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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

Avisha Shah

2019

ABSTRACT OF THE THESIS

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

by

Avisha Shah

Master of Science in Oral Biology

University of California, Los Angeles, 2018

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 has been used as the gold standard for pulp capping since decades, the development of mineral trioxide aggregate (MTA) and its derivatives, also known as hydraulic calcium-silicate cements (HCSCs) have ushered in a new wave of therapeutic pulp capping agents. Nonetheless, there is limited evidence about the pulpal toxicity and dentin regenerative capacity of these materials. In the current study, the effects of 4 of these HCSCs (PROROOT® MTA, TheraCal LC, EndoSequence BC RRM, Endo-Eze™

MTAFlow) along with the control groups, composite and UltraCal® XS (Dycal)on reparative dentin and dentinal bridge formation and periapical bone loss were evaluated radiographically and histologically in mice. Pulp exposure was induced bilaterally in maxillary first molars of C57/BL6 mice. The maxillae were harvested, fixed, and subjected to μCT scanning, three-dimensional volumetric analysis and Hematoxylin and Eosin staining (H &E) was done to evaluate the reparative potential of the pulp capping materials. Tartrate-Resistant Acid Phosphotase (TRAP) staining was performed, and osteoclasts were quantified. Among the five HCSCs used for pulp capping, only UltraCal® XS (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 UltraCal ® XS (Dycal) developed periapical radiolucency's. Histologically, dentinal bridge formation was observed in all HCSCs except UltraCal® XS (Dycal). Histologically, TRAP+ osteoclasts were absent in all the HCSCs. Although some samples of Theracal LC showed a few TRAP+ osteoclasts, significantly more were seen in the UltraCal ® XS (Dycal) group. These findings indicate that with the exception of UltraCal® XS (Dycal), all HCSC derivative materials evaluated are comparatively effective to the original PROROOT® MTA in promoting reparative dentin formation and pulpal repair.

The thesis of Avisha Shah is approved.

Mo Kang

Nadia Chugal

Bo Yu

Reuben Kim, Committee Chair

University of California, Los Angeles

2019

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1. Introduction:

1.1 Direct Pulp Capping as a Conservative Treatment for Pulp Exposure

The aim of vital pulp therapy is to treat reversible pulpal injuries in both permanent and primary teeth, maintaining pulp vitality and function. The dental pulp possesses the ability to form a dentin-like matrix (tertiary dentin) as part of the repair in the dentin-pulp organ. Vital Pulp therapy includes two therapeutic approaches: Indirect Pulp capping in cases of deep dentinal cavities and direct pulp capping or pulpotomy in cases of pulp exposure (Fuks 2008).

Direct pulp capping is the process of the removal of caries and applying pulp capping material over the pulp exposure site without removal of the inflamed tissue underneath the caries lesion. It is reasonable to assume that the completion of inflamed tissue removal is critical to the healing of vital pulp therapy (Aguilar et al., 2011). According to a study by Damashke et al., 80.2% of the teeth that underwent direct pulp capping treatment showed favorable outcomes (Damashke et al., 2010). In another study where, direct pulping was performed on carious-exposed pulp the success rate was 81.8% (Matsuo et al., 1996). Direct pulp capping is thus a successful conservative form of treatment for pulp exposure.

1.2 Utilization of Calcium Hydroxide as a Pulp Capping Material

Calcium Hydroxide (CH) is considered as a gold standard among the pulp capping materials. An advantages of calcium hydroxide is its ability to inhibit bacterial growth. This is due to the release of hydroxyl ions from CH in an aqueous environment, which is capable of causing bacterial cell death (Siquera 1999). The pH values of most current CH-based cements such as Dycal range from 10-12 (Schmalz G et al., 2009). This alkalinity stimulates reparative dentin formation and kills bacteria, but is also extremely toxic to pulp cells (Schroder et al., 1985). When in direct contact with the pulp, CH produces a superficial layer of coagulative necrosis up to 2mm in depth as well as inflammatory changes in deeper tissue (Mohammadi Z et al., 2011).

Two unfavorable consequences of CH pulp caps have been shown by studies. First, CH can produce a persistent stimulating effect on dentin formation, leading to pulpal obliteration (Mohammadi Z et al., 2011, Lim KC et al., 1987). If root canal treatment is needed in the future, the hypercalcification can make this procedure difficult if not impossible. Another potential adverse effect of direct pulp caps with CH is chronic inflammation, which can eventually lead to internal resorption (Mohammadi Z et al., 2011, Lim KC et al., 1987).

1.3 Utilization of MTA as a Pulp Capping Material

Mineral Trioxide Aggregate (MTA) was developed by Torabinejad in 1993. It was developed initially as a root end filling material and subsequently has been used for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, repair of root perforations, and as a root canal filling material. It is currently a popular choice among clinicians as a pulp capping material.

