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M.C. Sánchez-Quevedo

G. Ceballos

J.M. Garcíal

I.A. Rodriguez

M.E. Gómez de Ferraris

A. Campos

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M.C. Sánchez-Quevedo[1], G. Ceballos[2], J.M. García[1], I.A. Rodríguez[3], M.E. Gómez de Ferraris[3] and A. Campos[1]

[1]Departamento de Histología y Biología Celular, Facultad de Medicina y Odontología, Universidad de Granada, Granada, España,
[2]Servicio de Estomatología, Hospital Clínico Universitario, Universidad de Granada, Granada, España and
[3]Cátedra B de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, Argentina

Summary. Teeth fragments from members of a family clinically and genetically diagnosed as having amelogenesis imperfecta were studied by scanning electron microscopy and X-ray microprobe analysis to establish the morphological patterns and the quantitative concentration of calcium in the enamel of anterior (canine, incisor) and posterior (premolar and molar) teeth. The prism patterns in the enamel of teeth from both regions were parallel or irregularly decussate, with occasional filamentous prisms accompanied by small, irregularly rounded formations. Prismless enamel showed the R- and P-type patterns. Calcium levels in enamel of amelogenesis imperfecta and control teeth differed significantly between anterior and posterior teeth, indicating that the factors that influence normal mineralization in different regions of the dental arch are not altered in the process of amelogenesis imperfecta.

Key words: Amelogenesis imperfecta, Prism patterns, Calcification, Scanning microscopy, X-ray microprobe analysis, Anterior teeth, Posterior teeth

Introduction

Amelogenesis imperfecta (AI) is a term that identifies a group of hereditary alterations that affect the formation of enamel extracellular matrix (Dong et al., 2000). This disorder has been classified into 14 subgroups according to the characteristics of the enamel, clinical appearance and Mendelian mode of inheritance (autosomal dominant or recessive, and sex-linked dominant or recessive). The difficulties in establishing an acceptable correlation between the clinical classification and microscopic findings (Bäckmann et al., 1993) have brought to light the need to search for correlations between the mineral component of enamel in different teeth and microscopic observations of the prism and prismless structure of the enamel. To this end some studies have tried to measure the mineral content and distribution in enamel with quantitative microradiography and chemical analysis (Wright et al., 1993; Bäckmann and Angmar-Mansson, 1994; Wright et al., 1995) or with quantitative X-ray microanalytical histochemistry, a productive tool recently applied to mineralized tissues (Wright et al., 1991; López-Escámez et al., 1992, 1993; López-Escámez and Campos, 1994; Campos et al., 1994, 2000; Sánchez-Quevedo et al., 1998). The development of the tooth germ is influenced by the position of the tooth in the anterior or posterior part of the dental arch, where genetic and molecular factors along with interdental spacing, tooth inclination, arch growth and tooth size also come into play (Thomas et al., 1998; Avery and Stelle, 1999; Ferraris and Campos, 2000).

This study was designed to use quantitative x-ray microprobe analysis (EPMA) with scanning electron microscopy (SEM) as a histological approach to determine the morphological patterns of the enamel in different types of anterior (canine, incisor) and posterior (premolar, molar) teeth in the dental arch in teeth from persons who were clinically and genetically diagnosed as having AI. In addition, we measured the concentration of calcium, as the influence of the anterior or posterior location of the tooth on the morphological pattern and degree of enamel calcification have not been analyzed in patients with AI.

Materials and methods

The material consisted of 20 specimens from the coronal part of permanent teeth obtained in the course of preparation for crown placement from patients with autosomal dominant clinically and genetically diagnosed AI (5 incisors, 5 canines, 5 premolars and 5 molars). All

patients were members of the same family (Ceballos and Ceballos, 1988). As the control material we used coronal specimens from 20 normal teeth (5 incisors, 5 canines, 5 premolar and 5 molars) extracted during orthodontic or periodontal treatment.

