Original Article

The quality of etched enamel in different regions and tooth types and its significance in bonding and the development of white spot lesions

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ABSTRACT

Objectives: To quantify differences in the etch quality of enamel within and between human teeth, which has not previously been attempted.

Materials and Methods: The buccal right and left halves of 27 extracted human teeth were randomly allocated to scanning electron microscopy (SEM) or micro–computed tomography (μCT) for evaluation. The buccal surfaces were pumiced, etched with 37% phosphoric acid gel etchant for 15 seconds, rinsed, and air dried. Each tooth was divided into three regions (incisal, middle, and cervical) and viewed after etching at 1200× magnification with SEM. The μCT scans were taken before and after etching to calculate apparent and material mineral densities.

Results: SEM showed greater aprismatic enamel and poorer etch quality (ie, significantly less percentage enamel) for the posterior than anterior teeth and for the cervical region than for the incisal and middle regions of all teeth. Although there were no density differences prior to etching, μCT demonstrated that etching increased material density significantly more for the anterior than posterior teeth. Prior to etching, the enamel in the cervical regions was significantly less dense than the enamel in the middle or incisal regions. Etching significantly increased the material density of all three regions, which decreased initial regional differences. After etching, the apparent density of the cervical region remained significantly lower than the densities of the other two regions.

Conclusions: Based on SEM and μCT, there is greater aprismatic enamel and inferior etch quality in the cervical regions of all tooth types and is clinically significant in explaining the failure of sealant retention and the propensity for white spot lesions. (*Angle Orthod.* 2021;91:576–582.)

KEY WORDS: Etching; Enamel; Human; Bonding; Aprismatic

INTRODUCTION

Enamel is a homogenous structure composed primarily of inorganic matter, organized into hydroxyapatite crystal prism bundles.[1] It has two distinct layers: an outer "prismless" enamel layer and an underlying prismatic layer.[2] The prismless layer has an optic axis nearly parallel to the enamel's surface.[2–5] In contrast, prismatic enamel has distinct rod boundaries oriented perpendicular to the surface. The aprismatic enamel is believed to be due to decreased ameloblast activity during tooth development and the disappearance of Tomes' processes during the end of amelogenesis.[6]

Etching is supposed to remove the outer aprismatic layer and expose the underlying prismatic rods.[7] Scanning electron microscopy (SEM) images of etched buccal premolar and molar enamel have shown that the cervical region is composed primarily of prismless enamel; the incisal and middle thirds of etched premolar enamel exhibit distinct prism-end, honeycomb-type structures.[8,9] These studies were limited to the posterior teeth (ie, premolars and molars) and are qualitative in nature. Etch patterns evaluated using SEM images of silicone impressions indicate that the etch quality of the anterior teeth is superior to the quality of the posterior teeth,[10,11] but these studies were

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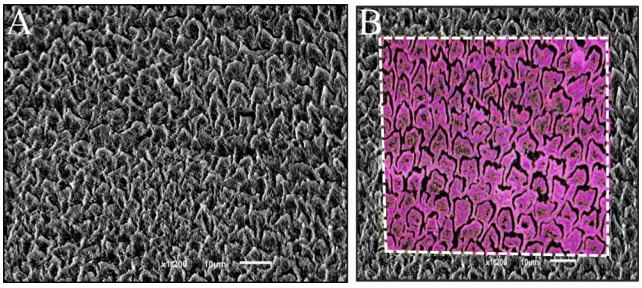

Figure 1. (A) Etch pattern from a representative sample. (B) BIOQUANT Osteo software selection of enamel present in the image for calculation of percentage of enamel remaining after etching (BV/TV).

also qualitative and evaluated only the central regions of the teeth.

