On the method of revealing enamel structure by acid etching. Aspects of optimization and interpretation

Steinar Risnes1 | Chunfang Li1,2

1Institute of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway
2School and Hospital of Stomatology, Wuhan University, Wuhan, China

Correspondence
Steinar Risnes, Institute of Oral Biology, Faculty of Dentistry, University of Oslo, PO Box 1052 Blindern, Oslo 0316, Norway.
Email: steinarr@odont.uio.no

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Abstract
The study aimed at finding an optimal combination of acid concentration and etching time when nitric acid is used as etchant for the study of the finer details of human dental enamel structure. Four hundred 2–3-mm-thick segments of facio-lingually sectioned human third molar crowns were assigned to 20 groups with 20 specimens in each group, each group differing with respect to acid concentration (0.1, 1, 2.5, and 5%) and etching time (15, 30, 45, 90, and 180 s). After etching and preparation, specimens were observed in the scanning electron microscope (SEM). Surface roughness/topography increased with increasing acid concentration and increasing etching time, but not in a linear fashion; generally, prisms tended to go from flat-surfaced to cone-shaped and prism sheaths from fissure-like to wedge-shaped. Intragroup variations and intergroup similarities were considerable. The two major enamel factors determining the etch effect are crystal orientation and prism sheath properties. Other factors, such as distribution of porosities and crystal quality, also contribute probably. Slight to moderate topography is best for observing the finer enamel structure, for example, etching with concentrations in the range 0.1–1% and with etching times in the range 15–90 s, the stronger the acid, the shorter the time. The depth effect of nitric acid is judged to be relatively small. Considerable variations in expression of prism cross-striations were observed. SEM observations of acid-etched enamel in carefully selected planes are a powerful method for the study of enamel structure, bearing in mind the artifactual aspects of the observed surface.

KEYWORDS
acid etching, dental enamel, interprism, nitric acid, prisms, scanning electron microscopy

1 | INTRODUCTION
Tightly packed hydroxyapatite crystals constitute about 87% of the volume of dental enamel (Brudevold & Söremark, 1967), making it the hardest tissue in the body, resistant to mechanical wear. The crystals are, however, chemically vulnerable, being soluble in acid. The acid dissolution of enamel hydroxyapatite crystals occurs preferentially in central defects along their long axis (c axis), evident both in carious and acid-treated enamel (Arends, 1973; Arends & Jongebloed, 1977; Daculsi & Kerebel, 1977; Frazier, 1968; Johansen, 1964, 1965; Johnson, 1966, 1967; Jongebloed, Molenaar, & Arends, 1975; Scott, Simmelink, & Nygaard, 1974; Simmelink, Nygaard, & Scott, 1974; Swancar, Scott, Simmelink, & Smith, 1971; Takuma, Tohda, Tanaka, & Kobayashi, 1987; Voegel & Frank, 1977). In accordance with this, human enamel shows solubility properties comparable to a slightly defective form of hydroxyapatite (Shellis, 1996; Shellis, Wahab, & Heywood, 1993). It has been...
suggested that crystal cores are rich in carbonate and that this may account for their preferential dissolution (Boyd, 1979).

Human enamel crystals are organized in two main phases, prisms and interprism (Boyd, 1965; Risnes, 1998, 1999), which differ only in crystal orientation. Prism crystals are generally oriented parallel to the prisms, while interprism crystals show a cervical deviation of about 45° relative to the prisms. In areas with arcade-shaped prism profiles in a pattern 3 configuration (Boyde, 1965), crystals deviate progressively in cervical direction from prism core to interprism (Poole & Brooks, 1961; Risnes, 1999). The differential orientation of the crystals in enamel of humans and other mammals seems to be related to the topography of the secretory surface of the ameloblasts, with a tendency for the crystals to orient themselves perpendicular to the cell secretory surface (Boyd, 1965; Risnes, 1999; Wakita & Kobayashi, 1983; Wakita, Tsuchiya, Gunji, & Kobayashi, 1981). However, the mechanism of crystal orientation may be different in rat enamel where crystals are oriented obliquely to the ameloblast secretory surface (Risnes, Septier, Deville de Periere, & Goldberg, 2002).