Sample preparation for EPMA

The tooth fragments were plunge-frozen in liquid nitrogen-cooled Freon 22. The samples were transferred to Polaron E 5350 the freeze-drying apparatus and dried at -80 °C for 48 h. All specimens were mounted on a carbon stub and sputter-coated with a thin layer of carbon in an argon atmosphere at 0.1 Torr for 30 s.

Electron probe X-ray microanalysis

Specimens prepared as described above were studied in a Phillips XL-30 scanning electron microscope (SEM) (operating voltage = 15 kV; spot size = 500 nm; tilt angle = 35°; take-off angle = 61.34°). An energy dispersive spectrometer (EDAX DX-4) was used for quantitative analyses (count rate = 1200 cps; live time = 50 s). Spectra were collected by pin-point electron beam at x40 000, and a total 200 spectra (10 for each of the 20 AI and control specimens) were obtained. The peak-to-background (P/B) ratio method (Statham and Pawley, 1978; Small et al., 1979) was used to measure the concentration of calcium.

Microcrystalline salt standards were used to quantify calcium as described in previous publications (Campos et al., 1992, 2000; López-Escámez and Campos, 1994; Sánchez-Quevedo et al., 1998). The standards were cryofixed in liquid N_2, freeze-dried and sputter-coated as described above, and analyzed in the microscope immediately after preparation to avoid contamination or chemical modification. Micro-crystalline standards were

mounted on 200-mesh nickel grids fixed with adhesive graphite lamina to SEM holders. The elemental weight percent (WP) of each salt standard was calculated as reported in previous publications (Warley and Gupta, 1991; Warley, 1993, 1997).

Morphological study

Carbon-coated specimens were gold-coated after EPMA analysis, and were examined in a Phillip XL-30 SEM and photographed.

Statistical study

The data were analyzed with one-way ANOVA and paired comparisons with the Bonferroni test, using the BMDP statistical package.

Results

Scanning electron microscopic images of enamel in teeth with AI showed a number of different patterns in both the anterior and posterior parts of the dental arch. In specimens with AI the outer enamel showed a prismless structure of variable size, and an irregular array of fissures. The P-type (prism-dependent) and R-type pattern (Retzius-dependent) of prismless enamel were observed (Figs. 1, 2).

The underlying prismatic enamel was arranged in two patterns: parallel prisms were more common, and a decussate pattern was seen only in certain areas. Parallel prisms showed a regular diameter and a keyhole outline. Decussate prisms were varied in their orientation and diameter, and showed a variable arcade-like outline delimiting an irregular pit structure (Figs. 1, 3, 4). Regardless of their architectural pattern, some prisms showed a filamentous configuration (Fig. 5); these were
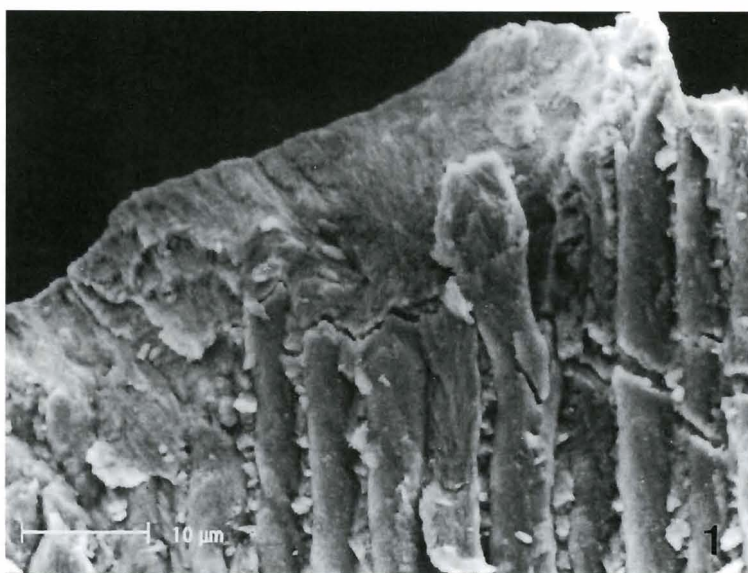

Fig. 1. P-type pattern in prismless enamel, and parallel pattern in prism enamel.

accompanied by irregularly rounded elements of variable size arranged, in some cases, in a beaded or ribbon-like pattern seen mainly in the region underlying the prismless enamel (Fig. 1).