The present study was designed to quantify how teeth respond to etching. No previous study has quantified differences in etch quality among the various regions of all teeth. Etching characteristics of one tooth or region cannot be extrapolated to the rest of the human dentition. How enamel responds to etching holds important implications for decalcification. Differences in etch quality could provide orthodontists with a better understanding of bracket bonding failures and sealant retention. Differences in enamel characteristics also make it possible to determine the regions and teeth most susceptible to white-spot lesion formation.

MATERIALS AND METHODS

Extracted human teeth were collected from various oral surgery offices, where they were all stored in a 0.1% thymol solution.[12] Once they were received, they were disinfected in a 10% sodium hypochlorite solution for 24–48 hours, sorted by tooth type, and again stored in a 0.1% thymol solution. Maxillary and mandibular incisors, canines, premolars, and molars were included. The teeth had to have intact buccal enamel surfaces free of restorations, caries, decalcification, fluorosis, and enamel defects. The study was approved by the Institutional Review Board and faculty advisors of Texas A&M University College of Dentistry.

Twenty-seven teeth (3 of each tooth type) that met the inclusion criteria were sectioned, and the roots were removed. The buccal right and left halves were randomly assigned, using random numbers generated with Microsoft Excel, to either SEM or micro-computed tomographic (μCT) analyses. There were 27 specimens for both the SEM and μCT analyses.

After the initial (T1) μCT scans were conducted, the surfaces of each specimen was cleaned using a slurry of nonfluoridated flour of pumice and water with a handheld rubber cup denticator, rinsed, and air dried. A 37% phosphoric acid gel etchant (Reliance Orthodontic Products, Itasca, Ill) was then applied to cover the entire buccal surface of each sample, left in place for 15 seconds, copiously rinsed with water, and then dried with an oil- and moisture-free syringe until a frosted appearance of the enamel surface was visually apparent.[13–15]

SEM Protocol

After etching, the samples were viewed with SEM, placed in a 100% ethanol solution for 1 hour, and then placed in a vacuum overnight.[16] They were mounted on aluminum stubs, sputter coated with gold for 2 minutes, and viewed with an SEM.[5,6,9,11] The incisal, middle, and cervical thirds of each sample were viewed with a JEOL (Tokyo, Japan) JSM-6010LA InTouchScope Analytical Scanning Electron Microscope at 1200× magnification.[17] Image contrasts were standardized using Preview by Apple photo editing software (Cupertino, Calif) and then analyzed with BIOQUANT Osteo (Nashville, Tenn) software (Figure 1). An area of enamel remaining after etching was selected to calculate the percentage of enamel present in the image, using the software's ratio of enamel volume to total volume, with lower percentages indicating more distinct prism-end, honeycomb-type structures. Each image was processed three times and the results were averaged. The intraclass correlation for average measures was 0.962 and method errors ranged from 2.5% to 2.8%.

μCT Protocol

Samples designated for viewing with μCT were prepared by placing a notch 1 mm from the edge with a diamond disc, which served as a reference for delimiting the region of interest selected for the final (T2) scans.[18] The samples were oriented vertically (incisal-apical) in a 12.3-mm diameter viewing tube filled with 70% ethanol and scanned with a μCT 35 Desktop Micro CT Scanner (Scanco Medical, Wangen-Brüttisellen, Switzerland) following the manufacturer's recommendation for dental tissue samples: energy/intensity of 70 kVP, 114 μA, 8 W; medium resolution; field of view/diameter of 12.3 mm; and voxel size of 6.0 μm. Before the final scan, the incisal, middle, and cervical regions of the samples were defined on the scout scan (Figure 2).

