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ORIGINAL RESEARCH

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Evaluation of the biological effect of mineral trioxide aggregate in inflamed pulp—*in vivo* analysis

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Abstract

The health of dental pulp tissue is critical to maintaining normal tooth function from the eruption of permanent teeth to the formation of the apex. The study evaluated the inflamed pulp response to the mineral trioxide aggregate (MTA) after direct pulp capping with the mechanical pulp exposure in rats' incisor. Forty-eight mandibular central incisors of twenty-four Sprague-Dawley rats which were prepared with the cavities of one mm diameter, and the pulp exposures were randomly assigned into two groups: MTA group and calcium hydroxide ($Ca(OH)_2$) group. The direct pulp capping was performed after three days and samples histological observations conduction within eight weeks. In both MTA and $Ca(OH)_2$ groups, dentin -like structures were observed in the pulp tissues of some teeth. The number of teeth with reparative tissue in MTA group was statistically significantly higher than that in $Ca(OH)_2$ group ($p = 0.041$). Inflammatory cell infiltration was found in the crown pulp tissues in two groups, and no statistical difference was observed between the two groups ($p = 0.243$). Pulp necrosis occurred in both groups, and there was no statistical difference between the two groups ($p = 0.622$). The results in this paper suggest that MTA promotes direct pulp capping and hence has certain potential clinical applications value in the treatments for the preservation of inflamed pulp.

Keywords

Pulpal tissue; Inflammation; Direct pulp capping; Mineral trioxide aggregate; Calcium hydroxide

1. Introduction

In humans, the eruption of the first permanent tooth typically occurs between the ages of six and twelve, and the transition from deciduous to permanent teeth is then complete [1]. Although the morphology of the crown has been completed when a permanent tooth erupts, its organizational structure is not yet fully mature, with the manifestation of short roots, thick root canals, rich blood supply of pulp tissue, and low mineralization level [2]. After a tooth erupts, its dental pulp tissue continues to differentiate into odontoblasts, forming dentin in the root, gradually elongating the root, and then forming a narrow apical foramen [3]. Therefore, the reparative and regenerative ability of dental pulp tissue between permanent tooth eruption and apical formation is of is critical for tooth development [4]. Teenagers with developing pulp tissues are vulnerable to the damage of newly erupted permanent teeth, particularly maxillary central incisors, due to a high level of physical activity and a lack of self-protection awareness [5, 6]. For the treatment of young permanent teeth after crown fracture, because the root is not completely formed, the choice of treatment plan should first consider whether the root can continue to develop. Therefore, preservation of vital pulp is the first choice for the treatment of young permanent teeth after crown fracture [7]. Uninfected exposed teeth can be directly pulp capping to form reparative dentin to preserve the remaining pulp [8]. Many teenagers, oblivious to the significance of timely treatment, overlook the injured teeth until the occurrence of pulp tissue necrosis and root development stalls [9]. However, some teenagers timely seek medical attention when their teeth are inflammatory but not yet necrotic [10]. The preservation of the pulp tissue with inflammatory response is for the functioning and continued development of the root. Therefore, preserving the inflammatory pulp is critical to preserving teeth after dental trauma [11]. Traditional treatment is to perform apexification on the affected tooth or pulp revascularization [12]. However, these two methods have certain limitations in the age of young permanent teeth [13, 14]. For example, the apexification often results in a thin root canal wall and insufficient root resistance. It often requires 3–6 months of root canal dressing, long-term calcium hydroxide sealing, and there is a risk of root fracture. The revascularization only has few cases. It lacks of case-control studies of large samples and long-term effect evaluation. Whether it will accelerate root canal calcification remains to be observed, and the success rate of long-term endodontic treatment is not yet available.

Gronthos *et al*. [15] (2000) described dental pulp stem cells as mesenchymal, derived from dental pulp tissue, with the potential of self-regeneration and multi-directional differentiation, and play an important role in maintaining the dynamic stability of dental pulp function. The biological characteristics of dental pulp tissue undergo a series of changes after stimulation from inflammation or injury [16]. Dominick J and Alongi (2010) argued that dental pulp stem cells derived from inflammatory pulp could still regenerative [17]. It has also been reported that dental pulp stem cells isolated from inflammatory pulp can form pulp dentin complex similar to that from normal dental pulp stem cells after transplantation into nude mice; suggesting that mesenchymal stem cells in inflammatory pulp tissue still differentiate and participate in pulp tissue repair and regeneration [18].

