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Microtomographic and histological evaluation of two bioceramics as pulp capping agents *in vivo*.

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ABSTRACT

Maintaining pulp vitality and function is a priority of the medicaments employed in pulp therapy to preserve tooth integrity. ***Aim:*** *This study evaluated inflammatory response and reparative dentin bridge formation after direct pulp capping with two different bioceramics.* ***Materials and Method:*** *This was an in vivo controlled experimental study on 12 male Wistar rats. Pulpotomies were performed and the exposed pulps were capped with Biodentine or Neo MTA. After 15, 45 and 90 days, maxillary segments were obtained and prepared for histologic analysis and Micro-CT. Hounsfield Units (HU) were quantified.* ***Results:*** *Micro-CT analysis showed greater mineralization at 90 days with Neo MTA than with Biodentine. HU did not differ significantly ($p > 0.05$) between molars treated with Biodentine and Neo MTA at 15 and 45 days, but at 90 days, there was statistically significant difference ($p < 0.05$) between them. Reparative dentin was observed near the pulp exposure and canal orifice with both bioceramics. At 45 and 90 days, molars treated with Neo MTA showed mineralized tissue filling the canal orifice. Molars treated with Biodentine showed mineralized tissue and dentin bridge at the site of exposure at 45 days, and total pulp exposure coverage and mineralized dentin matrix at 90 days.* ***Conclusions:*** *Biodentine and Neo MTA induce the formation of reparative dentin bridge after 45 days with inflammatory cell infiltrate.*

Keywords: *endodontic repair materials - bioceramics - cellular inflammatory infiltrate - reparative dentin*

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Evaluación microtomográfica e histológica de dos agentes biocerámicos como recubrimiento pulpar *in vivo*

RESUMEN

Mantener la vitalidad pulpar y su función es una de las prioridades de los medicamentos utilizados en la terapia pulpar con la finalidad de preservar la integridad del diente. ***Objetivo:*** *El objetivo de este estudio fue evaluar la respuesta inflamatoria y la reparación del puente dentinario con dos biocerámicas.* ***Materiales y Métodos****: Se realizó un estudio experimental in vivo en 12 ratas Wistar formando dos grupos de estudio (n = 6), en las que se realizaron pulpotomías. Posterior a 15, 45 y 90 días, se obtuvieron segmentos de los maxilares y se prepararon los especímenes para análisis histológicos y cortes microtomográficos. Las Unidades Hounsfield (UH) se cuantificaron.* ***Resultados:*** *El análisis microtomográfico mostró un incremento en la mineralización después de 90 días con Neo MTA comparado con Biodentine. No existió diferencia significativa ($p > 0.05$) entre las UH posterior a 15 y 45 días, sin embargo, a los 90 días hubo diferencia significativa ($p < 0.05$) entre Biodentine y Neo MTA. A los 45 y 90 días los molares tratados con Neo MTA mostraron la formación de tejido mineralizado en el orificio comunicados. Los molares tratados con Biodentine mostraron la formacion de tejido mineralizado, a los 45 dias se observó un puente de dentina en el sitio expuestos y una cobertura total de la exposición pulpar y una matriz de dentina mineralizada a los 90 días.* ***Conclusiones:*** *Biodentine y Neo MTA inducen la formación del puente dentinario reparador posterior a 45 días con infiltrado de células inflamatorias.*

Palabras clave: *materiales de obturación endodóntica - biocerámicos - infiltrado celular inflamatorio - dentina de reparación*

INTRODUCTION

The purpose of pulp therapy is to repair the pulp tissue to preserve its vitality and function after it has been damaged[1]. The treatment consists of removing the inflamed or damaged pulp tissue and sealing the pulp cavity with biocompatible bioactive materials and dental tissues to promote the formation of reparative dentin or dentinal bridge, thereby preserving pulp viability[2].

Treatments to promote dentin bridge formation can still be challenging. The dentin bridge is considered the histological indicator of a subsequent healing process after exposure of the pulp tissue. It should seal the pulp tissue to protect it and prevent microleakage, thereby preventing recurrent infections[1].

