

Collagen Analysis in Human Tooth Germ Papillae

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The extracellular matrix (ECM) performs a very important role in growth regulation and tissue differentiation and organization. In view of this, the purpose of this study was to analyze the collagen, the major organic component of dental pulp ECM, in papillae of human tooth germs in different developmental phases. The maxillas and mandibles of 9 human fetuses ranging from 10 to 22 weeks of intrauterine life were removed and 16 tooth germs (1 in the cap stage, 8 in the early bell stage and 7 in the late bell stage) were obtained. The pieces were processed for histological analysis and stained with hematoxylin-eosin, Masson's Trichrome and picrosirius staining technique. Both types of collagen in the dental papilla were only detected by the picrosirius staining technique under polarized light microscopy. Type III collagen was detected in all specimens. Type I collagen was present in focal areas of the dental papilla only in some specimens. In conclusion, the findings of this study showed that type III collagen is a regular component of the papillae of human tooth germs whereas type I collagen is present in a significantly lesser amount.

Key Words: dental papillae, extracellular matrix, odontogenesis, type I collagen, type III collagen.

INTRODUCTION

The dental pulp is a connective tissue with a peculiar organization and location. It is surrounded by dentin, which is a hard and inelastic tissue. The dental pulp is composed of cells (fibroblasts, odontoblasts and undifferentiated mesenchymal cells) in contact with a complex chain of macromolecules secreted extracellularly, which form the extracellular matrix (ECM) (1). In addition to providing a structure for the tissues, this matrix has a bioactive role in the regulation of cell behavior, influencing its development, migration, proliferation, form and function (2).

The main macromolecules in dental pulp ECM are collagenous proteins (especially types I and III collagen), non-collagenous proteins (fibronectin, tenascin, osteonectin, sialoprotein and osteocalcin) and

proteoglycans, including hyaluronic acid, chondroitin sulfate, heparan sulfate and phospholipid (1-4).

Collagen constitutes nearly 34% of the total ECM proteins and type I and III collagens are the most predominant types. Type I collagen, most commonly found in dense conjunctive tissues, is necessary for tissue architecture stabilization, while type III collagen, most commonly found in loose conjunctive tissues, has an important function in tissue elasticity (5,6).

Type I collagen is the most abundant protein in mineralized tissues, except for enamel, and is also the main ECM organic component. It has been suggested that this protein may be either involved in odontoblastic differentiation or a component of predentin secreted by polarized odontoblasts (7,8). Type I collagen has been identified in dental papilla during dental organ development, predentin, intertubular and reparative dentin and

human pulp fibroblast culture (9-13).

There are few studies addressing human dental pulp development. In addition, most previous studies were done in rodents and their application in humans is limited. There are no published data referring to the presence and distribution of ECM components, such as collagen, in human dental papillae.

Because ECM has a relevant role in growth regulation and tissue differentiation and organization, the purpose of this study was evaluate the main component of ECM, collagen, in the dental papilla of tooth germs of human fetuses.

MATERIAL AND METHODS

Tooth germs were obtained from 9 human fetuses (forensic *post-mortem*) ranging from 10 to 22 weeks of pregnancy. Parental written informed consent was given and the project was approved by the Ethics in Research Committee of the Metropolitan University of Santos.

In each case, the maxillas and mandibles were carefully dissected, fixed in 10% buffered formalin and demineralized with formic acid (14). The pieces were rinsed in distilled water, bisected into left and right halves, dehydrated, embedded in paraffin and serially sectioned longitudinally. Five-micrometer-thick cuts were obtained and stained with hematoxylin-eosin (HE) for routine histological examination.

As tooth formation occurs during a large time-span, the developmental stage of individual tooth germs is poorly related to gestation time. Therefore, in this study, the evaluation of the specimens was related to tooth germ developmental stages and not fetal age. Collagen was analyzed by Masson's Trichrome and Picrosirius (Sirius Red) histochemical methods (15) under light and polarized microscopy, respectively.

RESULTS

Sixteen tooth germs were obtained from the 9 human fetuses. No specimen exhibited germs in the bud stage. The sample was composed of 1 germ in the cap stage, 8 in the early bell stage and 7 in the late bell stage. The morphological aspects of the different human tooth germ developmental stages are illustrated in Figure 1.

The results of histochemical analysis are show in Figures 2 to 4. In the specimens stained by Masson's

Trichrome (Fig. 3), collagen fibers were not observed in the dental papilla, regardless of the phase of tooth germ development. These fibers were found in the connective tissue of gingiva, alveolar bone and dentin.

