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MTA as modulator of periapical tissue healing in rat molar: A histological study

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ABSTRACT

Background: Periapical surgery has been suggested as a treatment option for teeth with periapical lesions when those lesions continue despite receiving root canal therapy. Since sealing the apical region is the operation's primary goal, the choice of the root-end filling material affects how the surgery turns out. The retrofilling materials Zinc Oxide Eugenol (ZOE) and Mineral trioxide aggregate (MTA) are both known to have antibacterial characteristics. The purpose of this study is to determine how MTA affects as a Modulator of Periapical Tissue Healing through histological examination in Rat Molar.

Methods: A dental fissure bur measuring 0.7 mm is used to remove the buccal root apex from the buccal alveolar bone's surface, creating the cavity. One of the following is placed within each cavity: Group 1: MTA, Group 2: ZOE. For each material series, six samples were used. We classified the healing outcomes for each MTA and ZOE retrograde filling material into three groups based on histological analysis: the amount of newly generated bone, the number of fibroblasts, and the infiltration of neutrophils into the surgical site.

Results: On the 6th day of examination, fibroblasts were seen in the area around the wound. A significant inflammatory response, including neutrophil infiltration, was seen around the ZOE after retrograde filling. On the 16th day, the new alveolar bone structure showed a slight increase. After filling the MTA on the 6th day of examination, the immediate inflammatory response was insignificant. Neutrophils were observed to enter the region surrounding the retrofilled MTA, and a small number of osteoclasts were observed to be resorbing bone. Around the wound site, fibroblasts can also be detected. On the 16th day, unlike ZOE, a lot of new bone grows close to this material.

Conclusion: MTA has the ability to modulate periapical healing in rat molar.

1. Introduction

An inflammatory reaction to bacteria above the tooth root and the failure of the root canal causes a periapical lesion. Chronic inflammation is a painful side effect of the body's defense mechanism against germs from the diseased root canal system. One or more of the frequent causes of periapical radiolucencies include trauma, caries, or tooth wear. Microorganisms may colonize pulp tissue if it loses blood flow as a result of trauma, which can result in periradicular disease. Identifying periapical lesions is necessary. And the endodontic status must be evaluated prior to treatment. The biological, physical, and chemical characteristics of a root canal sealer should be sufficient. The kind and substance of the sealant, which distinguish one sealer from the other, determines the treatment's efficacy. Sealers emit substances that interact with the periradicular tissue in different ways and cause different effects.[1–4]

Periapical surgery has been suggested as a treatment option for teeth with periapical lesions when they don't respond to orthograde root canal therapy. Since sealing the apical region is the operation's primary goal, the material selected for the root end filling affects how the surgery turns out. As a result, the substance used should have the following properties: biocompatibility, excellent marginal seal and attachment to root dentin, antimicrobial activity, low cytotoxicity, dimensional stability and compression resistance, appropriate setting time, and biomimetic characteristics under operational circumstances.[5,6] Mineral trioxide aggregate (MTA), a biocompatible and hydrophilic endodontic cement, can encourage osteogenesis and healing. It consists of a thin

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trioxide powder made of tricalcium oxide, silicon oxide, and bismuth oxide that hardens in the presence of humidity, together with additional hydrophilic particles like tricalcium silicate and tricalcium aluminate. The pH of a colloidal gel is 12.5, is produced when the powder is moistened, and it solidifies into a structure in 3–4 h.[7,8]

The MTA has recently found use in dentistry, namely regarding conservative and endodontic procedures. Unpredictable events like coronal trauma are difficult for medical professionals to handle. In order to help a patient who has experienced dental trauma, the dentist should be ready to act. The prognosis of the trauma itself can frequently be improved with early management. Sealing dentinal tubules should be the main goal in cases with coronal fractures with exposed dentine. Because dentinal tubules are large and numerous, especially in young patients, A large number of bacteria and their metabolites can spread to the pulp below and cause inflammation with even a tiny quantity of exposed dentin. This kind of incident could, on occasion, quickly result in necrosis.[9–11]

