

COMPARATIVE STEREOLOGIC STUDY BETWEEN SECRETORY AND MATURATION AMELOBLASTS IN RAT INCISORS

ESTUDO ESTEREOLÓGICO COMPARATIVO ENTRE OS AMELOBLASTOS DE SECREÇÃO E DE MATURAÇÃO EM INCISIVOS DE RATOS

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The ameloblasts both in secretion phase and in smooth-ended ameloblasts in maturation phase were studied using stereologic methods in transmission electron microscopy (TEM). From secretion to maturation phase of amelogenesis, the nucleus volume decreased 23% and cytoplasm volume did not show significant changes; the total volume and surface of the rough endoplasmic reticulum (RER) decreased 74% and 90%, respectively, and of the mitochondria increased 742% and 384%, respectively; the surface-to-volume ratio for RER and mitochondria decrease 59% and 42%, respectively; and the predominantly apical secretory granules disappeared joined at Tomes' process and lysosomes and phagic vacuoles have appeared principally in supranuclear cytoplasm. Although significant morphologic variation occurs from the secretory to the maturation phase of the ameloblasts, their cytoplasm volume remains unaltered.

UNITERMS: Maturation and secretory ameloblasts; Rat incisors; Stereology; Ultrastructure.

INTRODUCTION

Ameloblasts are epithelial cells which play crucial role in synthesis, secretion and maturation of the dental enamel. For the fulfilment of these functions, ameloblasts undergo morphologic modulations. During the phase of protein matrix synthesis and secretion, ameloblasts are a longer cell, with ellipsoid nucleus in the basal portion and a great amount of membranes of both rough endoplasmic reticulum (RER) and the Golgi's complex. Secretory granules in the central and apical portion also are seen. The terminal apical portion of the cells is so called Tomes' process⁸. Afterwards, ameloblasts start modulation

pattern, which practices enamel maturation. Thus, cell height and volume decrease, the amount of organelles diminishes, Tomes' process disappears¹⁹ and digestive vacuoles and lysosomal enzymes increase in the cytoplasm²¹. In the maturation phase, ameloblasts are able to modulate, in cyclic pathways, for two morphofunctional types, one type is called smooth-ended ameloblast, whose apical portion is replete of microvesicles, it takes out role in water and organic material resorption; and other type is named rough-ended ameloblast, it has apical plasmalena with projections, this morphological type takes out role in introduction of inorganic material in maturing enamel^{4,8,19,21}.

Many trials about this issue look for elucidate amelogenesis^{8,19,21}, as well as more specifically the secretion and maturation phases, with focusing to elucidate dental enamel development, using either light microscopy^{7,14} and electron microscopy^{2-4,7,8,14-18}. Furthermore, these two stages of amelogenesis appear to be a comparable phenomenon among mammalian species. There are several important anatomical distinctions in enamel structure among the species that have been examined¹⁸. Consequently, secretory and maturation phases of amelogenesis are not having been investigated extensively up to now.

In order to improve our understanding about this issue, in the present study, we compared morphologically and stereologically the ameloblasts in secretion and maturation phases of amelogenesis, at ultrastructural level.

MATERIAL AND METHODS

Animals and Experimental design

Five male adults rats (Wistar) weighting around 150 g, were used. The rats were anesthetized with 0.5ml/kg body weight of xylazine plus and 1.5ml/kg body weight of Ketamine and the jaws were fixed by vascular perfusion with 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1M sodium cacodylate buffer, pH 7.3, for about 10 min at room temperature²². The lower incisors were then dissected from the jaws and demineralized in 4.13% EDTA adjusted to pH 7.2 with sodium hydroxide, during which time mature enamel was dissolved^{20,22}. Ameloblasts localization in secretory and maturation phase was made, using as reference molar tooth²⁰. Incisor fragments with regions of secretory and maturation ameloblasts were post-fixed with 1% osmium tetroxide in the same buffer for 1h at 4° C, dehydrated in ethanol and propylene oxide and embedded in SpurrTM resin. Ultrathin sections obtained in ultramicrotome Poter-Bloom-Sorval MT-2 were stained with uranyl acetate and lead citrate and examined in EM 900 Zeiss transmission electron microscope.

