Morphological and immunohistochemical aspects of the dental pulp in different functional phases

Hîncu Mihaela[1], Horea Manole[2], Petra Şurlin[2] , Mehedinţi Rodica[3]

Abstract

The special morphological structure of the dental pulp has enforced its differentiation from the dense tissues in order to obtain a barrier from the external environment and has also enforced modifications of the response of the dental pulp tissue to different stimuli.

The complex study of the dental pulp has imposed the usage of both classical demineralization studies – that can determine changes in the pulp tissue and also the study of interrelations with the hard structures of the tooth, and also the usage of advanced techniques like electronic microscopy and immunohistochemistry.

All of these techniques have allowed us to distinguish less discussed structures of the dental pulp.

Key words: Dental pulp, Electronic microscopy, Immunohistochemistry, Morphology.

Introduction

The dental pulp is a mesenchymal conjuctive tissue situated in a cavity in the central area of the tooth – the pulp chamber (cavum dentis) and includes the coronal cavity (cavum coronale) and continues in the roots of the teeth with the radicular channel (canalis radicis dentis); they communicate with the external environment at the apex level through the apical foramen (foramen apices dentis). The coronal pulp is rich in cells and vascular and nervous elements. The radicular pulp contains less cells and has feeble vascularisation and the apical pulp is poor in cells but is rich in fibers, similar to the periodontal conjuctive tissue structure. [3]

From a histological point of view, the pulp maintains the morphological characteristics of the embryonic tissue, containing fundamental substance, cells, fibers, blood vessels, lymphatic vessels and nervous fibers. The matrix of the dental pulp tissue appears as a colloidal, homogenous gel; the proteoglycans in its structure form numerous connections and their appearance varies in accordance with the physiological state in the dental pulp. [17]

The glycosaminoglycan chains (mostly hyaluronic acid, chondroitin-4-sulphate and chondroitin-6-sulphate) represent 50 – 90% of the proteoglycan molecules. [2]

Glycoproteins are formed by polypeptidic chains, and their proportion grows with the age, despite the proteoglycans. [14]

The cells composing the dental pulp are divided into 4 categories:

- basic (fundamental) cells: fibroblasts, fibrocytes and odontoblasts;

- relay – cells (round cells of Weil-Hohl) – this type of cells are to be found in the subodontoblastic layer and were described by Hohl as intermediate forms between fibroblasts and odontoblasts. They are elongated,

[1] Associate Professor, Headmaster of the Histology Department, Faculty of Dental Medicine, "Ovidius "University Constantza, Romania

[2] Assistant Professor DMD, PhD, University of Medicine and Pharmacy, Craiova, Romania,

[3] Associate Professor, Histology Department, Faculty of General Medicine "Ovidius " University Constantza, Romania

bipolar cells that form elements of a specific matrix of the reparative (reactional) dentin

- defensive cells (macrophages, lymphocytes, plasmocytes)

- mesenchymal cells – a special cell population identified through immunohistochemistry and characterized by a highly proliferation capacity [8]. They appear as polyhedral cells with a big central nucleus, poorly colored, with abundant cytoplasm presenting lots of prolongations. These cells are frequently linked with blood vessels. The number of mesenchymal cells decreases with age, thus reducing the self-renewing potential of the pulp. [11]

The pulp fibers are represented by collagen, reticulin, oxitalanic and Korff fibers.

Related to other types of tissues, the pulp tissue is poor in collagen fibers, with type I collagen predominant, especially in younger pulp tissue. Korff fibers are responsible in some measure for the formation of the dental matrix [9]. Oxitalanic fibers are dispersed in the pulp tissue, being thinner and fewer compared to collagen and reticulin fibers. The pulp receives blood from many arterial vessels that are organized in a dense network. The arterial vessels form anastomoses in the pulp root, between 2 roots or in the same root presenting more channels.

