HISTOLOGICAL ASSESSMENT OF PULP RESPONSE AND IT’S MORPHOLOGICAL CHANGES UNDER THE INFLUENCE OF MTA AS CAPPING AGENT, STUDIED ON LIGHT MICROSCOPE

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Summary

Multiple analyses discussed using MTA as direct capping material. These reports have shown that MTA is a promising material for the preservation of pulp tissue when used as a direct capping material. Many clinical studies have demonstrated successful outcomes after the use of MTA as a capping agent on mechanically or cariously exposed pulps. The studies performed by us in this research focused primarily on highlighting the changes happening at a cellular level in dental pulp obtained from human teeth whose caries had been treated with MTA (Mineral Trioxide Aggregate), as compared to pulp originating from healthy teeth. For the investigations there have been used teeth that had been extracted for orthodontic purposes 6, 14 and 30 days after treatment of caries with MTA, as well as a healthy premolar extracted for the same purposes, which served as a benchmark. It seems that MTA is the ideal material for direct capping in permanent teeth, compared to currently used materials, it being a bioactive material that has the ability to create an ideal environment for healing. Our study confirms some of the studies made on MTA, meanwhile it does not confirm other studies. In conclusion our study confirms the presence and the forming of collagen matrix in the predentine area. This matrix is getting mineralized in time, forming osteodentine, followed by a tertiary dentine bridge, a few months after capping with MTA. Inflamatory phenomena are persisting even after 2 weeks since capping, with a constant withdraw in the next period of time. The pulp-dentine plate appears formed 30 days after capping.

Key words: MTA, pulp-dentine plate, odontoblasts, pulp tissue, light microscope

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Introduction

From its appearance in November 1993, hundreds of experiments and studies have been done on the chemical and physical properties, on the antibacterial activity and biocompatibility of MTA (Parirokh and Torabinejad, 2010; Torabinejad and Parirokh, 2010).

Initially recommended as a root canal filling material, MTA has been subsequently used for pulp capping, pulpotomy, apexogenesis, repair of root perforations or furcations, having been recognised as a biocompatible, bioactive³, conductive⁴ and inductive material in hard bone tissue formation.

Multiple analyses discussed using MTA as direct capping material(Camp, 2008; Bakland, 2000; Ward, 2002; Joffe, 2003; Witherspoon, 2008). These reports have shown that MTA is a promising material for the preservation of pulp tissue when used as a direct capping material. Many clinical studies have demonstrated successful outcomes after the use of MTA as a capping agent on mechanically or cariously exposed pulps (Min et al., 2008; Nair et al., 2008; Kim and Jou, 2000; Aeinehchi et al., 2003; Patel and N. Cohenca, 2006; H.H. Hong et al., 2006; Iwamoto et al., 2006; Farsi et al., 2006; Bogen et al., 2008; Sawicki et al., 2008).
The studies performed by us in this research focused primarily on highlighting the changes happening at a cellular and sub-cellular level in dental pulp harvested from human teeth whose caries had been treated with MTA (Mineral Trioxide Aggregate), as compared to pulp originating from healthy teeth.

We intend to follow the steps of the tissue regeneration/healing of human dental pulp consequently to direct capping with white Angelus MTA (Brazil), by electron-microscopic study at regular intervals, in the period immediately following application of the material. We sought to identify the stages of healing and approximate the time required for each phase of the healing. We also sought to identify the cellular pulp changes from odontoblasts to elements of cellular immunity. Being knowledgeable on MTA’s biocompatibility, we wanted to observe how pulp cell elements relate to the material.

### Material and methods

For the investigations there have been used teeth that had been extracted for orthodontic purposes 6, 14 and 30 days after treatment of caries with MTA, as well as a healthy premolar extracted for the same purposes, which served as a benchmark.

Four teeth that were to be extracted for orthodontic purposes have been identified: two 3rd molars and two 2nd premolars. Plexal anesthesia has been done with 4% Articaine Ubistezin Forte® (3M ESPE GmbH). After the onset of the anesthesia, in the two molars and one of the premolars, 1st class cavities have been prepared under cooling, penetrating the pulp chamber. For this purpose, there have been used: a saline-cooled turbine, a size 018 globular diamond burr (DFS GmbH), to create access, and a size 018 reverse cone diamond bur (DFS GmbH), to remove the pulp cavity ceiling. After opening the pulp chamber, haemostasis was performed with sterile cotton pellets. After haemostasis, white Angelus MTA has been prepared, according to the manufacturer’s instructions, and applied with a special instrument over the small gap in the pulp chamber roof, in a 2-3 mm thick layer. 15 minutes have been granted for the material to set, after which a provisional Citodur ® (Doriot Dent) filling has been placed over. Then control radiographs have been taken.

