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Histological and immunohistochemical aspects of the atrophic dental pulp modifications of abutment teeth

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Abstract

Background: Reducing the thickness of hard dental tissues through the preparation of teeth for fixed prosthodontics represents an aggression for the dentin–pulp complex and may cause changes in dental pulp tissues, by means of acute or chronic inflammation, or by asymptomatic, atrophic modifications. *Aim*: The aim of the study was to histological and immunohistochemical evaluate samples of dental pulp selected from previously prepared teeth, which had been functioning as abutment teeth for some years. *Patients, Materials and Methods*: The starting point of the study was a statistical study conducted on a batch of 276 patients, of which 64 needed to change the fixed prosthetic restorations. Some of the existing abutment teeth were extracted, others presented previously performed root canal treatments and others required endodontic treatment. *Results*: Of the 21 samples taken, 12 showed atrophic pulp modifications, represented by low cellularity, collagen fibrosis, vascular congestion, and pulpal calcifications. *Conclusions*: Certain irreversible atrophic changes can be observed in abutment teeth's pulps, a fact that justifies the need of performing pre-prosthetic endodontic treatment.

Keywords: abutment teeth, dental pulp, atrophic modifications, histology.

Introduction

Over the last decades, complex oral rehabilitation has been performed by the conventional means of fixed prosthetic dentures (FPDs) [1]. In order to properly insert them on the abutment teeth and hence for their integration into the morpho-functionality of the dentomaxilar apparatus, it becomes necessary to remove a considerable amount of dental hard tissues, carried out in the clinical stages of the teeth's preparation.

This procedure is perhaps the most traumatic of all the other clinical interventions performed on the tooth surface, because, under poor conditions, it can cause the overheating of hard tissues, irreversibly damaging the nervous fibers in the dentinal canals, but also the entire dental pulp [2].

Removing the hard-dental tissues can trigger a painful syndrome, similar to that of the preinflammatory hyperemia, even in the presence of a well-adapted provisional restoration. Other procedures, such as cleaning the surface of abutment teeth before cementing, provisional restorations, temporary or final cementation, forced insertion of the FPD on the abutment teeth, excessively tight contact points, misbalanced occlusal relations, may cause injuries to the dental pulp [3].

Beside these favoring factors, even in the case of a balanced occlusion, the abutment teeth need to uphold all the occlusal forces that are transmitted through the pontic and the retainers of the FPD. Certain authors state that, in time, these forces will determine changes of the abutment teeth, particularly within their pulp tissues [4–6].

Dentin is the protective layer of the dental pulp, positioned immediately under the enamel layer, having an inferior hardness, but with demonstrated reparatory abilities in cases of acute trauma or chronic inflammation [7–10]. The dentinal–pulp complex concept is based on the interrelation between the dentin and the pulp, mediated by dentinal tubules and odontoblasts. It claims that any impact received by the dentin will affect the pulpal tissues and any pulpal pathology will determine a decrease in both the quantity and the quality of the produced dentin [11].

The dental pulp's reactions to the preparation of a full prosthetic crown are still a subject of interest for many researchers, as an inflammatory reaction of the pulp may cause the FPD's failure [12, 13].

The teeth selected as future abutment teeth for an FPD have their own history regarding caries, type and quality of restorative procedures, occlusal trauma, periodontal disease, etc., so when deciding to mill them for a fixed prosthetic restoration, their pulpal status is somewhat compromised, meaning that any supplementary aggression may irreversibly disrupt all the defensive mechanisms of

the pulp–dentin complex, causing a loss of any signs of vitality [3].

Certain studies have found relatively low failure rates of abutment teeth, due to endodontic reasons [13, 14].

Aim

The aim of our study was to histological and immunohistochemical (IHC) evaluate the atrophic pulpal changes in abutment teeth, on which endodontic treatment was performed for prosthetic reasons, without any clinical symptomatology.