Many different materials with appropriate physicochemical and biological properties are used to repair deciduous and permanent teeth. Materials based on calcium hydroxide (CH), such as mineral trioxide aggregate (MTA Plus (Prevest DentPro) and Biodentine (Septodont Ltd)), are considered regenerative. These cements can increase calcium ion concentration, aiding hard-tissue formation. CH has antimicrobial activity and promotes hard-tissue formation, but is highly soluble, has poor sealing ability and lacks adhesion. MTA has good sealing properties and biocompatibility, but becomes discolored, has long setting times and is expensive[3]. MTA-based cement and Biodentine have demonstrated high clinical success rates in treatments which would formerly have had reserved prognoses, such as root resorption, perforation, and direct pulp capping[4]. MTA Plus (Avalon Biomed Inc.) is similar in composition to the original MTA, but has been replaced by Neo MTA Plus, which contains tantalum oxide instead of bismuth oxide as a radiopaque agent, and an excellent inorganic powder of tricalcium and dicalcium silicate, which, when mixed with the water-based gel, initiates the setting reaction[5].

Biodentine, a calcium silicate-based dentin substitute, has a liquid phase and a powder phase. The powder contains tricalcium silicate, calcium carbonate and zirconium oxide, while the liquid contains water, calcium chloride as a setting accelerator, and a modified polycarboxylate[6].

Advances in restorative materials should promote the development of specific therapies and materials designed to regenerate dental pulp, even in complex clinical situations[7]. There is thus a need to evaluate the therapeutic potential of newly available bioactive materials by verifying and assessing their potential to promote tertiary dentin formation and tissue repair and regeneration. However, there is no consensus regarding how long after the application of Biodentine and Neo MTA to observe dentin bridging and inflammatory response.

This study evaluated the inflammatory response and reparative dentin bridge formation in an animal model 15, 45, and 90 days after direct pulp capping with two different bioceramics.

MATERIALS AND METHOD

The study design was approved by the Internal Committee for the Care and Use of Laboratory Animals of the Faculty of Dentistry of the National Autonomous University of Mexico (027-CIC-2019) and followed the parameters established in the Official Mexican Standard NOM-062-ZOO-1999[8].

Pulp capping procedure

Twelve male Wistar rats (weighing 240-300 g) were used for direct pulp capping experiments. Rats were sedated with an intramuscular injection of Ketamine (ANESKET®) (80 mg/Kg) and Xylazine (PROCIN®) (10 mg/Kg).

After cleaning and disinfecting the right and left maxillary first molars with 0.2% chlorhexidine digluconate solution for one minute, a class-I cavity with pulp exposure was prepared on the occlusal surface of each, using a steel round bur (FG1/4 MDT®) with a low-speed electric micromotor (NSK Surgic AP), under constant irrigation with sterile saline solution. One experienced operator performed all the procedures to establish a stable, standard-sized cavity (approximately 0.4 mm diameter and depth). Drilling time for each sample was about 10 seconds (Fig. 1a-b). Bleeding of the pulp communication was controlled by applying light pressure with sterile cotton pellets for a few seconds.

The exposed pulp was directly capped with Neo MTA (NuSmile, Ltd.) on the right maxillary first molars, and Biodentine (Septodont) on the left maxillary first molars, following the manufacturers' protocols. After direct pulp capping, dental etching was performed with Scotchbond™ Etchant Phosphoric Acid (3M ESPE) for 15 seconds. Single Bond Universal (3M™ ESPE) was applied as directed by