After six days, the 3rd molar (1.8) has been extracted, under plexal anesthesia, with the same 4% Articaine Ubistezin Forte®. After extraction, the tooth has been cut at the cement-enamel junction with a sintered 345 disc (DFS GmbH), mounted to the straight handpiece. After removing the root, glutaraldehyde 2.7% had been injected into the pulp chamber and, after 5 minutes, the pulp has been removed with a No. 3 probe. The same has been repeated in the case of molar 2.8, premolars 1.5 and 2.5. The last one has not been subjected to the treatment, serving as witness.

As intended, after tooth extraction the pulp was removed to be studied by optical microscopy, by the semi fine section technique. For optical microscopy studies, the semi-fine section technique has been used, sections cut with the ultramicrotome, with a thickness of 500 nm. These sections were subjected to specific colouring for synthetic resins with the help of epoxy tissue stain. Examination of the sections was performed with an Olympus BX 51 microscope, image capture was accomplished with a CCD Media Cybernetics camera using Image Pro Plus software.

### Results

According to the work protocol, all dental pulp tissue fragments were processed using the specific methodology for studies by transmission electron microscopy (Ward, 2002; Craciun and Horobin, 1989; Hayat, 2000; Kay, 1967; Kuo, 2007; Ploaie and Petre, 1979; Watt, 1997; Weakley, 1981).

**The healthy dental pulp**

(Fig. 1-4)
Dental pulp is composed of embryonic tissue, helping with the nutrition and maintenance of tooth vitality and dentin formation. It is well vascularised and innervated, being a soft tissue, which retains its embryonic appearance in adults too. The dental pulp is situated in the pulp chamber and root canal and presents three regions: crown, root and apical. Being a conjunctive tissue it is made up of 3 components specific to any tissue: fundamental substance, fibers and cells (Cristea, 1991) (Fig. 1-4).

The fundamental matrix is gelatinous, much more abundant in the dental pulp of young people than in adults and especially elderly people. It is rich in water, glycosaminoglycans, proteoglycans and glicoproteins. The pulp acts as a medium to transport nutrients from vessels to cells and metabolites from cells to vessels (Nita, 1992; Toader-Radu, 1997; Toader-Radu, 1996).

The fundamental substance contains distributed the pulp cells and fibers (Fig.1-3), the blood vessels and nerve fibers (Fig.4).

Pulp fibers are reticulin fibers in young people and, later in adults, they are collagen type I and III fibers and are scattered among the pulp cells (Fig.2, 3). The fibers interlock and form a three-dimensional network with meshes of different sizes that house the pulp cells. On the edge of the pulp, towards the dentin (Fig.1), the fibers are more abundant and form the so-called Korff’s layer (Fig.1), that presents upward branches which mix with the odontoblasts and then get to the predentin and the dentin.

Pulp cells consist mostly of fibroblasts, more numerous in the central pulp. Their extensions also make up a smaller mesh network in young people and larger in adults and elderly people. In the meshes are located the other cells of the pulp, represented by lymphocytes, macrophages, plasmocytes, mastocytes, eosinophyls and neutrophyls, all being migrated blood cells. In addition, there are embryonic cells also. In our images, the most obvious are fibroblasts (Fig.2,3).

Towards the pulp periphery, i.e. the dentin, there are odontoblasts, cells that play a role in the synthesis, secretion and formation of dentin. They are numerous, arranged in a palisade, side by side, column shaped, each having its core at the base of the cell (Zhang, 1999) (Fig.1-4) and in the apical area, usually, a long column shaped extension, which penetrates the predentin, called Tomes extension, and then all extensions go into the dentin tubes, bearing the name of Tomes fibers here.

The odontoblasts actively synthesize and secrete, at predentine level, a number of collagen precursors and an amorphous material composed of glycosaminoglycans and glycoproteins, which together with the mineral salts, primarily the calcium ones, will make up the dentin matrix in the form of hydroxyapatite crystals arranged in a parallel manner (Stevens, Lowe, 1997).

The images obtained by us on the histological structure and layout of odontoblasts do not show their extensions into the predentin and dentin, due to pulp detachment of the apical area of the odontoblasts from the dentin at the time when they were collected for study. In the pulp there are also blood vessels and nerve fibers, which show a normal histological structure (Fig. 4).

The data presented above represent a picture of the normal structure of dental pulp and its components, as we found on the images taken by the light microscope.
From a general perspective, considering the resolution limitations of optical microscopy, no major changes appear in the pulp cells and fibers after a 6 day treatment with MTA, as compared to the situation presented above about the healthy dental pulp. However, the changes that came up, and visible in semi-fine sections, will be mentioned minutely further on.

The dental pulp contains a fiber and cell density comparable to that of the healthy pulp, noting that the nerve bundles are more present (Fig.5). It is worth mentioning that small nerve fibers arranged in small groups, have slightly twisted axons, which means their capacity to transmit nerve influxes is affected (Fig.6). The fundamental substance of these fibers presents small areas of lysis.