The scans were postprocessed by defining a region of interest that was approximately 50-μm deep in the incisal, middle, and cervical regions of each tooth using the previously described notch (Figure 3). The three-dimensional reconstructions of both the pre- and post-etch scans were approximately 600–700 μm on each side.[19] Mineral density was then computed using the μCT 35 version 6.1 software (Scanco Medical) with

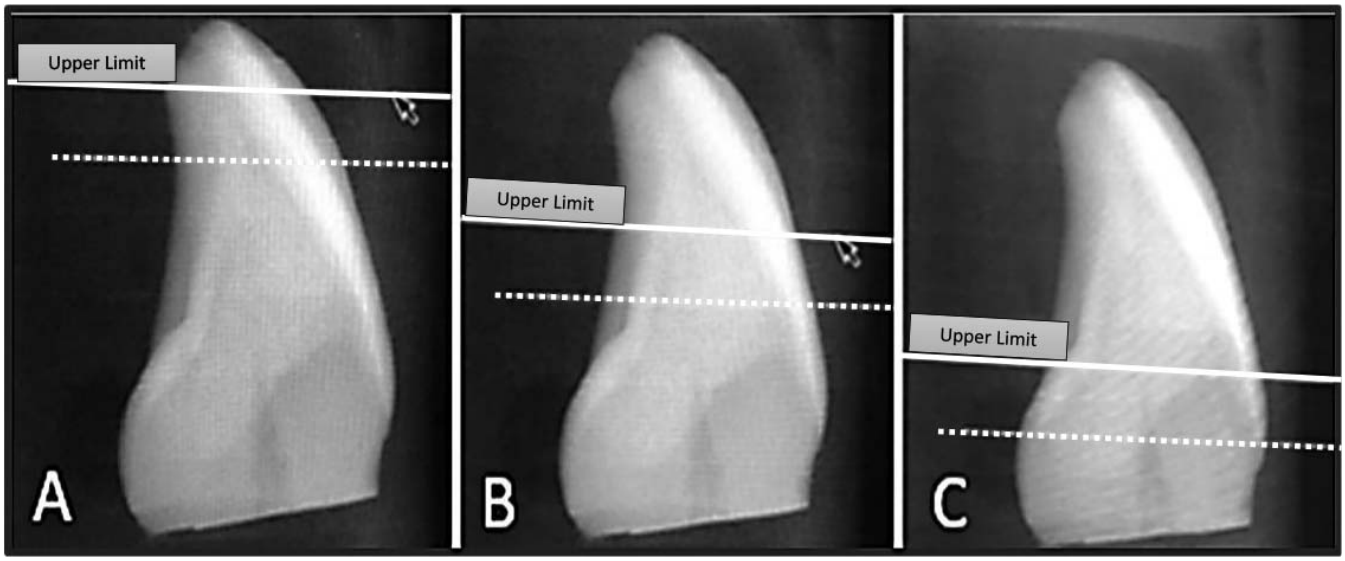

Figure 2. μCT scout scan used to identify the (A) incisal, (B) middle, and (C) cervical thirds of each tooth, with upper limit of each third identified.

threshold levels for enamel set between 580 and 1000.[20] Material and apparent densities were recorded for each sample.

Based on 15 randomly selected replicates of the μCT samples, there were no systematic differences for material or apparent density. The intraclass correlations were 0.77 and 0.94 for the apparent and material densities, respectively. The method errors for apparent and material densities were 59.0 and 20.2 mg/cm^3, respectively.

Statistics

To ensure standardization of the procedures, one blinded investigator performed the postprocessing of all images acquired for SEM and μCT. SPSS (IBM SPSS Statistics, Inc, Chicago, Ill) and a significance level of .05 were used for the statistical analysis. The skewness and kurtosis statistics showed normal distributions. For the SEM data, preliminary analyses showed that there were no between-jaw differences. Because there was no difference between the incisor and canine samples, or between the premolar and molar samples, they were combined. A paired *t*-test was used to evaluate differences between teeth and regions.