Related to the veins, the arterioles present a typically peripheral dental disposition justified by the high metabolic changes in the odontoblastic layer. [15]

In the pulp tissue the capillaries are very dense, especially in the subodontoblastic layer and even between the odontoblasts. When odontoblasts begin their secretory activity, the capillaries invade the odontoblastic layer near the predentin and finally the endotelial layer becomes discontinuous.

The veins follow the arterial vessels and leave the tooth through a central radicular vein in the apex. The lymphatic capillaries are formed in the subodontoblastic layer; their diameter varies and their walls present perforations in order to realize the exchange with the fundamental substance around them.

The capillaries are gathered in the center of the dental pulp where they form bigger vessels resembling the veins [16]. The lymphatic pulp vessels form anastomoses with the lymphatic vessels coming from the periodontal ligament**.** The nervous fibers are represented by sensitive and autonomic fibers. The sensitive fibers belong to the maxillary and mandibular branches of the trigeminal nerve, follow the blood vessels and ramify alongside the pulp walls. Most sensitive fibers (80%) are myelineted fibers but have no conjuctive sheath. The unmyelinated fibers reach the periodontoblastic layer and ramify openly, as the myelinated fibers do also.

Some of the nervous fibers penetrate the dentin through the dentinal tubules and surround the ends of the odontoblasts [5].

The autonomic fibers influence the muscle fibers in the blood vessels walls, being responsible for the regulation of the vascular debit. The sympathetic nervous fibers are more numerous in the radicular pulp and induce the contraction of the vessels [12].

Material and methods

The material used was represented by connective human tissue that was separated from the hard structures, retrieved from the oral cavity during pulpectomies performed for therapeutic or prophylactic reasons following the extraction of the tooth. The retrieving of the pulp material from the walls of the pulp chamber, alongside with the odontoblastic layer was performed from the pulp cavity using an adequate excavator. For the study of the radicular pulp we have used teeth with voluminous roots and large channels like canines, central superior incisors, etc, and special needles (T35/40). The retrieved material was fixed using 10% formaldehyde for 24 hours.

Calcium bicarbonate was added to the solution in order to neutralize the formic acid.

We have used the paraffin wax inclusion technique and the resulting blocks were

stained using H&E coloration and also immunohistochemistry techniques (Standard ABC (Avidin Biotin Complex) Method).

The immunohistochemical method was used on pulp tissue fixed with formaldehyde and embedded in paraffin wax and cut at 5 microns. The sections were displayed on Sigma slides (treated with poly-L-lysine). The use of the immunohistochemical technique has allowed us, through amplified antigen-antibody reactions, to locate in situ the specific components of some cells and tissues. In order to realize this, monoclonal antibodies that recognize one antigenic determinant were used.

The immunohistochemical technique was applied on the same material fixed with formaldehyde and embedded in paraffin wax. Through this technique we intended to identify the actin in the smooth muscle fibers from the blood vessels walls, the mesenchymal cells, the T and B lymphocytes and the macrophages. The immunohistochemistry technique used (Standard ABC (Avidin Biotin Complex) Method) is based on the fact that the usage of monoclonal 1A4 antibodies permits the recognition of the smooth muscle actin. The antibody stamps the smooth muscle cells, the myofibroblasts and the myoepithelial cells like the ones around acinus of the salivary gland[4]. The S100 antibody gives a positive reaction for the Langerhans dendritic cells, some of the T- lymphocytes and histiocytes.

Results and discussion

The examination of the slides obtained through classic histological techniques has shown a tissue with mesenchymal morphological characteristics, reach in fundamental substance in the form of a homogenous colloidal gel that contains cells, nervous fibers, lymphatic vessels and blood vessels (Fig.1). Among the cells we have identified odontoblasts – cells specialized in secreting the primary dentin, relay-cells, Weil-Hohl cells, and cells that have a role in the protection of the tissue alongside lymphocytes, macrophage and neutrophils (Fig.2).

