

# Mineralization of human premolar occlusal fissures. A quantitative histochemical microanalysis

A. Campos<sup>1</sup>, I.A. Rodriguez<sup>2</sup>, M.C. Sanchez-Quevedo<sup>1</sup>,  
J.M. Garcia<sup>1</sup>, O.H. Nieto-Albano<sup>2</sup> and M.E. Gomez de Ferraris\*

<sup>1</sup>Department of Histology and Cell Biology, School of Medicine and Dentistry, University of Granada, Granada, Spain and

<sup>2</sup>Cátedra B of Histology and Embryology, Faculty of Odontology, National University of Cordoba, Córdoba, Argentina

**Summary.** The mechanisms of cariogenesis in occlusal fissures remain elusive because of limited information about fissure structure and wall mineralization. The purpose of the present study was to determine the correlation between morphological patterns in occlusal fissures in human premolars and quantitative histochemical patterns of mineralization in the walls of these formations. We used scanning electron microscopy and quantitative X-ray microanalysis with the peak-to-local background ratio method and microcrystalline calcium salts as standards. We distinguished three morphological patterns of fissures in scanning electron microscopic images. The wall of the fissures was less mineralized than the control enamel in all three types of fissures. Because the fissure walls are hypomineralized, we suggest that practicing dentists should take into account the degree of mineralization when they are preparing the fissures for the application of sealant.

**Key words:** Mineralization, Premolar, Fissures, Microanalysis

## Introduction

Occlusal fissures are deep invaginations in the enamel surface of premolars and molars, whose origin has been related to the formation of enamel (Sakakura, 1986; Moss-Salentijn and Hendricks-Klyvert, 1990; Gaspersic, 1996). The morphological appearance of a cross-section through the fissure can vary or remain constant. The mechanisms of cariogenesis in occlusal fissures remain elusive because of limited information about the structure of fissures and the morphological features of carious lesions that arise in them (Hirano and Aoba, 1995). Although the enamel is thinnest at the base of the fissure (Gillings and Buonocore, 1961), caries appear preferentially at the entrance and on the lateral

walls (Fukuda and Suga, 1986). This implies that the morphology of the fissures, together with the mineralization of the walls, are factors which, in association with the accumulation of plaque and organic matter, may influence the appearance and progression of caries.

Because of the close relation between the profile of the fissure and the development of caries, different methodological approaches, including computer-assisted reconstruction, have been used to investigate them (Nagano, 1960; Fejerskov et al., 1973; Galil and Gwinett, 1975; Juhl, 1983; Rohr et al., 1991; Hirano and Aoba, 1995). The use of scanning electron microscopy (SEM) with electron-probe X-ray microanalysis (EPMA) to examine mineralized tissues is a productive tool for the histochemical study of both morphological features and the chemical elements that take part in the mineralization process (Engel and Hilding, 1984; Campos et al., 1994). It is only in recent years that a quantitative approach has been developed to investigate dental and other mineralized tissues with the peak-to-local-background (P/B) ratio method, using crystals of inorganic salts as standards (Lopez-Escamez et al., 1992, 1993a; Campos et al., 1994; Sanchez-Quevedo et al., 1998).

The purpose of the present study was to determine the correlation between morphological patterns in occlusal fissures of human premolars and quantitative patterns of mineralization in the walls of these formations. This quantitative approach was implemented using the techniques of histochemical electron probe microanalysis described above.

## Materials and methods

We studied three groups of three human erupted premolars extracted for orthodontic or periodontal reasons (total of 9 premolars). No clinical lesions were detected. In each group the morphological profile of the fissure was different: V-shaped, I-shaped and Y-shaped (Fig. 1). These profiles were identified in 1-mm-thick longitudinal cross-sections cut with a diamond-impregnated disc (Accuton, Struers, Denmark) at 700

Offprint requests to: Dr. A. Campos, Departamento de Histología y Biología Celular, Facultad de Medicina y Odontología, Universidad de Granada, 18071 Granada, Spain. Fax: 34-958244034 e-mail: [acampos@histolii.ugr.es](mailto:acampos@histolii.ugr.es)

rpm. The sections were examined with SEM, as will be described below.

#### Sample preparation for EPMA

All slices were plunge-frozen in liquid nitrogen-cooled Freon 22. The samples were transferred to the freeze-drying apparatus (Polaron E5350) at  $-100^{\circ}\text{C}$  for 24 h. Samples were left in the freeze-drying chamber to return slowly to room temperature.

#### Electron probe X-ray microanalysis

Tooth slices prepared as indicated above were studied in a Phillips X-L 30 scanning electron microscope (operating voltage: 15 kV; spot size: 500 nm; tilt angle:  $35^{\circ}$ ; take-off angle:  $61.34^{\circ}$ ). An energy dispersive spectrometer (EDAX DX-4) was used for quantitative analysis (count rate: 1200 cps; live time: 50 s). Spectra were collected by pin-point electron beam at  $\times 40\,000$ .