RESULTS

The SEM images showed consistent differences in the etch quality among the various regions and teeth (Figures 4 and 5). The posterior teeth showed poorer and lower etch quality (ie, greater aprismatic enamel) in all regions. The anterior teeth showed the best etch

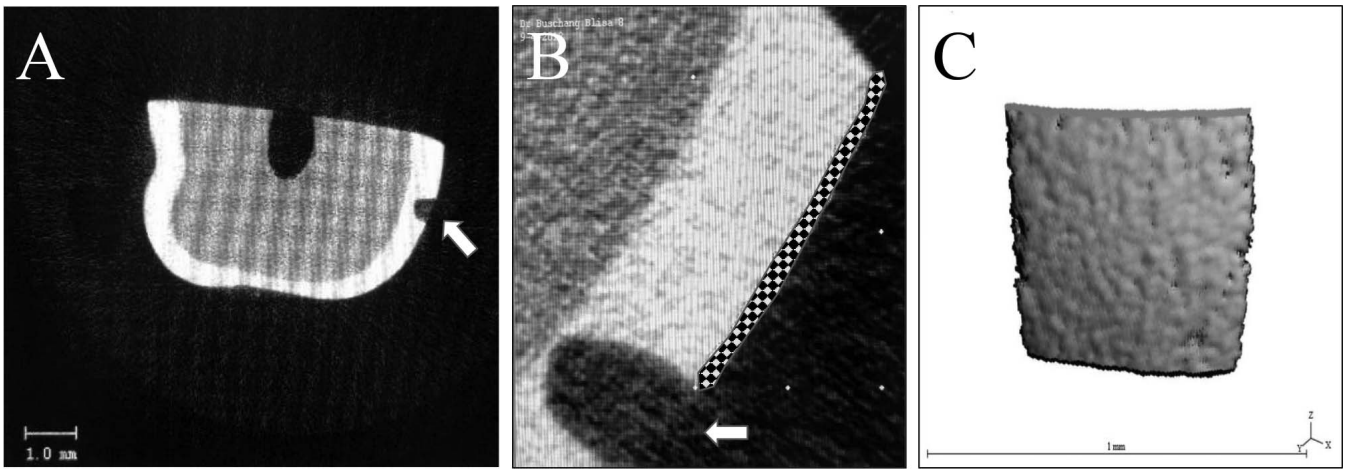

Figure 3. μCT (A) cross-sectional scan showing notch (white arrows), (B) region of interest selection (checkered), and (C) three-dimensional reconstruction of region of interest for density calculations.