MTA has many properties that make it a desirable pulp capping material. MTA is highly biocompatible and is chemically nearly inert (Torabinejad 2010). It induces limited tissue necrosis and inflammation in vivo and is also capable of inducing hard tissue formation at a faster rate and of greater thickness and quality than CH-based materials (Faraco et al., 2001). MTA is also able to form an excellent seal with tooth structure that protects against bacterial leakage (Torabinejad 2010). Finally, MTA has an antibacterial effect, which is less effective than that of CH.

Mente et al. conducted a large controlled clinical trial comparing long term outcomes of direct pulp caps performed with MTA and CH. They found direct pulp caps performed with CH had a failure rate 2.5 times that of MTA and concluded that MTA was a superior material (Mente et al., 2014). Another author

also reported higher failure rates for direct pulp caps carried out with CH (31.5%) vs. MTA (19.7%) (Hilton et al., 2013).

1.4 Utilization of MTA and its Derivatives as Pulp Capping Materials

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 according to Parirokh in 2018, there exists very limited scientific evidence as to any clinical superiority or improvements to toxicity or efficacy of these derivative materials. Due to the limited evidence available, 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 and its derivates using uCT 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 different HCSCs have differential regenerative potential in inducing reparative dentin formation and resolving pulp inflammation when used as dental pulp capping materials in vivo. 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 uCT 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. Mouse Model

C57/BL6 mice(six-week-old-female) purchased from Jackson Laboratory (Bar Harbor, ME, USA) 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 mice were 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. Toe

pinch test was used to confirm aesthesia. 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. Direct 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 handpiece 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 um 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.

2.3 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 by anesthesia, the mice were returned to the vivarium for housing.

2.4 Tissue Procurement

After 5 weeks, the mice were anesthetized using isoflurane and euthanized by cervical dislocation. The maxilla of these mice was 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.

2.5 μCT Scan and three-dimensional volumetric analysis

The fixed mouse maxillae were wrapped in 70% ethanol-soaked gauze and placed in a 15 mL contribute tube for uCT analysis. They were subsequently scanned in Scanco μCT 40 (Scanco Medical, Brüttisellen, Switzerland) at a voxel size of 20 μm^3 and a 0.5-mm aluminum filter at 55 kVp and 145 μA, with an integration time of 200 milliseconds using a cylindrical tube (field of view/diameter, 20.48 mm). Maxillary tissues were reconstructed and analyzed via the CTan and CTvol programs (Bruker microCT, Kontich, Belgium) to generate three-dimensional images and cross-sectional images. Bone loss was quantified by measuring the distance between the cementoenamel junction and the alveolar ridge on palatal and buccal roots of the first molars in all the groups on Dataviewer (Bruker microCT)

2.6 Hematoxylin and Eosin staining

Upon completion of μCT scan, mouse maxillae were decalcified using 5% EDTA and 4% sucrose in PBS (pH 7.4) for 5 weeks. The decalcified maxillae were trimmed by making a sagittal cut immediately anterior to the first molar and were sent to the University of California, Los Angeles Translational Procurement Core

Laboratory and processed for paraffin embedding. 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. The sectioned slides were deparaffinized at 60°C, then rehydrated in ethanol with an increasing concentration of water. The rehydrated tissue slides were stained with hematoxylin or 2.5 minutes, washed with water and 95% ethanol, and then stained with eosin for 1 minute. The stained slides were dehydrated in 70%, 95%, and 100% ethanol, followed by xylene. The slides were mounted using mounting medium (Permount; Fisher Scientific, Houston, TX). Digital images were taken through an Olympus microscope (model DP72; Olympus Corp., Tokyo, Japan) at ×100, x200 magnification.

2.7 Tartrate-Resistant Acid Phosphatase Staining

Tartrate-Resistant acid phosphatase staining was performed, as described previously (Williams et al., 2014). Briefly, the sectioned slides were incubated with tartrate-resistant acid phosphatase solution in a humidification chamber at 37°C in the dark for 1 hour. The slides were washed in water and counterstained in hematoxylin, then mounted with ImmunoHistoMount (Sigma-Aldrich, St. Louis,

MO). Osteoclasts were identified by the presence of multiple nuclei ($n > 5$). Osteoclast number quantification and surface area were measured using ImageJ software version 1.48 on digital images taken through an Olympus microscope (model DP72) at ×100 magnification.

2.8 Statistical Analysis

One-way analysis of variance and Tukey's post hos test were used to compare the volume of the periapical radiolucency and number of osteoclasts between the PCMs. All of the statistical analyses were performed with Prism software version 8 (Irvine, CA), with a significance level of 0.05.

3. Results

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

In order to determine the degree of dentinal bridge and reparative dentin formation induced by the different HCSCs 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. uCT analysis of the harvested mice maxilla demonstrated significant differences between the different HCSC materials (Figure 1).

In mice receiving PROROOT® MTA for pulp capping, ten out of twelve (83%) exhibited 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.

