

## ORIGINAL PAPER

# Considerations on the ultrastructural particularities of the dental pulp cells

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### Abstract

We realized an ultrastructural study of the cells of the dental pulp, having in view their particularities relative to other types of conjunctive tissue. For this purpose, we selected five cases represented by teeth without subjective or objective symptomatology. Within the paper there are exposed the morphological aspects observed by means of electron microscopy. The results are then discussed in relation with a series of observations made by other researchers regarding the particularities of the pulp cells structures.

**Keywords:** dental pulp, odontoblasts, dendritic pulp cells.

### Introduction

The dental pulp is a conjunctive tissue surrounded by hard dental tissues, which realize a barrier against the injuries of the external environment, but which determines also modifications on the type of response of the dental pulp, comparing to other conjunctive tissues, to various stimuli.

The study of the intimate structure of all the pulp components both in physiological and pathological conditions raises a huge interest for a more intimate knowledge of the mechanisms of response of the pulp to various stimuli and in various conditions having in view the elaboration of the most judicious materials and methods of treatment in the pulp pathology.

The electron microscopy has opened new ways of research in this domain too, allowing to evidence details of ultrastructure on the level of all the pulp constituents.

### Material and methods

For this electrono-microscopical study, we selected five cases on which vital pulpectomies were performed, and the necessary material, the pulp conjunctive tissue, was collected.

The selected cases were represented by teeth without subjective or objective symptomatology, without previous dental treatments on which the pulpectomy was done for prosthetic purposes.

The pulpectomies were performed under local

anesthesia with 3% mepivacaine, without vasoconstrictor. The collecting of the pulp tissue submitted to study was preceded by an opening as less brutal as possible of the pulp chamber by means of the micromotor and of some spherical drills of sizes adequate to the teeth on which we acted.

The real collecting was done from pulp chamber by means of some well-sharpened excavators, of adequate sizes.

In collecting the fragments, we took into account two conditions imposed by the electron microscopic study:

- the tissue for study must get into the fixation in maximum 2–3 minutes after the irrigation with blood ceased;

- the fragments of tissue collected must not be over the volume of 1 mm<sup>3</sup>.

If the collected piece was bigger, it was cut in cubes of maximum one cubic millimeter, by means of two new razor blades, joined and moved parallel so not to press the tissue.

The samples collected this way followed the classic stages necessary for the preparation of the human histological material having in view the study on the electronic microscope with transmission.

The examination was done by means of a JEOL JEM1010 electron microscope with CCD camera, within the Center for Electron Microscopy of the "Babeş-Bolyai" University, Cluj-Napoca.

The analysis of the images and the interpretation of the results were done in cooperation with a specialist in ultrastructural studies.

## ✚ Results

The odontoblasts are the pulp cells with the highest specialization, a fact that captivated numerous researchers.

The cellular bodies of the odontoblasts, elongated, present all over the surface of the pulpar chamber and of the radicular channels, contain a nucleus and different organites implied in the synthesis and transport of the proteins. As in all the secretor cells, these implied organites are polarized: the nucleus is situated at the basal pole of the cell, while the Golgi apparatus, the endoplasmic reticulum and the majority of the mitochondria are distributed between the nucleus and the secretor pole or the apical one (Figure 1).

The pulpal fibroblasts could not ultrastructurally differentiate from the usual fibroblasts. The observations done allowed the description of some minute details within the structure of the fibroblast. The intracellular organs are well developed in the case of the fibroblast.

The rugous endoplasmic reticulum is well-developed, with dilated cisterns which contain an amorphous or granular material, and which continues with the external sheet of the perinuclear membrane. The mitochondria are numerous, they prove the active character of the cellular metabolism, well-expressed mitochondrial cristae; they are in close relationship with the ergastoplasmic membranes.