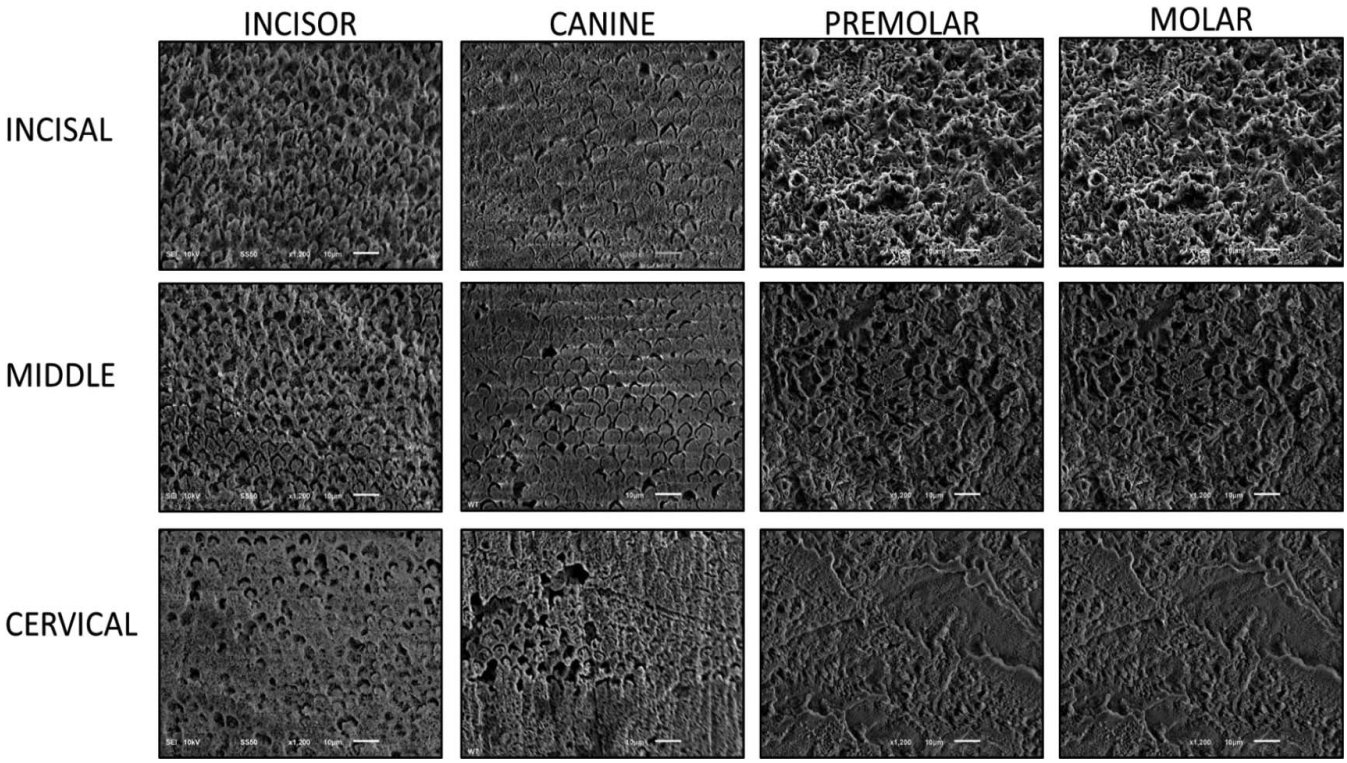

Figure 4. SEM images (1200× magnification) of etched maxillary teeth and regions.

patterns in the incisal and middle regions, with distinct prism-end and honeycomb-type structures. The etch quality in the cervical regions was inferior (ie, greater aprismatic enamel) across the tooth types.

The mean percentage enamel remaining after etching varied from 66.2% to 77.7%, depending on the tooth type and region sampled (Table 1), with a greater percentage of enamel representing a poorer