Calcium hydroxide is the most commonly used pulp capping agent [19, 20]. Some studies have found that calcium hydroxide as a pulp capping agent also has certain disadvantages, such as pulp chamber atresia, easy dissolution, and degradation after acid etching [21, 22]. Lee *et al*. [23] (1993) first reported the application of mineral trioxide aggregate (MTA) in dental treatment. In 1998, the Food and Drug Administration U.S. approved MTA for dental treatment. *In vitro* experiments have confirmed MTA to exhibit biocompatibility, sealing and antibacterial properties, with the potential induction of mineralization of dental pulp stem cells and their differentiation into odontoblasts [24, 25]. MTA can promote the proliferation and differentiation of dental pulp cells in permanent teeth and deciduous teeth [26].

The aim of this study is to base on the results of previous *in vitro* studies, this study conducted an *in vivo* experiment on the mandibular central incisor of rats to evaluate the biological effects of MTA in inflammatory pulp.

2. Materials and methods

2.1 *In vivo* study

The rats used in the analysis were 7-week-old and 200 grams each, and were provided by Changzhou Cavens Laboratory Animal Co. Ltd. Rats were kept in the laboratory animal house of Wuxi People's Hospital.

A total of 30 rats were used in the analysis, of which six were used for preliminary experiments. The other 24 rats were randomly and evenly divided into the MTA and the calcium hydroxide ($Ca(OH)_2$) group. Both mandibular central incisors of all 30 rats were selected for the experiment.

In the preliminary experiments, the histological observations were conducted on the mandibular central incisor of rats with mechanical pulp exposure after 2 hours, 3 and 5 days. The time point for the observations was determined from the time when inflammation occurs in dental pulp tissue. First, a rat model of inflammation and pulp capping was established. After weighing, the rats were anesthetized with small animal respiratory anesthesia with isoflurane inhalation. The oxygen flow rate was 5 L/min. After sedation, intraperitoneal injection of 1% concentration of pentobarbital sodium 40 mg/kg was performed. After successful anesthesia, the cuspis of the mandibular central incisor of the rat was removed with forceps until the pulp was exposed, and the diameter of the pulp hole was measured around 1 mm.

Three days after the crown fracture surgery, following successful anesthesia, the cotton rolls were used to isolate saliva. In the MTA group, pulp exposure holes were filled with self-cured The ProRoot® MTA (Dentsply Sirona, Germany), and then covered with glass ionomer cements (FUJI, Japan) after MTA solidified. In the calcium hydroxide group, Light-curing calcium hydroxide paste (VOCO, Germany) was used to fill the pulp exposure hole. After 20 seconds of light cure, the glass ionomer cements (FUJI, Japan) were covered on the paste. Upon the onset of anesthesia, the length of the test operation was 10 minutes.

After eight weeks, the rats were anesthesia and decapitated. The mandibular central incisor was peeled off with a scalpel.

2.2 Histological observations

The teeth were decalcified with Sodium Formate Citrate decalcification solution for 2 months. The decalcified samples were subsequently dehydrated in a graded ethanol series (70–100%), embedded in paraffin. Cross-section and longitudinal sections (5 μm) were performed for the use of histological staining and immunohistochemical staining. Hematoxylin and eosin (H&E) staining were used for the histological staining. Both groups of specimens were stained with immunohistochemistry to detect the expression of sialo-phosphoprotein (DSPP). For the immunohistochemical staining, slides were deparaffinized followed by antigen retrieval in heated citrate buffer for 10 min (10 mM citrate, pH 6.0 at 95–100 °C). Peroxidase was blocked by incubating sections in 3% hydrogen peroxide (H_2O_2) for 5 min. Non-specific antibody binding was prevented with a blocking solution (5% Blocking Buffer (BSA), 0.1% triton X-100 in phosphate buffered saline (PBS); 30 min at room temperature). Sections were decanted and incubated with primary sialophosphoprotein antibodies diluted 1:200 in blocking solution, for 24 hours at 4 °C. The corresponding secondary antibody was incubated for 1 hour at room temperature (~22 °C), rinsed with PBS, and allowed to develop together with the developer at room temperature for 10 min.