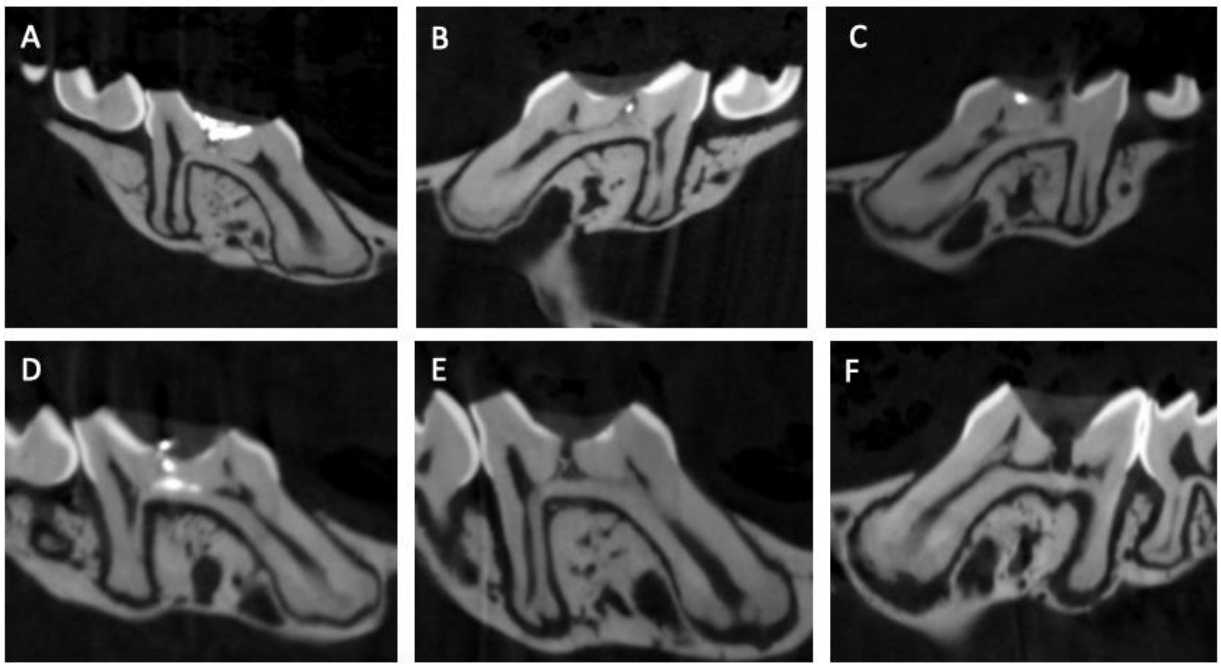


FIGURE 1 Direct Pulp-Capping with different HCSCs inducing variable degrees of dentinal bridge formation and periapical radiolucency. (A) PROROOT® MTA, (B) TheraCal LC, (C) EndoSequence BC RRM, (D) MTA Flow, (E) UltraCal® XS (Dycal), as well as (F) composite, which served as the control group.

In mice receiving TheraCal LC, twelve out of thirteen (92%) exhibited 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™ MTA Flow 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. The summary of these findings can be found in FIGURE 2.

(A)

PCM Type	Dentinal Bridge Formation
PROROOT® MTA	10/12
TheraCal LC	12/13
Endosequence BC RRM	13/14
Endo-Eze™ MTA Flow	13/14
UltraCal® XS	0/14
Control	0/10

(B)

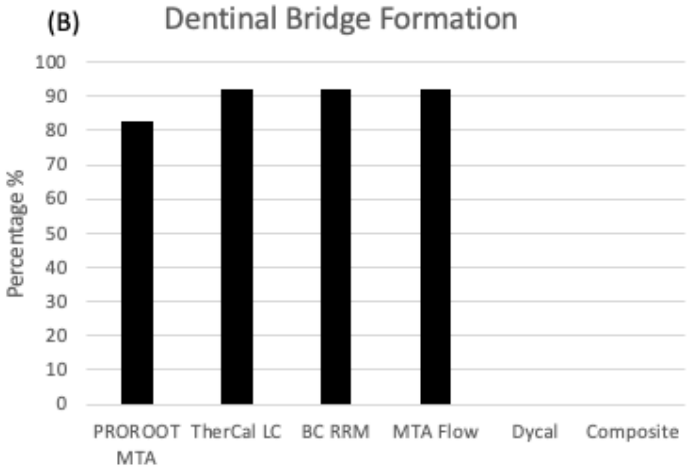


FIGURE 2 (A) Qualitative and (B) Quantitative Analysis of dentinal bridge formation between the PCMs.

