Bioceramics-based Intentional Replantation for Root Canal Therapy in Beagle Dogs

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ABSTRACT

The objective of the study was to assess the application value of bioceramics-based intentional replantation for root canal therapy (RCT) in Beagle dogs. Periodontitis models were established with 36 teeth from three beagle dogs. They were divided into three groups including control (conventional RCT), MTA group [intentional replantation (IR) based on mineral trioxide aggregate (MTA)], and iRoot BP Plus group (IR based on iRoot BP Plus), 12 teeth in each group. The parameters including periodontal indexes (loosening degree, periodontal pocket depth (PD), gingival recession (GR), bleeding index (BI), periodontal fracture, alveolar bone height and density and tooth discoloring) were observed. One-month post-operation, the MTA and iRoot BP Plus groups had higher proportion of degree 1 loosening, GR, bone mineral density, bone height, and treatment success rate than the control. The proportion of degree III loosening, PD, BI, C-reactive protein (CRP), interleukin-2 (IL-2), interleukin-6 (IL-6), and the incidence of repeated fracture were clearly lowered in MTA and iRoot BP Plus groups as against control (P<0.05). The MTA group had a clearly higher proportion of teeth with discoloration compared to control and iRoot BP Plus groups (P<0.05). IR based on iRoot BP Plus can effectively inhibit inflammation, induce bone formation, protect the periodontal ligament and promote the prognosis.


INTRODUCTION

Dental nerve inflammation, apical inflammation and pulpitis are common dental diseases in the department of stomatology and the main treatment method is Root Canal Therapy (RCT) (Asnaashari et al., 2022). In recent years, with the continuous improvement of instruments, materials and fillings of RCT, the first cure rate of RCT is reached to 90% (Baseri et al., 2023) and the cure rate of root canal retreatment is more than 50% (Elnaghy et al., 2023). However, due to the complex anatomy of root canal and the residue of bacterial biofilm, the infection of periapical periodontitis cannot be controlled in some patients with by RCT. Relevant studies believe that the causes of this phenomenon are actinomycetes infection, lateral root canal and/or apical divergence. (Pirani et al., 2018; Johnsen et al., 2023). For those teeth that could not be effectively treated by RCT, an intentional replantation was proposed to treat those cases clinically (Okaguchi et al., 2019). Intentional Replantation (IR) is the “last resort” for such teeth with pulpal and periapical inflammation to be retained (Santos et al., 2022). The main treatment process of IR can be divided into: preoperative evaluation of the affected teeth, minimally invasive extraction of the affected teeth, in vitro diagnosis and treatment of the affected teeth, alveolar replantation and fixation of the affected teeth. In addition to periapical periodontitis, IR can also be used in the treatment of oral diseases such as root fissure and root dysplasia (Untara et al., 2021; Niavarzi et al., 2022). IR can fully treat the affected teeth under direct vision in-vitro, in which first remove and control the infectious materials, then seal the root surface, and put back into the alveolar to achieve the purpose of preserving the affected teeth and restoring the chewing function of the teeth (Park et al., 2022). Some studies have found that the reasonable use of intentional replantation in the treatment of teeth with periodontitis can improve the probing depth, gingival atrophy, and proximal bone loss, and the loosening of the affected teeth will also be improved (Saida et al., 2018). Although, periodontal ligament healing is the most ideal therapeutic goal of IR, but the key to this healing method is the activity of periodontal ligament cells (Saber et al., 2023). The activity of periodontal ligament cells may be affected by in-vitro time of the affected tooth, preservation media and root filling materials (Campos et al., 2023). At present, it is widely accepted that teeth should not be separated for more than 15 minutes (Diniz et al., 2023), and suitable preservation media can retain the activity (such as Hank’s Balanced Salt...
Solution (HBSS) but the expenses (Yang, 2009) and sterility (Brier et al., 2020) remain as challenges. It has been found that the success rate of replantation can also be improved by improving root tip treatment methods and upgrading the apical closure of inverted filling materials (Lee et al., 2021). Bioceramic materials are currently the apical filling materials with better apical closure, which are mainly based on iRoot series materials and mineral trioxide aggregate (MTA) (Dibazar et al., 2023). Previous studies have shown that bioceramic materials have stable biocompatibility, good antibacterial activity, and can promote tissue regeneration (Akhtar et al., 2023). Among them, MTA is the most widely used bioceramic material with the most mature technology, but it can cause tooth discolor (ElSebaai et al., 2022). The iRoot series mainly includes iRoot BP Plus, iRootSP and iRoot FS. iRoot BP Plus is the most suitable for root tip backfilling, and it is easier to operate than MTA and it takes less time to cure (Qian et al., 2022). However, there is a lack of research on the application of bioceramic materials for intentional replantation. In order to evaluate the therapeutic effect of bioceramic intentional replantation, Beagle dogs were selected as an animal model of periodontitis and the intention replantation based on bioceramic materials was adopted to evaluate the success of the treatment and the occurrence of inflammation in the affected teeth.