The Golgi apparatus is developed, especially being situated perinuclearly. We met numerous free ribosomes, dense to the flux of electrons, but also associated to the endoplasmic reticulum. We also met within the cytoplasm of the fibroblast lysosomes, microtubule, microfilaments, and cellular residues. The nucleus of the fibroblast appears on the electronic microscope voluminous with regular contour, rich in euchromatin (Figure 2).

Within this study, we noticed a great variability of the number and the morphology of the constituents of the fibroblast cell, both from a case researched to another, but also within the same tissular substratum.

The quantity and the position of the cytoplasmic organs are variable according to the metabolic activity in which the cell is observed. Thus, the fibroblasts with activity dominantly fibrilogenetic have in cytoplasm numerous ribosomes and polyribosomes isolated or grouped around the endoplasmic reticule, and mitochondria concentrated perinuclearly (Figure 3).

The fibroblasts, which elaborate the fundamental substance, present a cytoplasm with an aspect slightly vacuolar, because of the presence of some large cisterns of rugous endoplasmic reticulum, and of the Golgi reticular apparatus, which contain numerous granular inclusions of different densities, representing more often, the structural glycosaminoglycans and newly synthesized glycoproteins (Figure 4).

The histology of the fibroblasts reflects their functional stage. The reduction of their metabolic activity is accompanied by a reduction of the cytoplasm volume and by the reduction of the number

of organites, as well as the diminishing of the development of the intracytoplasmic organites.

The general shape of the fibrocytes appears similar with that of the fibroblasts. Rugous endoplasmic reticulum is less developed and limited in the perinuclear area.

The mitochondria are small and contain a material dense to the flux of electrons. The cytoplasm contains little ribosomes and rare microtubules. The nucleus of the fibrocytes appears less voluminous with margin with dented aspect, with an aspect denser to the flux of electrons, because of the richness in heterochromatin (Figure 5).

Within the dental pulp, we were also able to identify a series of cells implied in the defense processes. The histocytes and the macrophages are two functional aspects of the same cell type. According to the pulp metabolic conditions, one type or the other can dominate.

The nucleus of these cells is well represented, round, normochrome, positioned more often centrally. The cell limits are very irregular, the cell membrane presenting pseudopods, microvilli or deep invaginations.

The lysosomal formations dominate the voluminous cytoplasm of the macrophages. The lysosomes are delimited at their boundaries by a tri-laminated membrane with dense aspect to the flux of electrons in the electron microscope.

The number of lysosomes and the quantity of enzymes grow with the stimulation of the macrophages by the antigens. By antigen stimulation, the most important morphological modification consists of the apparition of dense granules spread in the whole cytoplasm and which represent in essence primary lysosomes.

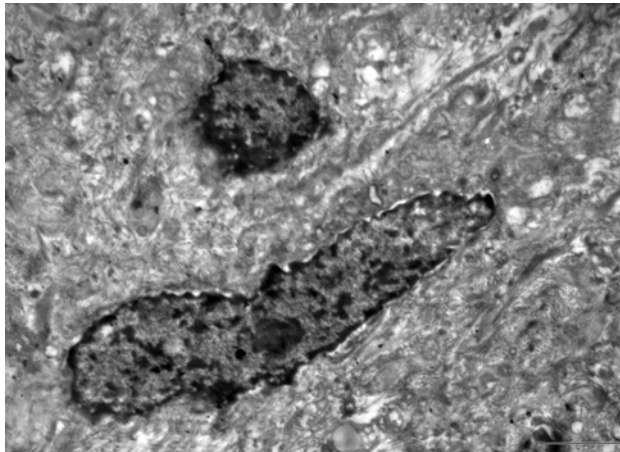
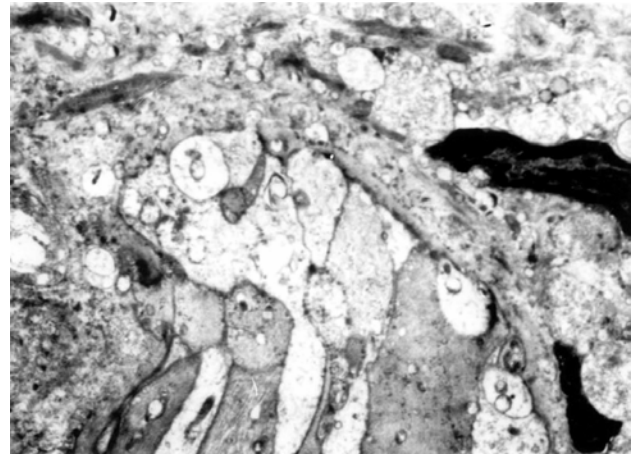
To some macrophages it was possible to evidence within the cytoplasm material ingested in the form of vacuoles of endocytosis which, during the phagocytosis, fuse with the primary lysosomes, forming the phagolysosomes or the secondary lysosomes, which offer the foamy aspect in the current histological techniques (Figure 6).