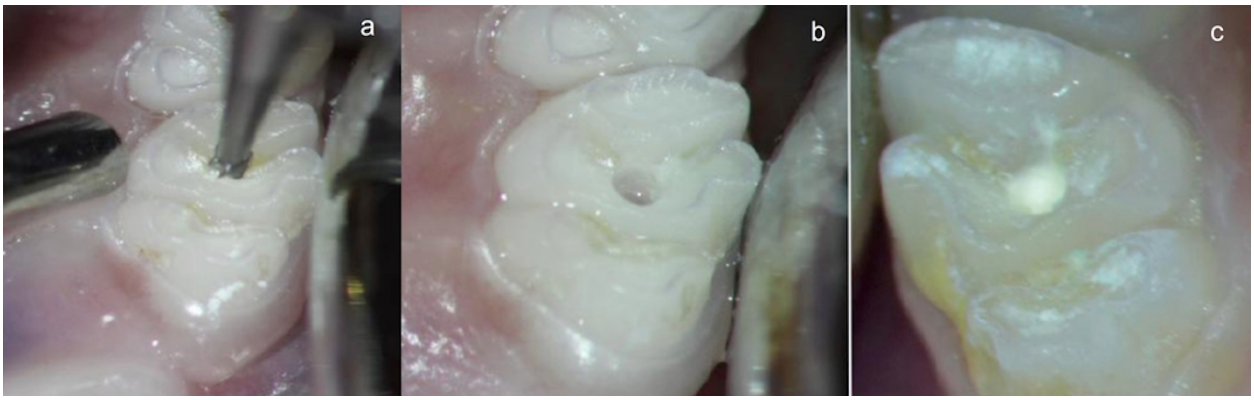

Fig. 1: Vital pulp therapy in a rat molar. ***a)*** *Preparation of the class-I cavity on the occlusal surface,* ***b)*** *Pulp communication,* ***c)*** *Placement of materials as direct pulp capping and resin filling.*

the manufacturer, and the cavity was restored with Filtek Flow Z350 XT (3M ESPE) (Fig. 1c).

After the restorations were completed, all the rats were evaluated clinically and weighed, and it was found that there was an increase in volume, bleeding, fistulization, and dental mobility. The rats were divided into three groups (n = 4) and euthanized at 15, 45 or 90 days after direct pulp capping.

Micro-CT Analyses

Micro-CT images were used to verify the presence of the bioceramic materials in the region of the pulp chamber, and the sealing they provided in the coronal portion of the molars, while the animals were under inhalation anesthesia with Isoflurane at a concentration of 2/100 oxygen. The images were acquired with a micro-CT scanner (Albira ARS II PET/CT). The field of view was the maxillary area, with current 0.4 mA, voltage 45 kV, and 1,000 projections to obtain high-resolution images (CT-High-Resolution). Hounsfield Units (HU) were calculated by tracing a circular ROI of 60 mm^2 on each micro-CT image on the axial plane, using the OsiriX MD DICOM Viewer program in the cavity area per period. Two-way ANOVA with Sidak's multiple comparisons test was performed with a p-value of 0.005. GraphPad Prism 9.4.1 was used.

Histological evaluation

After the established periods, the molars were dissected and fixed in 10% formaldehyde for 24 h at 4 °C. Then, they were demineralized in Evans and Krajian solution for 5 days at 4 °C. Finally, they were dehydrated in a series of alcohol solutions ranging from 50%, 60%, 70%, 80%, 90%, (10 minutes each) to 100% (three 15-minute changes) and embedded in paraffin. Sagittal sections (7-µm thick) were cut and stained with hematoxylin and eosin and evaluated at different magnifications.

RESULTS

Micro-CT analysis of dentin formation

No abnormal radio-dense region indicating the development of a periapical lesion was observed in any of the micro-CT images. The micro-CT images showed similar behavior at 15 and 45 days in the molars treated with Neo MTA (Fig. 2a-b) and Biodentine (Figure 2d-e). At 90 days, molars treated with Neo MTA (Fig. 2c) had greater mineralization than those treated with Biodentine (Fig. 2f).

The HU analysis demonstrated similar behavior at 15 and 45 days in the molars treated with Neo MTA and Biodentine, and a statistically significant difference at 90 days ($p < 0.05$), when HU was higher in molars treated with Neo MTA than in those treated with Biodentine (Fig. 3). In molars treated with Neo MTA, HU increased from an average of 18173.3 ±337.7 on day 15, to 19735.4 ±1588.5 on day 45, and 2209.4 ±2709.6 HU on day 90. In molars treated with Biodentine, HU increased from 19313.9 ±1133.7 on day 15, to 20168.0 ±623.8 on day 45, and decreased to 19450.1 ±2952.1 on day 90 with no statistical difference compared to day 45. Likewise, there were no statistical differences ($p > 0.05$) between molars treated with Neo MTA and those treated with Biodentine at 0,15, 45 and 90 days (Fig. 3).