MATERIALS AND METHODS

Study animals: Three 18-month-old healthy beagle dogs (all provided by National Canine Laboratory Animal Resource Center, Zengcheng, Guangzhou) weighing 13-18 kg were selected. Dental condition: in the initial stage of permanent dentition, the teeth were healthy, complete, without deformity and caries. All beagle dogs were housed in the Laboratory Animal Department of Jiangnan University Medical Center for adaptive feeding for 1 month and used in the subsequent study. Approval by the relevant animal experimental ethics committee was obtained to conduct the study.

Establishment of periodontitis model: During the first 2 weeks of modeling, the dogs’ teeth were brushed every 2 days with a soft briquetted toothbrush (for children) and toothpaste (for pets), and the surface of each tooth was brushed once to prevent the formation of plaque. Following 2 weeks, the model was established after confirming that the periodontal status of all dogs was normal. The first premolar, the first molar, and third incisor on both sides of the upper and lower jaw (n= 12 teeth) of each dog were selected as the experimental teeth and a total of 36 teeth of 3 dogs.

Experimental grouping: Total 36 experimental periodontitis teeth were established in 3 beagle dogs, which were randomly divided into control, MTA and iRoot BP Plus groups. The teeth in the MTA group were treated with MTA-based IR, and those in the iRoot BP Plus group were treated with iRoot BP Plus-based IR.

Treatment method: Before treatment, X-ray (Biodriver biotechnology, USA) was performed on all the affected teeth to evaluate the root canal and periapical condition, and the corresponding treatment was carried out after complete tooth cleaning. It should be noted that the experimental animals were required to fast for 12 hours before surgery. Firstly, all experimental animals were anesthetized by intramuscular injection of 0.04 mL/kg SIMendan I and 0.5 mL/kg 3% sodium pentobarbital (Kunming Chongerkang Pharmaceutical Co., LTD.). The control group was treated with conventional RCT. After anesthesia, the teeth were treated with rubber dam (Denteck/KSK, Japan), followed by detrenchment, pulp opening, pulp cavity cleaning, root canal exploration, mechanical preparation, and root canal irrigation with 3% sodium hypochlorite (Sichuan Xinyun Jinjing Technology Co., LTD.). After suction, the root canal was filled with gutta-percha tips (Dentsply) and root canal paste (Foshan Pingyu Trading Co., LTD.), and the root canal sealing was carried out with zinc oxide cement (Shanghai Rong Xiang Dental Material Co., LTD.). The MTA and iRoot BP Plus groups were treated with IR based on MTA/iRoot BP Plus. The affected teeth were held with minimally invasive extraction forceps (Guangdong Kuaian Dental Medical Technology Co., LTD.). Subsequently the extraction of the teeth by shaking, the inflammatory granulation tissue in the alveolar fossa and root tip was removed, while it was wet compress in sterile normal saline. In-vitro examination and management of the affected teeth were performed, and stones on the root surface and abnormally infected material such as inflammatory granulation tissue were scraped using a Gracey subgingival curette (HuFriedy group, USA). The apex was cut off by 2-3mm with a high-speed handpiece split drill (MANI, Japan) to eliminate the lateral canals and apical differences, reprepotation of the apex. The apical foramen was closed with MAT (Dentsply, USA)/iRoot Bp Plus (Innovative Bio Ceramix, Canada) backfilling. After the reduction of the affected tooth, the tooth was firmly fixed along the occlusal surface to the root, and the occlusion condition was checked. If no abnormality was found, a resin splint (Shanghai Huazhong Rehabilitation, China) was used to fix the adjacent teeth. Postoperative antibiotics (Penicillin G benzathine and penicillin G procaine 120,000 IU) were subcutaneously administered every day for 7 days for preventing the infection.