When dental trauma results in pulp exposure, managing the exposed pulp is the emergency intervention. In addition to endodontic therapy, other options include direct pulp capping or partial pulpotomy. Partial pulpotomy could be performed with MTA, because of the MTA's high compatibility, which is analogous to Zinc Oxide Eugenol (ZOE) action and makes it a potent antibacterial. Clinicians have been using zinc oxide eugenol sealers for a long time due to their antibacterial qualities; nevertheless, as they set, they shrink significantly and have a slow setting time. A pulp of blood vessels, nerves, and mature mucosal connective tissue consists of a cellular component. If a carious or traumatic injury to the tooth has resulted in an irreversible change of the pulp tissue and its necrosis, endodontic therapy is utilized. This approach can also be used in prosthetic rehabilitations where the dental component will almost certainly result in irreversible pulp change due to the significant decrease of dental tissue. To achieve a good root canal filling during endodontic therapy, the root canal system must be dry and all blood contamination must be avoided. In order to successfully complete pulp capping or perforation sealing, it is essential to limit bleeding and achieve a dry field.[12–15]

When MTA comes into exposure to human tissues, it can release calcium ions for cell development. Because of its alkaline pH, it also regulates the production of cytokines and supports an antibacterial environment. As a result, it promotes hard tissue-producing cells' migration and differentiation, which in turn results in the production of hydroxyapatite on the surface of MTA and the formation of a biological seal. Finally, the retrograde cavity needs to be fully dry before a surgical endodontic surgery. Because of its biocompatibility, antibacterial qualities, sealing abilities, and hydrophilic nature, this cement stands apart from all other materials now in use. It is crucial to comprehend how this biomaterial functions, how it behaves when in touch with other dental materials, and most importantly, what impact does it have on apical tissue and how do the cells around the material react.[4,16]

The purpose of this study is to determine how MTA affects as Modulator of Periapical Tissue Healing in Rat Molar.

2. Materials and methods

2.1. Ethics statement

This study has received approval from University Graduate Schools of Dentistry's Animal Care and Use Committees. All animal studies were conducted in accordance with the Animal Studies Guidelines and were conducted under sodium pentobarbital anesthesia with every effort made to minimize the suffering of the animals.

2.2. Animals

Male, 8 weeks old Wistar rats are a type of rats were used (n = 24).

2.3. Cavity preparation

Guideline for the first mandibular molar apicoectomy in rats. A dental fissure bur measuring 0.7 mm is used to remove the buccal root apex from the surface of the buccal alveolar bone, creating the cavity.

One of the following is placed within each cavity:

Group 1: MTA (Cerkamed bio MTA, Poland)

Group 2: ZOE (Endoseal Prevest DenPro, India)

For each material series, six samples were used.

2.4. Histochemistry

Rats were administered phosphate buffered saline with 4% paraformaldehyde, 6 days and 16 days following surgery. The samples were treated through a graded ethanol series and then embedded in paraffin after undergoing decalcification for around two weeks. Hematoxylin-eosin was used to stain four-micron serial sections. A light microscope (Nikon, Japan) was used to examine each segment.

2.5. Histological evaluation

We classified the healing outcomes for each MTA and ZOE retrograde filling material into three groups based on histological analysis: the amount of newly generated bone, the number of fibroblasts, and the infiltration of neutrophils into the surgical site.

2.6. Statistical analysis

The relevance of the data changes in Rat's alveolar bone after cavity preparation between the treatment and control groups was defined using a T-test.

3. Results

3.1. ZOE

On 6th day examination, fibroblasts were seen in the area around the wound (Fig. 1A). A significant inflammatory response, including neutrophil infiltration, was seen around the ZOE after retrograde filling (Fig. 1B). On the 16th day, the new alveolar bone structure showed a slight increase (Fig. 1C).

3.2. MTA

After filling the MTA on day 6, the immediate inflammatory response was insignificant. Neutrophils were observed to enter the region surrounding the retrofilled MTA (Fig. 2A), and a small number of osteoclasts were observed to be resorbing bone. Around the wound site, fibroblasts can also be detected (Fig. 2B). On day 16, unlike ZOE, a lot of new bone grows close to this material (Fig. 2C).

The mean and standard deviation of the amount of Fibroblast, Neutrophils and new Bone in Wistar Rats periapical tissue is shown in Table 1 and Table 2. Every studied group's result was $p > 0.05$, indicating that all of the data had a normal distribution. A boxplot diagram is used in Fig. 3 to summarize the research findings.