2.3 Statistical analysis

The data are presented as the mean ± Standard Deviation (SD) and the statistical analysis was performed using SPSS 18.0 software (International Business Machines Corporation, Armonk, NY, USA). Differences among groups were assessed using Chi-squared Test. If below 0.05, the p value was considered statistically significant.

3. Results

3.1 The inflammatory response of exposed dental pulp tissue

In the preliminary experiment, six rats with 12 teeth in total were dissected two hours, three days, and five days after the pulp exposure, and the mandibular central incisor specimens were obtained. All specimens were decalcified by H&E staining and observed under a microscope. Two hours after

the pulp was exposed, the pulp tissue below the pulp hole was normal in four specimens. Three days after the pulp was exposed, vascular congestion was observed with a small amount of inflammatory cell infiltration, but the root pulp were intact. Five days after the pulp was exposed, 1 spinal necrosis was observed in one specimen, and a large number of inflammatory cell infiltrations of the coronary pulp and partial dental pulp and partial dental pulp necrosis were observed in three specimens. Therefore, the 3rd day after pulp exposure was selected as the time point to observe the response of MTA to inflamed dental pulp tissue.

3.2 Tissue reaction after pulp capping in inflamed dental pulp tissue

Three days after the pulp of the mandibular central incisor was exposed, direct pulp capping was performed, and the material was placed 8 weeks after the operation. Four dental fillings fell off among the 24 teeth in the MTA group, while five dental fillings fell off among the 24 teeth in the $Ca(OH)_2$ group. The comparison results of histological observation are shown in Table 1. In the MTA group, 13 specimens exhibited dentin-like tissues under the perforation, while the calcium hydroxide group had seven cases.

The difference in dentin-like tissue development was statistically significant between the two groups ($p = 0.0415$). Four specimens in the MTA group showed pulp inflammation, while the $Ca(OH)_2$ group had seven specimens with pulp inflammation. There was no statistical difference between the two groups ($p = 0.243$). The MTA group and the $Ca(OH)_2$ group had three and four specimens with pulp necrosis. Nonetheless, the pulp necrosis development difference was not statistically significant between MTA and $Ca(OH)_2$ groups ($p = 0.622$).

In the MTA group, four dental fillings fell off. Among the remaining 20 specimens, 11 specimens showed irregular calcified tissue at the exposed pulp hole, a dentin-like tubule structure, and cell-rich pulp connective tissue (Fig. 1). Observing the same specimen in longitudinal and transverse sections. The transverse section exhibits abundant vascular regeneration in the pulp cavity, and a polarized odontoblast layer is observed around the tube wall below the perforation. The longitudinal section shows the pulp below the perforation. On the cavity wall, fresh dentin tissue was developed (Fig. 2). In two specimens, calcified bridges were repaired at the exposed pulp holes, and the calcified bridges showed normal dentin-like tubule structures (Fig. 3). The four specimens showed inflammation of the pulp and no new dentin tissue was observed. Three specimens showed pulp necrosis. Five out of the 24 teeth fillings in the $Ca(OH)_2$ group in fell off. Seven specimens showed the growth of fresh dental pulp tissue beneath the perforating hole (Fig. 4). Seven specimens showed large inflammatory cell infiltration in the crown of the tooth. Five specimens showed necrosis of the coronary pulp tissue. One specimen showed absorbed in the medullary cavity and one specimen showed local calcification and atresia in the medullary cavity (Fig. 5).

3.3 Expression of sialophosphoprotein in dental pulp tissue after pulp capping in inflamed dental pulp tissue

Both groups of specimens were stained with immunohistochemistry to detect the expression of sialophosphoprotein (DSPP). In specimens with dentin-like tissue formation, there was sialophosphoprotein (DSPP) expression in the cells below the perforating hole (Fig. 6). In specimens with severe pulp inflammation, only a small amount of sialo-phosphoprotein expression was seen below the pulp hole.