Morphological findings

At 15 days after pulp capping, hard tissue formation

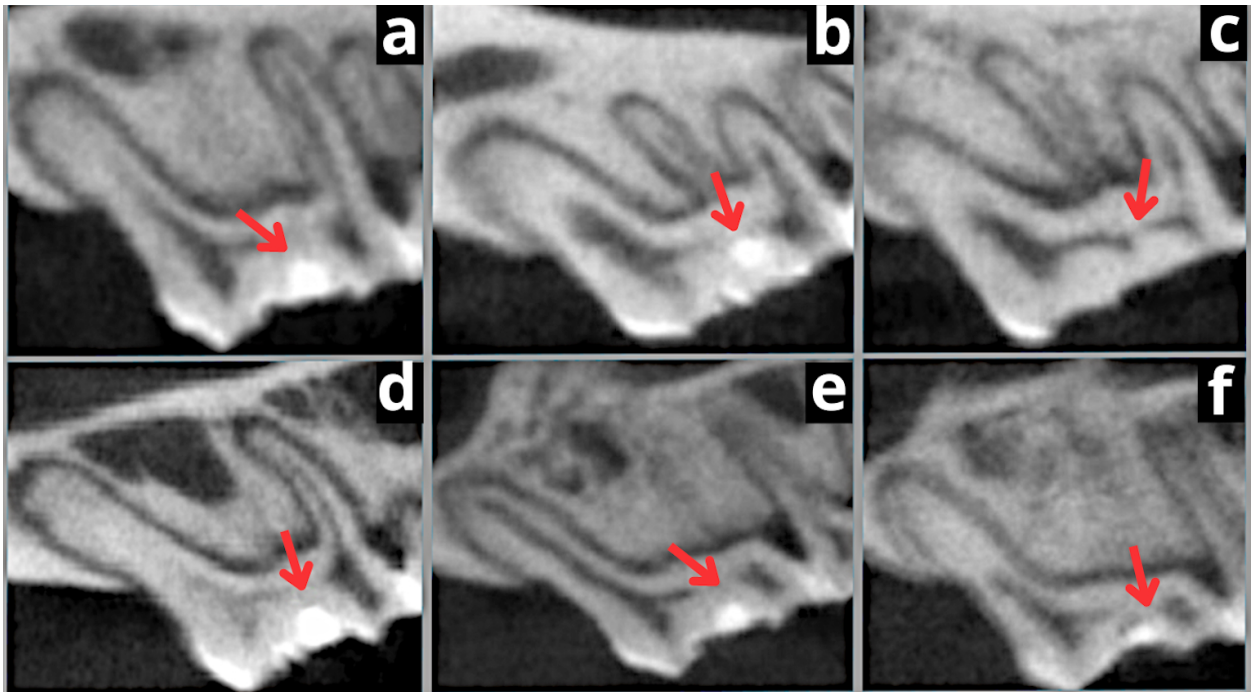

*Fig. 2: Micro-CT images of pulp-capped rat molars with Biodentine after 15 (**a**), 45 (**b**) and 90 (**c**) days. arrows indicate communication and radio-dense areas. images with Neomta after 15 (**d**), 45 (**e**) and 90 (**f**) days. Arrows indicate dental bridge formation.*

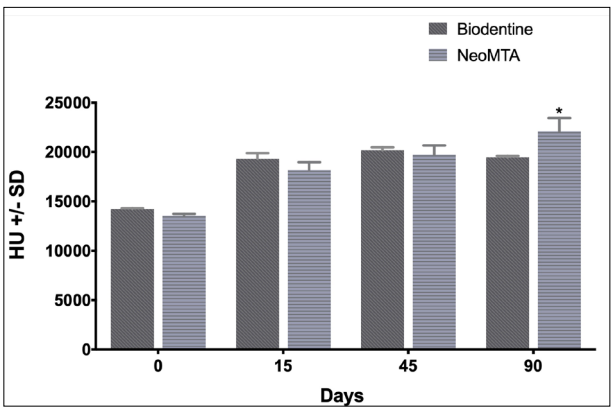

Fig. 3: Average HU values with Biodentine and NeoMTA.

and reparative dentin were observed near the pulp exposure site and adjacent to the existing dentine in molars treated with Biodentine and Neo MTA (Fig. 4a-f and 5a-f, respectively).

