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Evaluation of pulpal and dentin regeneration by different pulp-capping materials using mouse model

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Evaluation of pulpal and dentin regeneration by different pulp-capping materials using mouse model

A thesis submitted in partial satisfaction of the requirement for the degree Master of Science in Oral Biology

by

Sarah Saad Hussain

2019

ABSTRACT OF THE THESIS

Evaluation of pulpal and dentin regeneration by
different pulp-capping materials using mouse model

by

Sarah Saad Hussain

Master of Science in Oral Biology
University of California, Los Angeles, 2019
Professor Reuben Kim, Chair

The development of pulp capping agents has been instrumental in promoting reparative dentin formation and facilitating pulpal repair in response to pulp exposure during extensive caries excavation. Although calcium hydroxide was long the gold standard for pulp capping, the development of mineral trioxide aggregate (MTA) has ushered in a new wave of therapeutic pulp capping agents and MTA derivatives, also known as hydraulic calcium-silicate cements (HCSCs). Unfortunately, little is known as to the pulpal toxicity and dentin regenerative capacity of these materials. In the current study, the physiologic effects of four HCSCs on reparative dentin and dentinal bridge formation and periapical bone loss were evaluated radiographically and histologically in mice. Pulp exposure was induced in maxillary first molars of C57/BL6 mice, and reparative potential of pulp capping materials was evaluated by μCT analysis and

Hematoxylin and Eosin (H&E) staining. Among the five HCSCs used for pulp capping, only

Dycal failed to induce reparative dentin and dentinal bridge formation, instead inducing periapical bone loss. Although TheraCal LC also induced periapical bone loss, significantly more teeth treated with Dycal developed periapical radiolucency's. Histologically, dentinal bridge formation was observed in all HCSCs except Dycal. These findings indicate that with the exception of Dycal, all HCSC derivative materials evaluated are as effective, if not more so, than the original PROROOT® MTA in promoting reparative dentin formation and pulpal repair.

The thesis of Sarah Saad Hussain is approved.

Mo Kang

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Bo Yo

Reuben Kim, Committee Chair

University of California, Los Angeles

2019

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1. Introduction:

1.1 Utilization of Pulp Capping for Treatment of Pulp Exposure

The management and treatment of the dentin-pulp complex is one of the hallmarks of endodontics and restorative dentistry, as the elimination of dental caries and simultaneous preservation of the supporting dentin is critical in maintaining tooth vitality. However, circumstances exist in which pulpal trauma or mechanical pulp exposure during caries excavation may be inevitable, and thus methods of pulpal repair are critical to the long-term success of these teeth. Among these, the utilization of pulp capping, and more specifically direct pulp capping (DPC), has permitted pulpal healing and repair, protection from further insult, and abrogation of pulpal necrosis in response to pulp exposure (Aguilar *et al*., 2011).

Although many pulp capping materials exist, the hallmark of an effective pulp capping material is the preservation of pulp vitality and promotion of reparative dentin formation (Qureshi *et al.*, 2014). In order to achieve these goals, these pulp capping materials must be biocompatible and bioactive to prevent host rejection of the material and should allow for adhesiveness, dimensional stability and be radiopaque to ensure that the material is retentive, will remain in place and can be visualized radiographically (Kaur *et al.*, 2017). From a biologic standpoint, those pulp capping materials that are anti-bacterial, promoted re-mineralization, secrete fluoride and prevent further inoculation by pathogenic bacteria are by far the most effective (Araújo *et al.*, 2018). Ultimately, the utilization of direct pulp-capping using materials with the aforementioned properties is critical to the formation of reparative dentin and a physical barrier against pathogens, thereby facilitating pulpal repair and preventing pulpal necrosis in response to pulpal exposure (Song *et al*., 2017).

1.2 Evolution of Direct Pulp Capping Materials

Among the pulp capping materials, calcium hydroxide (CH) has become the most commonly used despite several notable drawbacks to its use. Among these include its inability to adhere to dentin (Pameijer & Staley, 1998), its potential dissolution by cariogenic acid and tissue borne fluids (Cox *et al.,* 1985; Olmez *et al.,* 1998; Stanley & Pameijer, 1985) and potential development of tunnel defects during dentinal bridge formation (Cox *et al.,* 1985). In light of these deficiencies, novel pulp capping materials were eventually developed, including mineral trioxide aggregate (MTA), whose use in endodontics was first identified by the Torabinejad group (Ford *et al.,* 1996; Torabinejad *et al.,* 1993). MTA, which is a combination of Portland cement and Bismuth Oxide powder, was originally developed for root-end filling but has since been utilized for other functions in endodontics, including apexogeneiss, repair of root perforations, and pulp capping, among others (Parirokh & Torabinejad, 2010). When compared to calcium hydroxide, MTA has been shown to be clinically superior (Cho *et al.,* 2013; Hilton *et al.,* 2013; Iwamoto *et al.,* 2006; Mente *et al.,* 2010; Nair *et al.,* 2009), despite a number of distinct disadvantages, such as potential for tooth and tissue discoloration, poor handling and setting time, and high cost (Islam *et al.,* 2006).

Since the patent expiration of MTA, several MTA derivatives, or hydraulic calcium-silicate cements (HCSCs), have become available for use for the aforementioned endodontic applications. Many manufacturers of these HCSCs claim their product to be superior to the original ProRoot MTA, however there exists a notable absence of scientific evidence as to any clinical superiority or improvements to toxicity or efficacy of these derivative materials. Thus, comparative studies

examining the effects of MTA and HCSCs are necessary to identify materials with the best clinical outcome and least toxicity.