4. Discussion

Healthy dental pulp tissue exhibit certain repair. It is vital to preserve the dental pulp function of young permanent teeth after injury in order to maintain root development. Rat has been proved to be an effective experimental model for evaluating the pulp reaction after pulp capping with different biomaterials [27, 28]. Rats have oral flora similar to humans [29]. Rat teeth are anatomically and histologically similar to human teeth. In addition, the healing process after direct pulp capping in rats is comparable to the healing process observed in humans. The formation of rat dentin bridge can be histologically displayed within four weeks after direct pulp capping, which is suitable for short-term evaluation [30, 31]. In this investigation, the mandibular central incisor of seven-week-old SD rats were selected as the research specimen. It was found that the pulp tissue showed inflammatory reaction after three days of pulp exposure, while the root pulp was normal. Therefore, third day after pulp exposure was selected as the inflammatory pulp model to explore the biological response of inflammatory pulp tissue in pulp preservation treatment.

For young permanent teeth with pulp exposure due to caries or trauma, how to preserve the vitality of the inflamed pulp tissue is a principle of treatment that meets biological standards. The choice of pulp capping agent plays a key role in it. The formation of restorative dentin is an important indicator for judging the effect of pulp capping and the successful preservation of dental pulp [32]. Which separates the dental pulp from the bad environment that may damage it, and creates a good environment for the restoration of dental pulp vitality [33]. A high-performance dental pulp capping material is the key to the success of pulp capping. The ideal pulp capping material should have the characteristics of eliminating inflammation and inducing differentiation of dental pulp cells [34]. The traditional calcium hydroxide pulp capping agent is an alkaline material, the physical properties are not stable enough, and the connection with the dentin is not tight. In addition, slight leakage is likely to occur sometimes. What's worse, a small amount of hyperemia and inflammatory cells can be seen under the formed calcified bridge, some of which can cause dental pulp necrosis or root canal calcification is impassable.

MTA is a bioactive material that is biocompatible, radiopaque, has low solubility, and induces dentin bridge formation. MTA has lighter inflammation after capping the pulp, causing less congestion and necrosis, and the sealing performance is significantly better than calcium hydroxide,

TABLE 1. Histological observation of dental pulp tissue sections in rats.

Group	Reparative Dentine	Inflammation	Necrosis	Intramedull-ary absorption
MTA (n = 20)	13 (65%)	4 (20%)	3 (15%)	0
Calcium hydroxide (n = 19)	7 (36.8%)	7 (36.8%)	4 (21.1%)	1 (5.3%)
χ^2	4.176	1.365	0.242	1.080
p	$p = 0.041$	$p = 0.243$	$p = 0.622$	$p = 0.299$

MTA: mineral trioxide aggregate.