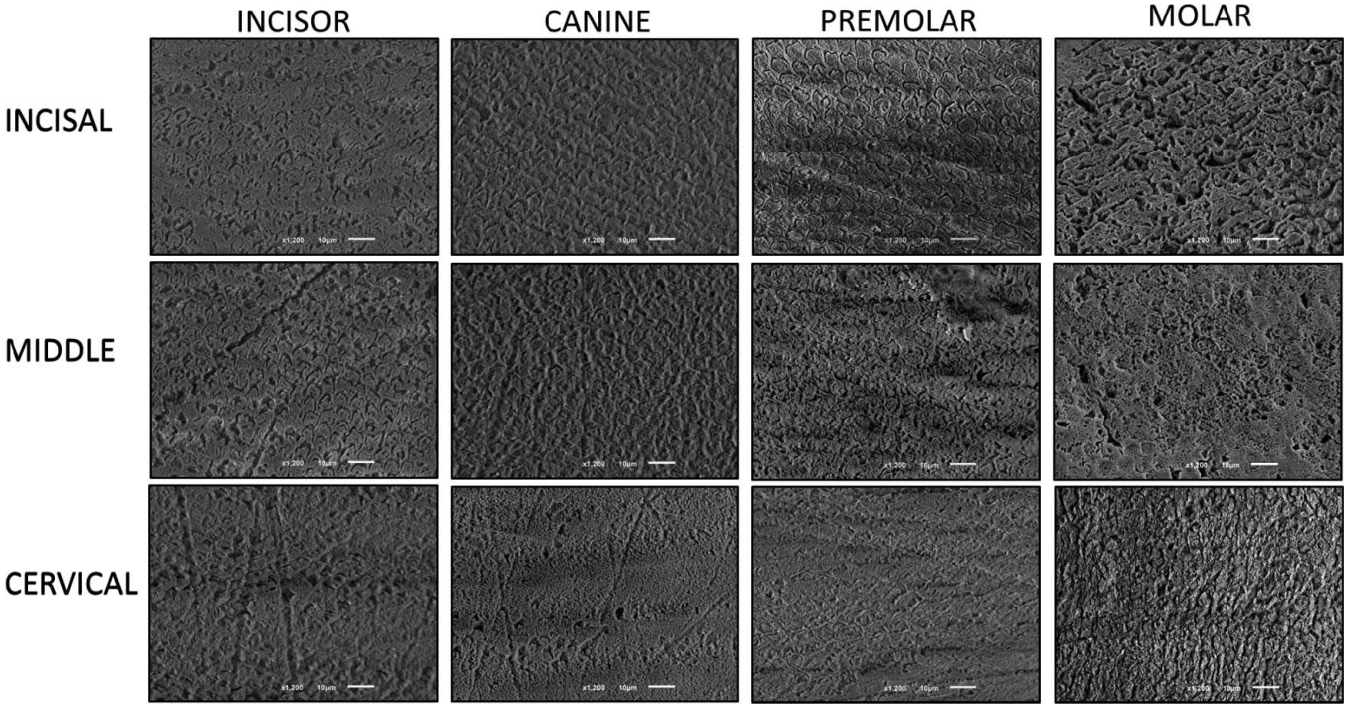

Figure 5. SEM images (1200× magnification) of etched mandibular teeth and regions.

Table 1. SEM/BIOQUANT–Derived Percentage of Enamel (BV/TV) Remaining by Tooth Type and Region[a]

	Anterior		Posterior		Group Difference	
	Mean	SE	Mean	SE	Mean	Probability
Cervical	69.5	1.16	77.7	1.49	−8.2	<.001
Middle	67.3	1.74	74.5	1.11	−7.1	.004
Incisal	66.2	1.62	73.7	1.77	−7.5	.005

[a] SE indicates standard error; BV, bone volume; TV, total volume.

etch quality (ie, fewer open enamel rods). The anterior teeth exhibited better etch quality than the posterior teeth, with statistically significant regional differences. The cervical region showed a higher percentage enamel remaining after etching as compared with the middle and incisal regions regardless of tooth type. Although there were no statistically significant differences between the middle and incisal regions, the percentage enamel remaining in the cervical regions was significantly greater than the amounts remaining in the middle and incisal regions (Table 2).

There were no significant differences in apparent or material densities between the anterior and posterior teeth prior to etching. Although there also were no differences in the changes of apparent density that occurred after etching, the anterior teeth showed greater increases in material density than the posterior teeth, with statistically significant differences in the cervical and middle regions (Table 3).

Prior to etching, the apparent and material densities of enamel in the cervical region of the teeth were significantly less than the density of enamel in the middle and incisal regions (Table 4). Although the apparent density did not change, material densities of the cervical, middle, and incisal regions increased significantly with etching (Figure 6). There were no statistically significant between-region differences in the changes in apparent or material density that occurred with etching. After etching, the apparent density of enamel in the cervical region was significantly greater than the densities in the middle and incisal regions (Table 5).

DISCUSSION

The qualitative and quantitative results showed no differences in etch quality between the maxillary and mandibular teeth. The only other studies that compared etch patterns among various tooth types came to a similar qualitative conclusion.[11,21]

In the present study, the SEM etch patterns were better for the anterior than posterior teeth. Previous studies investigating etch pattern quality have focused on specific teeth and extrapolated their results to the rest of the dentition.[8,9,22] One study that evaluated different tooth types focused on a small area at the center of each tooth[11]; another assessed etch at the center of teeth indirectly via impressions of etched tooth surfaces produced with an epoxy resin.[21] Both studies reported that posterior teeth exhibited inferior etch patterns, but the differences were not quantified. Aprismatic enamel remaining after etching may explain why the posterior teeth have an inferior etch quality. Whittaker et al[23] concluded that incisors have thinner layers of aprismatic surface enamel than posterior teeth prior to etching. If the etch does not remove the entire thickness of aprismatic enamel, it might be expected to produce an inferior etch quality.