1.3 Utilization of *In Vivo* Models for Evaluation of Direct Pulp Capping Materials

Several *in vivo* models, including the use of dogs, pigs, rats and monkeys, have been utilized over the last several decades to study the efficacy and safety of direct pulp capping materials (Dammaschke et al. 2010; Jegat et al. 2007; Koliniotou-Koumpia and Tziafas 2005; Paterson et al. 1981; Sela and Ulmansky 1970; Tarim et al. 1998; Tziafa et al. 2014). Although these *in vivo* models allowed for observational studies and enhanced our understanding of the clinical outcomes of these pulp capping materials, the advent of transgenic and gene targeted knockout mice have allowed for the study of molecular mechanisms of disease and have provided a novel and more detailed means by which to study pulpal wound healing and reparative dentin formation. The utilization of these mouse models have allowed for the quantitative and qualitative analysis of reparative dentin formation resulting from direct pulp capping *in vivo* (Song *et al.,* 2017; Hunter *et al.,* 2015). However, none of these animal models have examined the potential for dental bridge formation resulting from direct pulp capping by MTA. In light of this, we developed a unique direct pulp capping mouse model that replicate the exact steps being used clinically and examine both presence of periapical bone loss and reparative dentin formation by MTA using μCT analysis. Thus, this novel direct pulp capping mouse model may serve to predictably translate *in vivo* findings to support the use of MTA and HCSCs in a clinical setting.

Here, we hypothesize that MTA derivatives are superior to conventional MTA in regenerating dental pulp by enhancing reparative dentin formation in vivo. This hypothesis will be tested using

our recently established direct pulp-capping mouse model to evaluate commercially available MTA derivatives (eg, HCSCs) in inducing reparative dentin formation *in vivo* and also to assess periapical bone loss. This model will allow us to perform μCT and histologic analysis for reparative dentin formation and periapical inflammation in order to determine the difference between HCSCs in mice. The results of these findings will be instrumental in aiding clinicians in the selection of the direct pulp capping material with the best predictable outcome in performing direct pulp treatment.

2. Materials and Methods:

2.1. Animal Model

C57/BL6 mice purchased from Jackson Laboratory were used for experiments that were performed according to the approved institutional guidelines from the Chancellor's Animal Research Committee. C57/BL6 mice were housed in a pathogen-free vivarium in the UCLA Division of Laboratory Animal Medicine (DLAM). The following HCSC pulp capping materials were used: PROROOT® MTA, TheraCal LC, EndoSequence BC RRM, Endo-Eze™ MTAFlow, and Dycal. Composite restorations with no pulp capping material were used as controls. Direct pulp capping protocol as established previously in our laboratory (Song et al. 2017) was followed.

2.2. Mouse Anesthetization

Eight-week-old female C57/BL6 mice (n = 40) where anesthetized using ketamine (80-120 mg/kg of mouse weight)/xylazine (5 mg/kg of mouse weight), which was administered intraperitoneally (i.p.) at a dose of 10 mL/kg. Anesthesia was confirmed by performing a toe pinch.

2.3. Pulp-capping Procedure

The mice were divided into 6 groups, with each group containing 7 mice receiving a different pulp capping material (PCM) and 5 mice receiving only composite filling (no PCM placed), which served as a control. For each mouse, the mouth was held open using a retractor with the mouth facing upward, and the first maxillary molar was visualized using a 10x microscope. A ¼ round bur and high-speed headpiece at 200,000 RPM was used to perform access opening until the hue of pulp was visualized without pulp exposure. A #15 endodontic K-file with a diameter of 150 μm was used to perforate the dentin and expose the pulp so as to prevent displacement of dentinal debris into the pulp chamber and canals. PCMs were prepared and delivered onto the exposed pulp using the tip of an explorer and was packed into the exposed pulp using the flat, back side of a fine paper point to allow for proper condensation of PCM into the exposed pulp. The exposed dentin was etched for 15 seconds using a small amount of viscous, 35% phosphoric acid in order to roughen the tooth surface and allow micromechanical bonding of dental adhesives to the tooth structure. Etchant was removed using section and sterile water, and the tooth was dried using a compressed air duster. Dental adhesive was applied to the tooth structure on the backside of a paper point, thinned out using compressed air for 3 seconds, and cured for 20 seconds using a curing light. Flowable composite was gradually added on top of the MTA, the tip of an explorer was used to flow the composite to allow a complete seal, and the composite was cured for 30 seconds. An explorer was used to confirm that the composite had been fully cured.

2.4. Post-op Care

Carprofen (5 mg/kg) was administered subcutaneously (sc) to the mice immediately after the pulp-capping procedure. The mice were placed on a heating pad at low power to keep the animals warm as they awoke from anesthesia. Upon recovering from anesthesia, the mice were returned to the vivarium for housing.

2.5. Tissue Procurement

After 5 weeks, the mice were anesthetized using isoflurane and euthanized by cervical dislocation. The maxillae of these mice were carefully dissected and placed in a 50 mL centrifuge tube containing 4% paraformaldehyde in PBS, pH 7.4, at 4 °C overnight to allow for tissue fixation and then stored in 70% ethanol.

2.6. μCT Scanning

Isolated and fixed mouse maxillae were wrapped in 70% ethanol-soaked gauze and placed in a 15 mL contribute tube for μCT analysis. These tubes were mounted onto the μCT scanning stage, as outlined in the manufacturer's instructions. The following settings were using during μCT scanning: current of 145 μA, a voltage of 55 kVp, and an exposure time of 200 ms. Image acquisition was performed at a 20-μm resolution and with a 0.5 mm Al filter, and the image was reconstructed and visualized.