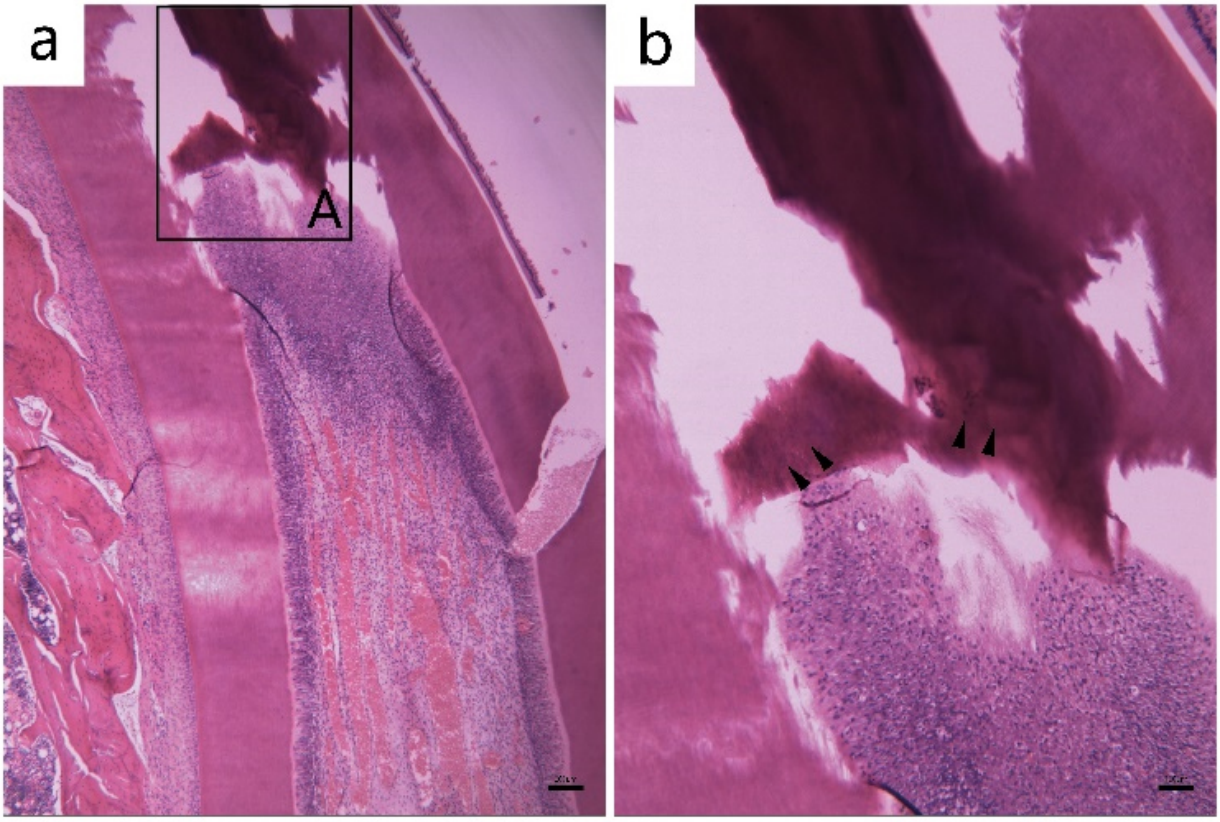

FIGURE 1. Histological appearance in MTA group after 8 weeks. Representative photomicrographs showing (a) a dentin-like tubule structure, and cell-rich pulp connective tissue. (b) Partially magnified image of the A section. H&E stain ×200 magnification. Arrows indicate the exposed pulp hole.

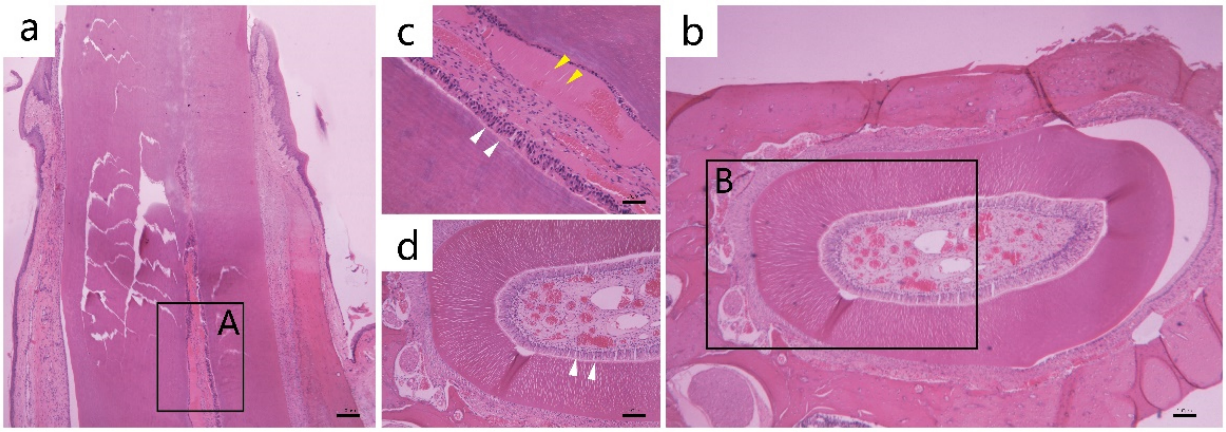

FIGURE 2. Histological appearance in MTA group after 8 weeks. Representative photomicrographs showing (a) longitudinal section; (b) transverse section; (c) Partially magnified image of the A section; (d) Partially magnified image of the B section. H&E stain ×200 magnification. Arrows indicate a polarized odontoblast layer and fresh dentin tissue.