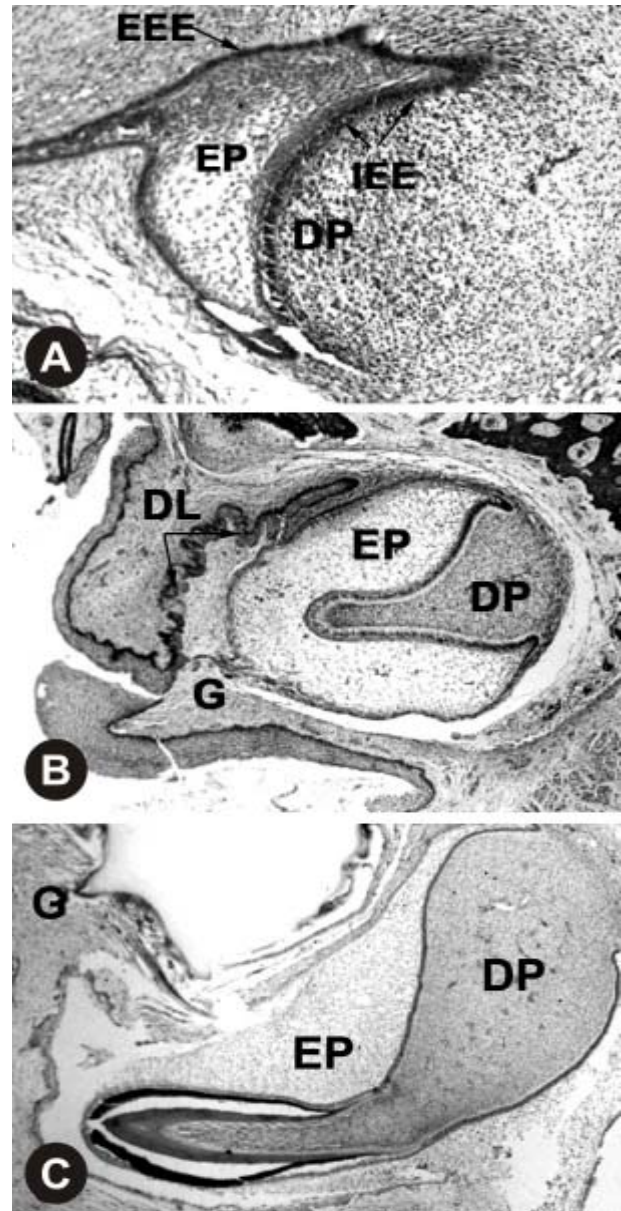


Figure 1. Photomicrographs of human tooth germs at three developmental stages . A (cap stage): external enamel epithelium (EEE), inner enamel epithelium (IEE), enamel papilla (EP) and dental papilla (DP). B (early bell stage): dental papilla (DP), enamel papilla (EP), dental lamina (DL) and gingiva (G). C (late bell stage): dental papilla (DP), enamel papilla (EP) and gingiva (G). HE (original magnifications: A: X100; B: X40; C: X40).

Collagen was found only in specimens stained by Picrosirius under polarized microscopy (Fig. 4). Type I collagen appeared as thick yellow or red fibers present in a large amount in dentin (Fig. 4A) and in the connective tissue of gingiva (Figs. 4A and B). Little amount of type I collagen was detected in the tooth germ papilla and only in some specimens (Figs. 2 and 4B). This collagen appeared as short red fibers in focal areas of tooth germ papilla, underneath the inner enamel dental epithelium (Fig. 4B).

Type III collagen was detected in all samples. This collagen appeared as delicate green-stained fibers and arranged in a parallel fashion and homogeneously distributed in tooth germ papilla (Fig. 4B). This type of collagen was also present in the connective tissue of the gingiva and dental follicle. Both types of collagen were absent in the enamel papilla.