2.7. Tissue Processing and Staining

Upon completion of μCT scan, mouse maxillae were decalcified using 5% EDTA and 4% sucrose in PBS (pH 7.4) for 2 weeks. The decalcified maxillae were trimmed by making a sagittal cut immediately anterior to the first molar, and were paraffin embedded with the longitudinal section of the first molar facing the cutting surface. Using the microtome, 5 μm-thick sections were prepared and mounted to slides, with the pulp-capping areas coinciding with the distopalatal (DP) root, which was used as a landmark. The precise area of interest was established by examining the histology under the light microscope and comparing them to the μCT images. In order to perform hematoxylin and eosin (H&E) staining, slides were deparaffined and rehydrated with xylene (2x) and serially-diluted ethanol (100% EtOH 2x, 95% EtOH 2x, and 70% EtOH 1x). These slides were subsequently ran through running tap water, and stained with hematoxylin solution for 2.5 minutes and rinsed again with tap water. The slides were then placed in 95% ethanol for 1 minunte, stained with Eosin solution for 1 minute and rinsed again with tap water, and dehydrate with serially-diluted ethanol (70% EtOH 1x, 95% EtOH 2x, and 100% EtOH 3x) and xylene (3x). These slides were then mounted.

3. Results

3.1 Direct pulp capping using HCSCs induce variable degrees of dentinal bridge and reparative dentin formation.

In order to determine the extent to which HCSCs can induce dental bridge and reparative dentin formation *in vivo*, pulp exposure was induced in 40 mice and pulp capping was performed in replicates using different HCSCs and using composite as a control. Five weeks post-pulp capping,

μCT analysis of harvest mice maxilla demonstrated significant differences between the different HCSC materials (**Figure 1**).

In mice that received PROROOT® MTA for pulp capping, ten out of twelve (83%) exhibited notable dentinal bridge formation, as well as partial calcification of the pulp chamber (**Figure 1A).** The other two mice (17%) did not show dentinal bridge formation, however narrowing of the pulp chamber was evident. Two additional mice were excluded from the sample set, as the composite filling and PCM fell out and no reparative dentin formation was possible.

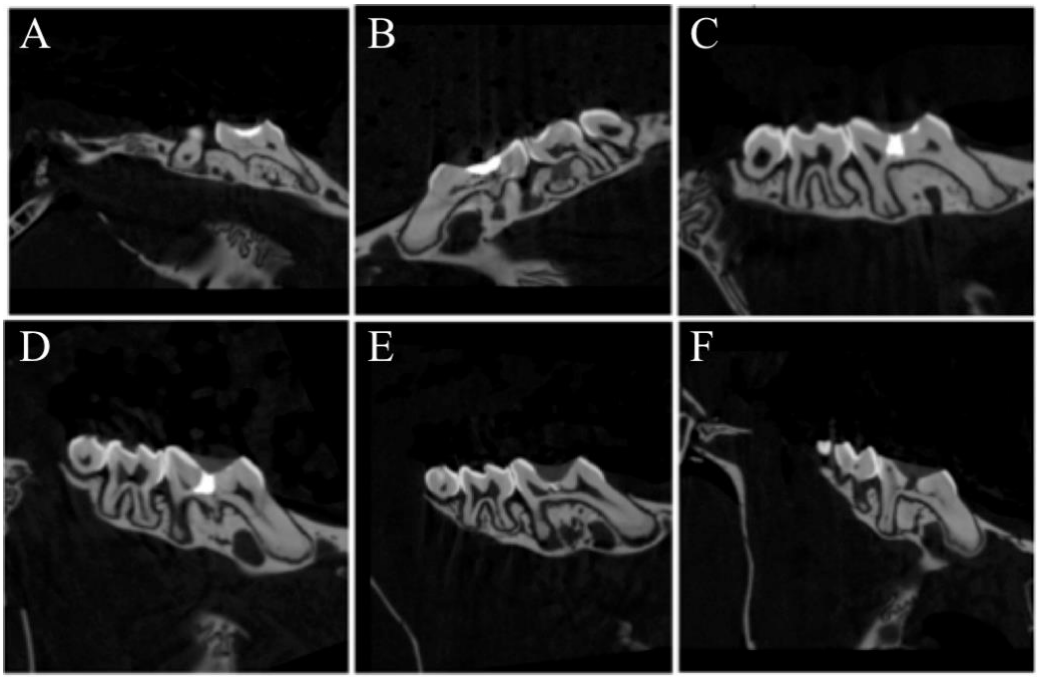

Figure 1: **Effect of HCSCs on pulp capping and pulpal/dentinal repair.**

Pulp exposure was induced in C57/BL6 mice. Mice were subsequently underwent direct pulp capping procedure using HCSCs, including (**A**) PROROOT® MTA, (**B**) TheraCal LC, (**C**) EndoSequence BC RRM, (**D**) MTA Flow, (**E**) Dycal, as well as (**F**) composite, which served as the control group.

In mice receiving TheraCal LC, twelve out of thirteen (92%) exhibited notable dentinal bridge formation, as well as partial calcification of the pulp chamber (**Figure 1B).** The one mouse (8%) did not show dentinal bridge formation, however narrowing of the pulp chamber was evident. One additional mouse was excluded from the sample set, as the composite filling and PCM fell out on the right side and no reparative dentin formation was possible.