Stereological analysis

Examination of ultrathin sections and acquiring of the cells images to electronmicrographs were executed using systematic randomization^{24,25}, in nominal 12000X magnification. To evaluate of the cytoplasmic and nuclear volume, semithin sections of 0.5mm were stained in methylene blue + azur II, and the images in

1000X magnification were acquired in Zeiss Axioshop photomicroscopy and sent to image analysis system KS300. The higher (D) and smaller (d) diameters of the nuclei were measured and the mean nuclear volume was calculated using the formula: $V = \frac{\pi}{6} (D \cdot d \cdot \sqrt{D \cdot d})$.

For obtain the cytoplasm volume, initially we obtained the nuclear and cytoplasmic volume density (Vv) and we made a proportional rule. In 20 electronmicrographs per animal the multipurpose test-system^{23,25} for counting the number of points and intersections was used to make evaluation of morphometric parameters. The volume density of each structure was calculated by quotient between number of points over each structure and number of points over the cytoplasm. The total volume of each structure was evaluated through their volume density multiplied by the cytoplasmic volume. The surface density of each structure was obtained by quotient between number of intersections with the structure four times and total number of points in the cytoplasm multiplied by size of straight line of the test-system used (0.9722mm)²⁴⁻²⁵. The total surface was calculated multiplying their surface density by cytoplasm volume. The surface-to-volume ratio of the structure was obtained by quotient between their total surface and total volume²³⁻²⁵.

Statistical analysis

The measures were compared by ANOVA one-way test, using the software SigmaStat – Jadel TM Scientific software for Windows and the level of significance was set at 0.05.

RESULTS

Morphological results

The secretory ameloblasts are tall and prismatic in shape. In the apical portion (Figure 1A) exhibited a great amount of RER, few secretory granules disposed throughout Tomes' process and a region more electrodense representing the junctional complex net. In the basal cytoplasm (Figure 1B) ellipsoid nucleus with one or more nucleolus is evident and a great number of small and longer mitochondria of several configurations occupy the suprabasal region. The small Golgi complex is seen in medial region of the cell.

The maturation ameloblasts present lower height and larger width in respect to secretory ones. Their apical plasmalema (Figure 2A) shows more electrodense projections due to tonofilaments of desmosomes associated to junctional complex. The

apical cytoplasm (Figure 2A), exhibits large number of mitochondria with several configurations. In the central portion of the cells (Figure 2A), various lysosomes and phagic vacuoles, microfilaments and few amount of membranes of RER and Golgi complex are observed. The basal cytoplasm (Figure 2B) exhibits ellipsoid nucleus, larger phagic vacuoles and spherical mitochondria in higher number than in secretory ameloblasts.

Quantitative results

All the stereologic results are shown in the Table 1. The nucleus volume of secretory ameloblasts showed 23% higher than maturation ones. For the cytoplasmic volume no significant statistical differences ($p > 0.05$) from secretory to maturation ameloblasts was observed.