Patients, Materials and Methods

The start point of this study was a statistical one, performed on 276 patients, of whom 64 had to have changed the existing FPDs. Patients were both women and men, between the ages of 37 and 54 years old. The research was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania. All patients gave their written informed consent to participate in the study.

After the removal of the FPD, all abutment teeth (n=348) were analyzed considering their support capacity through a direct clinical examination and a radiological observation, using orthopantomographs. The following elements were analyzed: the odontal status (lack of hard tissues due to deep decays under the crowns), the endodontic status of the teeth without root canal treatment, by testing the pulp vitality using thermal and electrical tests, as well as the periodontal status.

The non-endodontically treated abutment teeth were grouped as follows: abutment teeth with severe periodontal disease, abutment teeth with mobility because of high prosthesis loading, abutment teeth with necrosis and abutment teeth answering positively at vitality tests.

Some of the abutment teeth having a positive answer at the vitality tests presented carious lesions or needed supplementary preparation, so that endodontic treatment had to be performed, by removing the vital pulp tissues.

The inclusion criteria were: (*1*) patients with FPD needing to be changed; (*2*) positive response to the vitality tests of the abutment teeth; (*3*) exhibiting a carious lesion or requiring extensive preparation. The exclusion criteria consisted of: (*1*) patients with severe systemic disease; (*2*) endodontically treated abutment teeth; (*3*) abutment teeth with higher mobility than grade I.

The endodontic treatment was performed under magnification, using the operating microscope, in a single visit.

After applying the inclusion and exclusion criteria, the patient batch was narrowed to 21 patients, from whom 29 samples of dental pulp were collected. The process of collecting the tissues was performed after the patients signed the participating in the study agreement, after they were explained that nevertheless during the actual endodontic treatment, the pulpal tissue would be degraded and dissolved, under the mechanical and chemical action of instrumentation and lavage solutions.

The harvesting of tissues was performed under local anesthesia with Articaine and vasoconstrictor, using rubber dam isolation. The access cavity was created with mild, atraumatic moves of the burs and the removal of the pulp itself was done using sharp dental excavators and nerve extractor files (*tire-nerfs*).

The removed dental pulp sections were immediately stored in individual containers, coated with 10% neutral buffered formalin solution. This stops metabolic cell processes and cytolysis, also preventing bacterial contamination.

Afterwards, the samples were passed to the Department of Histology, University of Medicine and Pharmacy of Craiova, where the necessary steps for inclusion in paraffin blocks were made. The blocks were numbered for easy identification and consequently sectioned, using the rotary microtome, resulting in 5 μm sections. Samples were displayed on glass slides, dried and prepared for histological staining.

Of the total 29 samples, eight were damaged during the fixation and staining procedures, resulting in a final number of 21 samples, included in the study.

The samples were stained using the Hematoxylin–Eosin (HE) technique and trichrome one, following the Goldner–Szekely (GS) method. After the first histological analysis, IHC assessment was performed using anti-alpha-smooth muscle action (α-SMA) antibody (monoclonal mouse anti-α-SMA, clone 1A4, 1/100 dilution, Dako), and anti-S100 antibody (polyclonal rabbit S100, 1/1000 dilution, Dako).

The histological and IHC assessment was performed by a specialist of the Department of Histology, University of Medicine and Pharmacy of Craiova, using a Nikon Eclipse 90i (Tokyo, Japan) optical microscope.

Results

The study included samples of dental pulp, originating from clinically asymptomatic abutment teeth, which required endodontic therapy due to prosthodontic reasons, in order to set-up a new FPD.