Mice receiving EndoSequence BC RRM and Endo-Eze™ MTAFlow as pulp capping agents demonstrated dentinal bridge formation in thirteen out of fourteen mice (92%), with only 1 mouse in each group (7%) not exhibiting dentinal bridge formation **(Figure 1C,D**). In these mice, the PCM was unintentionally pushed into the pulp chamber, however reparative dentin formation was still evident below the PCM.

In response to the use of Dycal as a pulp capping agent, none of the mice demonstrated dentinal bridge formation (**Figure 1E)**. Seven out of fourteen of these mice did not show reparative dentinogenesis, and the remaining mice demonstrated pulp chamber calcification that was not consistent with dentinal bridge formation, with the pulpal calcification originating in the middle of the pulp and extending apically towards the canal space. The lack of dentinal bridge formation was consistent with that of the control group, in which composite was used without any PCM for pulp capping, resulting in sporadic pulp chamber and canal calcification. The summary of these findings can be found in **Figure 2** and **Table 1**.

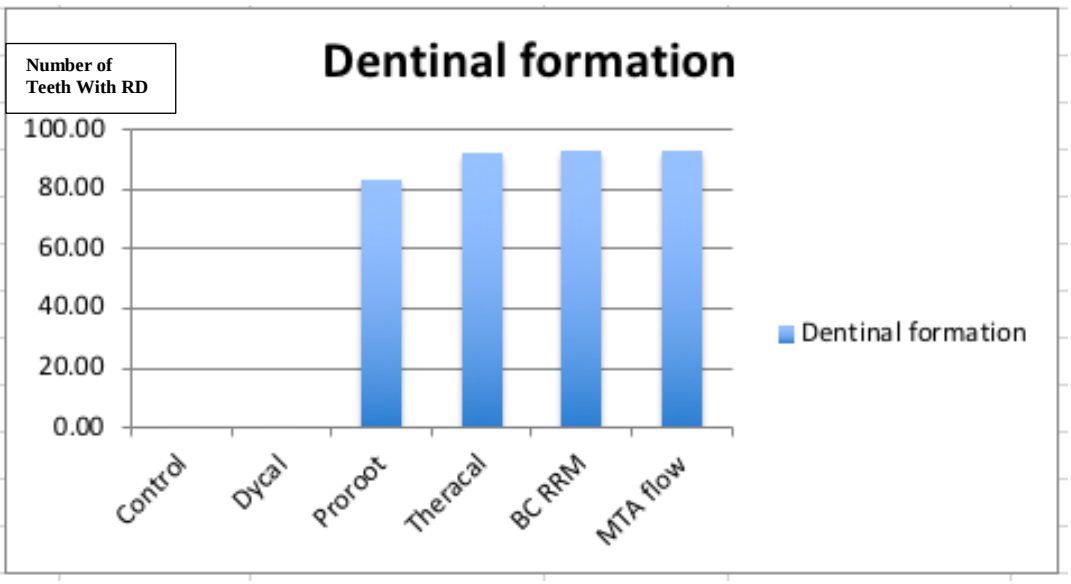

Figure 2: Quantitative effect of HCSCs on pulpal repair and dentinal bridge formation.

PCM Type	Dentinal Bridge Formation	Number of Teeth with PARL
PROROOT® MTA	10/12	0/14
TheraCal LC	12/13	1/14
EndoSequence BC RRM	13/14	0/14
Endo-Eze™ MTAFlow	13/14	0/14
Dycal	0/14	4/14
Control	0/14	2/14

Table 1. Quantification of dentinal bridge and PARL formation based on µCT analysis.

Reparative dentinal bridge formation and periapical bone loss resulting from the use of direct pulp capping materials was quantified.

In addition to dentinal bridge and reparative dentin formation, the development of periapical bone loss and periapical radiolucency (PARL) was also evaluated between PMCs. Among these groups, only TheraCal LC and Dycal exhibited PARL development. One out of fourteen (7%) of TheraCal LC treated mice developed PARLs, whereas four out of fourteen (29%) of Dycal pulp exposed teeth developed PARLs. In comparison, only two out of fourteen (14%) of control treated teeth developed PARLs. The summary of these findings can be found in **Table 1** and **Figure 3**.

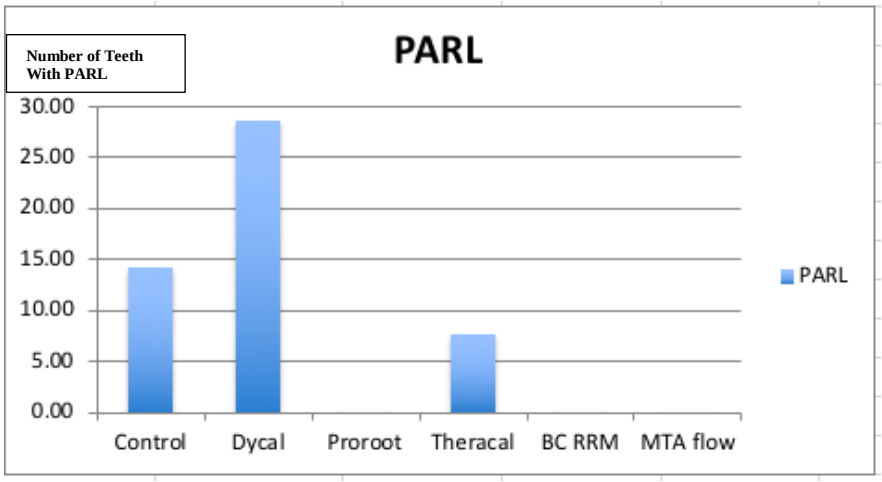

Figure 3: Quantitative effect of HCSCs on PARL development between PCM groups.