In respect to RER, there was a decrease from

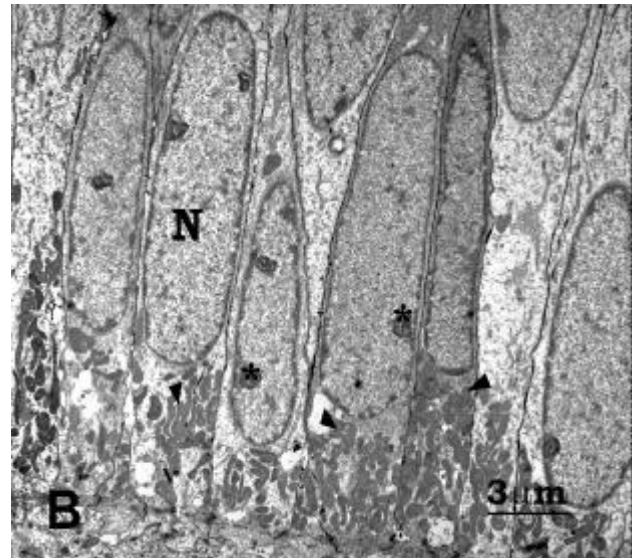
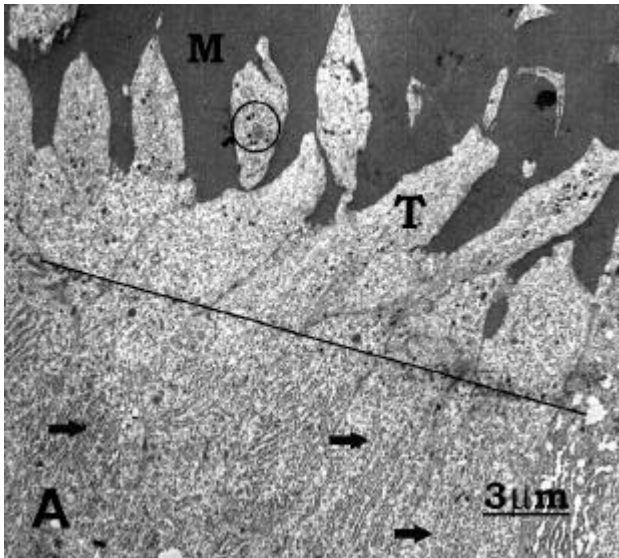


FIGURE 1- Secretory ameloblasts. **A)** Observe in the apical portion a great deal of RER (arrows), few secretory granules (circle) concentrated in Tomes' process (T), next the enamel matrix (M). In the Tomes' process, there were regions more electrodense, represented by junctional complex net (line). **B)** The basal portions is occupied by longer nucleus (N) showing an evident nucleolus (asterisk) and great deal of mitochondria (arrowheads) of several configurations. TEM; x12,000

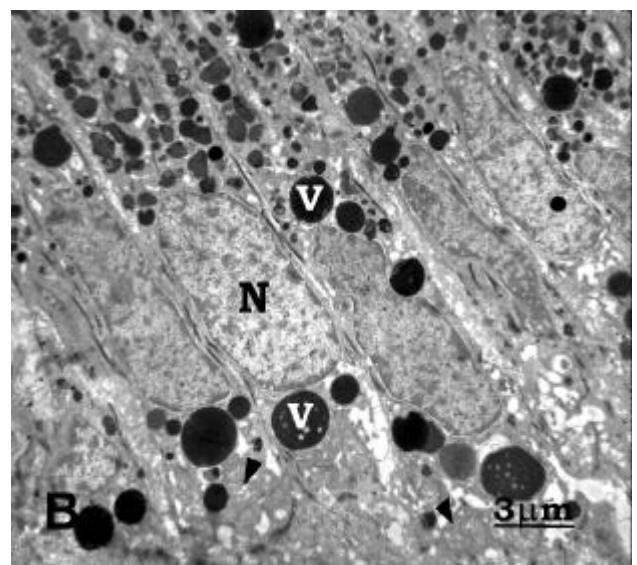
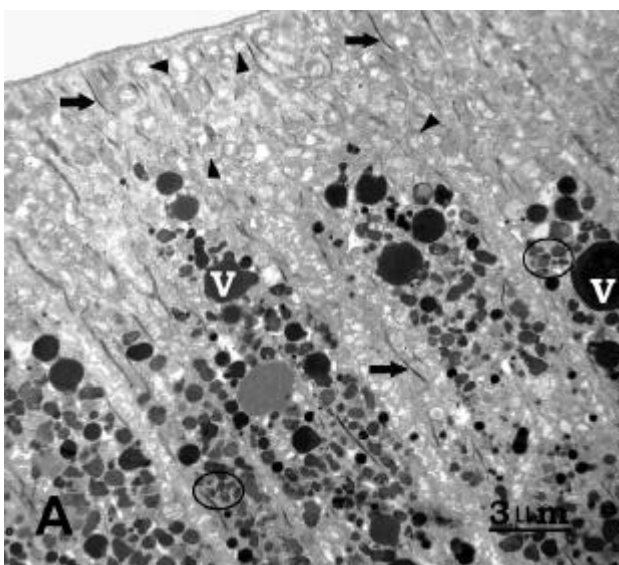