From the fact that crystals in prisms and interprism are differently oriented, it follows that any cut or ground enamel surface will have crystals of different orientation abutting on it. Since crystals are preferentially attacked by acid in central defects along their long axis, areas where crystals are oriented with their long axis perpendicular to the surface and crests where the crystals are oriented parallel or obliquely to the surface (Johnson, Poole, & Tyler, 1971; Poole & Johnson, 1967; Sharpe, 1967; Simmelen et al., 1974). The resulting topography reflects the crystal organization: depressions where crystals are oriented perpendicular to the surface and crests where the crystals are oriented parallel or obliquely to the surface. This is an important basis for observing the basic prism-interprism structure in acid-etched ground surfaces of enamel in the scanning electron microscope (SEM). Of importance is also the prism sheath, which represents the boundary between the differently oriented crystals in prisms and interprism and which is accentuated by acid (Crawford & Whittaker, 1977; Johnson, 1967). Thus, while enamel structure determines the effect of acid etching, it itself is revealed by the procedure.

Only few studies have addressed the problem of finding the most suitable etching procedures for studying dental enamel structure in the SEM, regarding type of acid, acid concentration, and etching time (Boyd, Jones, & Reynolds, 1978; Poole & Johnson, 1967). Our aim was to perform such a study with nitric acid, which is a well-known etchant for SEM studies of enamel (e.g., Fosse, Risnes, & Holmbakken, 1973; Li & Risnes, 2004; Risnes, 1979, 1990). There exists an extensive literature on acid etching of dental enamel that has a different main focus, namely to find the most suitable etching procedure for creating optimal surface roughness for mechanical bonding of resins to enamel (e.g., Gwinnett, 1988; Retief & Derys, 1989; Zhu, Tang, Matelinina, & Hägg, 2014). However, these two lines of research in acid etching of enamel, focus on enamel structure and focus on resin bonding, are of reciprocal relevance to each other.

2 | MATERIALS AND METHODS

The material and the methods used were described by Risnes and Li (2018a). In short, crowns of human third molars were cleaned, dehydrated, and embedded in Spurr’s resin. Focio-lingual segments, 2–3 mm thick, were cut with a rotating diamond wheel and randomly assigned to 80 groups with five specimens in each group, each group differing with respect to grinding/polishing procedure, concentration of acid, and etching time. Specimens were ground with water on silicone carbide paper (3 M Company) of grits 600, 1,000, and 1,200. One group was ground on grit 1,200 paper and then polished with 0.05-μm particle size Micropolish® alumina powder (Buehler) in water against the back side of the grinding paper. After cleaning/rinsing and drying, specimens were etched with four different concentrations of nitric acid: 0.1, 1, 2.5, and 5%, and etched for five different periods of time: 15, 30, 45, 90, and 180 s. The specimens were held by a pair of tweezers, dipped in a petri dish with 50 mL of the acid, and moved back and forth (about 1 cycle/s) for the whole etching period. The acid was changed for each 15 specimens etched. After rinsing and air-drying, the specimens were mounted on aluminum stubs with a cyanoacrylate glue, sputter-coated with 30-nm gold palladium, and observed in an SEM (Philips XL 30 ESEM) operated at 12–15 kV. Areas with transversely and areas with longitudinally sectioned prisms were identified and photographed at ×5,000 magnification.

3 | RESULTS

It should first be stated that the different grinding/polishing procedures were of no major concern for the evaluation of the effect of acid-etching on the basic prism-interprism structure; one could in most instances find suitable areas for observation between patches of a smear layer and between scratches. Thus, specimens obtained by different preparation procedures were pooled, yielding 20 groups with 20 specimens in each group, the groups differing only in acid concentration and etching time.

In midcoronal, facio-lingual longitudinal tooth sections, as in the present study, most of the prisms in the outer third of the enamel are cut longitudinally. Cross-cut or slightly obliquely cut prisms were sought in the region of the Hunter–Schreger bands.

The appearance of the basic prism-interprism structure after etching with nitric acid of different concentrations for different periods of time is shown in Figures 1 (0.1% acid), 2 (1% acid), 3 (2.5% acid), and 4 (5% acid). The identification of and distinction between prisms and interprism in areas where the prisms were sectioned transversely/obliquely (Figures 1–4a,b,d,e,g,h,i,j,k,m,n) was based on one or more of the three following criteria: (a) presence of prism sheaths separating prisms from interprism (e.g., Figures 1d, 2a, 3a, and 4a), (b) difference in crystal orientation between prisms and interprism (e.g., Figures 1h, 2b, 3e, and 4b), and (c) difference in topographic level of prisms and interprism (e.g., Figures 1j, 2j, 3j, and 4a).