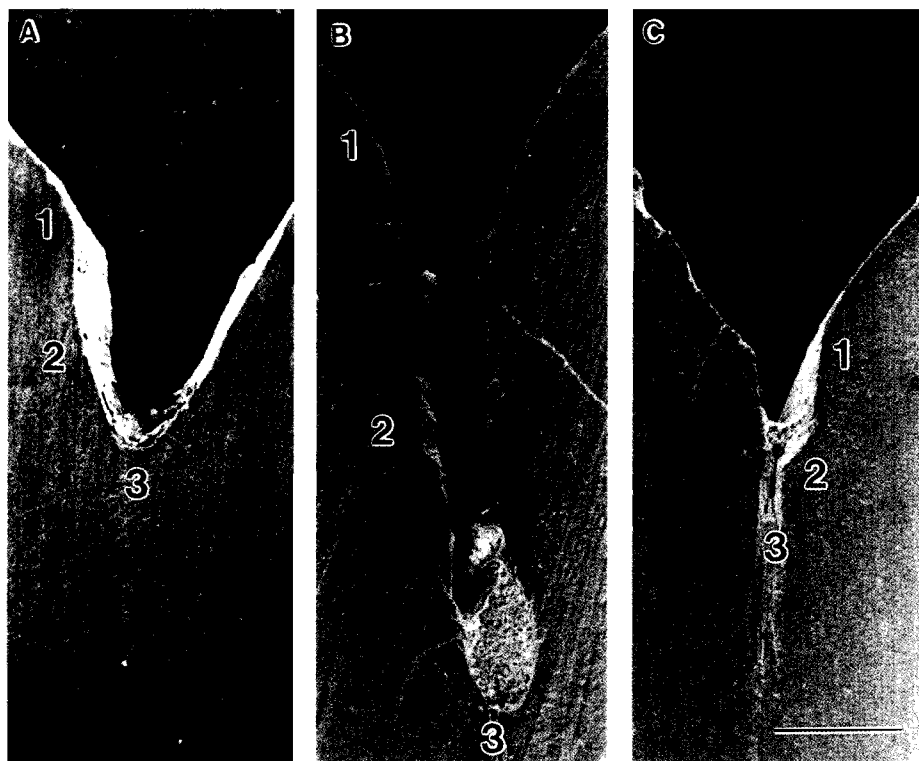
For each fissure we analyzed 10 points at the base, 5 points on each side of the medial wall, and 5 points on each side of the fissure close to the occlusal surface (entrance). Therefore we analyzed 30 sample points in each fissure (total of 270 analyses). The analyses were done at a distance of  $1\ \mu\text{m}$  from the surface of the fissure. As a control we sampled 10 randomly chosen points in different parts of the rest of the enamel (total of 90 analyses).

The microcrystalline salt standards used for calcium quantification (Campos et al., 1992; López-Escámez and Campos, 1994) were  $\text{Ca}_2\text{P}_2\text{O}_7$ ,  $\text{C}_6\text{H}_{11}\text{O}_7 \cdot 1/2\text{Ca}$ ,  $\text{Ca}_2\text{P}_2\text{O}_7$ ,  $\text{PO}_4\text{HCa}$ ,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ,  $\text{PO}_4\text{HCa} \cdot 2\text{H}_2\text{O}$ ,  $\text{C}_{12}\text{H}_{21}\text{O}_{12} \cdot 1/2\text{Ca}$ ,  $\text{CaH}_4\text{O}_8\text{P}_2 \cdot \text{H}_2\text{O}$ ,  $\text{CaSO}_2 \cdot 2\text{H}_2\text{O}$ .

Microcrystalline standards were mounted on 200 mesh nickel grids fixed with adhesive graphite lamina to SEM holders. The standards were analyzed in the SEM immediately after preparation to avoid contamination or chemical modification. The elemental weight percent (WP) of each salt standard was directly proportional to peak-to-local background, i.e.,  $\text{Cs} = k(\text{Ps}/\text{Bs})$  where Cs is the element WP of standards (known), (Ps/Bs) is the element peak-to-local-continuum X-ray intensity ratio from the analysis of salt standards (determined during the analyses), and k is the characteristic calibration constant for each element, calculated from the above equation. The WP was determined by the direct proportion method based on the following relationship between the enamel determinations and the salt standard:  $\text{Ce}/\text{Cs} = (\text{Pe}/\text{Be})/(\text{Ps}/\text{Bs})$ , where Ce is the element WP of a given region of the fissure wall or of the rest of the enamel (unknown), (Pe/Be) is the element peak-to-local continuum X-ray intensity ratio from the analysis of a given tooth, and Cs and Ps/Bs are the data from the standards.

#### Morphological study

Carbon-coated specimens and specimens that were



**Fig. 1.** Morphological profiles of fissures in human permanent premolars: V-shaped (A), I-shaped (B) and Y-shaped (C). The numbers denote the different sampling points along the wall of the fissure: 1: entrance; 2: midzone; 3: base. Bar: A,  $500\ \mu\text{m}$ ; B,  $170\ \mu\text{m}$ ; C,  $200\ \mu\text{m}$ .

gold-coated after EPMA analysis were examined in a Philips XL-30 SEM and photographed.