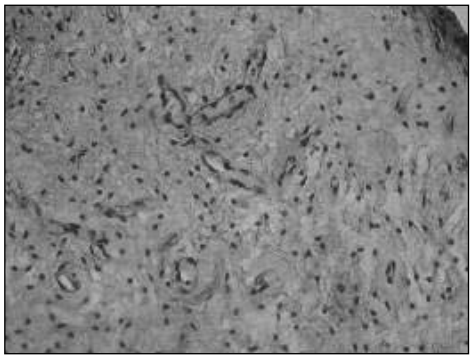

Fig.nr.1 Dental pulp-normal view.
Tricomic stain GS X 200

It was observed that fibroblasts (Fig.3) are a heterogeneous cell population with a structure similar to fibrocities in other young conjuctive tissues. They are columnar polarized cells with elongated cellular processes that form a network which contains fundamental substance and other types of cells.

They have a large nucleus with sprayed chromatin, easy basophile cytoplasm reach in organelles.

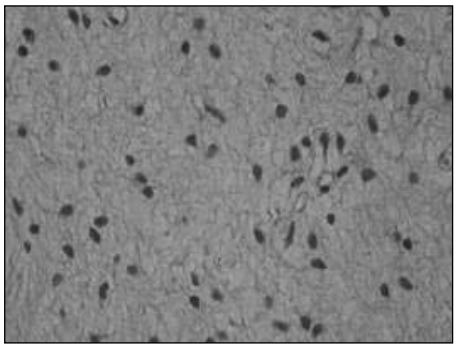

Fig.nr.2 Detailed image of Fig.nr.1 H-E stain X 400

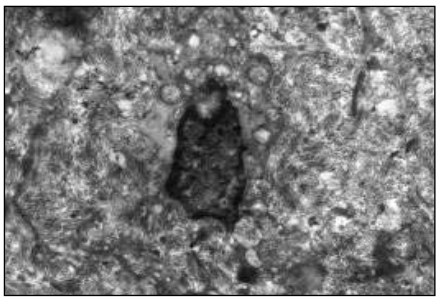

Fig.nr.3 E.M. Image: Active fibroblast

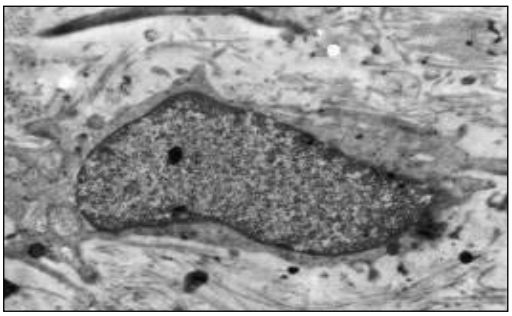

Fig.nr.4 E.M. Image: Dental pulp typical fibroblast

Odontoblasts are big cells (30-40 microns), elongated, present in the marginal area of the dental pulp. They have cytoplasmatic prolongations that are slowly elongating as the odontoblasts are retrieving behind the secreted material. Organelles that are responsible for the synthesis of primary dentin are present in their cytoplasm. The elongated ends of the cells have no organelles present but they contain microfilaments and microtubules that transport the synthesized material.

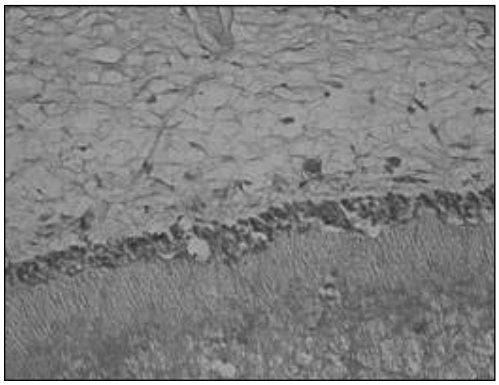

Fig.nr.5 Marginal dental pulp. Odontoblastic layer, predentine and dentine H-E stain, X 200

The Hohl cells represent an intermediate form between fibroblasts and odontoblasts (Fig.4,5). An area that contains a small number of cells – the acellular zone of Weil (Fig.6) – appears between the Hohl cells and the odontoblasts. Also in this area we can observe the vascular plexus of Weil.