At 15 days after treatment, an inflammatory infiltrate with polymorphonuclear cells, and macrophages were observed in both groups. After 45 and 90 days, both groups displayed chronic inflammatory-cell infiltration of the coronal pulp near the border of the newly formed dentin. However, the pulp tissue in other areas, such as the pulp horns, was similar to normal pulp tissue, and there was no sign of pulp necrosis. Molars treated with Biodentine exhibited less inflammation and the best pulp-tissue reaction. Both groups showed incremental lines of dentin throughout the experiment.

At 45 and 90 days, both groups exhibited reparative dentin formation. However, the quality of the reparative dentin was better witeh Biodentine than with Neo MTA.

With Biodentine, the histological results showed that after 15 days, the dentinal bridge of reparative dentin calcified above the pulp tissue. The border between the wound of the pulp chamber and the dentin was covered in incremental lines of reparative dentin (Figure 4a-b), and the pulp tissue under the newly formed tissue below the cavity floor consisted of odontoblast-like cells adjacent to the reparative dentin made of incremental lines of mineralized tissue and possible cell inclusions. The pulp area showed few inflammatory cells (Fig. 4b).

At 45 days after pulp capping, the area of the cavity was visible (Fig. 4c-d), there was an area of irregular mineralized tissue above the pulp wound area, and the pulp area had a rich cell zone of odontoblast-like cells with a degenerative area at the border of the dentin (arrowhead) with congested capillaries, and a mild-to-severe inflammatory reaction above vital pulp tissue. The pulp tissue contained elongated, columnar odontoblast-like cells. Regarding morphology, the cell bodies extending from the