Material density, as measured by μCT, showed that the anterior teeth should respond better to etching than the posterior teeth. Anterior teeth exhibited greater changes in material density than posterior teeth did. The lower-quality etch and inferior response of posterior teeth may help explain bracket bond failure

Table 2. Paired Regional Differences in SEM/BIOQUANT–Derived Percentages of Enamel Volume to Total Volume (EV/TV) Remaining After Etching[a]

Tooth Region	Mean Difference	SE	Probability
Cervical, middle	2.59	0.99	.015
Cervical, incisal	3.57	1.19	.006
Middle, incisal	0.98	0.98	.325

[a] SE indicates standard error; EV, enamel volume; TV, total volume.

Table 3. Changes (T1–T2) in Enamel Densities (mg/cm^3) of the Anterior (Incisors, Canines) and Posterior (Premolars, Molars) Teeth, Along With Statistical Comparisons of Group Differences[a]

	Anterior		Posterior		Group Difference	
Density	Mean	SE	Mean	SE	Mean	Probability
Apparent						
Cervical	−12.5	33.4	−28.3	37.4	−53.8	.361
Middle	−12.4	28.8	−14.4	37.5	−2.03	.966
Incisal	−43.1	36.5	−47.2	35.5	−90.2	.092
Material						
Cervical	85.5	14.3	10.9	23.4	−74.6	**.010**
Middle	65.0	19.0	20.5	6.9	−44.5	**.043**
Incisal	57.1	18.3	19.0	9.8	−38.2	.092

[a] Bold indicates prob < .05.

Table 4. Pre-etch Between-Region Differences in Apparent and Material Densities[a]

Tooth Region	Mean Difference	SE	Probability
Apparent			
Cervical, middle	51.0	16.1	**.004**
Cervical, incisal	74.5	21.6	**.002**
Middle, incisal	16.1	18.8	.400
Material			
Cervical, middle	32.7	9.7	**.003**
Cervical, incisal	29.1	12.7	**.031**
Middle, incisal	−3.6	10.4	.730

[a] SE indicates standard error; bold indicates prob < .05.

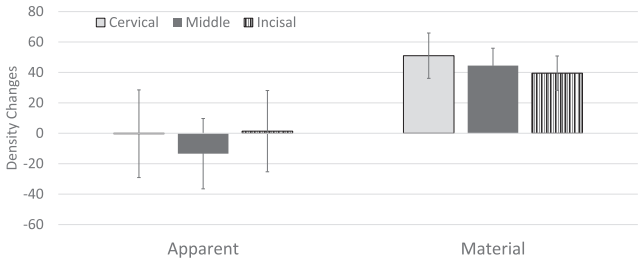

Figure 6. Changes (T1–T2) in apparent and material density with etching.

in orthodontics, which has been reported to be greater for the posterior than anterior teeth.[24–26]

The etch quality of cervical enamel is inferior to enamel in the middle and incisal regions. Previous studies have qualitatively demonstrated that the cervical region does not exhibit a prism-end pattern after etching,[8,9] but they examined only one tooth type and/or region. The present study confirmed this relationship across all tooth types and regions. The inferior etch quality in the cervical region can be explained by aprismatic enamel, which is orientated parallel rather than perpendicular to the enamel surface.[2,4,5] If the etchant cannot penetrate the full thickness of prismless enamel, a poor etch quality should be expected. This is why pretreatment mechanical abrasion of the tooth enamel, which removes the aprismatic enamel and exposes the underlying prismatic enamel, improves etch quality.[9] The presence of aprismatic enamel after etching and the lack of a distinct etch pattern explains why sealant retention is poor in the gingival region of teeth.[27] Aprismatic enamel could also explain orthodontic bracket debonds. Because of their shorter crown heights, a greater percentage of the brackets are bonded in the cervical regions of molars and premolars, where the etch patterns are inferior.