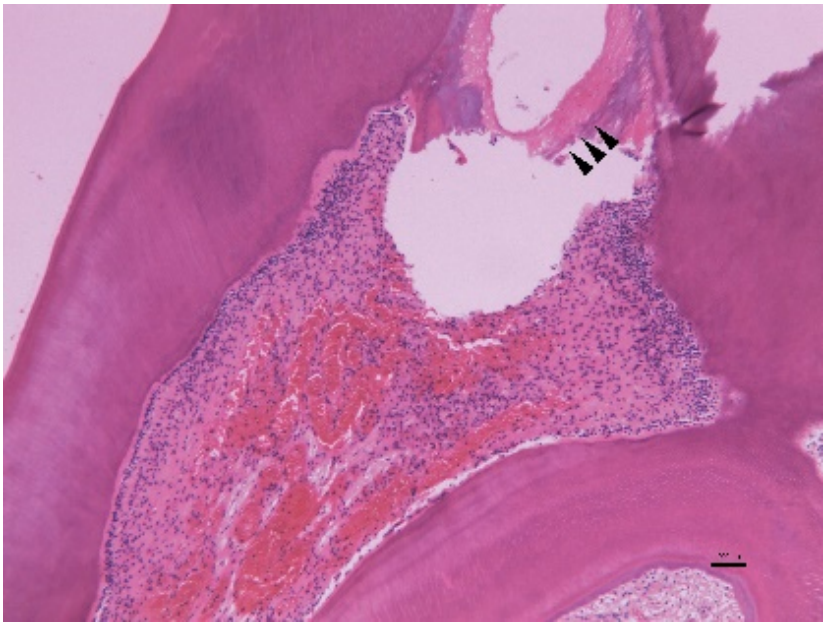

FIGURE 3. Histological appearance in MTA group after 8 weeks. Representative photomicrographs showing calcified bridges. H&E stain ×200 magnification. Arrows indicate normal dentin-like tubule structures.

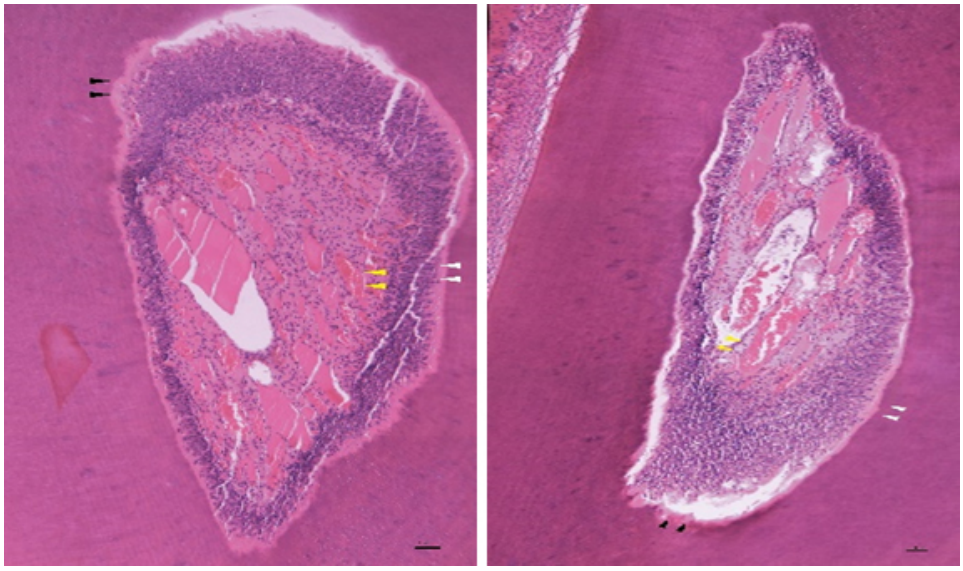

FIGURE 4. Histological appearance in $Ca(OH)_2$ group after 8 weeks. Representative photomicrographs showing the fresh pulp tissue. H&E stain ×200 magnification. Arrows indicate the tissue beneath the perforating hole.