In the cytoplasm, we can also find mitochondria, a Golgi apparatus increased in dimensions and centrosomes situated, usually, perinuclearly. Within the cytoplasm there are also numerous filaments made of contractile proteins, present more frequently in the peripheral area of the cell (Figure 7).

The morphology of the macrophages appeared extremely heterogeneous depending on the functional stage in which the cell was surprised, stage which depends on its turn on the tissular conditions.

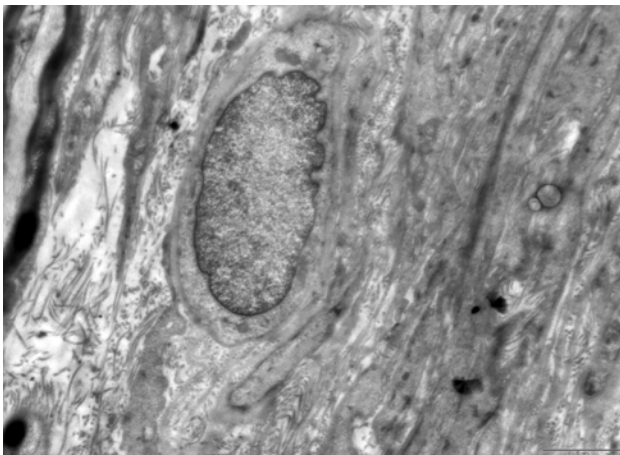
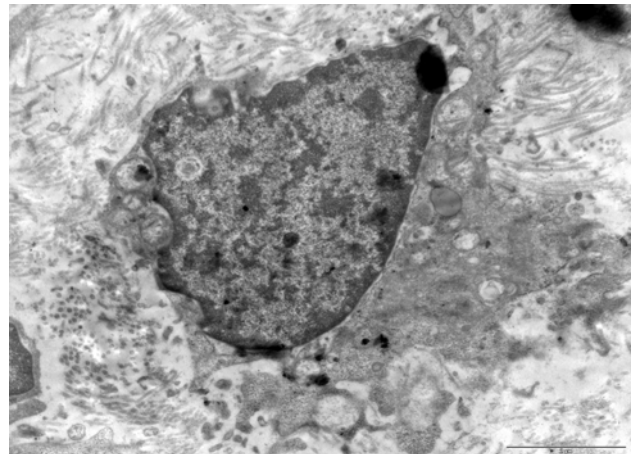
A different cell category identified within this study is represented by the dendritic cells which take part into the immune response and which have as common characteristic the presence of numerous cytoplasmic prolongations. They are also called antigen presenting cells. The evidencing of these cells can be done only by means of electron microscopy and by immunohistochemical tests, as they are difficult to be differentiated by the rest of the pulp cells by means of photon microscopy.