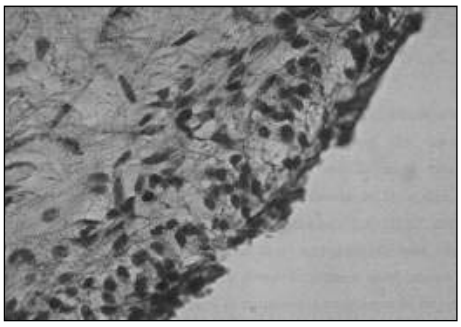

Fig.nr.6 Detail of Fig.nr.5 The acellular zone of Weil. Tricromic stain GS, X400.

The macrophages are big cells variable in number in accordance with the local metabolic conditions. They have a wavy cellular membrane with cytoplasmatic prolongations. The central area of the dental pulp contains usually few cells (Fig.7,8); during inflammatory or other types of physiological processes (resorption of the temporary root of the tooth) [1] their number and the number of immunocompetent cells grows.

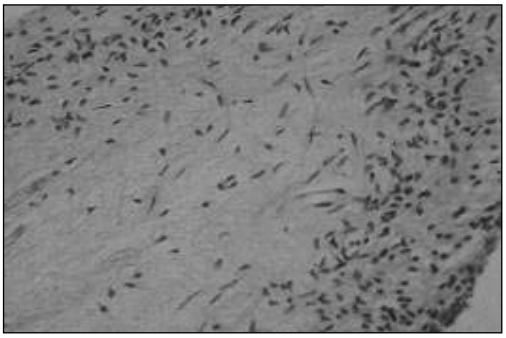

Fig.nr.7 Central zone of the dental pulp poor in cells. H-E stain, X 200

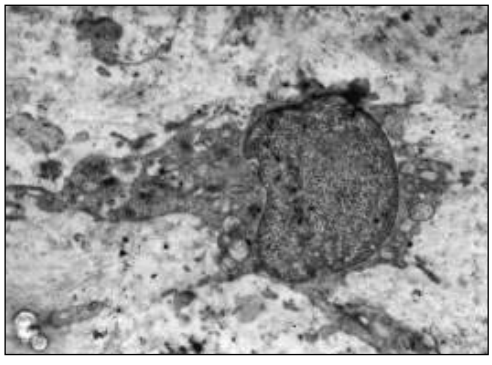

Fig.nr. 8 E.M. Image: Macrophage

The dendritic cells (cells with many cytoplasmatic prolongations, observed through ME and immunohistochemical tests) (Fig.9) are frequently disposed along blood vessels and come in direct contact with the cellular membrane of the endotelial cells. This intercellular relationship demonstrates a functional interaction between the two cell types. [13]

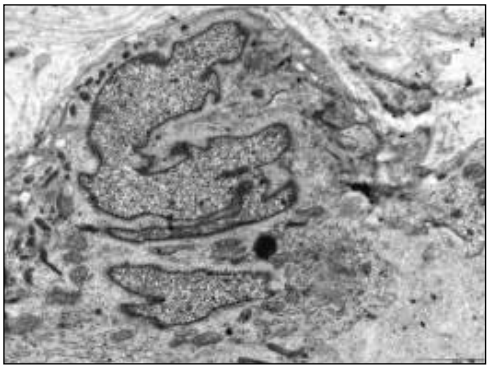

Fig.nr.9 E.M. Image: Dendritic pulpar cell.