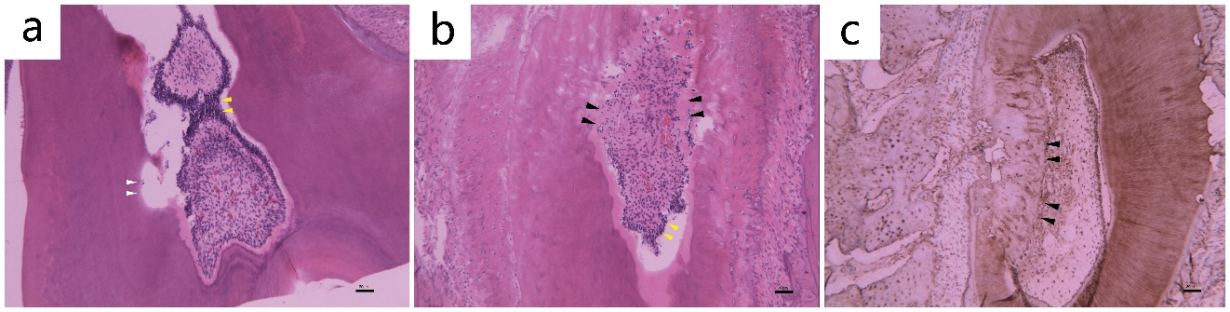

FIGURE 5. Histological appearance in $Ca(OH)_2$ group after 8 weeks. Representative photomicrographs showing (a) large massive inflammatory cell infiltration; (b) necrosis of the coronary pulp tissue; (c) local calcification and atresia. H&E stain and immunohistochemical stain ×200 magnification. Arrows indicate the tissue beneath the perforating hole.

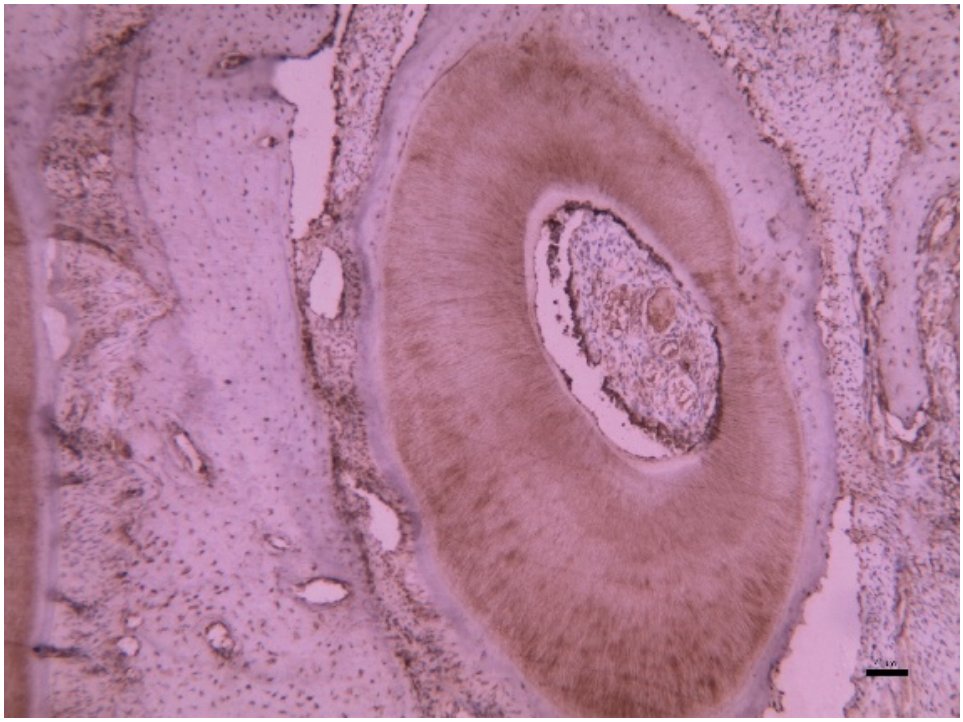

FIGURE 6. Expression of DSPP beneath the perforating hole in the specimen with dentin-like tissue formation. Immunohistochemical stain ×200 magnification.