Tables 1, 2 summarize the microanalytical data for calcium content in control and AI specimens in the anterior and posterior parts of the dental arch. Statistical analysis revealed significant differences (Levene's: F test: = 4.35, p = 0.0384; Welch approximation: df (1,193), F = 6.25, p = 0.0134; Bonferroni test: n = 100; p < 0.05) in calcium weight fraction between teeth with AI from the anterior (canines and incisors, $\bar{x}$ = 32.16) and posterior part (premolars and molars, $\bar{x}$ = 33.46) of the dental arch. The difference between the anterior and posterior regions was also significant in control teeth (Levene's: F test: = 29.07, p < 0.0001; Welch approximation: df (1,154), F = 56.05, p < 0.0001; Bonferroni test: n = 100; p < 0.001).

Table 1. Percent weight fraction and mean weight fraction of calcium in teeth from the anterior region of the dental arch.

	INCISORS	CANINES	$\bar{x}$±SD
Amelogenesis imperfecta	32.63±3.81	31.68±3.31	32.16±3.36
Control	29.93±1.85	30.19±1.45	30.06±1.65

Table 2. Percent weight fraction and mean weight fraction of calcium in teeth from the posterior region of the dental arch.

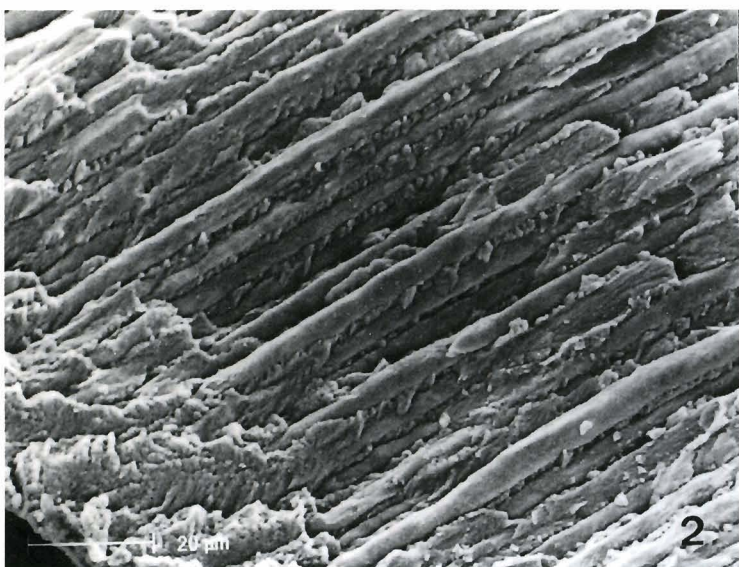|	PREMOLARS	MOLARS	$\bar{x}$±SD
Amelogenesis imperfecta	32.66±3.31	34.25±4.42	33.46±3.97
Control	31.16±2.24	34.04±3.00	32.61±3.00