After etching with the weakest acid (0.1%) for only 15 s, a darkish, amorphous surface layer partly masked the enamel structure.
(Figure 1b). However, it was possible to find areas where the prism-interprism structure could be observed (Figure 1a,c). By increasing the etching time to 30 and 45 s (Figure 1d–i), the surface generally became cleaner and distinction between prisms and interprism became clearer (Figure 1d,h). Surface roughness increased when the etching time was increased to 90 s (Figure 1j,k) and 180 s (Figure 1m, n). In general, surface roughness increased with increasing etching time for the same acid concentration, more evident for the weakest acid (e.g., Figure 1b,e,h,k,n) than for the three stronger acids (e.g., Figures 2–4b,e,h,k,n). Surface roughness also increased with
increasing acid concentration for the same etching time, more evident for the shortest etching time (Figure 1a,b) than for the longer etching times (e.g., Figure 1m,n). Variation in etch effect could be considerable within groups (compare for instance Figure 1a and b, d and e, g and h, j and k, and m and n) and similarities in etch effect could be considerable between groups (compare for instance Figures 1h and 3m, Figures 2e and 4e, and Figures 2j and 4j). The clearest distinction between prims and interprism was achieved when surface roughness was moderate with flat-surfaced prisms delimited by distinct prism sheaths and surrounded by an interprism whose crystals exhibited an
orientation clearly different from prism crystals (e.g., Figures 1d, 2j, 3g, and 4b).

The increased surface roughness with increasing acid concentration and etching time was mainly characterized by three features: (a) prisms tended to go from flat-surfaced to cone-shaped, (b) prism sheaths were accentuated, and (c) the difference in topographic level between prisms and interprism increased. However, deviations from these general trends were observed: flat-surfaced prisms could be found after etching with stronger acids for longer periods of time (e.g., Figure 4j), prism sheaths could remain narrow (e.g., Figure 3m),

FIGURE 3  Transversely (Transv.) and longitudinally (Long.) sectioned prisms etched with 2.5% nitric acid for 15, 30, 45, 90, and 180 s. Bar is 5 μm. Arrows indicate prism cross-striations. P, prism; IP, interprism
and prisms and interprism could be at the same topographic level (e.g., Figures 3n and 4m).

Variation in etch effect was also observed within prisms. Prisms could exhibit both flat and cone-shaped domains (e.g., Figures 1k,n, 2e,h, and 3b), and these domains could be separated by intraprismatic clefts. There was a tendency for prism cores to be more attacked than prism peripheries (e.g., Figures 2b,k,n, 3a,e, and 4b). With the weakest acid, the prism crystals closest to the prism sheath seemed less attacked (e.g., Figure 1g,h,j). The membraneous structures that occasionally delimited prisms (Figures 1e,m, 2d, 3d,h, and 4d), appeared to

**FIGURE 4** Transversely (Transv.) and longitudinally (Long.) sectioned prisms etched with 5% nitric acid for 15, 30, 45, 90, and 180 s. Bar is 5 μm. Arrows indicate prism cross-striations. P, prism; IP, interprism.
constitute or belong to the interprism and possibly including the prism sheath.

Examples of transversely sectioned (perpendicularly oriented) prism crystals being more readily etched than the more obliquely oriented interprism crystals were numerous (e.g., Figures 1j, 2g, 3g, and 4j). Examples of transversely sectioned (perpendicularly oriented) interprism crystals being more readily etched than obliquely oriented prism crystals were also observed (e.g., Figures 2n and 4n). Instances were also encountered where crystals oriented more obliquely to the surface were equally or even preferentially etched compared with crystals oriented more perpendicularly (e.g., Figures 2k, 3k, and 4k). There was a tendency that the difference in topographic level between prisms and interprism decreased toward the open cervical part of the prisms (e.g., Figures 1d, 2a,n, 3a, and 4g), in accordance with the gradual