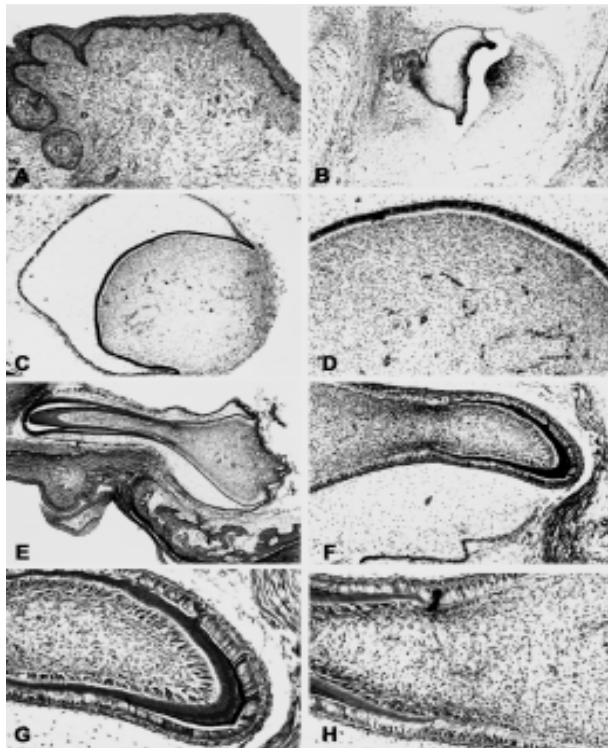


Figure 3. Photomicrographs of human fetuses stained by Masson's Trichrome. A: oral mucosa with blue-stained collagen fibers. B: dental germ in cap stage. C: tooth germ in early bell stage. D: close-up view of C depicting the dental papilla devoid of blue-stained collagen fibers; E-F: late bell stage showing collagen only in dentin, dental follicle, gingiva and alveolar bone. E-H: absence of collagen fibers in dental papilla (original magnifications: A, C and F: X100; B and E: X40; D, G and H: X200).

Specimens (n=16)	Developmental phase	Dental Papilla			Gingiva			Dental Follicle		
		MT	PS(I)	PS(III)	MT	PS(I)	PS(III)	MT	PS(I)	PS(III)
1	EB	-	-	+	+++	+	+	+	+	+
2	EB	-	-	+	++	+	+	+	+	+
3	EB	-	-	+	++	+	+	+	+	+
4	EB	-	+	+	++	+	+	+	+	+
5	EB	-	-	+	+++	+	+	++	+	+
6	EB	-	-	+	+++	+	+	+	+	+
7	LB	-	-	+	++	+	+	+	+	+
8	LB	-	+	+	+++	+	+	++	+	+
9	LB	-	-	+	+++	+	+	+	+	+
10	LB	-	+	+	+++	+	+	+	+	+
11	LB	-	-	+	+++	+	+	+	+	+
12	LB	-	-	+	+++	+	+	+	+	+
13	LB	-	-	+	+++	+	+	++	+	+
14	LB	-	-	+	+++	+	+	+	+	+
15	LB	-	-	+	++	+	+	+	+	+
16	C	-	-	+	++	+	+	+	+	+

Figure 2. Histochemical analysis of the tooth germs. EB: early bell stage; LB: late bell stage; C: cap stage; MT: Masson's Trichrome; PS(I): type I collagen by Picrosirius staining technique; PS(III): type III collagen by Picrosirius staining technique; (-) absent; (+) scarce; (++) present; (+++) abundant.

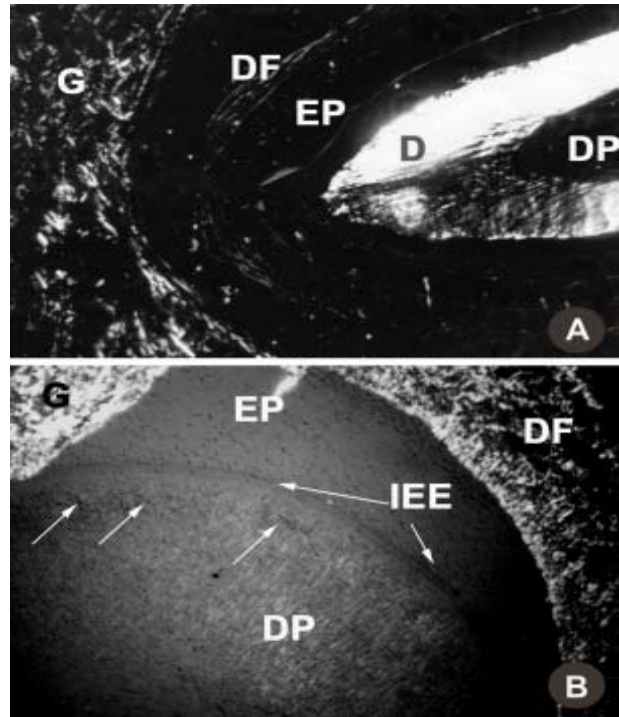


Figure 4. Polarization photomicrographs of human tooth germs in late (A) and early (B) bell stages. A: grey-stained type III collagen in gingiva (G) and dental follicle (DF). White-stained type I collagen in dentin (D). These fibers are absent in dental papilla (DP) and enamel papilla (EP). B: white-stained type I collagen in gingiva (G) and focal areas (arrows) of dental papilla (DP) underneath the inner enamel epithelium (IEE). In the dental follicle (DF) and gingiva (G) white type I collagen fascicles are intermingled with more delicate grey type III collagen fibers.