4. Discussion

The biomechanical preparation, root canal system disinfection, intracanal medications, kind of root canal sealers, excessive sealer extrusion, and apical amount of obturation are just a few of the variables that affect the healing of the periapical healing lesion. It is believed that intimate sealing ability, The essential qualities of an appropriate retrograde filling material include insolvability, mechanical strength, biocompatibility, and encouragement of tissue regeneration without causing inflammation. Bone healing is regulated by two interconnected

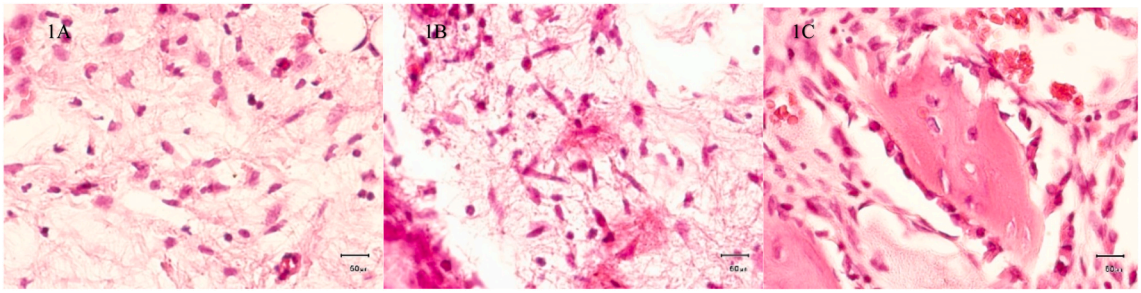

Fig. 1. Fibroblasts (1A) and Neutrophils (1B) were seen in the wound area on the 6th day following the administration of ZOE, and new alveolar bone formation (1C) was visible on the 16th day.

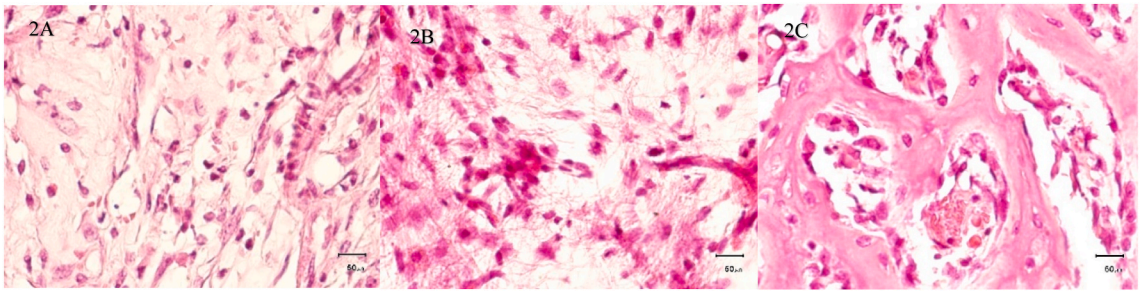

Fig. 2. Fibroblasts (2A) and Neutrophils (2B) were seen in the wound area on the 6th day following the administration of MTA, and new alveolar bone formation (2C) was visible on the 16th day.

Table 1
Mean amount of Fibroblast and Neutrophils on day 6th in MTA group and ZOE group.

Group	X±SD	X±SD
	Fibroblast	Neutrophil
MTA	27.33^{a}±1.03	6.00^{a}±1.41
ZOE	19.66^{b} ± 1.86	16.33^{b} ± 1.96

Note: different superscript showed significant differences ($\alpha < 0.05$).

Table 2
The mean number of new alveolar bones on day16^{th} in MTA group and ZOE group.

Group	X±SD
	Day 16
MTA	3.50^{a}±0.54
ZOE	1.50^{b} ± 0.54

Note: different superscripts showed significant differences ($\alpha < 0.05$).

mechanisms: bone resorption and new bone formation, which are controlled by osteoclasts and osteoblasts. These two processes are involved in the healing of the periapical area including alveolar bone.[17,18]

In this study, we looked into a few key traits of different retrograde filling materials. Histology data from the MTA and ZOE groups were analyzed to see whether there was any possibility of finding a correlation or difference between the two sets of data. This study employed a histological technique, wherein the amount of neutrophils, fibroblasts, and new bone in ratio can be estimated from histology data, to employ parametric statistical computations. As was previously described, when we used ZOE as a retrograde substance, many macrophages formed around it, this could have been brought on by the ZOE itself or by the humidity before setting may have been what caused these consequences. Additionally, there were many neutrophils seen in this tissue. The initial dissolving is caused by the ZOE susceptibility by the host's defense mechanism against the foreign object. However, it was believed that the ZOE did not prevent bone development because the new bone was seen two weeks following the operation. It was observed that in the ZOE materials, an intense inflammatory reaction was present at 1 week but was gone at 2 weeks. These outcomes were nearly identical to those from a prior study when rats' tibias were implanted with ZOE. When in contact with connective tissue, the irritating ZOE cement is known to cause an inflammatory response. Eugenol leaching from ZOE complexes may be responsible for this outcome. Inflammatory cytokines may be generated in an inflammatory response as a result of eugenol production's unintended consequences.[19,20]