3.2 Histological analysis of direct pulp capping using HCSCs exhibit distinct reparative dentinogenesis.

Having radiographically evaluated reparative dentin formation between different HCSCs, we next sought to histologically evaluate and confirm the development of true reparative dentin among mice undergoing pulp capping procedures. Five weeks after the pulp capping procedure and after μCT analysis of harvest mice maxilla was performed, these maxillae were demineralized, paraffin embedded and H&E stained for histologic analysis.

Mice that received PROROOT® MTA as a pulp capping agent exhibited clear histologic evidence of reparative dentin formation (**Figure 4A, B**). The observed dentinal bridge completely spanned the extent of the pulp exposure, and the reparative dentin was both homogenous in nature and in close proximity with the PCM. The periapex of these teeth appeared normal, with no noticeable periapical bone loss observed.

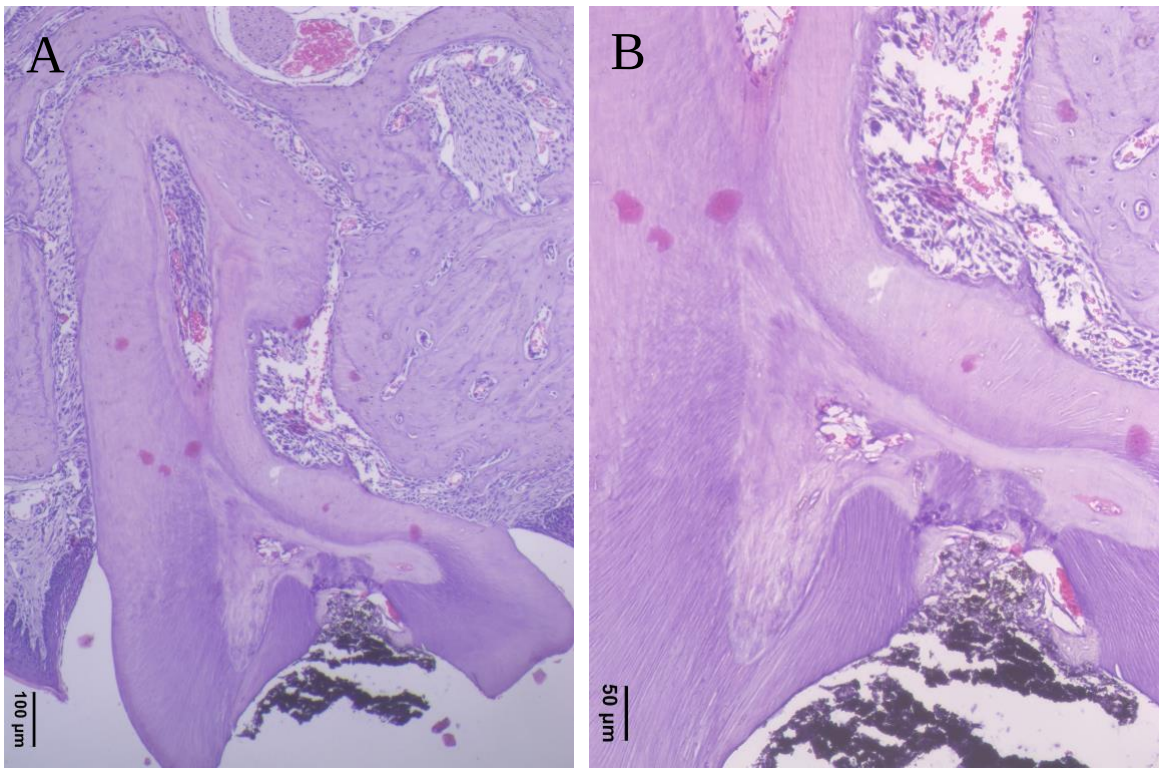

Figure 4. Histologic analysis of reparative dentin formation by PROROOT® MTA pulp capping agent.

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using PROROOT® MTA. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

Mice that received TheraCal LC as a pulp capping agent exhibited clear histologic evidence of reparative dentin formation, similar to that found with PROROOT® MTA (**Figure 5A, B**). The observed dentinal bridge similarly spanned the extent of the pulp exposure, and the reparative dentin was both homogenous in nature and in close proximity with the PCM. The periapex of these also did not exhibit noticeable periapical bone loss.