FIGURE 5  Schematic representation of theoretical considerations concerning etching of enamel related to orientation of prisms. To the right is shown a schematic representation of a block of enamel cut out from the tooth crown by a tangential tooth section (Ta) oriented transversely to the prisms (P), a longitudinal tooth section (L), and an obliquely transverse tooth section (oTr). The prisms in row 1 are parallel with both planes L and oTr. The prisms in row 2 change direction in plane oTr so that they are parallel with plane L only in the lower half of the block. Acid is applied to the tangentially sectioned plane (Ta). Here, the crystals of prisms in row 1 are oriented perpendicular to the etched surface, while the crystals of the interprism (IP) are oriented obliquely to the etched surface. These prisms will, therefore, be etched more than the interprism as indicated by the stippled lines in plane L. The prisms in row 2 are, together with their crystals, oriented obliquely to the etched surface, and more so than the interprism (IP) crystals. These prisms will, therefore, initially be etched less than the interprism as indicated by stippled lines at Levels L1–L3 in plane oTr. However, as the prisms change course their crystals will attain an orientation perpendicular to the etched surface and will subsequently be etched more readily than interprism crystals, which may lead to a reversal of the etch pattern at level L6. The table to the left indicates the etch depth for various concentrations of nitric acid and for various etching times (based on Fosse (1968) and own measurements) with a scale comparable to the schematic enamel block to the right. Cross-cut or obliquely cut prisms may be observed, either (a) in a direction toward the dentin or (b) in a direction away from the dentin. Which is the case may be determined by the orientation of the interprism crystals (arrows) relative to the direction in which the prisms arcades are oriented; at (a), the interprism crystals (arrows) approach the cut/etched surface with an orientation opposite to that of the prism arcades, indicating that prisms are viewed "approaching" from the dentin; at (b), the interprism crystals (arrows) approach the cut/etched surface with the same orientation as the prism arcades, indication that prisms are viewed "moving away" toward the enamel surface. SEM micrograph inserts are from Figure 2a at (a) and from Figure 2j at (b)
change in crystal orientation from prism core to cervical interprism as seen in longitudinally sectioned prisms (e.g., Figure 1l).

Transversely sectioned prisms observed in Hunter–Schreger bands are either approaching from the enamel-dentin junction (Figure 5a) or departing toward the enamel surface (Figure 5b). Which is the case can be deduced from the orientation of the interprism crystals relative to the orientation of the arcade-shaped prism profiles (Figure 5). In Figures 1-4, there are fewer examples of prisms approaching from the enamel-dentin junction (e.g., Figures 1d, 2a, and 3a) than examples of prisms departing toward the enamel surface (e.g., Figures 1n, 2j, 3e, and 4b).

The appearance of longitudinally sectioned prisms depends on the orientation of the plane of section relative to the arcade-shaped prism profiles in a predominantly pattern 3 configuration (Figure 6). Generally, surface topography was less pronounced than in areas with transversely/obliquely sectioned prisms. Prism sheaths were variably exposed, but tended to become more pronounced with increasing acid concentration (e.g., Figure 1c) and increasing etching time (e.g., Figure 1c,f,l,o). Prism cross-striations were variably expressed, from distinct (Figures 1o, 2c,o, 3i, and 4l,o), to vague or questionable (Figures 1f,l, 2f,l, 3o, and 4c,f), and indiscernible or absent (Figures 1c, i, 2i, 3c,f,i, and 4l).

4 | DISCUSSION

The sectioning/grinding/polishing procedures affected the observation of the enamel structure in two ways. First, they produced an amorphous smear layer of some microns thickness (Boyd, Switsur, & Stewart, 1962; Risnes & Li, 2018a) that had to be etched away for the enamel structure to be disclosed. In favorable locations, the enamel structure was well demonstrated after etching for 30–45 s with the weakest acid (0.1%), sometimes even after 15 s etching, which removes an enamel layer considerably thinner than 5 μm (Figure 5). This indicates that the smear layer may be of uneven thickness. Second, the sectioning/grinding procedures also produced scratches. An enamel layer of about 20–35 μm thickness has to be etched away to obtain a scratch-free enamel surface with the present grinding procedure (Risnes & Li, 2018a). For the purpose of revealing enamel structure, it would probably be better to use a grit of silicon carbide paper finer than 1,200, for example, 2,400 before polishing.