The pulpar tissue contains collagen, elastic, reticulin and oxitalanic fibers. The younger pulp contains a fine meshwork formed by collagen fibers (Fig.10,11,12). This meshwork is mostly present around the blood vessels. The Korff fibers are thicker, mostly present in the subodontoblastic layer; they pass through the odontoblasts, reach the dentin layer and participate to the formation of the matrix of the intercanalicular dentin. The oxitalanic fibers are fewer in numbers and dispersed in the pulp tissue (Fig.13).

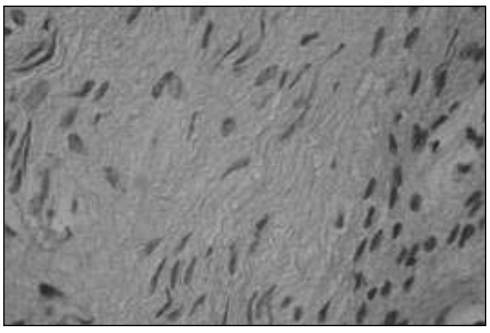

Fig.nr. 10 Increased number of collagen fibers nearby a inflammatory process. H-E stain, X 400

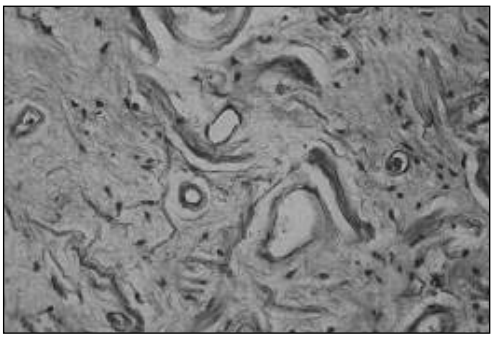

Fig.nr. 11 Collagen fibers- sectioned in different plans. Tricromic stain GS X 200

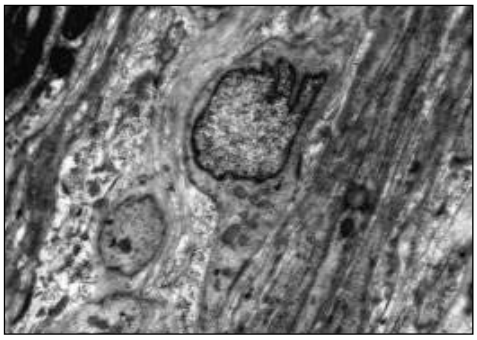

Fig.nr.12 E.M. Image: Collagen fibers into the extracelular matrix

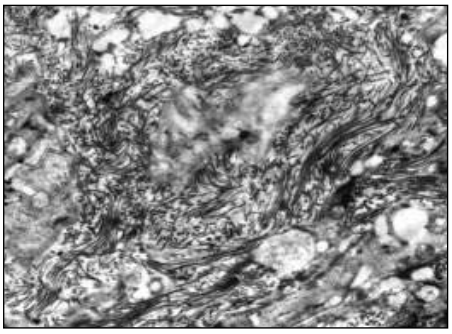

Fig.nr.13 E.M. Image: Extra cellular pulpar matrix

The blood vessels form a dense subodontoblastic net and the capillary vessels are distributed in the odontoblastic layer near the primary dentin, in the area where the endothelium becomes discontinuous and holey (Fig.14). The pericytes form a partial sheath around the capillary wall and contribute to the response of the capillary [6] and the regulation of the blood flow. The motor neurons that control the blood vessels are located in the arterioles, metarterioles

and the pre-capillary sphincters. In the dental pulp tissue we have also identified some atypical pulp calcifications that appear in isolated point – denticules and diffuse calcifications. Denticules are lamellar or tubular-shaped calcifications associated with some pathological manifestations such as profound cavities, chronic marginal periodontitis, profound obstructions, etc. the diffuse calcifications are usually present along blood vessels and collagen fibers.