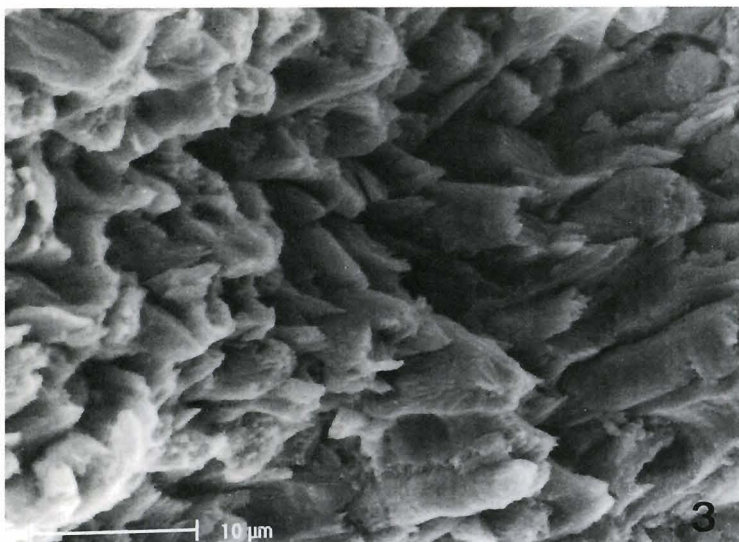**Fig. 2.** R-type pattern in prismless enamel.

Fig. 3. Area showing the decussate pattern in prism enamel.

Discussion

Scanning electron microscopy has been used by different authors, who have identified a wide range of morphological alterations in enamel structure in AI. In our material there were no differences in the microscopic patterns between teeth of the anterior and posterior part of the dental arch. The frequent presence of prismless enamel in the outer region of the enamel in different teeth we studied suggests that amelogenesis probably proceeds to its final phase of maturation despite the structural alterations that may appear in the affected ameloblasts at different stages of their development. Specimens of teeth with AI have R-type and P-type prismless enamel, which are related respectively with an alteration in the configuration of the striae of Retzius and with prism atrophy (Kodaka et al., 1991). In the former situation, the formation of prismless enamel is related with the activity of ameloblasts arranged along the edge of the enamel on the striae of Retzius. In the latter case, prismless enamel appears because of the diminished activity of ameloblasts and impairment of the Tomes process during the final phase of prism development (Fejerskov and Thylstrup, 1989). The appearance of each type of prismless enamel in different teeth shows that in AI the ameloblasts reach the final stages of development regardless of the qualitative results of their biological activity.

With regard to prism structure, the patterns we saw may reflect differences in the duration, nature and rate of ameloblast secretory activity during development of the affected enamel (Wright et al., 1991). Over large extensions of the enamel structure, the prisms were oriented parallel to each other and were normal in
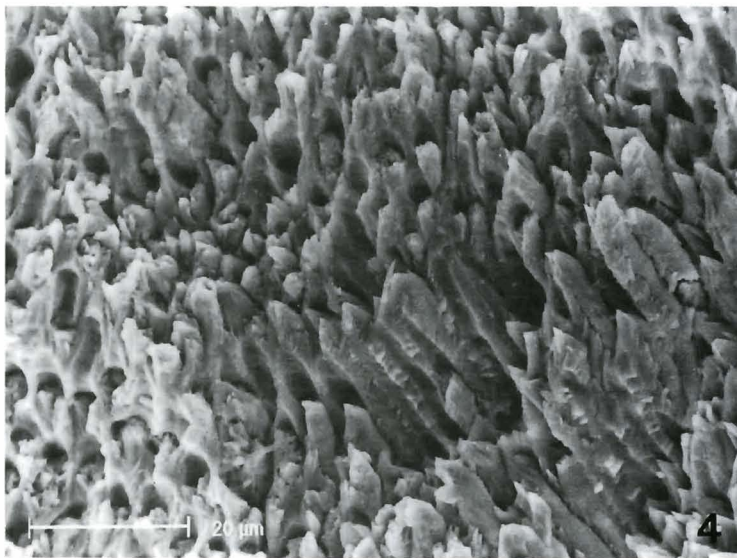

