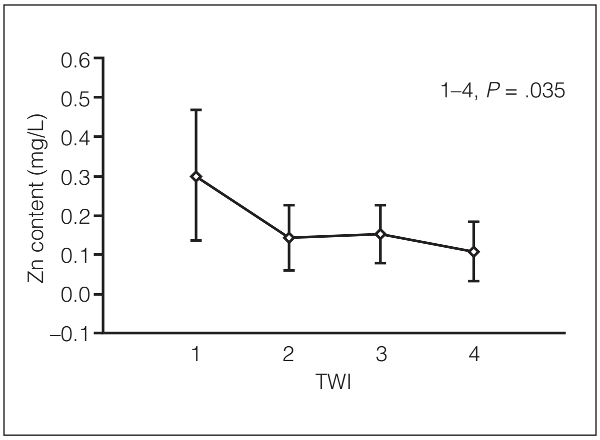

Fig 1 Correlation between degree of wear of incisal margins of maxillary central incisors and content of Zn in enamel bioptates.

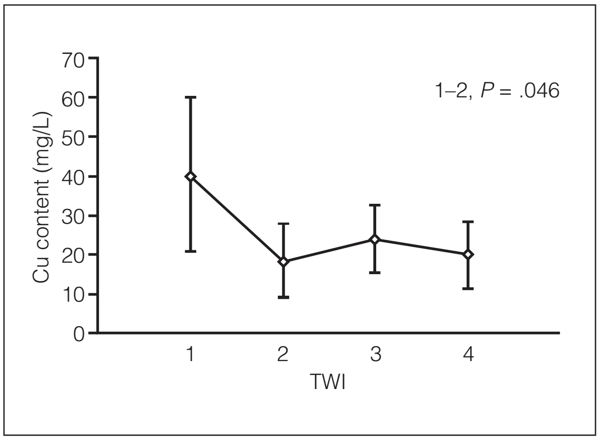

Fig 2 Correlation between degree of wear of incisal margins of maxillary central incisors and content of Cu in enamel bioptates.

Table 2 Comparison* of Ca, Mg, Zn, and Cu Content in Enamel Bioptates

	Group		
	Study (n = 50)	Control (n = 20)	
Mineral	Mean ± SD	Mean ± SD	*P*†
Ca (mg/L)	1.88 ± 1.38	1.85 ± 1.24	NS
Mg (mg/L)	0.30 ± 0.14	0.33 ± 0.15	NS
Zn (mg/L)	0.14 ± 0.04	0.08 ± 0.06	< .05
Cu (μg/L)	22.03 ± 17.45	36.67 ± 22.66	< .05

SD = standard deviation.
*Student *t* test.
†Statistical significance is < .05.

Discussion

Qualified patients were recruited for this study from those patients who decided to undergo treatment due to advanced loss of tooth hard tissue and low vertical dimensions. These patients had not been previously treated with prosthetics, nor had they taken any clinically applied preventive care measures; thus, it was possible to perform precise clinical examinations and other additional tests on these patients.[11]

It was difficult to establish one single etiologic factor of advanced tooth wear in the study group. There are three basic physical and chemical mechanisms involved in the etiology of tooth surface lesions. The mechanisms are attrition, erosion, and abrasion, which result in compression, flexure, and tension. These three basic mechanisms and their areas of overlap and interaction are the initiating and perpetuating etiologic factors in producing tooth surface lesions. The structure and composition of teeth as well as their environment are additional determinants of dental lesions.[16] Attempts at differential diagnosis, which gives primacy to a single mechanism, are likely to fail because they miss the interactive synergy of the various coactive mechanisms.

Acid biopsy is an invasive method but avoids the need for more invasive histologic preparations that would have required tooth extraction.[15] In addition, it is not possible to prepare typical histologic specimens of tooth enamel because the decalcification of enamel to prepare thin fragments makes it vulnerable to dissolving.