The histopathological (HP) changes of the dental pulp were very varied from one tooth to another, probably due to the age of the patient, but also to some physical aggression and suffering from the tooth in the antecedents. Dental pulp fragments from younger people showed the presence of an increased number of fibrocytes/fibroblasts, the presence of a moderate inflammatory infiltrate consisting of lymphocytes and an increased amount of collagen fibers (Figures 1 and 2). The collagen fibers were small in size and were ordered relatively orderly, from the apex of the tooth to the coronary area. The presence of an increased amount of fibrillar collagen causes deformation and congestion of blood vessels in the dental pulp. For these reasons, the blood vessels appeared of various sizes, with irregular lumen and uneven distribution in the structure of the dental pulp (Figure 3). In some dental pulps, the collagen fibers were dense, organized into bundles, with a variety of orientations, which resulted in the reduction of the blood vessel network (Figure 4).

The large concentrations of collagen fibers lead to a competition between various pulpal structural elements for

the available physical space. A consequent reduction of the blood vessels' number was observed, that altered their normal functions, causing vascular stasis and congestion, which were distinguishable on the histological samples.

In our study, of the 21 samples, 12 showed signs of intense cellular and vascular atrophy, with a massive fibrosis of the dental pulp. The arrangement of collagen fibers was totally disorganized, on the same histological section showing collagen fibers with longitudinal, transverse orientation or nodular organization. We found that as the amount of collagen fibers increased in the structure of the dental pulp, the network of blood vessels and the number of connective cells (fibrocytes, lymphocytes) were greatly reduced (Figure 5).

On some histological preparations, the dental pulp appeared with intensely hyalinized areas, where it could hardly be revealed the collagen fibers, the blood vessels or the connective cells (Figure 6).

Another HP aspect highlighted in the structure of the dental pulp, especially in the elderly, was the salt deposits between the conjunctive fibers. These calcifications had very varied shapes, locations and dimensions (Figure 7).

Knowing that some particular cells, called myofibroblasts, appear in the processes of collagenous fibrosis, we used the anti-α-SMA antibody to highlight these cells in the dental pulp. The microscopic study of the preparations showed that in the incipient processes of collagenous fibrosis, in the dental pulp originating from abutment tooth, there is an increased number of myofibroblasts (Figure 8), but these are reduced numerically in the areas with sclerosis or hyalinized pulp. These cells have contractile capabilities, due to their cytoplasmic actin and myosin content, and also have the ability to produce collagen.

To highlight the nerve fibers in the dental pulp, we used anti-S100 antibody. We found that in the incipient collagenous fibrosis processes present in the dental pulp, the nerve fibers maintain their longitudinal (Figure 9), relatively regular arrangement, whereas in the advanced processes of collagenous fibrosis, the nerve fibers appear in smaller numbers, they are deformed and dislocated by the excessive development of collagen fibers (Figure 10).

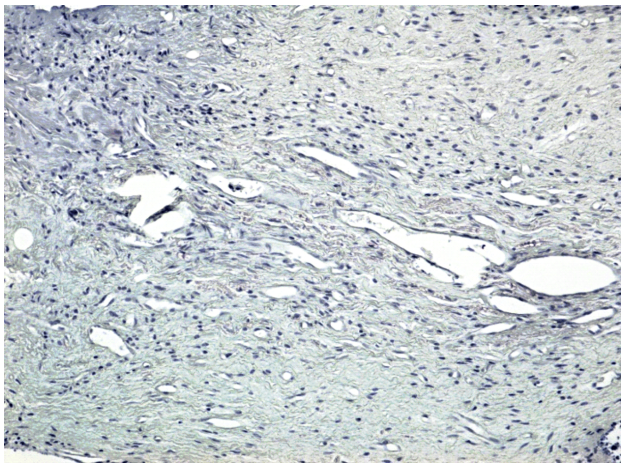

Figure 1 – ***Overview of dental pulp. The presence of a relatively dense connective tissue, rich in collagen fibers, with numerous fibroblasts, blood vessels and a moderate inflammatory infiltrate of lymphocytes can be observed (GS trichrome staining, ×100).***