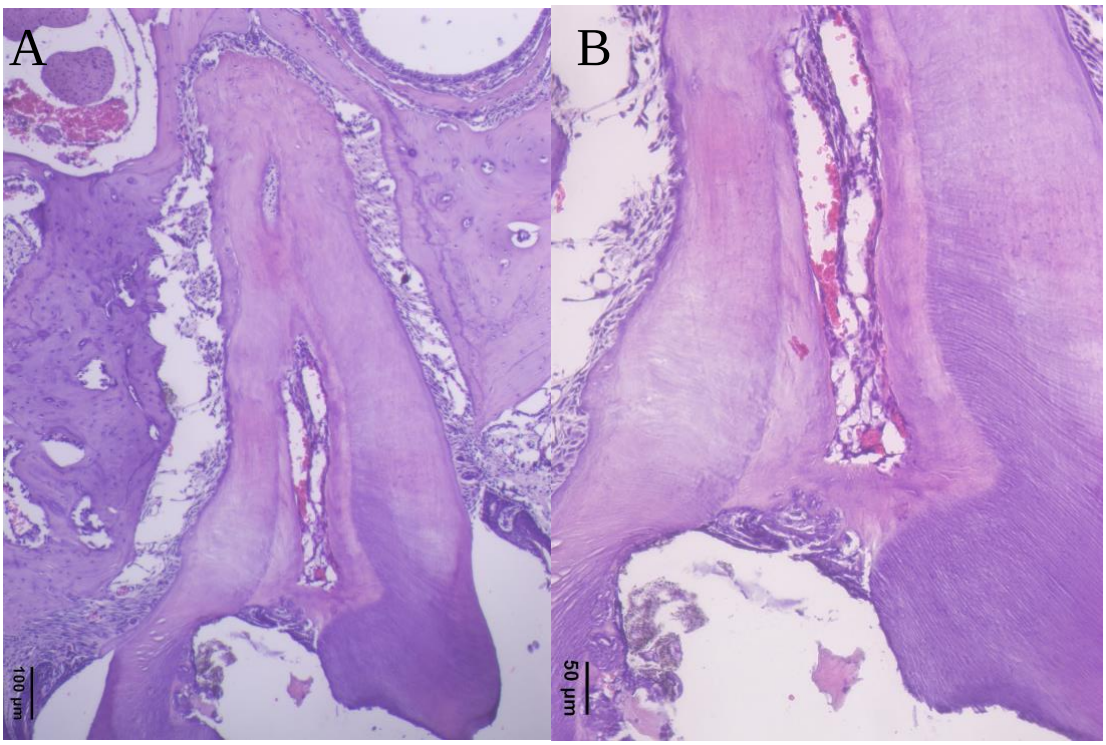

Figure 5. Histologic analysis of reparative dentin formation by TheraCal LC pulp capping agent.

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using TheraCal LC. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

Mice that received EndoSequence BC RRM as a pulp capping agent demonstrated histologically the presence of PCM beyond the pulpal roof and into the pulp chamber, as observed in the μCT analysis (**Figure 6A, B**). Reparative dentin was also observed below the PCM, however the dentinal bridge did not completely span the extent of the pulp exposure nor was it completely connected.

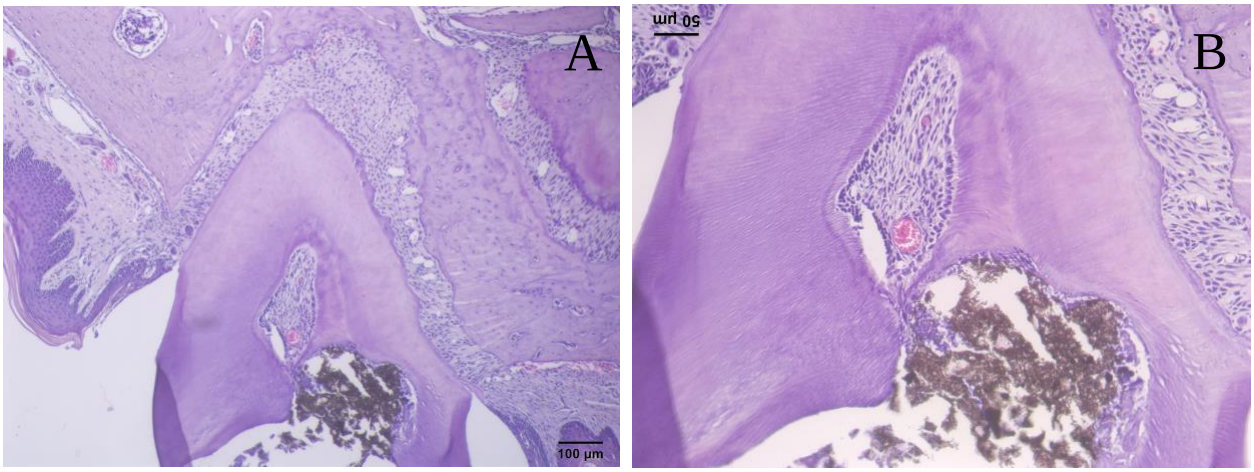

Figure 6. Histologic analysis of reparative dentin formation by EndoSequence BC RRM pulp capping agent.

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using EndoSequence BC RRM. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

Histologically, mice that received Endo-Eze™ MTAFlow as a pulp capping agent demonstrated homogenous, complete dentinal bridge formation beneath the PCM (**Figure 7A,B**). No periapical abnormalities were noted, and periapical bone remained intact. Further, the PCM in this group was cut smoothly during sectioning of the paraffin embedded sample, suggesting that Endo-Eze™ MTAFlow may have possibly been decalcified.

Unlike the other HCSCs, the use of Dycal as a pulp capping agent in mice did not demonstrate reparative dentin and dentinal bridge formation (**Figure 8A, B**). The pulp chamber within these teeth remained intact, with the pulp tissue continuous with the access opening and reactionary dentin observed in the apical third of the canal space. These findings were consistent with those found in the control group (**Figure 9A, B)**.