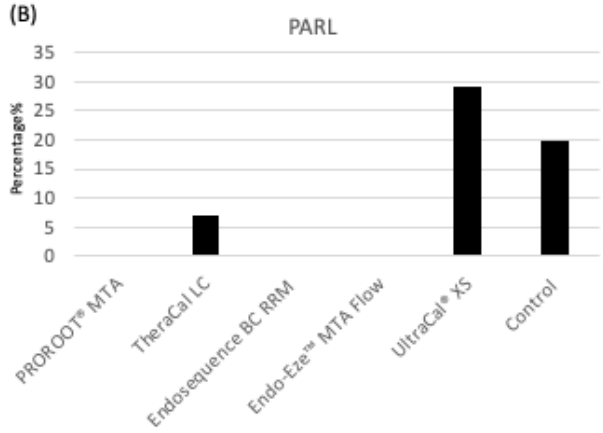
In mice receiving UltraCal® XS (Dycal) as a pulp capping agent, none of the mice demonstrated dentinal bridge formation (Figure 1E). Seven out of fourteen of these mice did not show reparative dentinogenesis, and the remaining mice demonstrated pulp chamber calcification that was not consistent with 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.

3.2 Analysis of periapical bone loss and periapical lesion development after direct pulp capping with the various HCSCs

In addition to dentinal bridge and reparative dentin formation, the development of periapical bone loss and periapical radiolucency (PARL) was also evaluated between PCMs. Among these groups, only TheraCal LC and UltraCal® XS (Dycal) exhibited PARL development. One out of fourteen (7%) of TheraCal LC treated mice developed PARLs, whereas four out of fourteen (29%) of UltraCal® XS (Dycal) pulp exposed teeth developed PARLs. In comparison, only two out of ten (20%) of control treated teeth developed PARLs.

(A)

PCM Type	Number of teeth with PARL
PROROOT® MTA	0/14
TheraCal LC	1/14
Endosequence BC RRM	0/14
Endo-Eze™ MTA Flow	0/14
UltraCal® XS	4/14
Control	2/10



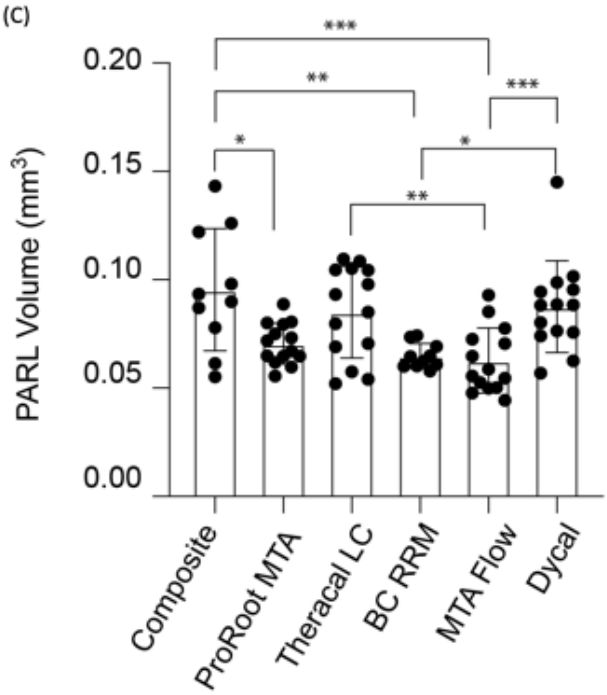


Figure 3 (A), (B) Qualitative and (C) Quantitative analysis of PARL between the PCM groups.

Significant differences was observed in the volume of the PARL between the control group and the PROROOT® MTA, EndoSequence BC RRM and Endo-Eze™ MTA Flow groups. Significant differences in the volume was also observed between the UltraCal® XS (Dycal) and the EndoSequence BC RRM and Endo-Eze™ MTA Flow groups. Significant difference was also observed between the Endo-Eze™ MTA Flow group and the TheraCal LC group. This Indicates that the volume of the PARL was significantly less or absent in the HCSC groups.

3.3 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 □CT analysis of harvest mice maxilla was performed, these maxillae were demineralized, paraffin embedded and H&E stained for histologic analysis.

In Mice that received PROROOT® MTA as a pulp capping agent exhibited clear histologic evidence of reparative dentin formation (Figure 4A, B, C). 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.