**Figure 1 – Predentin zone in which odontoblastic processes are noticed**

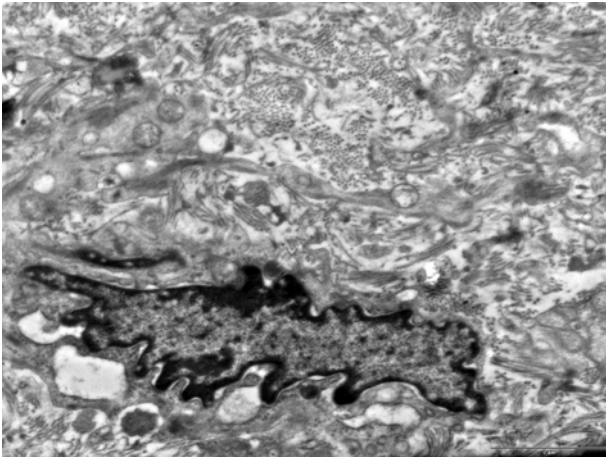


**Figure 2 – Typical pulpar fibroblast. Nucleus with normal distribution of the heterochromatin and of the euchromatin. It is observed the doubling of the nuclear membrane. Well-organized cytoplasm, rich in cellular organites, well-evidenced endoplasmic reticulum**

**Figure 3 – Fibroblast with a large nucleus with a lot of euchromatin, with rich cytoplasm, with numerous large-sized mitochondria with well-contoured mitochondrial cristae, at the periphery of the cell, upper right, there can be noticed fibrils of young collagen, freshly synthesized**



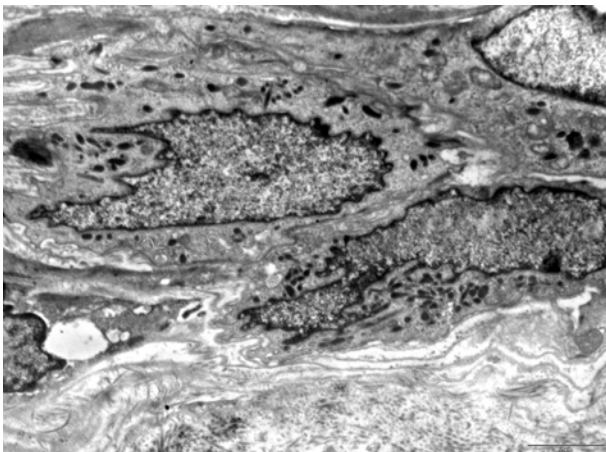
**Figure 4 – Fibroblast with a large nucleus with a lot of euchromatin, with rich cytoplasm; at the left of the image, the myelin sheath of a nervous fiber**



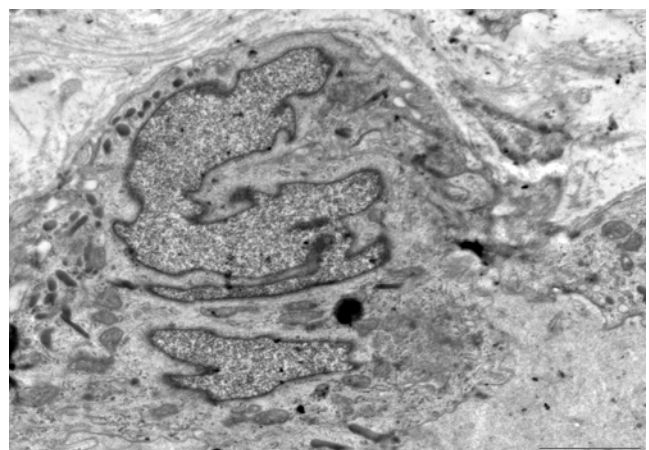
**Figure 5 – Fibrocyte. Nucleus with dented aspect; it is noticed the large quantity of heterochromatin at the periphery of the nucleus; points of heterochromatin are noticed also in the surface of euchromatin. Little cytoplasm with intracytoplasmic vacuolization suggests decreased cellular activity**



**Figure 6 – Macrophage in active stage, with numerous lysosomes within the cytoplasm, mitochondria of large dimensions, with well-expressed mitochondrial cristae, many free ribosomes**



**Figure 7 – Active macrophages with abundant morphoplasm with numerous lysosomes which appear very dense; it is also noticed the presence of the intracytoplasmic vacuoles**



**Figure 8 – Dendritic pulp cell. Abundant cytoplasm with well-developed morphoplasm with the presence of numerous well evidenced granules with aspect similar to the Birbeck granules within the Langerhans cell**

Generally, the dendritic cells do not have the capacity of phagocytosis and this is why they represent a distinct cell line of macrophages. Their dendritic aspect is characteristic, and from the point of view of the cell organites they differentiate from macrophages by the lack of lysosomes and of vacuoles of endocytosis.