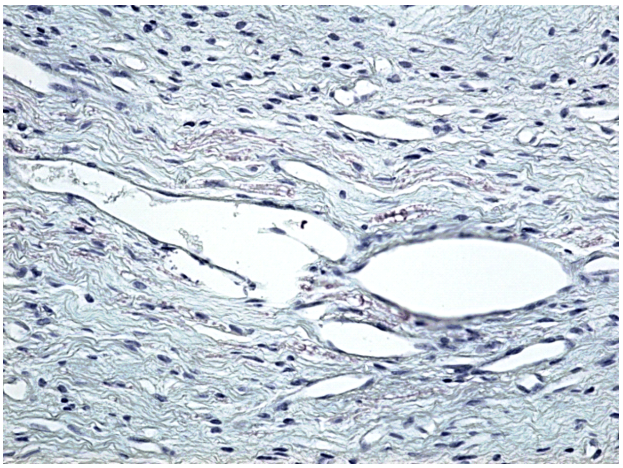

Figure 2 – ***Detail from the previous figure in which the presence of congested vessels is observed (GS trichrome staining, ×200).***

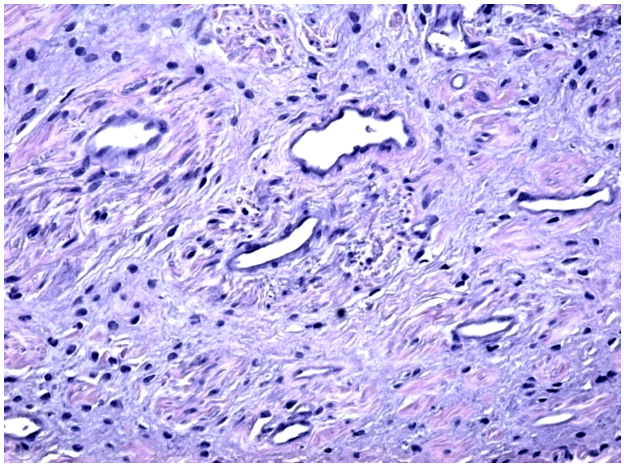

Figure 3 – ***Image of dental pulp with blood vessels with inhomogeneous distribution and the lumen deformed, due to the development of a local fibrosis process (HE staining, ×200).***

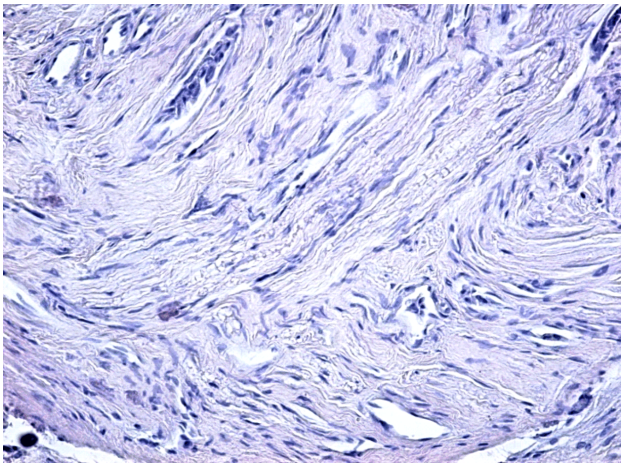

Figure 4 – ***Dental pulp with collagen fibers arranged in bundles with different orientation (HE staining, ×200).***

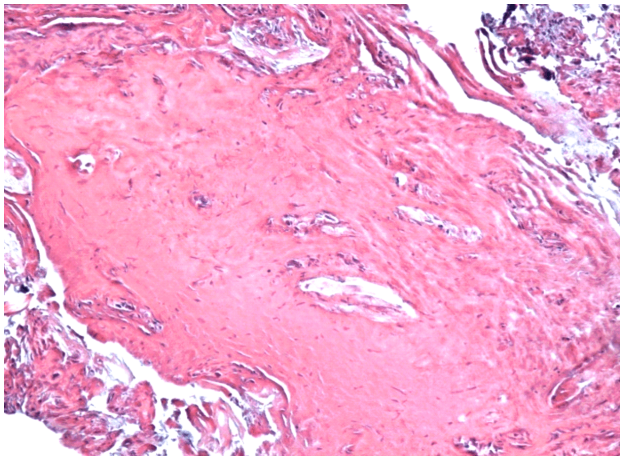

Figure 5 – ***Sclerotic dental pulp, rich in collagen, with a small number of blood vessels (HE staining, ×100).***