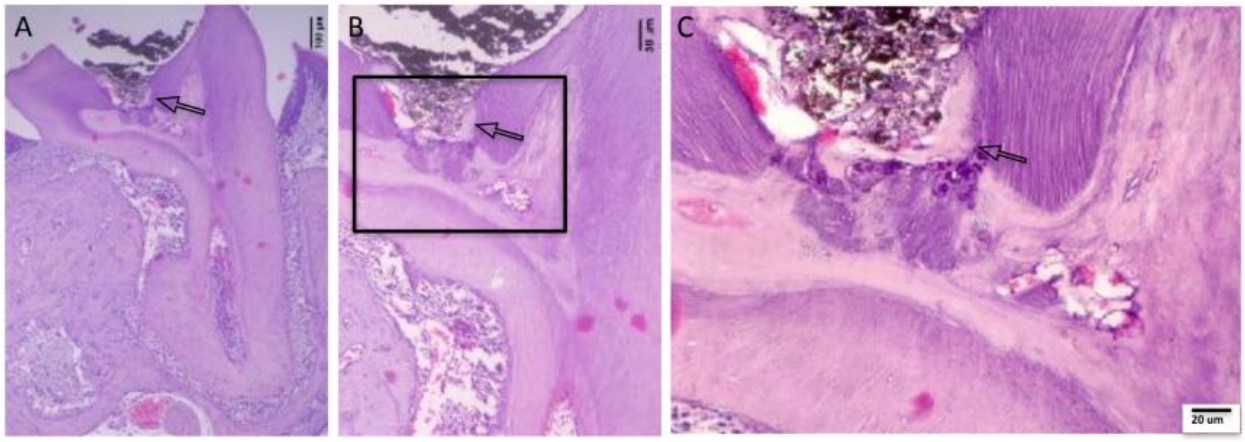


FIGURE 4 Histological evidence of dentinal bridge formation in groups receiving PROROOT® MTA as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

Mice receiving 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, C). 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 pulp tissue below the dentinal bridge however shows signs of pulpal inflammation. The periapex of these also did not exhibit noticeable periapical bone loss.

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 uCT analysis (Figure 6A, B, C). 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.

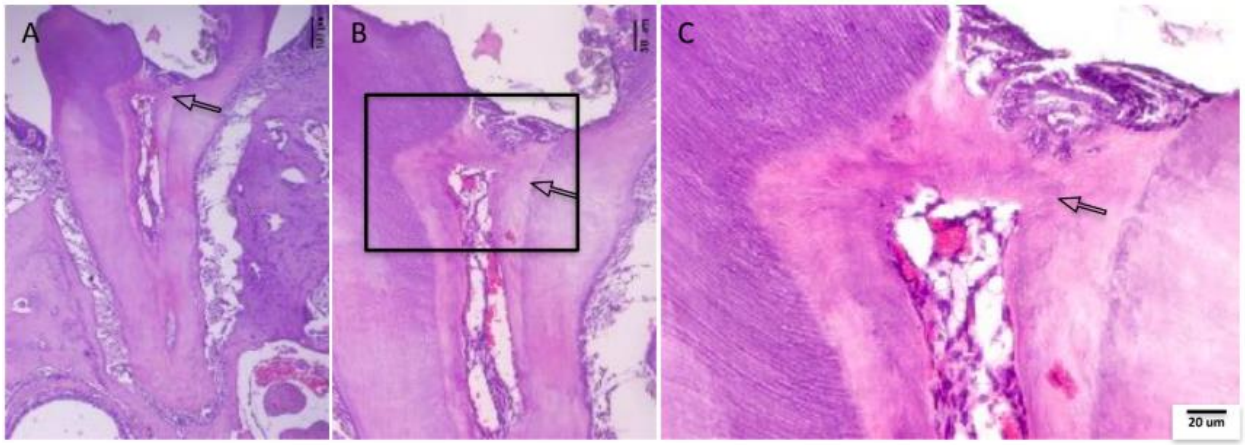


FIGURE 5 Histological evidence of dentinal bridge formation in groups receiving TheraCal LC as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

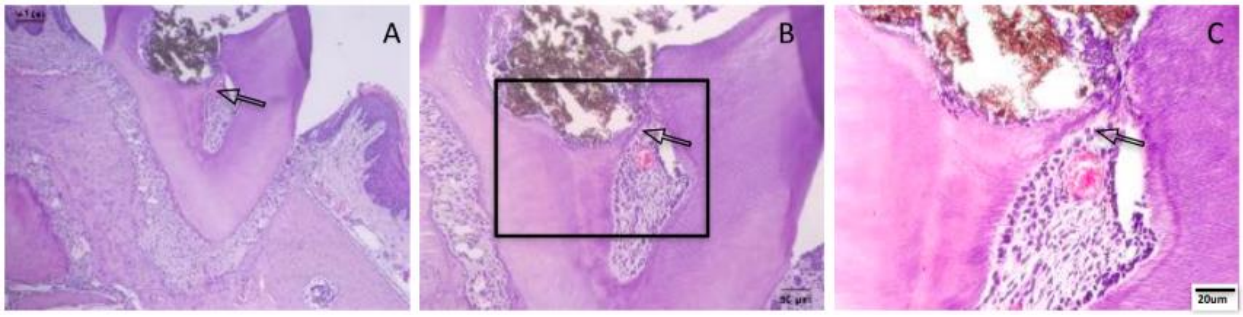


FIGURE 6 Histological evidence of dentinal bridge formation in groups receiving EndoSequence BC RRM as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

Mice that received Endo-Eze™ MTAFlow as a pulp capping agent demonstrated homogenous, complete dentinal bridge formation beneath the PCM histologically

(Figure 7A, B, C). No periapical abnormalities were noted, and periapical bone remained intact.