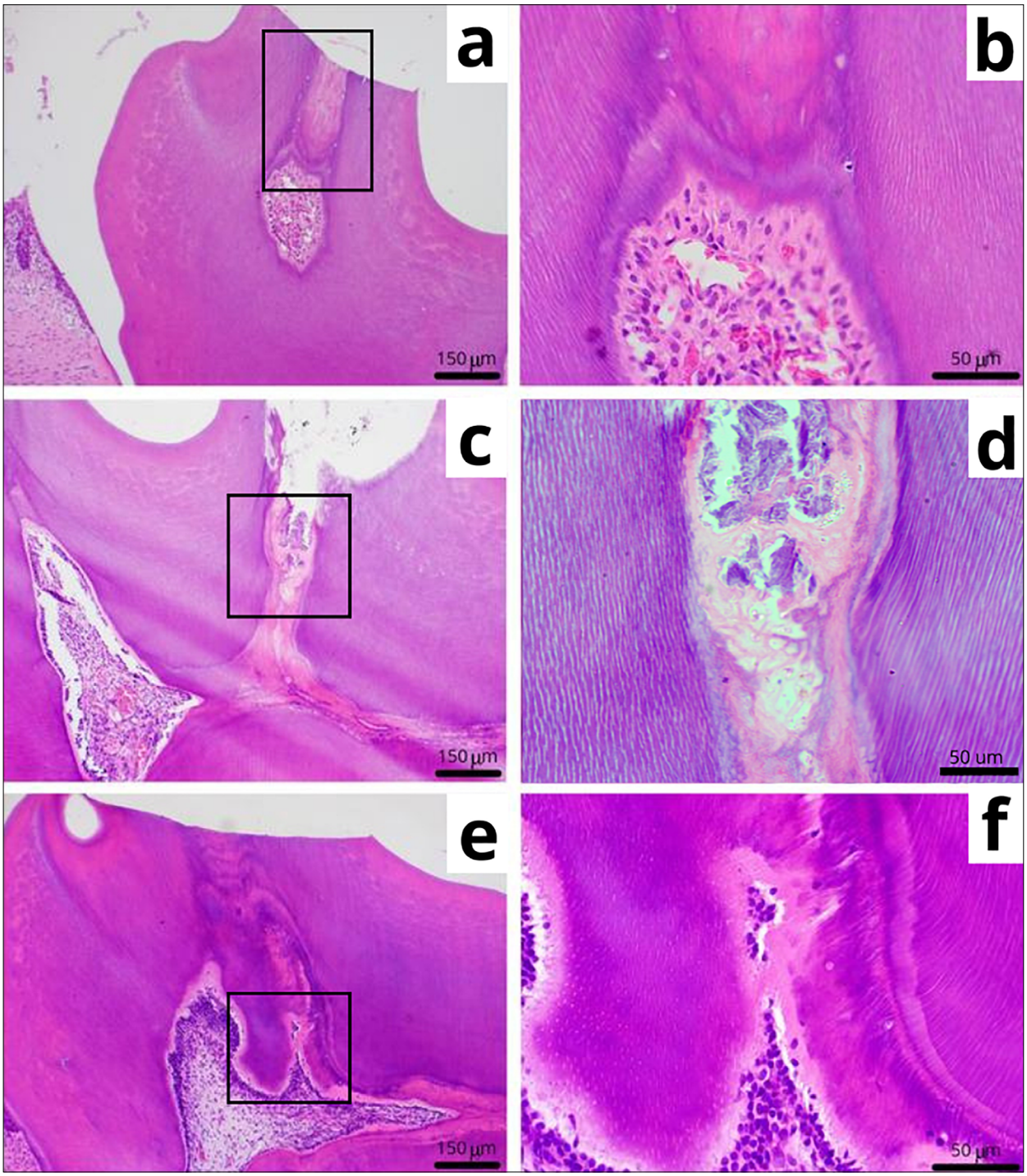

*Fig. 4: Histological features of pulpotomy with Biodentine. The boxes indicate the subsequent magnification (x20 and x40) after 15 days (**a-b**), 45 days (**c-d**) and 90 days (**e-f**). Magnification bar = 150 µm, 50 µm.*

pulp tissue to the dentin-like tissue contained large nuclei, and there were small capillary blood cells, and fibroblasts surrounded by collagen fibers.

At 90 days after direct pulp capping (Fig. 4e-f), there was a mineralized matrix of dentin at the pulp border, with irregular shape and different degrees of mineralization, and with sparse, twisted dentinal tubules in the area of the cavity. The pulp area had decreased in size, and the inflammatory response had finally resolved near the border of the dentin area and the pulp area. A zone comprising dentin, odontoblasts and odontogenic progenitor cells was

observed. The odontogenic-like cell area was a rich cell zone, and the underlying pulp tissue contained progenitor cells, fibroblasts with large oval nuclei, as well as small capillary blood cells, and loosely arranged collagen fibers.

With Neo MTA, at 45 days after direct pulp capping, the histological results showed a bridge of mineralized tissue resembling bone, with different levels of mineralization above the pulp tissue. The pulp chamber had shrunk. At higher magnifications (x20) (Fig. 5c), there were incremental lines of dentin at the pulp-tissue border of dentin resembling bone. Figure 5d (at x40) shows pulp tissue under the newly formed tissue beneath the