It seems that variations in etch pattern may be ascribed to two main factors: (a) variations related to the tooth and (b) variations related to the etching procedure. These will be considered separately below.

4.1 | Variations related to the tooth

These variations encompass variations in enamel structure and variations in enamel composition. Expression and orientation of prisms vary within the enamel cap. The superficial enamel is prismless to a variable extent (Gwinnett, 1967; Kodaka, Kuroiwa, & Higashi, 1991; Newman & Poole, 1974; Ripa, Gwinnett, & Buonocore, 1966; Risnes & Li, 2018b; Whittaker, 1982), which greatly affects the results of etching intact tooth surfaces (Carstensen, 1992; Hobson, Rugg-Gunn, & Booth, 2002; Meola & Papaccio, 1986). The general orientation of prisms in the longitudinal plane shows regional variations (Radlanski, Seidl, Steding, & Jäger, 1989, 1990). In addition, in the inner two thirds of the enamel cap, the prisms pursue a sinusoidal path toward the enamel surface in the transverse plane, creating the Hunter–Schreger bands (Boyd, 1989; Osborn, 1973; Risnes, 1998). Thus, any plane cut through the enamel will show regional variations in prism orientation and hence in orientation of prism and interprism crystals relative to the sectioned/ground plane. Since areas with crystals oriented perpendicular to the etched surface are attacked more readily than areas with crystals oriented obliquely or parallel with the surface (Johnson et al., 1971; Poole & Johnson, 1967; Sharpe, 1967; Simmelink et al., 1974), a topography related to crystal orientation will be created: in areas where the prism crystals are cross-cut, the prisms will be etched to a lower level than the interprism (e.g., Figure 2j), while in areas where the interprism crystals are cross-cut, the interprism will be etched to a lower level than the prisms (e.g., Figure 2n). Also, the difference between prism crystal level and interprism crystal level tended to decrease toward the open cervical part of the arcade-shaped prism (e.g., Figure 2a), in accordance with the gradient in crystal orientation from prism core to cervical interprism (Poole & Brooks, 1961; Risnes, 1999), as demonstrated in longitudinally cut prisms (e.g., Figure 1l). However, in some areas, it was more difficult to relate the etch pattern to crystal orientation (e.g., Figures 2e,k, 3e,k,n, and 4e,k). This may occur where prism and interprism crystals meet the ground plane at approximately identical angles. Another possible explanation is that prisms may change direction within the enamel that was etched away by the acid (Figure 5). Hence, the final etch result will not only depend on the crystal configuration at that level.
but also on the crystal configuration at preceding levels. This is in accordance with the finding that sequential etching of surface premo- lar enamel showed variable and changing etching patterns with pro- gressive etching of the same area (Kodaka, Mori, & Miyakawa, 1993).

The prism sheaths represent boundaries between prisms and inter- prism, that is, where crystals of different orientations meet (Orams, 1966; Risnes, 1999). Here minute amounts of organic material remain in micropores, probably created by imperfect packing of crystals. In the intact enamel, it is a zone of reduced hardness (Ge, Cui, Wang, & Feng, 2005) and seems to be a preferred route for penetration of fluids (Meira, de Mattos Brito, & de Sousa, 2015). The prism sheaths are accentuated by acidic solutions (Gustavsen & Silness, 1969; Shellis, 1996; Xu, Li, & Swain, 2009) and by carious (Johnson, 1967; Worawongvasu, 2015) and erosive processes (Meurman & Frank, 1991; Worawongvasu, 2015), and are expanded in hypoplastic enamel (Xie, Mahoney, Kilpatrick, Swain, & Hoffman, 2007). Enamel etched with stronger acids or for longer periods of time creates prism sheaths with a wedge-shaped profile, widest at the top, with slanting sides of prisms and interprism. In the present study considerable variations in prism sheath exposure were encountered for the same acid concentration and etching time (compare for instance Figures 1d and e, 2g and h, 3m and n, and 4j and k). Preferential etching of enamel through the prism sheath route may explain some of the instances where interprism with obliquely oriented crystals are etched to a lower level than prisms with more perpendicularly oriented crystals (e.g., Figures 2k, 3k, and 4k), the simple explanation being the less bulky mass of the interprism (Figure 7). It is not clear whether the preferential dissolution at prism sheaths by acid is due to the presence of microporosities or more soluble crystals at the prism peripheries (Shellis, 1996). On the other hand, large, seemingly more acid-resistant crystals bordering the prism sheaths have been observed in acid-etched and carious enamel (Johansen, 1965; Scott et al., 1974; Simmelink et al., 1974), evidence of which was also found in the present study after etching with 0.1% nitric acid (Figure 1g,h,k). It has been suggested that these crystals are not indigenous to normal enamel, but may be due to reprecipitation/recrystallization occurring during acid etching and in the carious process (Johnson, 1967). However, such crystals have also been found in normal enamel (Scott et al., 1974), and we found no evidence for reprecipitation artifacts in our material. The membraneous or sheet-like structures that occasionally were seen to delimit prisms in restricted areas (Figures 1e,m, 2d, 3d,h, and 4d), probably represent residues of organic material left behind in amelogenesis. It seems primarily to belong to the interprism domain, but prism sheaths and peripheral prism regions may also be involved (Simmelink et al., 1974).