The dendritic cells detected in the samples of pulp tissue taken into study presented some characteristics common with those of the Langerhans cells in the epidermis. They have a different dendritic aspect and are located especially on the margin the tissue, in a similar way to the Langerhans cells (Figure 8).

## ✎ Discussions

The electron microscopic studies conducted and cited in the literature allowed to evidence the intimate organization and the disposition of some components of the dental pulp.

Numerous authors described the ultrastructural aspects of the odontoblasts. The studies of electron microscopy evidenced the degree of development of the odontoblastic organites, their relationship with the neighboring structures, but also the way these structures respond to the various injuries to which the pulp is subjected. Thus, it could be observed that the odontoblastic Golgi apparatus present a convex face or the forming face on which small vesicles can be observed. The concave face or the maturation face presents elongated vesicles with content dense to the flux of electrons, granular or filamentous. There can be observed vesicles, which originate directly from the Golgi apparatus.

Marion D *et al.* observe that within the cytoplasm there also are present numerous filaments and microtubuli. The microtubuli are implied in the transport of the molecules synthesized by the odontoblasts [1].

On the apical extremity of the cellular body the groups of microtubuli and filaments are arranged transversally on the big longitudinal axis of the cell. They form the borderline between the properly so called cellular body and the odontoblastic prolongation. The cytoplasmic prolongation lacks organites and contains especially vesicles, microtubuli and microfilaments, sometimes-rare mitochondria [2].

The morphology of the odontoblasts reflects their metabolic activity and varies from an active stage of synthesis to a stage of metabolic quiet, of aged cell. The electron microscopy allowed describing of another stage in the cycle of life of the odontoblasts, a transitional stage, intermediary between the secretor stage and the one of inactivity.

Whereas in the secretor stage there can be seen intracytoplasmic organites, numerous and well-developed, in the transitional stage the decrease of the quantity of intracellular organites reflect the decrease of the functional activity of the odontoblast.

The nucleus is migrated from the basal extremity, expressing high quantities of condensed chromatin. The volume of the endoplasmic reticulum is reduced, and it surrounds the nucleus; there are present autophagic vacuoles [3].

The aged odontoblasts are smaller cells, which are found closely united one to another. The nucleus of such a cell is situated more apically, and the cellular organites are much lesser, in the cytoplasm prevailing tubular or filamentous structures and large vacuoles filled with lipids [4].

Although the pulpar fibroblasts could not ultrastructurally differentiate from the common fibroblasts, the electron microscope researches cited in the literature evidenced their intimate relationship with other components of the dental pulp, as well as the structural modifications of the fibroblasts, which appear as a response to the physiological or pathological transformations of the pulp [5]. Thus, it cannot be ignored their role in the formation of the intrapulpar calcifications and their potential shown *in vitro* to transform into odontoblastic-type cells [6].

The observations allowed the description of some intimate details into the structure of the fibroblast. It was frequently observed a cilium near the nucleus; by means of electron microscopy, it was noticed that this cilium results from the elongation of the tubuli, which originate in the centriole [2].

The Golgi apparatus is developed, it was the most frequently observed in the perinuclear area and it presents numerous vesicles delimited by a material dense to the flux of electrons [7].

Between the fibroblasts there can be observed a variable number of intercellular contacts, contacts of desmosomal type [3].

In the pulpar conjunctive tissue, the dendritic cells form a reticular array in the entire pulp, but they present accumulations especially in the perivascular areas in the centre of the pulp and in the periodontoblastic area at its periphery [8].