Observation indicators: The periodontal indexes of the affected teeth were observed before and 1 month after operation, including the degree of loosening of the affected teeth (Fig. 1). Periodontal pocket depth (PD), gingival recession (GR), bleeding index (BI) and whether the periodontal fixation was broken. The treatment effect was evaluated by observing the height and density of alveolar bone by X-ray film about one-month post-operation. The specific evaluation criteria for the treatment are given in Table 1. The tooth discoloration of each group was observed after the treatment.

Inflammatory factor detection: The changes of inflammatory factors before and 1 month after operation were detected. Peripheral venous blood about 2-3 ml was drawn in the fasting state and centrifuged at 3,200 r/min for 15 min in a serum separation centrifuge (Beckman Coulter International Trade, Shanghai). After blood stratification,
the supernatant was taken. The canine C-Reactive Protein (CRP), Interleukin-2 (IL-2) and IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Zhonghao Biological) was used to detect the concentration changes of CRP, IL-2, and IL-6 by double antibody sandwich method (Yin et al., 2022).

Statistical method: The SPSS 22.0 software was employed for statistical analysis. The measurement data conforming to normal distribution were expressed as mean ± standard deviation (X±s) and the t-test was adopted for contrast among groups. Count data were expressed as the number of cases (percentage) [n(%)], and chi-square test was applied for contrast among groups. P<0.05 was considered statistically meaningful.

RESULTS

Evaluation of tooth mobility: Before operation, there were 3 (25%), 2 (16.67%) and 2 (16.67%) degree III loosening, and 9 (75%), 10 (83.33%), and 10 (83.33%) degree III loosening in control, MTA and iRoot BP Plus groups, respectively. There was similar proportion of teeth with degree II and III loosening among the three groups before operation (P>0.05). One-month post-operation, there were 5 (41.67%), 9 (75%) and 9 (75%) teeth with degree I loosening, 3 (25%) with degree II loosening in each group, and 4 (33.33%), 0, 0 with degree III loosening in the three groups. The MTA and iRoot BP Plus groups had a clearly higher proportion of teeth with degree I loosening and a clearly lower proportion of teeth with degree II loosening as against control (P<0.05). There was similar proportion of teeth between the MTA and iRoot BP Plus groups (P>0.05) (Table 2).

Results of periodontal index evaluation: The figures (Fig. 2A-D) illustrate the measurements of periodontal depth (PD), gingival recession (GR) and bleeding index (BI) before and one month after surgery, as well as the incidence of periodontal fixation fractures one month after surgery in three groups.

Before the surgery, the PD, GR, and BI of the control group, MTA group, and iRoot BP Plus group were similar, with no significant differences between them (P>0.05). The respective measurements were as follows:

PD: Control: 6.65±1.03 mm, MTA: 6.52±0.92 mm, iRoot BP Plus: 6.81±1.01 mm
GR: Control: 1.01±0.72 mm, MTA: 1.02±0.77 mm, iRoot BP Plus: 1.00±0.80 mm
BI: Control: 3.53±0.39, MTA: 3.39±0.32, iRoot BP Plus: 3.57±0.30

One month after the surgery, significant differences were observed between the groups. The measurements were as follows:

PD: Control: 4.97±1.22 mm, MTA: 3.52±1.02 mm, iRoot BP Plus: 3.59±1.01 mm
GR: Control: 2.01±0.72 mm, MTA: 2.59±0.69 mm, iRoot BP Plus: 2.51±0.61 mm
BI: Control: 3.01±0.62, MTA: 2.01±0.51, iRoot BP Plus: 1.98±0.52

During the first month after surgery, the control group had the highest incidence of repeated fractures (41.67% of the teeth), while the MTA and iRoot BP Plus groups had lower incidences (8.33% each). Additionally, the MTA and iRoot BP Plus groups showed significantly lower PD, BI, and recurrent breakage compared to the control group, while exhibiting significantly higher GR (P<0.05). However, there were no significant differences (P>0.05) between the MTA and iRoot BP Plus groups regarding these measurements.