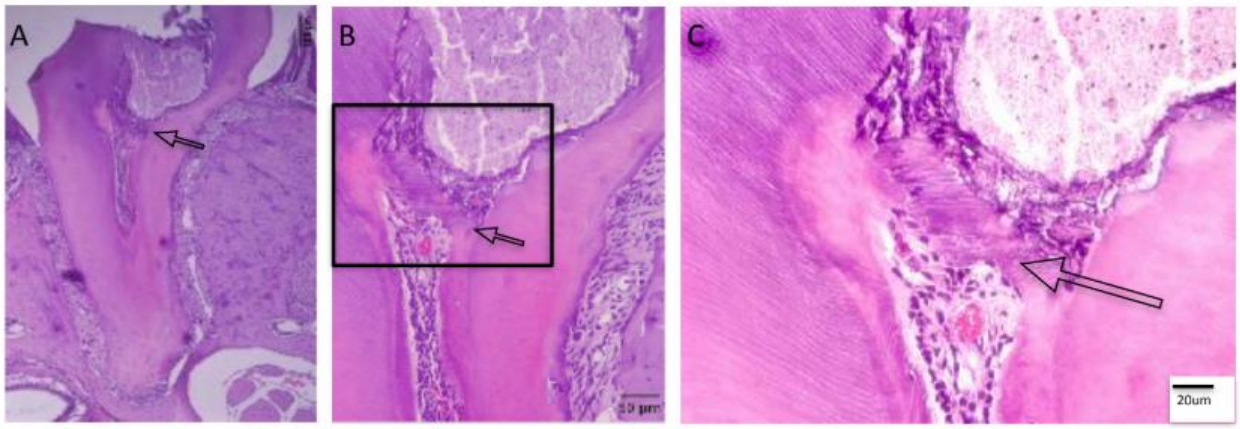


FIGURE 7 Histological evidence of dentinal bridge formation in groups receiving Endo-Eze™ MTAFlow as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

Unlike the other PCMs, the use of UltraCal® XS (Dycal) as a pulp capping agent in mice did not demonstrate reparative dentin and dentinal bridge formation (Figure 8 A, B, C). The pulp chamber within these teeth remained intact, however the pulp tissue showed signs of inflammation. These findings were consistent with those found in the control group (Figure 9 A, B, C).

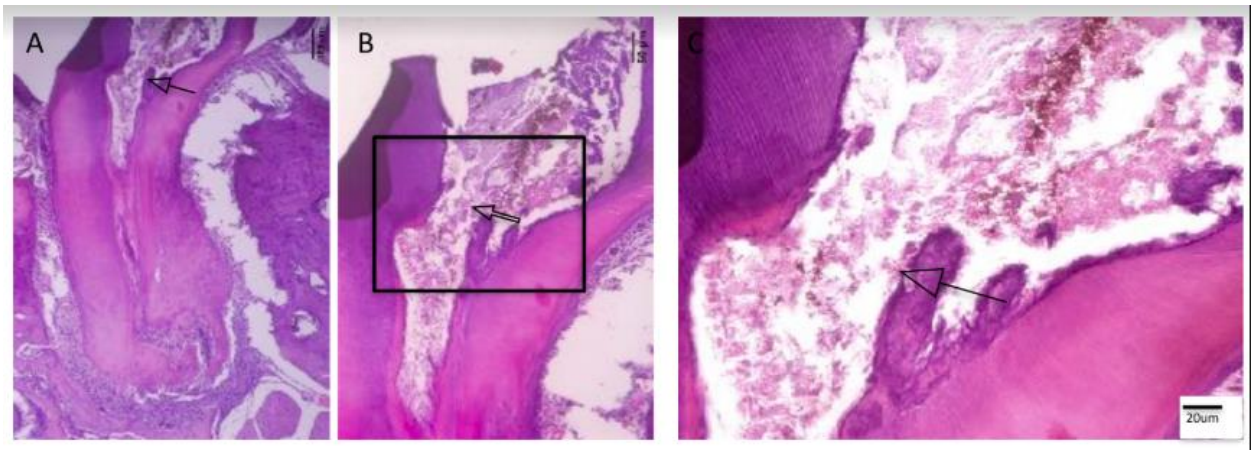


FIGURE 8 Histological evidence of absence of dentinal bridge formation in groups receiving UltraCal® XS (Dycal) as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