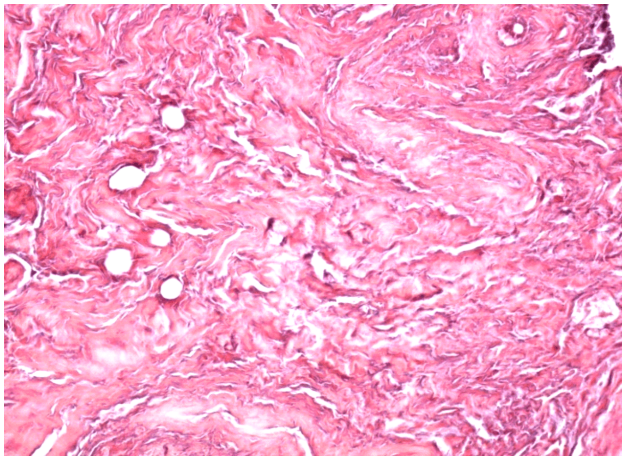

Figure 6 – ***Area of hyalinized dental pulp (HE staining, ×100).***

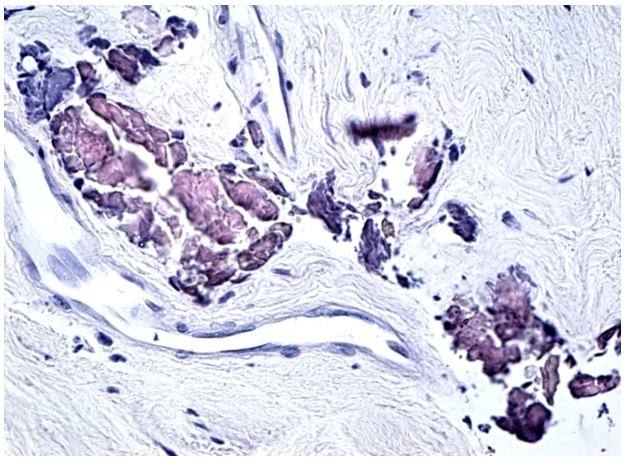

Figure 7 – ***Image of dental pulp with non-homogeneous calcium deposits (GS staining, ×200).***

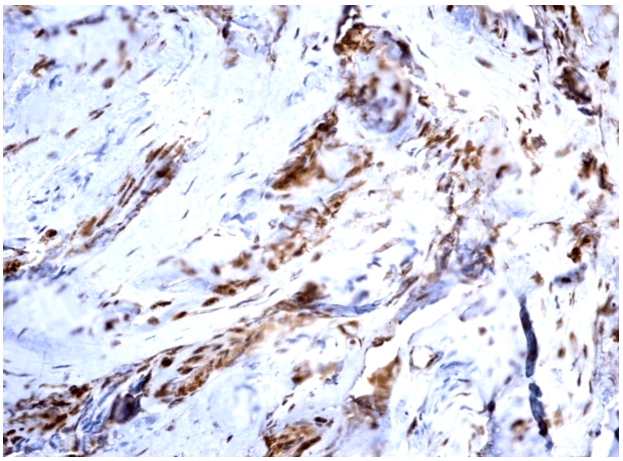

Figure 8 – ***Myofibroblasts in large numbers present in the dental pulp in an abutment tooth (Immunostaining with anti-α-SMA antibody, ×200). α-SMA: Alpha-smooth muscle actin.***