The cervical enamel was consistently less dense than the enamel in the middle and incisal regions. A previous study found significantly lower mineral content in the cervical than incisal or middle thirds of incisors that were not etched.[28] The lower density of enamel in the cervical region could be due to the presence and orientation of aprismatic enamel.[2] Histological and developmental studies showed that enamel mineralization begins at the incisal areas and extends apically toward the cervical region.[29] The fact that the cervical region is more aprismatic and less dense than the other regions suggests that the process of amelogenesis may be changing at the end of enamel formation. In addition, the cervical region is the thinnest. Enamel is thickest in the working areas of the teeth, such as the occlusal and incisal aspects, and tapers to a knife edge at the cemento-enamel junction.[30] The regional differences are clinically important because less dense enamel makes the cervical region more susceptible to demineralization and may contribute to white-spot lesions being most common in the gingival third of the teeth.[31,32]

Table 5. Post-etch Between-Region Differences in Apparent and Material Densities[a]

Tooth Region	Mean Difference	SE	Probability
Apparent			
Cervical, middle	64.6	24.8	**.015**
Cervical, incisal	79.3	28.9	**.011**
Middle, incisal	14.7	22.3	.516
Material			
Cervical, middle	26.1	15.8	.112
Cervical, incisal	17.5	16.8	.308
Middle, incisal	−8.6	9.8	.390

[a] SE indicates standard error; BV, bone volume; TV, total volume; bold indicates prob $<$ .05.

The SEM and μCT quantitative analyses performed in the present study provide novel approaches for assessing the quality of enamel etch. Although SEM has been previously used to qualify enamel, no other studies have attempted to quantify enamel etch patterns with SEM. The present study showed that software designed to study bone can be used to evaluate SEM images of enamel, the most mineralized material in the human body. The present study also showed that, using appropriate thresholds for hydroxyapatite, μCT analyses can also be used to quantify the mineral density of teeth and regions of teeth. Both approaches are valid because the quantitative results in the present study were consistent with the visual assessments, in both the present and past studies.[8,9,11,21]

Etching for 15 seconds with 37% phosphoric acid gel etchant does not always remove the outer aprismatic layer of enamel. He et al.,[20] who evaluated the hydroxyapatite density of enamel, reported the mean buccal enamel density to be 2228.1 $\pm$ 85.5 mg/cm^3. The enamel material densities obtained in the present study fall within this range, both prior to and after etching. Because the post-etch material density remained within accepted limits, it suggests that etching with 37% phosphoric acid etchant for 15 seconds does not remove a critical amount of mineral from the enamel surface, at least for some of the teeth and regions.

Clinically, the results imply that the cervical region of teeth may require a different etching protocol than the middle and incisal regions. The cervical regions, particularly of the posterior teeth, may require a longer etching time and/or a more concentrated etch to remove more of the aprismatic enamel. Studies should also be performed to determine the impact fluoride

application has an on enamel density, which could make the teeth less susceptible to white-spot lesions.

CONCLUSIONS

- There are no differences in etch quality between maxillary and mandibular teeth.
- Posterior teeth have an inferior etch quality and poorer response to etching than anterior teeth.
- Cervical enamel has an inferior etch quality and is less dense than the middle and incisal regions.
- There is greater aprismatic enamel and inferior etch quality in the cervical regions of all tooth types and is clinically significant in explaining the failure of sealant retention and the propensity for white spot lesions.
- BIOQUANT Osteo (Nashville, Tenn) software and μCT mineral density analyses are valid methods to quantify etch quality.

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