On the base of the electron microscopic aspect, there were identified at least two distinct types of cells. One type presents a prominent dendritic aspect; it never contains phagosomes, these representing most probably the line of the real dendritic cells. The other cellular type presents morphological characteristics similar to those of the macrophages.

The dendritic cells are frequently disposed along the blood vessels, with their longitudinal axis parallel to that of the endothelial cells. The observations done by means of electron microscopy of transmission with three-dimensional rotations have proven that the dendrites of these perivascular pulp cells are in close contact with the cellular membranes of the endothelial cells [9].

Another electron microscopic study has proven that the dendritic pulp cells located in the odontoblastic stratum of the pulp of the rat incise are frequently associated with the capillary endothelial cells. This close spatial association indicates a functional interaction between the endothelial cells and the dendritic pulp cells [10].

The dendritic pulp cells are also concentrated at the periphery of the dental pulp, where the pulp can be exposed to noxious external stimuli [9].

It appears logical that these cells with capability of immune surveillance to be strategically concentrated where the chances are highest to meet external antigens. In humans, these peri-odontoblastic dendritic cells usually present a typical dendritic aspect and are disposed within and under the odontoblastic stratum, as if each cell would delimitate its own area of immune surveillance.

Some of them send cytoplasmic prolongations up to the dentinal tubuli. Moreover, the observations done by means of scanning electron microscopy have shown that these peri-odontoblastic dendritic cells, both in humans and rats, send prolongations towards the nervous fibers, reactive to neuropeptides [11].

Generally, the dendritic cells do not have the capacity of phagocytosis and thus they represent a distinct cellular line from macrophages. Their dendritic aspect is characteristic, and from point of view of the cellular organites they differentiate from the macrophages by the lack of the lysosomes and of the endocytosis vacuoles. They have a different dendritic aspect and are located especially at the periphery of the tissue in a manner similar to the Langerhans cells. Thus, it could be suggested that the dendritic pulp cells have similar functional capabilities, being able to be responsible for the initiation of the immune response in the dental pulp by presenting the alien antigens to the T-helper cells [9].

Although, on studies on rats, there were occasionally detected plasmocytes in the dental pulp and their number grew with age [12], their presence seems to be caused, at least partially, by the chronic exposure by means of the dentinal tubuli to a series of external antigens.

Such a long exposure is characteristic to chronic marginal periodontitis, and the electron microscopic identification of different functional aspects of the plasmocytes underlines the importance of the immunological component in the response of the dental pulp in this disease [13].

The apparition of plasmocytes, which present Russell bodies within their cytoplasm, yet proves an inflammatory status of the dental pulp, in most of the cases, in an irreversible stage [14].

The T-lymphocyte differentiates from the B-lymphocyte, electron microscopically, by a much flatter plasmalemma, extremely rarely presenting small-sized microvilli and rare receptors for antigens. Although for a long time it was considered that within the normal dental pulp there are not lymphocytes, at the moment, the T-lymphocytes, even if they are not in great number, are considered normal residents of the normal dental pulp, their presence being evidenced by more and more numerous studies. They are situated predominantly along the vessels in the ventral area of the pulp [9, 15].

The Rouget's pericytes are found associated with the blood vessels and are situated in doublings of the

basal membrane that surrounds the capillaries. These are the ovalar cells, which seem to surround the vessels, forming a three-dimensional array around them, yet without to form a continuous blanket [12].

## Conclusions

The electron microscopic study allowed us to evidence details of ultrastructure on the level of the cells of the pulpar conjunctive tissue.

We noticed a great variability of the number and of the morphology of the fibroblasts and of their cell constituents, both from one researched case to the other, but also within the same tissular substratum, according to their functional stage.

In our study, we found some cells that presented characteristics common with those of the Langerhans cells in the epidermis; they can be considered dendritic pulp cells with similar functional capabilities, responsible for the initiation of the immune response in the dental pulp.

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