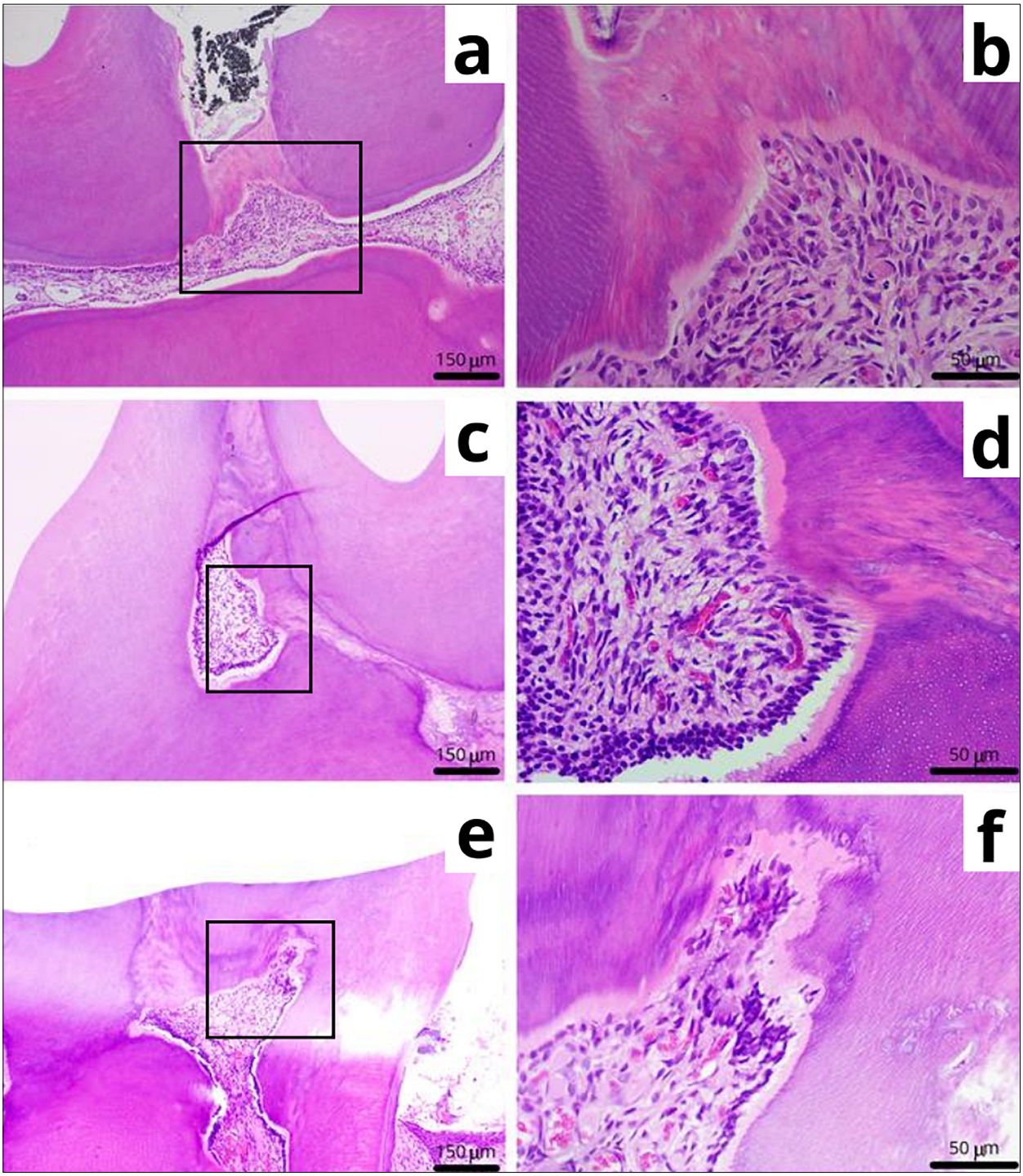

*Fig. 5: Histological features of pulpotomy with NeoMTA. The boxes indicate the subsequent magnification (x20 and x40) after 15 days **(a-b)**, 45 days **(c-d)** and 90 days **(e-f)**. Magnification bar =150 µm, 50 µm.*

cavity floor, consisting of odontoblast-like cells with few inflammatory cells.

At 90 days, reparative dentin was observed in localized areas under the cavity preparation, and the dentin-like tissue appeared irregular with sparse twisted tubules Fig. 5e (x20). Fig. 5G (x20) shows odontoblast-like cells adjacent to reparative dentin with incremental lines. The odontoblast-like cells appear columnar and elongated in morphology. At higher magnification (Fig. 5f x40), an area of incremental lines of dentin with different degrees of mineralization was evident, as well as disorganized pulp tissue with loose connective tissue and small capillary cells with mild congestion in otherwise healthy pulp tissue.

DISCUSSION

Previous studies have examined direct capping materials according to their ability to induce repair by forming mineralized tissue and the dentin bridge, which indicates a pulp healing process[9]. In the present study, the bioceramic materials Biodentine and Neo MTA were utilized as direct capping materials in an in-vivo model. Both materials resulted in the formation of a dentine bridge with an inflammatory cell infiltrate, and absence of bacterial contamination.