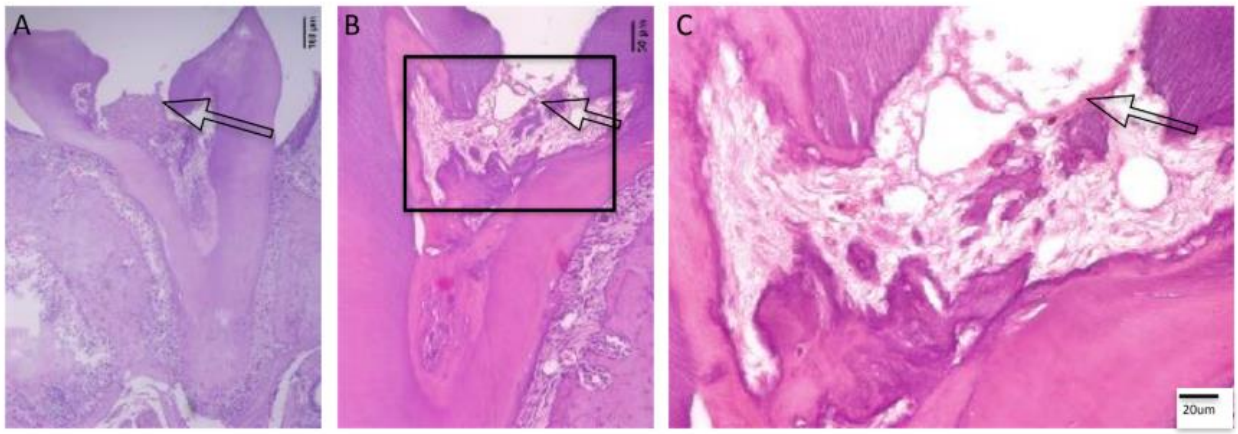


FIGURE 9 Histological evidence of absence of dentinal bridge formation in groups receiving Composite (control) as the pulp capping material using H & E staining under (A) 40 X (B) 100 X (C) 200 X magnification.

3.4 Histological analysis of direct pulp capping using HCSCs exhibit absence of osteoclasts in the periapical region

Having histologically evaluated reparative dentin formation between different HCSCs, we next sought to histologically evaluate and confirm the bone loss and development of periapical radiolucency's among mice undergoing pulp capping procedures. Five weeks after the pulp capping procedure and after uCT analysis and H & E staining, TRAP staining was performed for further histological evaluation.

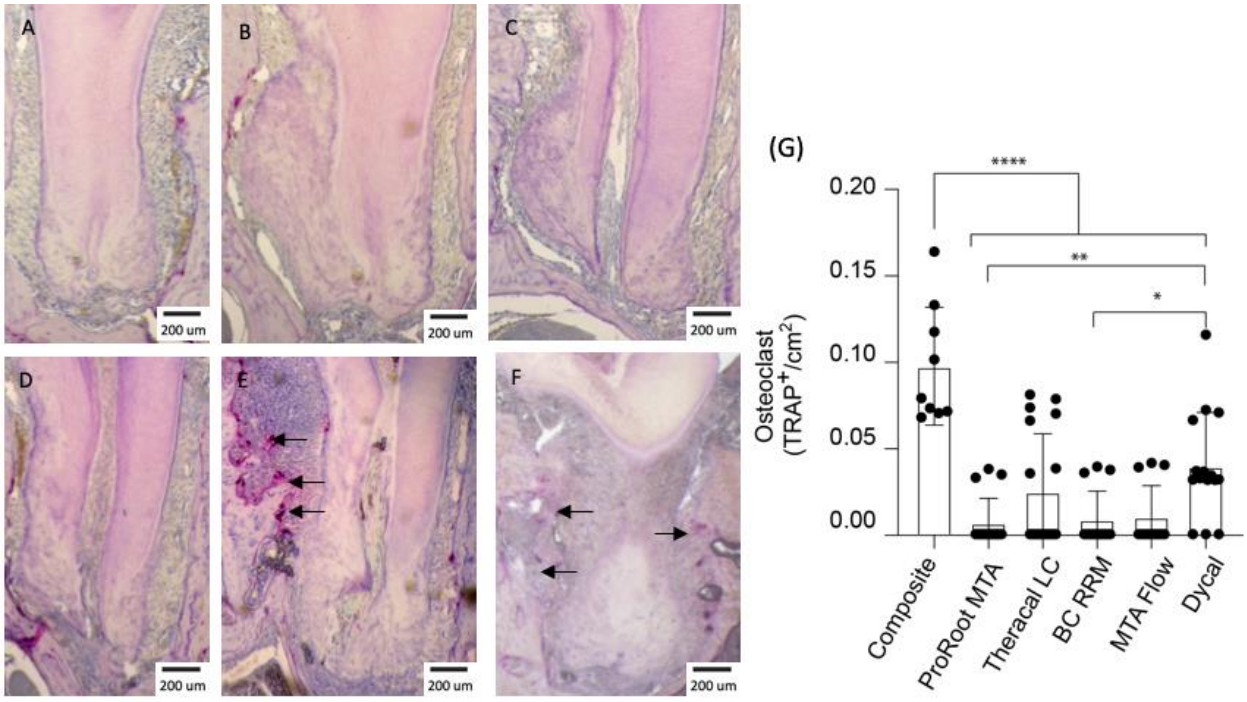


FIGURE 10. Histological evidence of TRAP + osteoclasts in (A) PROROOT® MTA, (B) TheraCal LC, (C) EndoSequence BC RRM, (D) MTA Flow, (E) UltraCal® XS (Dycal), as well as (F) composite, which served as the control

group. (G) Quantitative analysis of TRAP + osteoclasts between the pulp capping materials.