Within the arcade-shaped prisms, the core tended to be attacked more than the peripheral part. The vulnerability of the prism cores, also observed by others (Johnson et al., 1971; Poole & Johnson, 1967), seemed to be associated with an increased porosity. The pro- gressive change in crystal orientation from prism core to cervical interprism (Figure 1) implies less ideal packing of crystals and allows for more pores between them (Hamilton, Judd, & Ansell, 1973; Shellis & Dibdin, 2000). When etching cross-cut prisms, the combined effect of both crystal orientation and presence of pores will be greatest close to the prism core. Preferential etching of prism cores was less evident when etching with the weakest acid (Figure 1).

In areas where prisms were running parallel to the etched surface, the change in surface topography with increasing acid strength and etching time was not as evident as in areas where prisms were sectioned transversely. Although a great number of different orientations of the section relative to the arcade-shaped prisms are possible (Figure 6), a facio-lingual longitudinal section will tend to cut the prisms parallel with the central axis of their cross-cut arcade-shaped profiles. In such cases, both prism crystals and interprism crystals will be oriented parallel with the etched surface, resulting in less differential etching of crystals and therefore less surface topography (e.g., Figures 1l, 2f, 3o, and 4f). However, accentuated prism sheaths could allow penetration of resin (Crawford & Whitaker, 1977). Shimada and Tagami (2003) found that resin bonding to phosphoric

**FIGURE 7** Schematic representation of suggested influence of prism sheaths on etching of enamel. To the left, three prisms, separated by interprism, are oriented perpendicular to the surface where acid is applied. A possible result of the etching after about 30–45 s with 1% acid is shown to the right. Although prism domains are etched away more readily than interprism domains due to crystal orientation, the acid that invades the prism sheaths may create wedge-shaped clefts, eliminating interprism more effectively than prisms due to the lesser bulkiness of the former, resulting in pointed prisms projecting above the interprism. Inset: SEM from Figure 2e.
acid-etched longitudinally sectioned enamel was weaker than in areas of cross-cut prisms, probably because prisms were undermined through the prism sheath route and more prone to be detached from the surface. However, Jung, Wehlen, and Klimek (1999) found no difference in bonding strength between phosphoric acid-etched tangentially sectioned and longitudinally sectioned enamel.

The variable expression of prism cross-stria tions, relatively unrelated to etching procedures, is striking and corroborates earlier findings (Li & Risnes, 2004). Studies into the relationship between enamel/crown formation time and enamel geometry has strongly suggested that prism cross-stria tions represent a circadian rhythm in enamel formation (Antoine, Hillson, & Dean, 2009; Bromage, 1991; Risnes, 1986). Evidence for the presence of such a rhythm in enamel formation has been supported by the demonstration of circadian rhythms in the expression of clock genes and enamel protein genes in ameloblasts (Lacruz et al., 2012; Nirvani et al., 2017; Zheng et al., 2013). However, a basic circadian rhythm that affects ameloblast function to such an extent that it leaves incremental markings in the mature enamel, would be expected to exert its influence on all ameloblasts at all times, not only some ameloblasts sometimes. Considering the potential usefulness of prism cross-stria tions in studies of enamel formation chronology, their variable expression in histologi cal acid-etched SEM specimens, and their different expression/visibility in light microscopy compared to SEM (Warshawsky, Bai, & Nanci, 1984) indicates a need for more research into their nature and occurrence. It has been suggested that cross-stria tions may be related to variations in crystal concentration (Desoutter et al., 2019; Li & Risnes, 2004). Also the other incremental markings in enamel, the Retzius lines, show an enhanced visibility in light microscopy compared with SEM, possibly due to a summation effect with the former technique in a specimen of some thickness (Risnes, 1985). This explanation may not apply to prism cross-stria tions with an extension limited to individual prisms. However, in a thin longitudinal facio-lingual section, neighboring prisms may have cross-stria tions in synchrony, enhancing their visibility. This would be in line with an assumption that neighboring ameloblasts in the transverse plane are more in synchrony with respect to enamel elaboration than neighboring ameloblasts in the longitudinal plane, cf. the extension rate of Shellis (1984).