DISCUSSION

The presence and distribution of collagenous proteins in human dental papilla was analyzed. Types I and III collagens were observed in the tooth germ papilla of human fetuses. However, type I collagen was found in a lesser amount than type III and observed only in some specimens.

Two histochemical methods were used for detection of collagen: Masson's Trichrome and Picosirius staining under polarized microscopy. In the tooth germ papilla, this protein was only detected by Picosirius staining, which is a specific method for collagen detection. This technique allowed detecting the presence of type III collagen, which was abundantly distributed in a homogeneous fashion, regardless of the stages of tooth germ development. This type of collagen was present in the dental papilla, dental follicle and gingiva. These results obtained for type III collagen were already expected because the presence of type III collagen in tooth germ papillae and dental pulp of erupted teeth has been widely reported. However, most of those studies were performed in non-human species (7,12,16). The findings of the present confirmed the presence of type III collagen in the ECM of human dental papilla.

Birefringent type I collagen fibers were abundantly detected in the gingiva as red or yellow thick fascicles on sections stained by the Picosirius method. On the other hand, in the dental papilla, these collagen fibers were found in a small number and only in focal areas of some specimens. The results of the sections stained by Masson's Trichrome did not confirm these findings. Although in these sections blue collagen fascicles were abundantly found in the gingiva, tooth germ papilla collagen was not detected. This probably occurred because Masson's Trichrome technique is not specific for collagen detection, especially when this protein is present in small amounts.

Type I collagen appeared in focal areas of the dental germ papilla, underneath the inner enamel dental epithelium. It is exactly the region of the dental papilla where the pre-odontoblasts are situated. This result support the idea that this collagen synthesis could be a significant step in odontoblastic differentiation process (16,17).

Type I collagen was found in the predentin, which was expected, because this protein has already a well established function. A large number of studies

showed that collagen works as a guide in mineralization progression throughout its long axis (11,18).

The presence of type I collagen is common in tissues subjected to tension, such as skin and bones (2). During odontogenesis, the enamel organ is probably not subject to tissue tensions as erupted teeth or the tooth germ during the eruptive process (16). This might possibly explain why the examined dental papillae presented only minor type I collagen expression.

Hillmann and Geurtsen (11) investigated the distribution of the extracellular matrix molecules in human dental pulps of various ages using indirect immunofluorescence and polarized microscopy. The authors found both type I and type III collagens. Other authors observed type I collagen not only in erupted teeth, but also in tooth germ papillae. However, those authors used more sensitive histological techniques, such as the immunohistochemistry. Using this technique, Garcia et al. (13) observed that type I collagen labeled the pulp of tooth germs less intensively than pulps of erupted teeth.

The concomitant analysis of type I and type III collagen in the human dental papilla has not yet been done. Most information available about collagen expression in tooth germ papilla refers to type I collagen because, in general, type I collagen is the protein of choice to be evaluated in immunohistochemical studies. In this study, however, the examination of sections by the Picosirius-polarization method showed, by fiber color and morphology, both types of collagen in the same section. Type I collagen was situated under the pre-odontoblasts whereas type III collagen was homogeneously distributed throughout the tooth germ papilla. These results are consistent with the roles of each type of collagen on dental papilla, i.e., induction of odontoblastic differentiation (type I) and tissue elasticity (type III), which a property needed during the dental development. Therefore, further studies are required to better understand the participation of these proteins in tooth formation process.

In conclusion, the findings of this study showed that type III collagen is a regular component of the papillae of human tooth germs whereas type I collagen is present in a significantly lesser amount.

RESUMO

A matriz extracelular (MEC) tem um papel importante na

regulação do crescimento e na diferenciação e organização dos tecidos. Com base nestes aspectos o objetivo do deste estudo foi analisar o colágeno, maior componente orgânico da MEC da polpa dentária, na papila de germes dentários humanos, em diferentes fases do desenvolvimento. Foram obtidos fragmentos de maxilas e mandíbulas de 9 fetos humanos com 10 a 22 semanas de vida intra-uterina, dos quais foram analisados 16 germes dentários (1 em estágio de capuz, 8 em estágio de campânula precoce e 7 em estágio de campânula tardia). Secções histológicas seriadas foram coradas com hematoxilina e eosina, tricrômico de Masson e técnica de coloração do picrosírius. Ambos os tipos de colágeno na papila dentária foram somente detectados pela técnica de coloração do picrosírius usando microscopia de luz polarizada. Colágeno tipo III foi detectado em todas as amostras. Colágeno tipo I estava presente em áreas focais da papila dental em algumas amostras. Concluiu-se que o colágeno tipo III mostrou-se um componente regular da papila de germes dentários humanos, enquanto o colágeno tipo I esteve presente em quantidade significativamente menor.

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