The literature has reported that dentin-bridge formation is detected in human teeth at 6 weeks. It involves different mechanisms, including cell response to calcium, differentiation of odontoblasts, odontogenesis, dentinogenesis, and dental mineralization[10]. In animal models, the formation of a dentin bridge has been observed at 120 days after placing Biodentine in furcation perforations in beagle dogs[11]. In the current study on rat teeth, when Neo MTA was used, a dentine bridge was observed at 90 days in Micro-CT images. Other studies have demonstrated that Biodentine effectively induces reparative dentin when it is placed directly on mechanically exposed pulp tissue in rat teeth, exhibiting well-defined characteristics at the damaged site[12]. Kim et al.[13] placed Biodentine in rat teeth for 4 weeks and observed irregular heterogeneous distribution of mineralization nodules within a uniform thickness of a rigid tissue barrier. In contrast to our results, Candeiro et al. found that Biodentine and Neo MTA showed mineralized pulp healing process[9].

In the present study, both Biodentine and Neo MTA provided homogeneous seal (Figure 5a and b). This agrees with Ricucci et al.[14], who demonstrated with histological evidence that when the primary odontoblast dies or is absent due to pulp exposure, it does not regenerate. Therefore, the tissue resulting from reparative dentinogenesis lacks the tubular structure typical of dentin, and fibroblasts from the pulp calcify the new tissue during this repair process. Shayegan et al.[15] examined the pulp response after a pulpotomy in primary pig teeth at 7, 28 and 90 days using Biodentine, white mineral trioxide aggregate (WMTA) or formocresol. They found that Biodentine and WMTA are both suitable biocompatible materials. Nowicka et al.[16] evaluated the volume of reparative dentin bridges formed after direct pulp capping in human third molars and concluded that the complete reparative dentin bridge dependent on the material used demonstrated that Biodentine and MTA resulted in the formation of bridges with a significantly higher average volume compared to dentin bonding systems. Therefore, each material had different degrees of influence on dentin bridge formation, and both Biodentine and MTA generated reparative pulp responses. De Rossi et al.[17] performed pulpotomies on dog teeth, achieving success rates of 96.8% in treatments with Biodentine and 72.2% in those using MTA. Other articles also conclude that there is no significant difference between Biodentine and MTA in terms of clinical success[18,19].

Quiñones et al.[20] compared the biocompatibility of the regeneration of the dentin–pulp complex in a murine model with MTA Angelus, Neo MTA, and TheraCal PT. Histologically, they observed no cellular inflammatory infiltrate, but after 15 days, inflammatory cell infiltrate was slightly higher in teeth treated with Neo MTA than with MTA or TheraCal PT. However, at 30 and 45 days, all three materials had grade 1 of slight inflammatory infiltrate. Tziafa et al.[21] reported in an animal study that the dentin bridge thickness produced with use of Biodentine, at three and eight weeks, was significantly higher than that produced with use of MTA, which is consistent with the findings of the present study.

The differences found between the bioceramic materials could be related to the handling of the materials, which may have affected treatment outcomes. Biodentine presentation is in a capsule, which is eventually mixed with an amalgamator;

whereas the Neo MTA powder and liquid must be mixed by hand, which increases the chance of potential errors in the final preparation of the cement[22]. The setting time of MTA cement is longer, and moreover, the initial setting releases toxic substances into the cells, increasing the toxicity of the cement[23,24].

After setting, Biodentine has denser microstructure and less porosity than MTA cement, enabling more calcium ions to be released, and ultimately, more hard tissue was formed[6]. The results of the present study agree with previous reports that found superior results in teeth treated with Biodentine. Concerning cell biological response, the proposed mechanism of action that regulates the induction of reparative dentin and its quality is currently an active area of research. However, the molecular mechanisms remain poorly understood.

CONCLUSION

Based on the results and methods used in this study, Biodentine and Neo MTA induce the reparative dentin bridge formation with differences in the thickness and morphology of the hard tissue formed, and both produce a cellular inflammatory infiltrate.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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