with high hardness and small microleakage when compared with calcium hydroxide [35–38]. Wen *et al*. [39] examined the calcified bridge by tissue biopsy of 30, 60 and 90 days after direct pulp capping of 30 orthodontic premolars with MTA. The performance was significantly better than calcium hydroxide, and the inflammatory microenvironment will have a certain degree of influence on the osteogenic ability of dental stem cells [40]. Therefore, this study investigated MTA as a direct capping material to assess its biological effect on inflammatory pulp tissue. The formation of dentin bridge at the junction of pulp tissue and pulp capping material can be considered as a sign of healing. An earlier study by Monea and Stioca (2014) reported that MTA had a higher level of dentin bridge formation than calcium hydroxide in CBCT scan and histological evaluation [41]. Therefore, calcium hydroxide was selected as the control group in this study.

In this paper, MTA was used as a pulp capping agent for direct pulp capping in SD rat dental pulp model with mechanical exposure for three days. Histological observation at eight weeks after operation showed irregular dentin-like tissue formation above the pulp exposure hole, including dentin tubule-like structure, and no obvious inflammation of pulp tissue. Among them, seven cases were observed to exhibited inflammatory cells in the pulp tissue. It is thought that after direct pulp capping, microleakage of the filling material, such as the air combined in the mixing process or the excessive water incorporated, will increase the porosity of the material, leading to an increase in the solubility of the material and an increase in the cracks, affecting the differentiation and regeneration ability of the material to the inflammatory pulp [42].

The formation of dentin-like tissue is one of the criteria for evaluating pulp tissue repair. After pulp capping, the inflammatory pulp tissue first forms an irregular layer of reparative dentin, which may form a porous, heterogeneous calcified structure. In this study, MTA as a pulp capping agent has shown to induce inflammatory pulp cells to form reparative dentin. The number of dentin-like tissue specimens in the MTA group was statistically significantly higher than that in the $Ca(OH)_2$ group ($p < 0.05$). This finding is consistent with previous studies, confirming the mineralization of formed tissues after using MTA to cover the mechanically exposed dental pulp. Another factor evaluated in this study was the quality of newly formed calcified bridges. The founding in this study have shown that the calcium bridge in the dentin-like tissue formed after MTA pulp capping has a dentinal tubule-like structure, and a neatly arranged functional-like odontoblast layer can be observed. The dentin-like tissue formed in the calcium hydroxide group was relatively loose and porous. By comparison, it was found that the calcified tissue structure formed in the MTA group was due to the calcium hydroxide group.

Currently dentin phosphoprotein (DPP) and dentin sialoprotein (DSP) are the only two dentin-specific non-collagen proteins synthesized and secreted by odontoblasts [43, 44]. DPP and DSP are secreted at the beginning of mineralization and contribute to initiating dentin mineralization and regulating the size and growth rate of hydroxyapatite crystals. It has been reported that DSPP can induce the formation of uniform reparative mineralized tissue when implanted directly on mechanically exposed dental pulp, suggesting its contribution in reparative dentin formation [45, 46]. In this study, it was found that after pulp capping, there was sialo-phosphoprotein deposition under the perforation hole, suggesting the formation of new dentin-like tissue. The findings in this study confirm that the deposition of DSPP in MTA group was significantly

higher than that in calcium hydroxide group, suggesting that MTA could induce inflammatory pulp tissue to produce new dentin-like tissue.

5. Conclusions

The results from the current *in vivo* results show that MTA, as a pulp capping agent, directly acts on mildly inflammatory pulp tissue, inducing the formation of almost complete dentin bridges, effectively reducing pulp inflammation, and exerting the repair of pulp tissues. Therefore, MTA exhibits biological properties with potential clinical applications in the treatment of retaining inflammatory pulp.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

YZ, BTS and XYX—designed the research study. YZ and BTS—performed the research; wrote the manuscript. XDL—analyzed the data. XYX—reviewed and edited the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study on animals was approved by "Wuxi Children's Hospital Medical Ethics Committee" (WXCH2021-03-008). All experiments were in accordance with the guidelines of the China Ministry of Science and Technology Guide for the Care and Use of Laboratory Animals.

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Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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