FIGURE 2- Maturation ameloblasts. **A)** Observe in apical portion a good deal of mitochondria with several configurations (arrowheads), and filaments (arrows) associated to desmosomes in all cytoplasm. In the media portion observe lysosomes (circles) and phagic vacuoles (V). **B)** In the basal portion observe ellipsoid nucleus (N), little mitochondria (arrowheads) and little phagic vacuoles (V). TEM; x12,000

secretory to maturation ameloblasts in their volume density (for the 16.7% to 3.8%), absolute volume (74%), total surface (90%) and surface-to-volume ratio (59%). Whereas, for mitochondria, there was an increase in volume density (for the 3.64% to 28.22%), absolute volume (742%) and total surface (384%), and a decrease in their surface-to-volume ratio (42%).

DISCUSSION

Despite the various studies of the ameloblast biology using the remarkable techniques of molecular biology^{5,6,11,13}, few studies in electron microscopy are available^{2-4,7,8,14-18} and morphometric investigation

practically doesn't exist¹⁴.

In the present study, we found out some stereologic differences from secretory to maturation ameloblasts. Thus, the nuclear volume decreases, the cell height reduces and their width increase. According to earlier studies of Boyde; Reith² (1977); Josephen; Fejerskov⁷ (1977) and Skobe¹⁸ (1980) probably, during amelogenesis, the nucleus accompanies the morphologic cytoplasm changes. As there is only one monolayer to produce and secrete several protein quantities and qualities and yet afterwards lead the mineral deposition, the ameloblasts show a longer form to increase the efficiency of all this activity. After the secretion of all the genetically programmed enamel finishes, the cell diminish their height and increase

TABLE 1- The mean and standard deviation of morphometric parameters of the secretory and maturation ameloblasts

Parameters	Secretory (A)	Maturation (B)	A x B*
Absolute Volume (µm ³)			
Nucleus	398.6 ± 10.12	305.8 ± 8.64	P<0.05
Cytoplasm	2064.2 ± 40.16	2244.5 ± 157.76	P>0.05
Volume Density (%)			
REG	16.7 ± 1.44	3.8 ± 1.92	P<0.05
Granules	0.4 ± 0.06	**	
Lysosomes	**	2.4 ± 0.53	
Phagic Vacuoles	**	8.8 ± 4.35	
Mitochondria	3.6 ± 0.07	28.2 ± 7.53	P<0.05
Total Volume (µm ³)			
REG	344.4 ± 28.85	88.5 ± 23.82	P<0.05
Granules	9.0 ± 1.21	**	
Lysosomes	**	54.0 ± 16.14	
Phagic Vacuoles	**	199.9 ± 101.74	
Mitochondria	75.2 ± 2.42	633.1 ± 99.41	P<0.05
Total Surface (µm ²)			
REG	3001.2 ± 160.85	289.4 ± 107.86	P<0.05
Granules	53.3 ± 7.27	**	
Lysosomes	**	287.8 ± 80.73	
Phagic Vacuoles	**	848.1 ± 511.79	
Mitochondria	553.4 ± 117.95	2677.7 ± 388.07	P<0.05
Surface-to-volume ratio (µm ² / µm ³)			
REG	8.9 ± 0.85	3.6 ± 0.82	P<0.05
Granules	6.8 ± 2.12	**	
Lysosomes	**	5.4 ± 0.55	
Phagic Vacuoles	**	4.2 ± 0.06	
Mitochondria	7.4 ± 0.52	4.2 ± 0.06	P<0.05

* Statistical probability level

** Don't appear or appear little.

their width enhancing the withdrawal power of the matrix proteins. Although evident changes occur in this cell shape and in their intracytoplasmic structure, the cytoplasm volume remains unaltered.