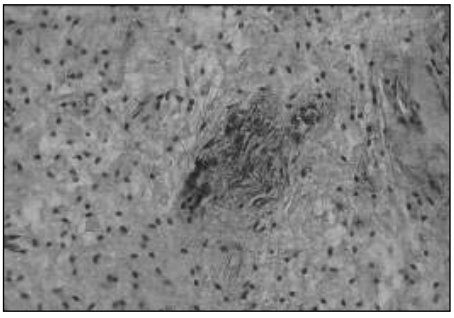

Fig.nr.14 Dental pulp tissue with nerves endings and a blood vessel. Tricromic stain GS X 200

The aging of the pulp (Fig.15,16) is a biological process that determines changes in the volume and structure of the pulp; the number of fibroblasts decreases gradually; they reduce their dimensions and migrate to the margins, the intra-cytoplasmatic organelles also decrease in numbers, especially the RER, the mitochondria and the Golgi apparatus. The odontoblasts show involution signs and their cytoplasm becomes vacuolar. This results in the formation of an irregular, atubular dentin. The density and thickness of the collagen fibers grows with the age, and the quantity of Glycoproteins and proteoglycans decreases. The coronarian fibrosis and irregular calcifications appear[10].

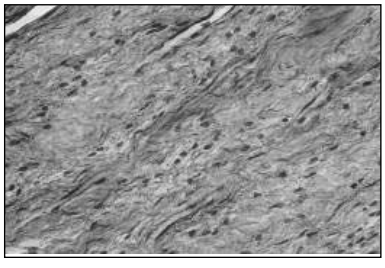

Fig.nr.15 Aging of the pulp. Tricromic stai, GS, X200

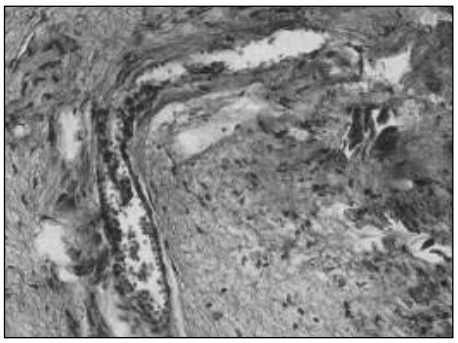

Fig.nr.16 Pulpar blood vessel with sclerohialinosis. Tricromic Stain GS, X200

The analysis of the immunohistochemical images shows a positive reaction for α-SMA in the arterioles and capillaries of the dental pulp, especially in the metarterioles where the muscular component is represented by an incomplete layer of muscle fibers disposed in a spiral manner. A positive reaction is also present in the precapillary sphincters that are composed of 2 -3 muscle fibers with a circular disposition, having an important role in the regulation of the blood flow. Apart from the myocytes, at the outskirts of the capillaries, in the vascular wall, between the basal membrane and the pericapillary layer we have observed some cells having contractile properties like pericytes – their cytoplasm present a positive reaction for the α-SMA.

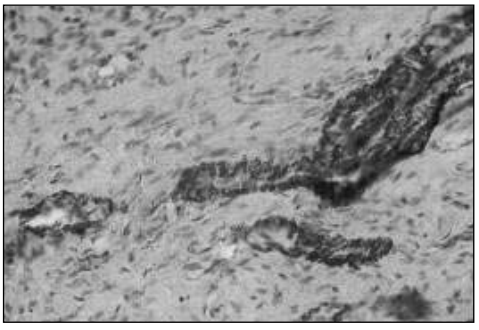

Fig.nr.17 Positive αSMA at the level of the blood vessels walls. X200

These cells function as a precapillar sphincter involved in the regulation and redistribution of the pulp blood flow.

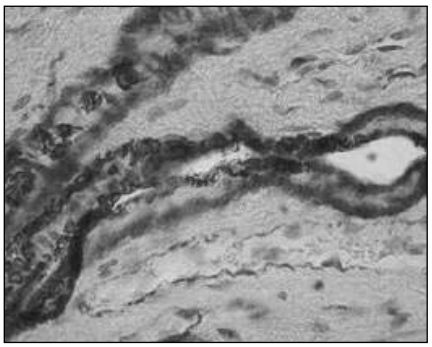

Fig.nr.18 Positive αSMA at the level of the blood vessels walls. X400

The actin in the smooth muscle fibers is also found in the non-muscular cells, but the α-SMA is a marker that can differentiate the fibroblasts from the smooth muscle cells [7] (Fig.18).