Measurement results of alveolar bone mineral density and bone height: The bone mineral density and bone height of the alveolar bone were measured by X-ray. Before operation, the alveolar bone mineral density of control, MTA and iRoot BP Plus groups were (102.19±11.82)g/cm², (101.95±10.21)g/cm² and (101.27±11.01)g/cm², respectively. Alveolar bone height before the surgery was (9.77±3.65) mm, (9.61±4.09) mm, (9.70±3.89) mm, respectively for control, MTA and iRoot BP Plus groups. There was similar in bone mineral density and bone height among three groups (P>0.05). One month after operation, the alveolar bone mineral density of control, MTA and iRoot BP Plus groups were (133.09±18.98) g/cm², (150.09±19.65) g/cm², and (149.43±19.32) g/cm², respectively. The alveolar bone height was (10.09±3.43) mm, (13.01±3.25) mm, and (12.79±3.03) mm, respectively. The alveolar bone mineral density and bone height of MTA and iRoot BP Plus groups were clearly higher as against control (P<0.05), but there was no significant difference (P>0.05) between MTA and iRoot BP Plus groups (Fig. 3A-B).

Comparison of treatment effect and tooth discoloration: Table 3 shows the treatment statistics. In control, 7 cases (58.33%) were successful in the treatment, 5 cases (41.67%) failed in the treatment, and the tooth discoloration was 0. In MTA group, 11 cases (91.67%)...
were successful, 1 case (8.33%) failed and 3 teeth (25%) had discoloration. In iRoot BP Plus group, 10 cases (83.33%) were successful, 2 cases (16.67%) failed, and 0 tooth had discoloration. The success rate of treatment in MTA and iRoot BP Plus groups was higher as against control, and the proportion of teeth with discoloration in MTA group was clearly higher as against control and iRoot BP Plus groups (P<0.05) (Fig. 4A-C).

**Inflammatory factor detection:** Fig. 5 shows the concentration changes of CRP, IL-2, and IL-6. Before the surgery, the concentrations of CRP (C-reactive protein), IL-2, and IL-6 in the three groups were as follows: The control group had a CRP concentration of 9.77±1.92 mg/L, the MTA group had a concentration of 9.91±1.82 mg/L, and the iRoot BP Plus group had a concentration of 9.81±1.69 mg/L. Additionally, the IL-2 concentration in the control, MTA, and iRoot BP Plus groups were (0.14±0.08) pg/mL, (0.14±0.09) pg/mL, and (0.15±0.10) pg/mL, respectively. The concentrations of IL-6 were (0.12±0.08) pg/mL, (0.11±0.07) pg/mL, and (0.12±0.07) pg/mL, respectively. There were no significant differences (P>0.05) in the concentrations of CRP, IL-2, and IL-6 among the three groups. One month after the surgery, the concentrations of CRP, IL-2, and IL-6 were measured again with the following results: The control group showed a CRP concentration of (4.29±1.01) mg/L, the MTA group had a concentration of (2.01±1.21) mg/L, and the iRoot BP Plus group had a concentration of (1.98±1.13) mg/L. In terms of IL-2 concentration, the control, MTA, and iRoot BP Plus groups had concentrations of (0.05±0.02) pg/mL, (0.05±0.01) pg/mL, and (0.05±0.03) pg/mL, respectively. The concentrations of IL-6 were (0.05±0.02) pg/mL, (0.03±0.01) pg/mL, and (0.02±0.01) pg/mL, respectively.
It was observed that the concentrations of CRP, IL-2, and IL-6 in both the MTA and iRoot BP Plus groups were significantly lower compared to the control group (P<0.05). This finding suggests that the MTA and iRoot BP Plus groups exhibited reduced levels of these inflammatory markers one month after the operation, which indicates a potential benefit of using these materials in reducing inflammation.

**DISCUSSION**

RCT is a common treatment method for a variety of dental diseases, and it has good clinical implications but the phenomenon of infection cannot be controlled. Therefore, IR has become the last means of RCT for oral diseases that cannot be cured by other methods. Relevant studies have found that the three-year survival rate of intentional replantation in the treatment of pulpitis and periodontal diseases is more than 80%. Vertical broken teeth also have a survival time of 2 to 6 years (Abdulsamad et al., 2022; Yadav et al., 2023), and the apical closure exerted by backfilling materials will affect the healing of periodontal ligament. Based on this, we used bioceram-based IR for the treatment of periodontitis and compared it with conventional RCT.

