

# NORMAL AND INFLAMMATORY HUMAN DENTAL PULP: A MORPHOHISTOCHEMICAL APPROACH

Luminita Maria D. Nica<sup>1</sup>, Marius Raica<sup>2</sup>

## REZUMAT

**Obiectiv:** Scopul acestui studiu este de a caracteriza structura țesutului conjunctiv pulpar pe probe histologice de pulpă dentară umană și dinți recent extrași.

**Material și metodă:** S-au folosit metode morfologice și histochimice de colorare pentru a evidenția celulele, fibrele și alte structuri prezente în țesutul pulpar normal și inflammat.

**Rezultate:** Rezultatele indică prezența de mastocite, depozite de glicozaminoglicani, absența fibrelor elastice și prezența depozitelor de fosfat și carbonat de calciu, în țesutul pulpar inflammat.

**Discuții și concluzii:** Fenotipul celulelor pulpare este încă o problemă controversată. Angiogeneza sincronă infiltratului inflamator este un aspect particular, evidențiat pe probele histologice.

**Cuvinte cheie:** pulpa dentară, modificări inflamatorii, mastocite, glicozaminoglicani, denticuli falși

## ABSTRACT

**Objective:** The aim of this study was to characterize the structure of normal and inflamed pulpal connective tissue on samples of human dental pulp and recently extracted teeth.

**Materials and Methods:** Morphological and histochemical staining methods were used in order to reveal the particular cell types and fibers present in dental pulp, in normal and inflammatory conditions.

**Results:** In the pathological pulp tissue we demonstrated the presence of mast cells, deposits of glycosaminoglycans, the absence of elastic fibers, and massive deposition of calcium carbonate and phosphate, disposed in three patterns: massive and compact, linear, and fine granular.

**Discussions and Conclusions:** One particular aspect is represented by the vascular proliferation around nerve fibers, an aspect that is similar to that of the angiogenesis model, especially in areas with abundant inflammatory infiltrate. The phenotype of pulpal cells is still a matter of controversy.

**Key Words:** dental pulp, inflammatory changes, mast cells, glycosaminoglycans (GAG), false denticles

## INTRODUCTION

Dental pulp is a connective tissue situated in a low-compliance environment represented by mineralized dentin. The pulp is considered together with the dentin as the pulpo-dentin complex due to anatomic, developmental, and functional relationships. The extracellular matrix is the major constituent of the connective tissue. This is composed of ground substance and fibrillar proteins such as collagen and elastin.<sup>1-3</sup>

Ground substance is mainly composed of macromolecules called proteoglycans and glycosaminoglycans.<sup>4,5</sup> The main cells of the connective tissue are the fibroblasts. The pulp also contains odontoblasts (the highest differentiated cells), undifferentiated mesenchymal cells, and immunocompetent cells (lymphocytes, macrophages, dendritic cells).<sup>6</sup>

The phenotype and specific functions of these cells are matter of controversy, as they are largely unknown.

## AIM

The aim of our study was to identify the particular structure of the pulpal connective tissue by using morphological and histochemical staining methods.

## MATERIALS AND METHODS

The histological samples consisted in human dental pulp, removed from teeth using the technique of vital

<sup>1</sup>Department of Odontology- Periodontology, Faculty of Dental Medicine,

<sup>2</sup>Department of Histology-Cytology,

Victor Babes University of Medicine and Pharmacy Timisoara

Correspondence to:

Nica Luminita

Ion Ghica Str. Nr.3; 1900- Timisoara

Phone: 0256-499181; E-mail: purcro@yahoo.com

pulpectomy, and in dental organs (recently extracted teeth).

The samples obtained were washed with a saline solution and fixed immediately in 4% formalin solution, buffered at pH 7.4. The fixation lasted for about 24 hours for dental pulp, and for 72 hours for extracted teeth, in order to allow the penetration of the staining solution through dentin and pulp.

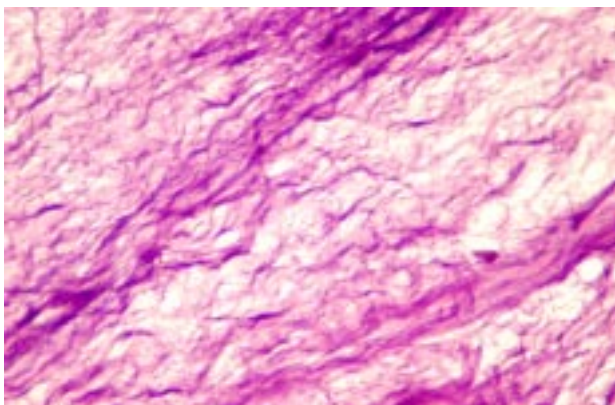
Morphological staining methods applied were: *hematoxylin-eosin* standard technique, and the *Masson trichrome* method, based on aniline blue and Biebrich scarlet; the latter colors the basophilic substrates in violet and red and the acidophilic substrates in light blue.