On the other hand, the RER showed evident decrease in total volume (74%) and total surface (90%) from secretory to maturation ameloblasts. This role is likely explained by the fall in enamel protein production. The surface-to-volume ratio of the RER decreased 59% showing that besides more volume and surface, the secretory ameloblasts RER cisterns are more flattened. During the secretion phase the quantity and diversity of structural and enzymatic proteins produced and secreted show a well-developed RER system and a well adapted Golgi complex. RER distributes its flattened cisterns to adequate its shape in the longitudinal way of the all cell and mainly to increase the efficiency in the protein production. Several formations of small Golgi complex are distributed in the ameloblast supranuclear portion and are difficult to be analyzed through stereology due to sparse distribution of their dictyosomes. Though subjective analysis at TEM it is difficult to check some quantitative difference of this structure between two studied modulation ameloblast, but some authors affirm that this difference doesn't exist^{9,10}.

We have noticed too the presence of secretory granules in secretory ameloblasts, as observed by Yamamoto²⁶ (1984). Their small diameters make their movements easier in the narrow cytoplasm in this phase and because they are released constitutively, present a very low frequency, that doesn't reach 1%. The movements are coordinated by the filaments, mainly of actin¹².

As soon as the cells get modulated in the maturation ameloblasts, we have found out a great number of lysosomes and phagic vacuoles that are engaged in digestion of enamel matrix for latter enamel mineralization¹⁵⁻¹⁷. These ultrastructures occupy around 12% of the ameloblast cytoplasm in maturation activity and are practically not seen in the secretion one. During the transition period between these two phases the lysosomes and the phagic vacuoles play an important role digesting selectively most of the organelles related to the proteic synthesis¹⁶.

Whereas, the total volume and total surface of mitochondria there was an increasing of 742% and 384%, respectively, and their surface-to-volume ratio decreased 42%. This last data shown that mitochondria of maturation ameloblasts are larger than in secretory ameloblasts, increasing their efficiency to obtain energy. As the RER quantity diminishes and the width of the cells increases the mitochondria can expand to

meet the greater energetic demand. Although they are found in all the cytoplasm, of the secretion ameloblast mitochondria are found mainly in the basal region and the ones of the maturation phase concentrate more in the apical region due the metabolic features of each phase.

The preservation of the ameloblasts perpendicular shape mainly in the secretion phase and the several morphological differences that the cellular surface goes before and during the modulations are the results of constant morphogenetic induction¹ and are dependent of the actin filaments activity and of the heat shock protein-25 (Hsp-25)¹².

The results described here confirmed that significant morphologic changes occur in ultrastructure ameloblasts during their modulation from the secretory to the maturation phase of enamel development, adapting to their new cellular function but although some evident changes in this cell shape and in their intracytoplasmic structure occur, the cytoplasm volume remains unaltered.

RESUMO

Ameloblastos são células de origem epitelial, que apresentam funções de síntese, secreção e maturação do esmalte dental. Assim sendo, ameloblastos passam por mudanças morfológicas durante o seu curso de desenvolvimento. Desse modo, cinco ratos adultos Wistar foram utilizados para avaliar a morfologia de ameloblastos da fase secretora e em maturação, usando de métodos estereológicos em microscopia eletrônica de transmissão. Os dados foram analisados pela análise de variância a um critério (ANOVA). Os resultados mostraram que da fase de secreção para a de maturação da amelogênese: a) ocorreu uma redução de 23% no volume absoluto nuclear e o volume absoluto citoplasmático não mostrou diferença estatisticamente significativa ($p > 0.05$); b) o volume total e a superfície total do retículo endoplasmático rugoso (RER) diminuíram 74% e 90%, respectivamente; c) o volume total e a superfície total das mitocôndrias aumentaram 742% e 384%, respectivamente; d) a relação superfície-volume do RER e das mitocôndrias diminuíram 59% e 42%, respectivamente; e) os pequenos grânulos de secreção, dispostos principalmente na área apical, desapareceram junto com o Processo de Tomes e apareceram os lisossomos e vacúolos digestivos principalmente na área supranuclear. Portanto, apesar de ocorrer uma significativa adaptação morfológica durante a modulação da fase secretora para a de maturação do

desenvolvimento do esmalte, o volume citoplasmático do ameloblasto permanece inalterado.

UNITERMOS: Ameloblastos de secreção e de maturação; Incisivos de ratos, Estereologia; Ultraestrutura.

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