The results of this study show that there were no significant differences in the Ca and Mg contents in enamel bioptates between the study and control groups. This suggests that the enamel of patients with advanced tooth wear is properly mineralized. However, analyses of the Zn and Cu contents in the enamel bioptates revealed startling results. Moreover, an essential difference was noted between the Zn and Cu contents and the degree of tooth wear. The changes in Cu and Zn content between the various TWI scores are quite similar. A possible reason may be the different layers of enamel where the bioptates were taken. When considering the results of tooth bioptate analysis, it must be noted that the content of a certain element in these samples can indicate its real content in the enamel or reflect the susceptibility of the enamel to dissolving under acid treatment.[15]

The observed changes may have resulted from processes occurring at early stages of enamel development prior to tooth eruption. During the final stage of development, tooth enamel crystals are covered with a delicate net of matrix proteins (mainly enamelin), thus creating a scaffold that protects hydroxyapatite crystals and reduces their fragility.[1,17] During enamel development following secretion, phase matrix proteins are decomposed, reabsorbed, and removed in liquid form.[18] This liquid substance is replaced with minerals that grow in length and thickness.[1,2,19] Excess amounts of certain metal ions can limit the activity of some proteolytic enzymes.[20–23] Thus, metal ions, such as Zn and Cu, at too low or too high concentrations in the enamel matrix can interfere with the activity of enamel proteinases and negatively affect amelogenesis. The influence of improper content of polyvalent metal cations (ie, Zn and Cu) during enamel formation on its final resistance to wear is little known. But in general such a dependence could exist when examining the effect of trace elements hypoalimentation on enamel formation in mice.[1]

There is no available data regarding the metabolism of Zn and Cu in tooth enamel after its maturation. However, it is well known that the environment of the oral cavity can modify the surface composition of the tooth enamel. Saliva is considered to be an important biologic factor that can influence the process of demineralization and enhance remineralization by transporting calcium, phosphate, and fluoride to eroded enamel and dentin.[3,4,13,24] Brookes et al[8] suggested that the precipitation of a protective copper phosphate layer on the enamel surface could reduce or inhibit demineralization. It is possible that Cu has a direct inhibitory effect on demineralization via stabilization of the enamel[8] while also having a detrimental effect on remineralization.[24]

Zn can interact with hydroxyapatite by adsorption onto crystal surfaces and/or incorporation into the crystal lattice.[10] Low concentrations of Zn can both modify or inhibit remineralization, but can also markedly reduce dissolution.[25]

The presented data reveal that trace elements such as Zn and Cu could influence the resistance of tooth enamel to wear, but this mechanism is unclear. The role of pre-eruptive conditions and post-eruptive modifications of tooth enamel surface due to the environment in the oral cavity must be considered. Although it is very speculative, it is worthwhile to consider the possible role of residual proteins remaining in the mature enamel; in some conditions, this very thin covering of crystals may be too weak to keep them tightly packed during an individual's lifespan. Some of these crystals could be removed from the enamel surface under different, specific oral conditions, and can be clinically observed as less resistance to wear; however, this aspect of tooth wear requires further research.

Conclusion

The composition of enamel compromised by altered Zn and Cu contents seems to offer a possible reason for the presence of excessive tooth wear. However, the results obtained in this work necessitate further, more detailed research to examine the composition of enamel before and after tooth eruption.

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Literature Abstract

Mandibular reconstruction using computer-aided design and computer-aided manufacturing: An analysis of results

The aim of this retrospective study was to investigate the accuracy of using virtual surgical planning (VSP) alongside computer-aided design/computer-assisted manufacture (CAD-CAM) in the reconstruction of the mandible. The reconstruction involved either vascularized iliac crest bone grafts (ICBG) or free fibula osteomyocutaneous flaps (FFF). The ideal mandibular reconstruction, after trauma or pathology, would require an ideal orthognathic relationship, flushed bone to graft/flap contact, bony fixation, and sufficient vascularized soft tissue coverage. Eight consecutive patients who underwent mandibular reconstruction using VSP were operated on by one surgeon, with a second surgeon performing FFF harvesting and microanastomoses. Comparing a virtual plan of the preoperative CT scan with the surgical plan, linear measurements (two transverse and one anterior-posterior (A–P) dimension) were made. The data obtained from the ICBG VSP technique revealed an average of 1.7 mm, 1.6 mm, and 0.2 mm that was detected to be surgically different from the virtual plan in the two transverse and A–P dimensions. As for the FFF VSP technique, the surgical result obtained was, on average, 2.5 mm, 2.7 mm, and 0.9 mm different from the virtual plan in two transverse and A–P dimensions, respectively. Comparing the results of ICBG and FFF reconstruction, VSP for ICBG reconstruction was more dimensionally accurate. In conclusion, the authors believed that VSP allowed for more accurate surgical control in achieving the planned orthognathic relationship as opposed to traditional free hand methods of reconstruction. It could also be a useful, additional training procedure for residents as it not only aided in the planning and surgery performance, but also allowed for anticipation of complications.

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