Both fibroblasts and fibers mainly located at the center of the pulp, have a structurally normal look (Fig.7). The blood capillaries either have many red blood cells in their lumen (Fig.8) or their lumen is devoid of blood elements (Fig.9).

As we approach the dentin, the number of fibroblasts and especially of the fibers increases, forming a denser and denser network (Fig.10).

In the area close around the dentin, many odontoblasts appear arranged in a palisade, with oval nucleus placed at different levels (Fig.11). The nucleus of each odontoblast has an oval, elongated shape in the longitudinal plane of the cell, it is placed towards the base of the cell, while the cell body is column shaped and extends towards the predentine, emitting extension that will penetrate the predentine. We mention the fact that in our images, the apical extensions of the odontoblasts seem interrupted, because of the pulp having been scraped and removed from the dentin during harvesting. In the odontoblasts’ cytoplasm, especially in the immediate supra-nuclear area lipid accumulation occurs (Fig.12), which may be due to the existence of damage in response to an inflammatory
process or as a result of the aging process or as an effect of a combination of the two.

The images taken of the area of the pulp that comes in contact with the floor of the cavity, respectively with MTA, reveal, 6 days after the treatment, bleeding areas here, with many extravased red blood cells from broken blood vessels (Fig.13). Their resorption and elimination by the macrophages is long lasting, but already there is an increase in the number of fibroblasts in the area and an intense collagen fiber synthesis (Fig. 14) to isolate the area and continue to participate in the formation of the roof/ceiling (bridge) in the coronal area of the pulp, towards the bottom of the cavity.

The conclusion is that 6 days after the treatment the effects of an inflammatory process in the pulp components are still present, especially in the contact area between the bottom of cavity, that is, the MTA and the neighboring pulp. Hence, to reach a workable balance between the two areas and restore full viability of the pulp with the formation of a roof/ceiling covering the MTA, a period longer than six days is required.
The histological structure of the dental pulp, 14 days after cavity treatment with MTA (Fig. 15 - 19)

In an overall look, in a cross-section performed at the border between the predentine and the pulp cavity, in the upper part towards the bottom of the caries, we notice the presence of the Tomes extensions, of the odontoblasts comprised in the mass of the predentine, under the bottom of the cavity (Fig.15). In a longitudinal section, we notice the area of the predentine containing these Tomes extensions (Fig.16). These images suggest that 14 days after treatment of the tooth decay with MTA, in the area of contact between cavity and the dental pulp, a plate is formed consisting of predentinal material, which also includes extensions of the subjacent odontoblasts.

The dental pulp contains numerous fibroblasts with an apparently normal structure, but also fibroblasts with a slightly affected structure, interspersed with active macrophages and neutrophyls (Fig.17). The appearance of the blood vessels and of most nerve fibers looks normal. There are, however, slightly affected nerve fibers, having not so straight disposal (Fig.18).

In the mid-marginal area of the dental pulp, towards the dentin, there are many odontoblasts with a normal structure, arranged in a palisade and with their cytoplasmic extensions directed towards the dentine from which the pulp was scraped (Fig.19). Probably, 14 days after treatment, the pulp-dentine plate below the caries is not totally rebuilt, only partially synthesized, as reported by Zarrabi et. al, 2010.
The histological structure of the pulp, 30 days after cavity treatment with MTA (Fig. 20-24)

The semi-fine sections made obliquely in the pulp tissue, taken from the contact area between the pulp and the dentine (Fig.20), show the Tomes extensions of the odontoblasts, which are apically made like Tomes fibers, that will enter the dentine tubes. At the base of the predentine, there are the odontoblasts, wrapped in a large mass of conjunctive fibers of the Korff layer (Cristea, 1991) (Fig.20). In the section where small areas of dentine have been discovered, we can see dentine tubes (Fig.21). Under these dentine areas there are the odontoblasts whose extensions penetrate the dentine.

In the dental pulp we find its cells and fibers, with normal representation in numbers (Fig.21), but with a slightly altered structure, especially the fibroblasts that do not have extensions.

In some peripheral areas of the dental pulp situated towards the dentine, the odontoblasts’ extensions seem broken because of the scraping during sampling (Fig.22).

In the coronal part of the dental pulp that comes in contact with the bottom of the cavity and with the MTA, it has been noted that, after 30 days of treatment with MTA, an insulating layer was formed that separates the two areas, the layer consists of numerous densely arranged collagen fibers, among which are found fibroblasts that synthesized them (Fig.23), fibroblasts that some authors have called "odontoblast-like" (Min et al., 2008; Zarrabi et al., 2010). Fibroblasts and the collagen fibers in this area were oriented parallel to the bottom of the cavity, that is, perpendicular to the side walls of the dentin (Fig.24). Certainly, out of the many fibers in this upper part of the dental pulp, some penetrate through the elements of MTA, there being a good biocompatibility.