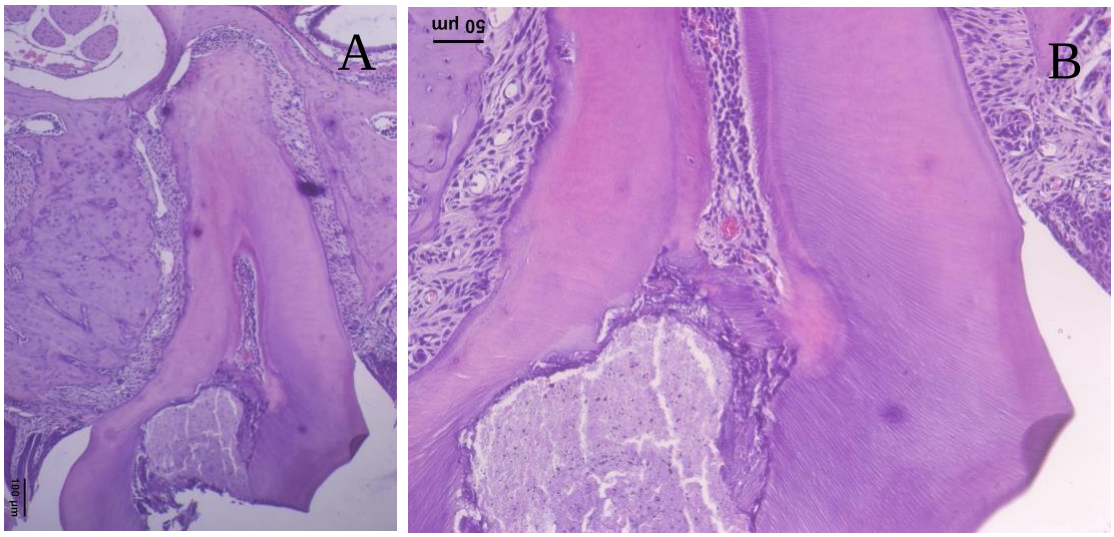

Figure 7. Histologic analysis of reparative dentin formation by Endo-Eze™ MTAFlow pulp capping agent.

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using Endo-Eze™ MTAFlow. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

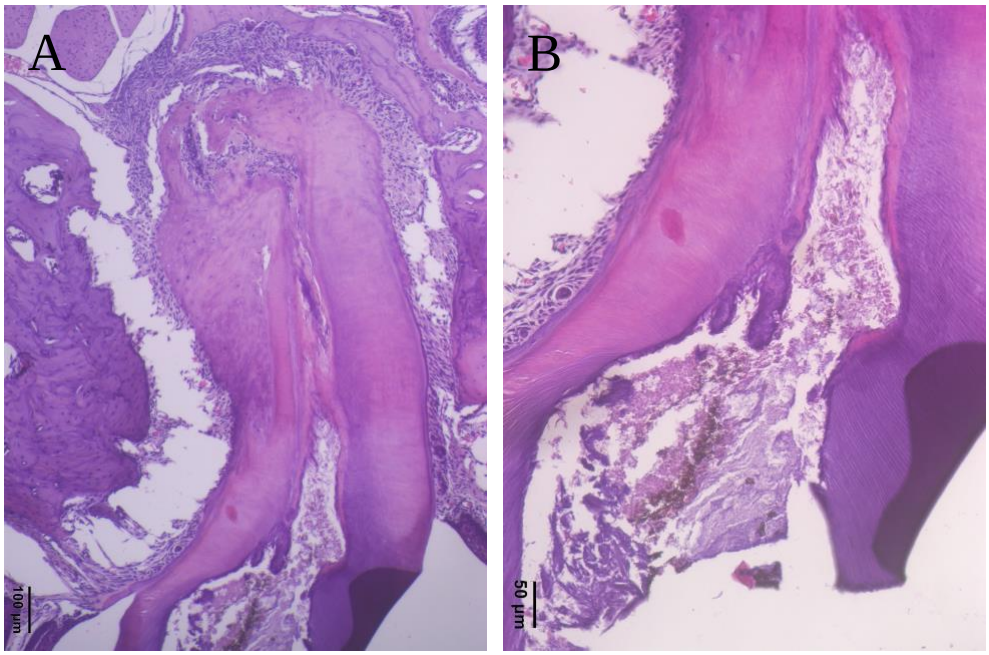

Figure 8. Histologic analysis of reparative dentin formation by Dycal pulp capping agent.

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using Dycal. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

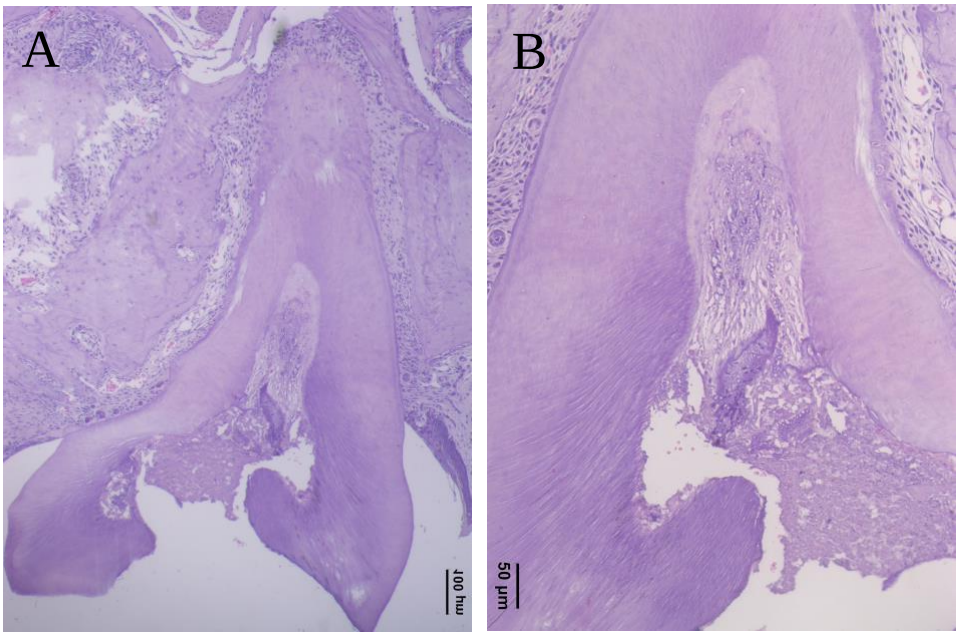

Figure 9. Histologic analysis of reparative dentin formation in the absence of a pulp capping agent (control group)

Pulp exposure was induced in maxillary first molars of C57/BL6 mice, which was subsequently capped using composite as a control. Reparative dentinogenesis was evaluated at (**A**) 10X and (**B**) 20X magnification.