MTA is a substance with physicochemical characteristics that promote matrix synthesis and mineralization while promoting restorative dentinogenesis through the recruitment and activation of cells that produce hard tissue. By isolating soluble cytokines entrenched in the surrounding root dentine from growth factors in the extracellular matrix that mediate wound repair of the dentine-pulp complex, it induces the creation of restorative hard tissue. Fibroblasts move from the central pulp to the wound site when MTA is present. It encourages the differentiation of progenitor cells into odontoblast-like cells and the multiplication of progenitor cells without inducing cell death. Additionally, MTA raises the protein expression of mineralized matrix genes, which are crucial for mineralization, and cellular indicators. It also stimulates the synthesis of in vitro mRNA. Along with transcription and angiogenesis factors, the biocompatibility of MTA that is set controls the expression of genes that produce dentin sialoprotein, osteocalcin, and alkaline phosphatase. Odontoblast signal proteins play a critical role in the differentiation of progenitor cells into cells that accumulate and repair hard tissues. Following the treatment with MTA, sialoprotein and osteopontin were found in the calcified hard tissue matrix in the pertinent area.[21,22]

Among the materials we looked at, the specimens packed with MTA displayed the most favorable histology reaction. Compared to ZOE, better outcomes were seen in the amount of fibroblasts and the growth

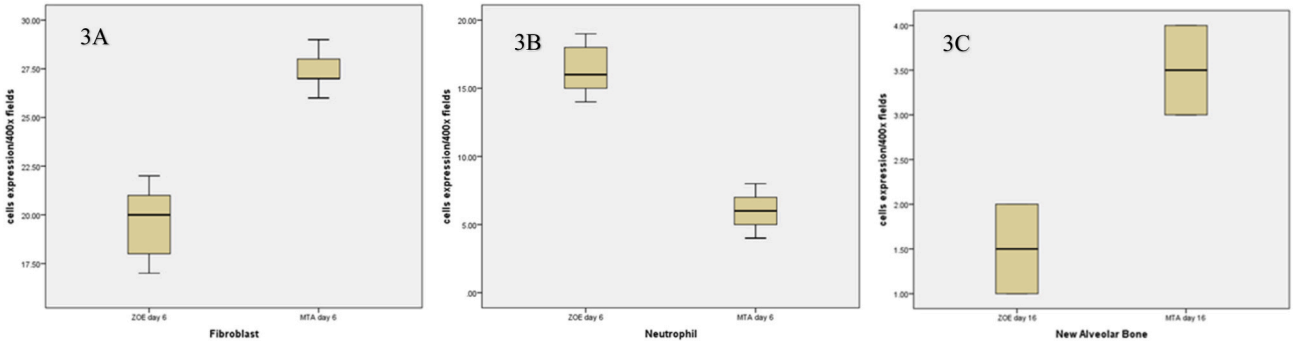

Fig. 3. Data for each treatment group between ZOE and MTA on days 6 and 16 are shown in a boxplot diagram as fibroblasts (3A), neutrophils (3B), and new bone growth (3C).

of new alveolar bone. This filling materials appeared to be biocompatible and did not cause significant inflammation or prevent the growth of new bone. MTA might not prevent tissue healing and might even be biocompatible. Some substances that were released from these compounds might hasten tissue healing. The findings of this study indicate that MTA has biocompatible qualities, making it a potentially useful material for retro fillings.

Even though the study's 16-day histological examination revealed an increase in the amount of alveolar bone formed, the researchers think that more research is necessary to fully understand the impact of MTA on bone repair. This research should look at bone formation over a longer time period and analyze other parameters.

5. Conclusion

MTA has the ability to modulate periapical healing in rat molar, seen in its capacity to regulate the periapical region's alveolar bone regeneration. The use of MTA in the apex area of the tooth shows that this material is very biocompatible, even though this material is in contact with the soft tissue mucosa and hard tissue around the apical area of the tooth, it does not show a negative response, there is faster and denser alveolar bone formation.

Declaration of competing interest

None.

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