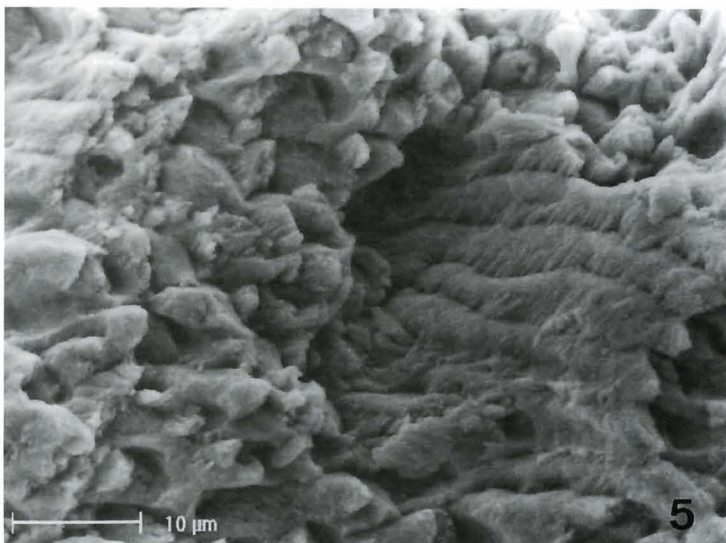**Fig. 4.** Arcade-shaped outlines in prism enamel.

Fig. 5. Prism enamel showing the filamentous pattern.

appearance. However, in some areas we observed anomalous patterns, i.e., a decussate pattern, a superimposed filamentous pattern or an arcade-shaped outline. Because these features have been described in SEM studies of normal developing human enamel, immature morphological patterns can be said to persist in the enamel of persons with AI (Risnes et al., 1998).

Selective alterations may affect some ameloblasts during enamel formation. An alteration in the expression of the genes that control enamelin secretion or the phenomena of apoptosis during ameloblast differentiation are probably involved in the etiology (Hu et al., 2000) and in the origin of these morphological patterns (Vaahtokari et al., 1996). In this regard, the differences in ameloblast apoptosis in different types of tooth during the prolonged modulation phase, when the enamel is hardening, may contribute to the microscopic heterogeneity between teeth seen in the present study, and to the differences between our observations and earlier descriptions of the microscopic features of enamel (Smith and Warshawsky, 1977; Joseph et al., 1994).

One of the most characteristic features of AI that SEM detects is the presence of noncrystalline-appearing, irregular rounded formations located in interprismatic areas. Different authors have related these formations with organic matter that is not removed during maturation, and which might be normal unprocessed or structurally abnormal enamel proteins (Wright and Butler, 1989). In our tooth specimens these formations were seen mainly in the region underlying the prismless enamel, a location that appears to support this hypothesis.

Several authors have investigated the correlation between morphological alterations and calcification with qualitative microprobe analysis. These studies have failed to find significant differences in mean calcium levels between normal and AI teeth, and suggested that the mineral in both cases is probably similar, at least in its primary composition (Wright et al., 1991). However, internal variations in calcium levels through the thickness of the enamel have been reported, and the nature of qualitative X-ray microprobe analysis is such that it causes different degrees of tissue excitation depending on tissue density. These technical limitations complicate determinations of the real concentration of calcium in the enamel, and make it necessary to use an appropriate quantitative approach.

With our quantitative method of X-ray microprobe analysis for mineralized tissues, which have been validated in several studies (López-Escámez et al., 1992, 1993; Campos et al., 1994; López-Escámez and Campos, 1994; Warley, 1997; Sánchez-Quevedo et al., 1998), we found that the degree of calcification differed significantly between the anterior and posterior parts of the dental arch in AI. Moreover, calcification also differed significantly between the anterior and posterior regions in normal teeth. Amelogenesis imperfecta causes morphological alterations in both regions, with evident

signs of immaturity in the process of differentiation. However, these morphological changes were not accompanied by alterations in the foreseeable process of mineralization in anterior and posterior teeth. Although enamel calcium levels are higher in AI as previously shown (Sánchez-Quevedo et al., 1999) the finding that calcium levels in the enamel of both AI and control teeth differed significantly between anterior and posterior teeth indicates that the factors that influence normal mineralization in different regions of the dental arch are not altered in the process of amelogenesis imperfecta.

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