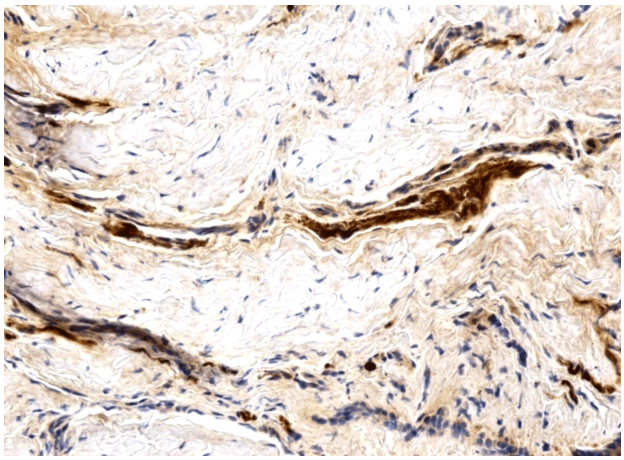

Figure 9 – ***Linear disposition of nerve fibers in a dental pulp with incipient fibrosis (Immunostaining with anti-S100 antibody, ×200).***

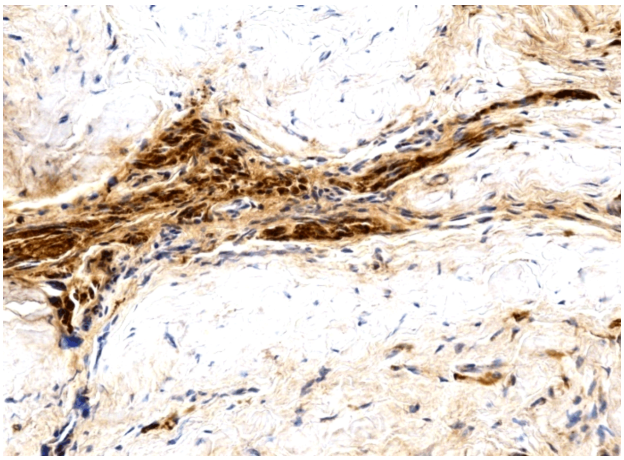

Figure 10 – ***Dental pulp with an advanced fibrosis process that deforms and dislocates nerve fibers (Immunostaining with anti-S100 antibody, ×200).***

Discussions

As previously stated in the literature, the pulp–dentin complex undergoes certain changes during the preparation of abutment teeth, which will be used in dental bridges. These changes can trigger defensive or inflammatory reactions within the pulpal tissue [3, 13–15].

The teeth that will undergo coronal preparation for prosthodontic reasons, as part of an oral rehabilitation treatment plan, frequently have carious lesions, correctly and incorrectly adapted coronal restorations, endodontic treatments or have been previously used as abutments [12]. Therefore, it may be quite difficult to objectively assess the tissular changes caused by the mechanical

preparation of the abutment teeth, as two main conditions should be simultaneously fulfilled: (*a*) the teeth should originate from young patients/or be newly erupted, so as not be affected by carious lesions or other pathologies, and (*b*) the dental pulp should be extracted from the tooth immediately after its preparation [16].

On the other hand, not only the tooth preparation itself can have damaging effects on the pulpal tissue, but also complementary elements such as the preparation technique, the use of cooling systems, the type of burs and the thickness of the removed enamel and dentin layers. The removal of a consistent dentin layer will lead to an extensive exposure of the dentin tubules, increasing dentinal permeability to external stimuli and to a consequent augmented painful sensation for the patient [17, 18].

Furthermore, pulpal changes can occur after the tooth's preparation, in the period of time until the testing of the bridge's fitting and its definitive luting. Considering the patients' possible local and systemic conditions, the teeth can suffer from irreversible pulpal changes, prior to their preparation. Their preparation can have an additional damaging effect on the dental pulp, disrupting the existing balance and causing an acute irreversible inflammatory reaction [19, 20].