#### Statistical study

Data for enamel were analyzed with two-way ANOVA and the Tukey test. All statistical analyses were done with the BMDP statistical package.

#### Results

Type V profiles were characterized by a wide entrance that narrowed progressively toward the base. Type I fissures had a more or less constant width throughout their depth. Type Y fissures initially showed a tendency to narrow from the entrance toward the base, but the most basal part of their profile was of constant width. In some profiles we saw amorphous, granular material adhering to the wall or at the base of the fissure (Fig. 1B).

Table 1 shows the calcium concentration (WP) in the different levels of the fissure in each morphological profile. In Table 2 we compare the significance of the differences in calcium concentrations between control enamel and the values at each level of the fissure.

#### Discussion

The use of quantitative histochemical electron probe microanalysis provided accurate data on the presence of the elements involved in mineralization processes and other biological processes (Warley, 1997; Roomans, 1988). In previous studies we established the suitability and precision of this approach for analyzing mineralized tissues, including enamel and dentine (Campos et al., 1992, 1994; López-Escámez et al., 1992, 1993a,b; López-Escámez and Campos, 1994; Fermin et al., 1998; Sánchez-Quevedo et al., 1998). We distinguished three types of morphological profile on the basis of SEM observations. These three types accounted for all the profiles we observed in sections of human permanent premolars. Some authors have used light microscopic observations to classify molar and premolar fissures into numerous subtypes (Nagano, 1960; Fejerskov et al., 1973); however, these subtypes were not evident in our material. Our findings are similar to those of Hirano and Aoba (1995), who identified V-, Y-, I- and U-shaped fissures using computer-assisted reconstruction. These

authors distinguished between I- and U-shaped fissures only on the basis of width; in our material we considered both wide and narrow fissures as I-shaped patterns as long as the walls were parallel throughout their depth. Although a fissure can change shape in different cross-sections of the same tooth, the purpose of this study was not to establish the mineralization pattern throughout a single fissure. Rather, our intention was to search for correlations between the morphological profiles and the patterns of mineralization in their walls.

We found that the wall of the fissures was less mineralized than the control enamel, regardless of the profile of the fissure. These differences were significant, with the exception of the entrance in Y-shaped fissures. Our data show that the degree of mineralization in the fissure walls is clearly lower than in the rest of the enamel. This is a confirmation, based on quantitative data obtained with histochemical EPMA, of earlier descriptive findings (Suga, 1989; Ekstrand et al., 1999).

Studies in different species have shown that during the final stage of enamel maturation, mineralization of the outermost layer increases markedly, so that at the end of the process the outer enamel is much more mineralized than the inner enamel (Suga, 1983, 1989). It is believed that fissures are formed by incomplete coalescence of separate centers of morphogenesis and ameloblastic activity during enamel development. Our findings with quantitative histochemical EPMA show that the walls of fissures are less mineralized than the enamel in the rest of the premolar. We suggest that the lower degree of mineralization in the fissure walls may reflect the persistence of an immature stage of incomplete coalescence during enamel development (Sakakura, 1986; Moss-Salentijn and Hendricks-Klyvert, 1990; Gaspersic, 1993).

The development of caries is influenced not only by the shape of the fissure but also by the creation of an acid environment produced by the accumulation of plaque and organic material (Ekstrand and Bjorndal, 1997). Furthermore, many authors have identified remains of enamel organs in occlusal fissures (Gilling and Buonocore, 1961; Galil and Gwinnett, 1975; Ekstrand et al., 1991). These factors may also contribute to the decreased mineralization we observed in the fissure walls.

We cannot say whether the lesser mineralization of the fissure wall is the result of incomplete maturation, demineralization caused by acid, or both. However,

**Table 1.** Calcium concentration (WP) in different levels of the fissure in each morphological profile. All values are mean  $\pm$  standard deviation. (n=30).

	V-SHAPED	I-SHAPED	Y-SHAPED
Control enamel	30.564 $\pm$ 2.384	30.942 $\pm$ 2.601	32.091 $\pm$ 1.917
Entrance	25.558 $\pm$ 3.317	28.157 $\pm$ 3.494	30.121 $\pm$ 3.219
Midzone	26.558 $\pm$ 3.179	28.197 $\pm$ 3.792	29.412 $\pm$ 2.875
Base	27.541 $\pm$ 2.699	27.008 $\pm$ 4.419	28.082 $\pm$ 3.080

**Table 2.** Significance of the differences (p values) in calcium concentrations between control enamel and different levels of the wall of the fissure.

	V-SHAPED	I-SHAPED	Y-SHAPED
Control-entrance	0.01	0.05	-
Control-midzone	0.01	0.05	0.05
Control-base	0.01	0.01	0.01

demineralization caused by acid is more likely to be involved, as the teeth we studied had been exposed to the oral environment. Our data point toward the possibility that the walls of the fissures are especially susceptible to the appearance and progression of dental caries. Because the fissure walls are hypomineralized, we suggest that practicing dentists should take into account the degree of mineralization we have documented when they are preparing the fissures for the application of sealant (Van Dorp and Ten Cate, 1987).

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