The histochemical staining methods used were: *von Kossa* silver staining, which specifically evidences carbonates and calcium phosphates stored in cells and tissues; *alisanine S* which stains in orange red deposits of calcium phosphate and is highly specific for hydroxyapatite; *toluidine blue* at pH 4.2 that evidences orthochromatic (blue) the non-sulphated organic substances, and metachromatic (violet) sulphated substances; *alcian blue* at pH 0.2 highlight in deep blue only the highly sulphated glycosaminoglycans; *orcein*, the method Unna-Taenzer, which stains elastic fibers and pathological deposits of elastin in brown-red.

All microscopical aspects were examined at a Nikon Eclipse 600 microscope, at the original magnification  $\times 200$ .

## RESULTS

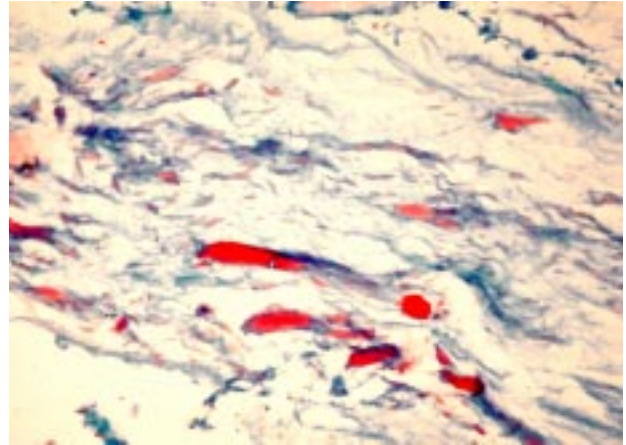
Normal dental pulp extracted from mature teeth is characterized by the presence of a loose connective tissue, with few cells in the central part of the dental crown, and with higher density in the radicular canal and at the apex. Collagen fibers are numerous, and fundamental substance is reduced (Fig. 1).



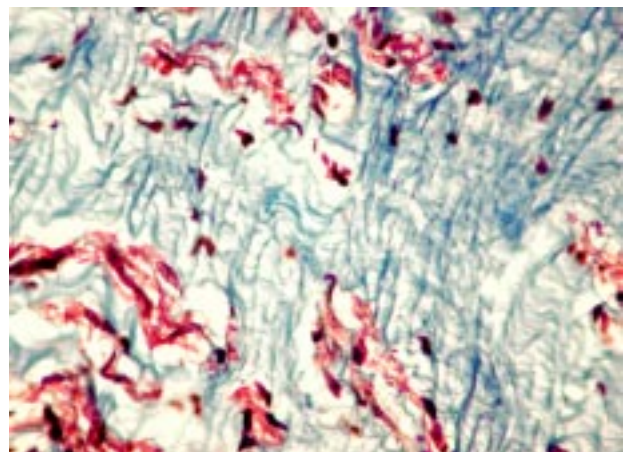
**Figure 1.** Normal dental pulp represented by loose connective tissue, with plenty of collagen fibers and rarely cells of fibroblastic type. (H&E stain, original magnification 200).

Deposits of glycosaminoglycans, involved in the lateral aggregation of collagen fibers, were present, and

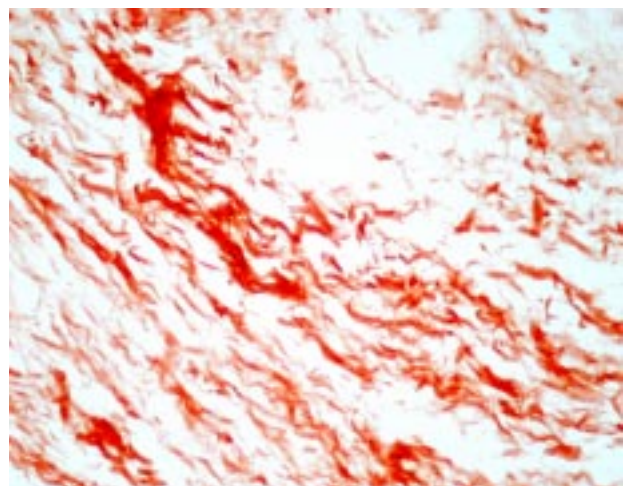
therefore, alcian blue reaction was positive (Fig. 2). Typically, collagen fibrils are arranged in sinuous fascicles, disposed among nerve fibers (Fig. 3). Except for fibroblasts, no other cell types were identified. Contrary to the pulpal tissue of mesenchymal type, elastic fibers were identified, which were positive at orcein reaction (Fig. 4).