Another possible situation could be explained by the recovery capabilities of the pulp–dentin complex that may cause atrophic tissues changes, with no clinical symptoms, but obvious histological changes. These atrophic tissues changes can consist of collagen fibrosis, a decrease in cellular density, occurrence of diffuse calcification processes and vascular stasis and congestion [21, 22].

In this study, atrophic tissues changes were detected within the pulp samples originating from abutment asymptomatic teeth. These changes may be associated with the challenges manifested in the dental pulp during the teeth's function, as well as with the patients' age.

A frequent histological change was the existence of a rich collagen fiber network, grouped in fascicles. This is often associated with the decrease of pulp cell density, as a result of constant secondary and tertiary dentin formation that reduces the size of the pulp chamber.

The excessive synthesis of collagen is stimulated by the presence of myofibroblast cells, a special type of fibroblasts that possess contractile capabilities and have been shown to exist within the dental pulp [23]. Their contractile activity is stimulated by the increased levels of the α-SMA protein [24].

The observation of the myofibroblasts was possible by means of anti-α-SMA antibody immunostaining, despite the fact that similar studies have concluded that this marker can also cause the staining of vascular walls [25]. A study on rats from 2017, detailed the role that myofibroblasts have during the pulpal regeneration, consequent to pulp removal, as well as stimulators of collagen synthesis [26].

α-SMA is found within the dental follicle, during the formation stages of the tooth, as well as within the mature dental pulp. This protein is synthesized by pericytes and other perivascular cells, including in the tooth's apical area [27]. A study on mice outlined the elevated capabilities of α-SMA-positive pulpal stem cells to generate a new vascular system, in cooperation with the endothelial cells [28].

The thick collagen fibers form a supportive network for the adherence of minerals that will calcify the dental pulp. These mineralization processes have been intensively studied in a series of histological and microscopic studies, but the cause of their formation and their exact role has not been clearly determined. It is believed that a tooth can have between one to 12 pulp stones, mostly being situated within the coronal pulp [29, 30]. Other authors state that about 50% of teeth contain at least one pulp stone [31].

Within most dental pulps, the degrees of pulp mineralization vary and can be correlated to the presence of carious lesions or coronal restorations, but not in all situations [32]. Regarding their localization, the pulp stones can be embedded in the dentin, attached to it or unattached. The information about the formation of the pulp stones in physiological settings or as a result of a pathological challenge is so far inconclusive. Nevertheless, the difficulties that they bring to endodontic treatments are a fact [2, 11].

The nervous fibers of the dental pulp also undergo certain changes during the life span of an abutment tooth, as degenerative processes that will influence their abilities to convey sensitive impulses and the dentin's sensibility to stimuli. Therefore, the tooth will become very slowly responsive or unresponsive to thermal pulp vitality tests [33].

In our study, the protein S100 marker was used to highlight the nervous components of the dental pulp, as previously described in similar studies [25, 34]. In the event of pulp inflammation, this marker triggers a positive response by other cellular types, especially immunological ones. Therefore, it cannot be regarding as being specific for the nervous fibers [35, 36]. Taking into consideration that the assessed pulp samples showed no signs of inflammatory reactions, S100 protein marker immunohistochemically targeted existing nervous fibers, exhibiting their linear or fasciculate pattern of distribution.

Conclusions

Fixed partial dentures' design often requires the usage of abutment teeth, which may have been previously used for this purpose or which may exhibit an important dental history. In our histological and immunohistochemical study, the pulp of abutment teeth exhibited tissular changes including collagen fibrosis, reduction of cellular number and existence of diffuse calcification processes. All these tissular changes are characteristic for the dental pulp's atrophic mechanisms. Considering the irreversible nature of these atrophic mechanisms, the initial endodontic treatment of abutment teeth can be justified.

Conflict of interests

The authors declare that they have no conflict of interests.

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