4. Discussion

The aim of this study was to utilize an *in vivo* mouse model to induce pulp exposure and study the radiographic and histologic effects of different HCSCs on reparative dentin formation and PARL development. To this end, our study demonstrated the effectiveness of PROROOT® MTA, TheraCal LC, EndoSequence BC RRM, and Endo-Eze™ MTAFlow in inducing reparative dentin and dentinal bridge formation, although TheraCal LC demonstrated PARL formation in some PMC treated teeth. Unlike these HCSCs, Dycal did not demonstrate reparative dentin formation

and many of the pulp capped teeth developed PARLs, with data consistent with findings in the control group.

Although the data in this study were very convincing, there were a number of challenges experienced during sample preparation. For instance, many of the PROROOT® MTA group samples were not completely decalcified, which resulted in the scratching of the paraffin films by the remaining mineralized hard tissue during microtomy and paraffin sectioning. This difficulty with sectioning may or may not have contributed to the clear discoloration observed during the sectioning of samples for histological analysis. Nonetheless, the results for the PROROOT® MTA group were consistent with previous studies by Dammaschke *et al* demonstrating the biocompatibility and hard tissue regeneration capacity of PROROOT® MTA in direct pulp capping in rats (Dammaschke *et al.,* 2010). Ford *et al.* also demonstrated the capacity of MTA to promote dentinal bridge formation with minimal inflammation (Ford *et al.,* 1996). However, not all teeth in the PROROOT® MTA group demonstrated reparative dentin formation, which may be the result of the poor handling properties of this material and the small size of the access opening which may have compromised the accuracy and sufficiency of the pulp capping of the pulp exposure.

In the TheraCal LC group, dentinal bridge formation was observed in most samples, however PARL formation did occur in one of the teeth. TheraCal LC is a resin modified Portland cement that is 45% resin (Gandolfi *et al.,* 2012), and the PARL formation experienced in this study is consistent with previous observational animal studies. Lee *et al.* demonstrated that TeraCal possess poor biocompatibility and induces extensive pulpal inflammation when used as a pulp

capping agent after partial pulpotomy in dogs (Lee *et al.,* 2015). This pulpal inflammatory reaction is likely due to the presence of acrylic monomer Bis-GMA in TheraCal.

In the third group in our experiment, the EndoSequence BC RRM group, thirteen out of fourteen pulp capped teeth demonstrated notable reparative dentin formation. These findings are consistent with a previous study by Chen and Karabucak, in which EndoSequence BC RRM was shown to possess superior tissue healing as a root-end filling material in dogs when compared to grey MTA, suggesting that biocompatibility of EndoSequence BC RRM may be similar, if not superior, to MTA (Chen *et al.,* 2015). Our study demonstrates similar biocompatibility and healing response to pulp tissue in a mouse model, with no notable PARL development or periapical bone loss associated with any of the pulp capped teeth. However, it is important to note that in this group, PCM material was inadvertently pushed into the pulp chamber, which made it difficult to visualize a distinct dentinal bridge, and instead, clear reparative dentin formation was observed on either side of the PCM.

The findings of this study in regard to the use of Endo-Eze™ MTAFlow were consistent with previous observational studies, including that of Bueno *et al.* in which the biocompatibility of MTAFlow was shown to be superior to ProRoot MTA (Bueno *et al.,* 2018). In our study, the application of Endo-Eze™ MTAFlow did not induce PARL development or periapical bone loss, and all samples exhibited dentinal bridge formation adjacent to the pulp capping material. Due to the soft consistency of MTAFlow, this material could easily be pushed into the pulp chamber with minimal pressure, and in cases where the MTAFlow was pushed into the pulp canal space, reparative dentin formation was observed on either sides of the pulp capping agent.

Among the pulp capping materials used in this study, Dycal demonstrated the poorest treatment outcome in terms of pulp vitality and reparative tissue regeneration. Kundzina *et al.* demonstrated a significantly reduced 36 month survival rate of 52% in adult human teeth treated with Dycal, as compared to 85% survival rate of MTA (Kundzina *et al.*,2017). In our study, the use of Dycal as a pulp capping agent yielded no reparative dentin formation, with four teeth demonstrating PARL development and periapical bone loss. Use of this pulp capping material resulted in poor prognosis and formation of reactionary dentin formation inconsistent with dentinal bridge formation. This reactionary dentin was found sporadically throughout the canal space and was not in close proximity with the Dycal material, suggesting that this material did not permit stimulation of viable cell growth over the dentinal bridge and irritated the pulp tissue sufficiently to induce reactionary dentin formation.

This study demonstrated radiographic and histologic evaluation of several prominent and commercially available HCSCs in pulp repair and reparative dentin formation. The findings of this study may play an important role in improving our understanding of the cell and tissue toxicity, pulpal and dentin reparative capacity and possible periapical bone loss potential induced by these HCSC materials.

5. Conclusions

All HCSC derivative materials are biocompatible and as effective as the original PROROOT® MTA in their ability to enhance reparative dentinogensis, with the exception of Dycal.

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