Chen et al. (2015) compared grey mineral trioxide aggregate (MTA) and EndoSequence Root Repair Material (RRM), while our study focused on comparing intentional replantation (IR) based on MTA and iRoot BP Plus in Beagle dogs. Their study highlight the potential benefits of bioceramics-based materials, such as MTA and RRM, in root-end surgery and intentional replantation procedures same a out study. These materials exhibit biocompatibility, good sealing ability and favorable tissue healing responses. The use of advanced imaging techniques, such as CBCT and micro CT, allows for more accurate assessment of healing outcomes. Yang et al. (2018) study investigates the effects of platelet-rich plasma (PRP) on periodontal healing, while our study examines the application of bioceramics-based intentional replantation using mineral trioxide aggregate (MTA) and iRoot BP Plus. It is worth noting that a direct comparison between the two studies may be challenging due to differences in methodologies, materials, and outcome measures. However, both studies contribute to the understanding of techniques and materials that can potentially improve periodontal healing in replanted teeth, albeit through different approaches.

One month after surgery, the MTA and iRoot BP Plus groups had clearly higher proportion of teeth with degree Ⅰ loosening and GR, and clearly lower proportion of teeth with degree III loosening, PD, BI, and incidence ratio of repeated fracture than the control (P<0.05). The results indicate that the IR based on MTA/iRoot BP Plus can clearly promote the healing and recovery of the affected teeth. Many studies have shown that MTA has good
sealing, biocompatibility and osteogenic induction, which can promote healing response and reduce inflammatory response (Moradzadeh et al., 2017). iRoot BP Plus has good sealing, stability, bacteriostasis, good fracture resistance, unique hard and solid properties and good biocompatibility (Zeng et al., 2023). Therefore, both can promote bone formation after tooth replantation, inhibit the growth of bacteria, and reduce the occurrence of inflammatory response. The concentrations of CRP, IL-2, and IL-6 in the MTA and iRoot BP Plus groups were clearly lower (P<0.05). Studies have shown that the serum inflammatory factor CRP in periodontitis is clearly increased (Sütü et al., 2015). Therefore, the results suggest that IR based on MTA/iRoot BP Plus can effectively reduce the occurrence of inflammatory response in the surrounding tissues of the affected teeth.

In addition, X-ray examination showed that the alveolar bone mineral density and bone height of MTA and iRoot BP Plus groups were significantly higher (P<0.05), suggesting that MTA and iRoot BP Plus could induce bone tissue formation, which was in line with the research results of Patrawalla et al. (2023). Therefore, IR based on MTA and iRoot BP Plus can effectively inhibit the occurrence of tooth tissue inflammation, protect the periodontal ligament and promote the prognosis. The treatment success rate of MTA and iRoot BP Plus groups was significantly higher (P<0.05). In addition, many studies have found that MTA treatment leads to tooth coloring, and the edge of dental fillers will appear gray black. Previous studies proposed that the reaction between bismuth oxide in MTA and dentin collagen (Shokouhinejad et al., 2020), ultraviolet (Madani et al., 2019), and sodium hypochlorite (Yang et al., 2021), is the main cause of tooth discoloration. It was found that the proportion of tooth discoloration in the MTA group was clearly higher (P<0.05), suggesting that MTA could cause tooth discoloration.

Conclusions: In conclusion, IR based on MTA and iRoot BP Plus can effectively inhibit tooth inflammation, induce bone formation, protect the periodontal ligament and promote the prognosis of the teeth. Furthermore, it was confirmed that MTA caused tooth discoloration, but the application value of IR based on iRoot BP Plus is higher. Actually, it was not a clinical trial, therefore the application of this method for clinical purpose needs further in-depth studies.

Conflict of interest: None

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Author contribution: Lixue Yin and Guoxin Yan designed and conducted the in vivo study, performed the experiments and acquired the data. Ye Cao contributed to data analysis and interpretation. Chenchen Liu assisted with data collection and provided critical input throughout the study. All authors were involved in manuscript preparation, reviewing, and editing.

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