4.2 Variations related to the etching procedure

Variations related to the etching procedure concern type of acid, acid concentration, etching time, and method of acid application. A great number of different acids have been used for etching dental enamel. These encompass strong inorganic acids, such as hydrochloric acid (Boyd e et al., 1978; Johnson et al., 1971; Simmelink et al., 1974; Whittaker, 1982) and nitric acid (Blosser, 1990; Fosse et al., 1973; Gardner & Hobson, 2001; Risnes, 1990). Also several carboxylic acids (weak acids containing the group –COOH), such as acetic, citric, formic, lactic, maleic, oxalic, and pyruvic acids, have been used (Galil & Wright, 1979; Johnson et al., 1971; Nichol, Judd, & Ansell, 1973; Poole & Johnson, 1967; Silverstone, Saxton, Dogon, & Fejerskiov, 1975; Tyler, 1976). Phosphoric acid is the acid most commonly employed as an enamel etchant, mainly due to its preferred use in clinical procedures. It is weaker than hydrochloric and nitric acid, but stronger than the carboxylic acids. It is mostly used in the concentration range 30–50%, which gives adequate etching combined with an acceptable low tendency for precipitation of calcium phospahte (Galil & Wright, 1979; Gwinnett, 1971; Silverstone, 1974; Soetopo, Beech, & Hardwick, 1978; Wang, Yeh, Fang, Sun, & Arvystas, 1994). However, etching sectioned and ground/polished enamel specimens with low concentrations (0.5–2%) of phosphoric acid for short periods of time (5–10 s) have given excellent results for SEM observations of enamel structure (Lester, Boyd, Gilkeson, & Archer, 1987; Lester & Hand, 1987; Lester & von Koenigswald, 1989).

In the laboratory situation, it has been suggested that prolonged etching under static dissolution conditions favors a precipitation of mineral on the etched surface, especially in the prism sheath region (Boyd e, 1989; Boyd e et al., 1978; Tyler, 1976) where the local concentration of calcium is most prone to increase due to calcium transport from deeper parts and due to the relatively isolated and protected environment of the sheath space. Stirring seems to be imperative for obtaining etched surfaces without reprecipitation. Constant stirring during etching promotes transportation of etched material away from the surface, preventing local supersaturation of calcium and phosphate. Enamel dissolution has been found to increase with increasing stirring rate (Linge & Nancollas, 1973). A great number of scanning electron micrographs of enamel acid-etched under static conditions, including clinical applications methods, resemble those obtained by Simmelink et al. (1974) and Tyler (1976), indicating that a reprecipitation had occurred. Even in slowly stirred systems, such an etch pattern may result (Boyd e et al., 1978). We could not detect any tendency toward reprecipitation in our enamel samples etched with nitric acid under constant stirring.