The positive reaction for α-SMA appears in different intensity in the apical region of the pulp, where the middle tunic is better developed.

Other type of cells in the dental pulp also present a positive reaction for α-SMA[18]. These are stem-cells, pluripotent mesenchymal cells that undergo a differentiation process to odontoblasts, passing through different intermediate stages of myofibroblasts.

The S-100 protein is a special marker used for the staining of the nervous tissue: glial cells, neurons (Fig.19). The study of this protein has allowed us to determine the nervous disposition at this level. The positive structures for S-100 protein appear as continuous structures along the blood vessels in the radicular pulpar tissue (Fig.20).

These structures are ramified at the coronary pulp level and some other finer positive S-100 structures cross the subodontoblastic layer and reach the primary dentin along the dental tubes. A growth in the density of the immunoreactive nervous fibers can be observed in the inflamed pulp tissue.

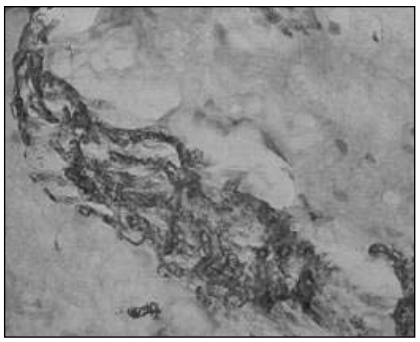

Fig.nr.19 Positive reaction at the pulpar nerves endings at S100 protein. X400

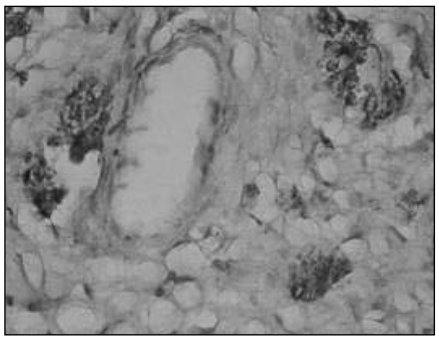

Fig.nr.20 Protein S100 positive structures nearby a pulpar blood vessel. X400

Conclusion

1. The dental pulp is a conjuctive, mesenchymal tissue that undergoes changes that affect the cell population, the fibers and the fundamental substance, but affect also the neuro-vascular component. These changes appear in time and are reflected also in the neighboring periodontal tissues.

2. The smooth muscle fibers are only present in the vascular wall, and their number and disposition varies with the type and caliber of the blood vessel.

3. The usage of the antibody markers for the actin of the smooth muscle has determined positive reaction in the vascular

walls, but also in the stem cells that in certain conditions can present contractile properties (in the transformation to myofibroblasts).

4. The number and morphology of the fibroblasts varies from one case to another depending on their functional degree.

5. The most specialized dental cells are the odontoblasts, that undergo morphofunctional changes following the activity of external factors.

6. The histiocytes and macrophages present a very well developed heterogeneity to citochemical markers as against the functional stage of the cell.

7. In the dental pulp we have identified cells that present characteristics specific to the Langerhans cells that are responsible for the initialization of the immune response.

8. The pulp tissue has a well organized immune system represented by B-lymphocytes, but the T-lymphocytes are more numerous, present especially in the perivascular area.

9. Using immunohistochemical reactions (S 100 protein) we were able to identify at the level of the dentinal canaliculi fine ramified nerves endings positive at S 100. These ramifications cross the subodontoblastic layer and get into the predentine by the side

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Correspondence to: Associate Professor Hîncu Mihaela, e-mail: teofil_mehedinti@yahoo.com

of the dentinal tubes.