**Figure 2.** Deposits of sulphated glycosaminoglycans evidenced in blue with alcian blue at pH 0.2 (original magnification 200).



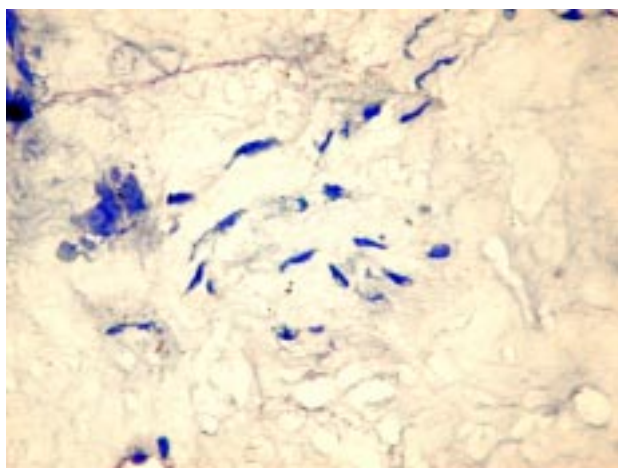
**Figure 3.** Sinuous collagen fibers (blue) and elastic fibers (red) (Masson trichrome stain, original magnification 200)



**Figure 4.** Elastic fibers evidenced in brown-red with orcein (original magnification 200).

No deposition of calcium salts could be demonstrated in the two histological forms of pulp tissue, with the histochemical reaction von Kossa being negative.

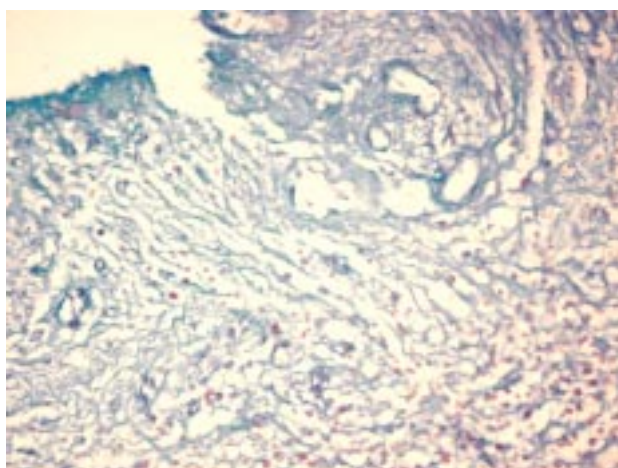
A uniform lesional aspect, at coronal and apexian level, characterized the pathological pulp. The presence of a chronic inflammatory infiltrate consisting in lymphocytes, macrophages and cells that morphologically are fibroblasts-like was observed. The density of the infiltrate was greater near the odontoblasts layer, which presented severe degenerative changes. Different from normal pulp, numerous mast cells were identified, orthochromatic at toluidine blue staining (Fig. 5).



**Figure 5.** Orthochromatic mast cells. Toluidine blue stain at pH 4.2 (original magnification 200).

In areas with great density of the inflammatory infiltrate, four particular aspects were observed:

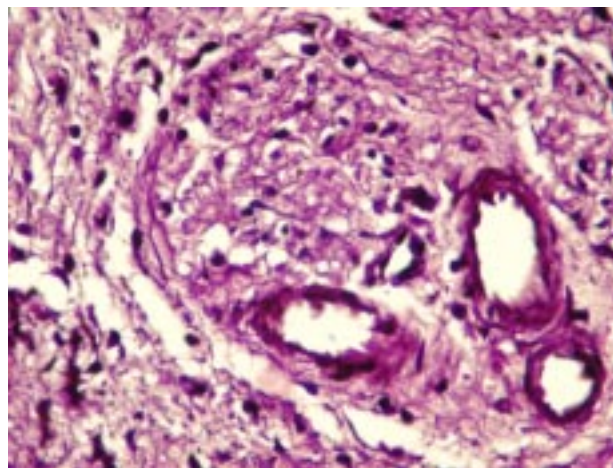
- Large deposits of GAG, intense positive for alcian blue staining method (Fig. 6); their presence is correlated with mast cells, which are active cells in the inflammatory response.



**Figure 6.** Deposits of GAG identified with alcian blue at pH 0.2 in areas of chronic inflammation (original magnification 200).