Considering surface topography/roughness, intragroup (same acid concentration and etching time) variations and intergroup similarities were considerable. The weakest nitric acid concentration (0.1%) produced a gradient in surface topography within the timeframe used (15–180 s). Progressive etching with the stronger acids (1, 2.5, and 5%) increased surface topography, but interpretation of the enamel structure was not enhanced/facilitated. However, the structure remained relatively intact, considering the fact that up to about 130–140 μm were etched away (Figure 5). An increase in surface roughness with increasing acid concentration and/or etching time has been observed (Boyd e et al., 1978; Retief, 1975; Watari, 1999), but the valley to peak height amounted to only about 2–4 μm within an etching time frame of 3 min. Blosser (1990) and Gardner and Hobson (2001), using 2.5% nitric acid, found a gradient in surface roughness with increasing etching time up to 60 s, which is not contradictory to our findings. However, prolonged etching under static conditions with hydrochloric acid for up to 5 hr (Johnson et al., 1971; Poole & Johnson, 1967; Simmelink et al., 1974), with phosphoric acid for up to 10 min (Silverstone et al., 1975) and with lactic acid for up to 5 days (Silverstone et al., 1975) resulted in an exaggerated surface topography not very suitable for studying the finer details of the basic enamel structure.

There are varying reports on the depth of action of acid etchants. One method of evaluating this depth effect is to measure the length of resin tags that penetrate into the enamel beyond the etched surface. Tags up to 100–170 μm have been observed (Diedrich, 1981;
Silverstone, 1974), but most are in the range of 5–50 μm (Crawford & Whittaker, 1977; Gwinnitt & Ripa, 1973; Hicks, 1984; Jörgensen & Shimokobe, 1975; Pahlavan, Dennison, & Charboneau, 1976; Retief, 1973; Soetopo et al., 1978). This is in accordance with the results of another method where detection of radioactive phosphorous indicated that phosphoric acid penetrates to a depth of about 50 μm (Smith, Spinelli, & Tartakow, 1976). Evaluation of the presence of porosities by polarized light microscopy, microradiography, and scanning electron microscopy has indicated a depth effect of from a few to about 15 μm (Boyde et al., 1978; Gwinnitt, 1971; Irimoda et al., 2000). The depth of action of acid beyond the etched surface varies with type of acid, acid concentration, and etching time; it seems to increase with etching time, but not necessarily with acid concentration (Boyde et al., 1978; Soetopo et al., 1978). Our impression from the present study is that nitric acid does not affect the subsurface enamel to a great extent within the time frame used (<3 min). Indirect evidence for this is the fact that surface topography showed relatively little change with increasing acid concentrations and increasing etching time (beyond 180 s etching with 0.1%). When etching for the same time with comparable concentrations of hydrochloric acid, increasing etching time showed relatively little change with increasing acid concentrations and etching time (beyond 180 s with 0.1%). When etching for the same time with comparable concentrations of hydrochloric acid, Etching showed relatively little change with increasing acid concentrations and etching time (beyond 180 s etching with 0.1%). Indirect evidence for this is the fact that surface topography showed relatively little change with increasing acid concentrations and increasing etching time (beyond 180 s etching with 0.1%). When etching for the same time with comparable concentrations of hydrochloric acid, Boyde et al. (1978) found subsurface porosities to a depth of only about 1–3 μm. Thus, it may seem that nitric acid dissolves surface crystals so effectively that it almost keeps track with the acids ability to penetrate into enamel porosities beyond the surface. In agreement with this, etching with nitric acid has been shown to give weaker bonding to enamel than phosphoric acid (Brown & Barkmeier, 1996; Gray, MacMillan, Payne, & McGadey, 1996). It should be mentioned, however, that bond strengths comparable to phosphoric acid have been obtained with a commercially available etchant containing a nitric acid–NPG (N-phenylglycine) combination (Berry, Barghi, Knight, & Conn, 1990; Reifeis, Cochran, & Moore, 1995; Saunders, Strang, & Ahmad, 1991).

5 | CONCLUSIONS

Enamel structure is revealed by acid etching. Prisms and interprisms are well demonstrated and defined by crystal orientation and prism sheaths. Variations in etch pattern were observed within groups and similarities in etch pattern were observed between groups. Variations related to the tooth and to the etching procedure are discussed. Surface topography increased with increasing acid concentration and increasing etching time, but not in a linear fashion. Slight to moderate topography is best for observing the enamel structure. With nitric acid, enamel should be etched with concentrations in the range 0.1–1% and with etching times in the range 15–90 s, the stronger the acid, the shorter the time. Prism cross-striations were variably expressed in all groups, stressing the need for more studies into their nature and occurrence. SEM observations of acid-etched enamel in carefully selected planes are a powerful method for the study of enamel structure, bearing in mind the artificial aspects of the observed surface.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Steinar Risnes https://orcid.org/0000-0002-9765-3862

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