In conclusion, after 30 days of treatment, the pulp-dentine plate is formed. Other authors (Min et al., 2008; Zarrabi et al., 2010) found that this plate was not complete two weeks after the treatment with MTA, but they also reported that, after a period of 60 days, it was completely formed, under the plate being present, besides fibroblasts and fibers, odontoblast-like cells, too, which tended to be arranged in a palisade.

Our results, at least the histological ones, suggest that, 30 days after treatment with MTA, the pulp-dentine plate would be almost established.
Discussions

Studies have shown that placing MTA on pulp tissue causes proliferation, migration and differentiation of odontoblast-like cells that produce a collagen matrix (Tziafas et al., 2002; Kuratate et al., 2008). The formed matrix is then mineralized and produces osteodentine and is followed by a tertiary dentin bridge formation, a few months after pulp capping.

Bortoluzzi et al., (2008) compared a mixture of MTAA powder and 10% calcium chloride (CC) or distilled water as pulpotomy agents on dog teeth. After 90 days, both groups showed favorable results in terms of hard tissue formation. However, in both groups the pulp exhibited inflammatory cells and angioblast proliferation. Nair et al (2008) have capped
intact maxillar and mandibular III molars with MTA and have observed them for 1, 4, and 12 weeks. Histological findings from this study showed that most samples treated with MTA were inflammation free after 1 week.

Based on these results, Sarkar et al. (2005) suggested that the biocompatibility, sealing ability, and the dentinogenic activity of MTA emanates from the physicochemical reactions between MTA and tissue fluids during the formation of hydroxyapatite.

Other studies have certified that MTAA induces odontoblast-like and undifferentiated pulp cell proliferation in rodents (Vajrabhaya et al., 2006). Investigators have reported that MTAA induce increased DNA synthesis in both cell types. It's been concluded that continuous release of ions from MTA provides optimal amounts of calcium for cell proliferation (Melegari et al. 2006). Koulaouziidou et al. (2005) have determined that MTA has the lowest antiproliferative activity. De Deus et al. (2005) have revealed that both Angelus MTA and gray MTA Angelus significantly inhibit endothelial cell viability in the first 24 hours.

Conclusions

Based on available information, it seems that MTA is the ideal material for direct capping in permanent teeth, compared to currently used materials, it being a bioactive material that has the ability to create an ideal environment for healing.

Data revealed, following this research, may provide new avenues of investigation for a better understanding of the mechanisms of action and manifestation of the processes that accompany the evolution and the long-term stability, and, respectively, the success of dental caries treatment with MTA or other hi-tech materials.

Our study can confirm the presence and formation of the collagen matrix in the predentine area, also described by Tziafas et al.(2002) and Kuratate et al. (2008). We cannot confirm the findings of the Nair et al.(2008) study either, which describes an inflammation free pulp phoenomena, a week after capping with MTA, given the fact that we found inflammatory elements even after 2 weeks.

Our research confirms the study made by Zarrabi et al.(2010) and Min et al.(2008), which describes the pulp-dentine plate formation after 60 days, emphasizing that we have shown the same thing, just 30 days after capping.

The findings of the study can be summarized as follows:

1. 6 days after the treatment, the effects of an inflammatory process in the pulp components are still present, especially in and around the area of contact between the dental pulp and MTA. The pulp only partially presents a normal structure, alterations being noticeable everywhere, both in the pulp matrix and in the vascular-nervous package from the coronal area of the coronal pulp. The presence of elements involved in phagocytosis indicates that the tissue is in a complex process of reconstruction

2. The structure of fibroblasts and odontoblasts, after 6 days, is still affected, as well as their physiological function;

3. After 14 days, in contact area between the MTA and the pulp, a plate made up of predentinal material has been formed, which includes extensions of the underlying odontoblasts.

4. After 14 days the pulp-dentin plate is almost completely restored, on the side towards the MTA, by the extensions of the odontoblasts depositing the predentinal material.

5. The ordered odontoblast mass in the lesion area, after 14 days, is meant to limit, it also through the neo dentinogenesis activity/process started, to repair it, the prerequisites and conditions of pulp healing being given.

6. After 30 days, the pulp-dentine plate is almost entirely rebuilt. The vascular and
nerve infrastructure is almost completely rebuilt.

7. The trend of odontoblasts, after 30 days, is to form a compaq layer. Predentine is disposed in a considerable layer, while odontoblasts’ extensions are piercing it through heading towards the capping agent.

References


Camp J.H., Diagnosis dilemmas in vital pulp therapy: treatment for the toothache is changing, especially in young, immature teeth, J. Endod. 34 (2008), pp. S6–S12.