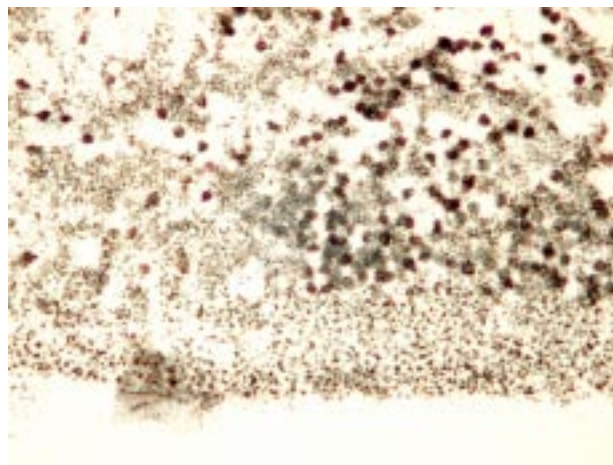
- Absence of elastic fibers
- The vascular proliferation especially around nerve

fibers is of particular interest (Fig. 7), and reveals the *angiogenesis model*. The observed blood vessels were represented by capillaries, with a high density on surface unit, and postcapillary venules with a thickened wall, concentrated especially in areas with abundant inflammatory infiltrate.



**Figure 7.** Vascular proliferation in the perinervous space. (H&E stain, original magnification 200).

- Massive deposits of calcium carbonate and phosphate disposed in three patterns: massive and compact deposits (*false denticles*); linear deposits disposed along the collagen fibers, and fine granular deposits, with great density in the odontoblastic layer (Fig.8).



**Figure 8.** Granular deposits of calcium carbonate and phosphate, abundant in the odontoblastic layer. Von Kossa reaction (original magnification 200).

## DISCUSSIONS

The inflammatory process in human dental pulp consists in vascular changes and migration of inflammatory cells to the site of inflammation. No mast cells are normally present in human dental pulp, but they are known as active cells in the inflammatory response. According to the studies of Miller, Sternberg et al. in 1978, mast cells are occasionally found in inflamed pulp, although degranulation cannot be

observed histologically because these cells lose their characteristic features after degranulation.<sup>7</sup>

Vascular proliferation in the perinervous space of human dental pulp is an aspect that supplements the description of the angiogenesis model, and remains to be clarified. In human dental pulp, angiogenesis is a phenomenon less described in the literature, but is mentioned after orthodontic tooth movement, by Derringer, Jagers and Linden, in 1996.<sup>8</sup>

The presence of calcium carbonate and phosphate deposits in pulpal tissue is frequently observed, and it sometimes represents a major problem of the endodontic treatment. According to Weine, 1989, calcifications are found in both healthy and aging pulps, although their incidence increases with age. False denticles are formed when a degenerating tissue structure serves as a nidus for deposition of concentric layers of calcified tissue.<sup>9</sup>

The mechanism of denticles formation in human dental pulp remains to be clarified.

## **CONCLUSIONS**

Morphohistochemical investigations performed on samples of normal and inflammatory human dental

pulp have revealed special structural characteristics of this particular tissue. The phenotype and specific functions of human dental pulp cells, and the mechanism of pulpal response after injuries are still matter of controversy.

## **REFERENCES**

1. Van Amerongen JP, Lemmens IG, Tonino GJ. The concentration, extractability and characterization of collagen in human dental pulp. *Arch Oral Biol* 1983;28:339-45.
2. Uitto VJ, Antila R. Characterization of collagen biosynthesis in rabbit dental pulp in vitro. *Acta Odontol Scand* 1971; 29:609-17.
3. Bradamante Z, Pecina-Hrnčević A, Ciglar I. Oxytalan fibers in human dental pulp. *Experientia* 1980;36:1210-11.
4. Linde A. A study of dental pulp glycosaminoglycans from permanent human teeth and rat and rabbit incisors. *Arch Oral Biol* 1973; 18:49-59.
5. Embery G. Glycosaminoglycans of human dental pulp. *J Biol Buccale* 1976;4:229-36.
6. Pulver WH, Taubman MA, Smith DJ. Immune components in normal and inflamed human dental pulp. *Arch Oral Biol* 1977; 22:103-11.
7. Miller GS, Sternberg RN, Piliero SJ, Rosenberg PA. Histologic identification of mast cells in human dental pulp. *Oral Surg Oral Med Pathol* 1978;46:559-6.
8. Derringer KA, Jagers DC, Linden RW. Angiogenesis in human dental pulp following orthodontic tooth movement. *J Dent Res* 1996;75:1761-6.
9. Weine FS, *Endodontic Therapy*, 4<sup>th</sup> Edition, The Mosby Co, 1989; p.148.