In mice receiving PROROOT® MTA as the pulp capping agent there was no bone loss and periapical radiolucency observed. Absence of TRAP + osteoclasts was also noted. Similar results were observed in groups receiving EndoSequence BC RRM and Endo-Eze™ MTAFlow (Figure 10 A, C, D). In mice receiving TheraCal LC as the pulp capping material osteoclasts were observed in some of the samples. Bone loss and periapical radiolucency was also noted in those samples (Figure 10 B). Mice receiving UltraCal® XS (Dycal) as the pulp capping material bone loss and periapical radiolucency was observed along with TRAP + osteoclasts which was comparable to the control group (Figure 10E, F).

After histologically observing the presence of osteoclasts on TRAP staining, the number of osteoclasts were quantified. There was significant difference in the number of osteoclasts between the composite and the PROROOT® MTA, EndoSequence BC RRM and Endo-Eze™ MTAFlow groups. Significant differences was also noted between the UltraCal® XS (Dycal) and PROROOT® MTA, EndoSequence BC RRM and Endo-Eze™ MTAFlow groups (Figure 10 G).

4. Discussion

The aim of this study is to evaluate the effects of different HCSCs on pulpal wound healing, pulpal inflammatory reaction and reparative dentin regeneration after direct pulp capping in vivo mice model using radiographic and histological evidence. To this end, our study confirmed 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 and TRAP + osteoclasts in some PMC treated teeth. Unlike these HCSCs, UltraCal® XS (Dycal) did not demonstrate reparative dentin formation and many of the pulp capped teeth developed PARLs, with similar findings observed in the control group.

MTA has been well studied in experiments and has shown good sealing ability (Torabinejad et al., 1993) and bio- compatibility (Holland et al., 1990). Dentine bridge- like hard tissue was consistently observed when MTA was used as a pulp–capping agent in monkeys (Pittford et al., 1996), dogs (Asgary et al., 2006) and rats (Salako et al., 2003). Ford et al. also demonstrated the capacity of MTA to promote dentinal bridge formation with minimal inflammation (Ford et al., 1996). Beuno et al stated that the biocompatibility of MTA Flow was superior to ProRoot MTA

(Bueno et al., 2018). These findings are consistent with the PROROOT® MTA and Endo-Eze™ MTAFlow groups observed is our study.

In the mice that received TheraCal LC as the pulp capping material, dentinal bridge formation was observed in all the samples however the pulpal tissue below the pulp capping material showed signs of mild inflammation. PARL was also noted in one of the samples with TRAP+ osteoclasts observed as well. In a study conducted by Bakhtiar et al. it was noted that TheraCal LC treatment resulted in disorganized pulp tissue (Bakhtiar et al., 2017). Jeanneau et al. studied the consequences of adding resins to tricalcium silicates by investigating TheraCal LC, their work showed that TheraCal is toxic to pulp fibroblasts and induces inflammation (Jeanneau et al., 2017). Lee et al. demonstrated that TheraCal possess poor biocompatibility and induces extensive pulpal inflammation when used as a pulp capping agent after partial pulpotomy in dogs (Lee et al., 2015). The study attributed this to the acrylic monomer Bis-GMA present in the material.

Mice receiving Endosequence BC RRM as the pulp capping agent all showed reparative dentin formation. No notable periapical bone loss observed in any of the mice. Chen et al. stated that RRM is a biocompatible material, and its biocompatibility and sealing ability is comparable to that of MTA. They also

showed superior healing tendency of RRM after root- end surgery in dogs as compared to MTA (Chen et al., 2015). A study by Liu S et al also showed that bioceramics have good biocompatibility to pulp tissue and induced proliferation of the dental pulp cells and formation of reparative dentin bridge (Liu S et al., 2015).

In groups that received UltraCal® XS (Dycal) as the pulp capping material none of the teeth demonstrated reparative dentin formation and in four of the teeth notable periapical bone loss and PARL was observed. UltraCal® XS (Dycal) showed the poorest treatment outcome amongst all the pulp capping materials tested in our study and was comparable to the control group. Use of this pulp capping material resulted in reactionary dentin formation and inconsistent with dentinal bridge formation. This reactionary dentin was found sporadically throughout the canal space and was not in close proximity with the UltraCal® XS (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. It has been reported that 89% of 192 dentin bridges formed by calcium hydroxide cement in monkeys contained tunnel defects that might fail to provide a permanent barrier and a long-term biological seal against bacterial infection (Cox et al., 1996). In a study conducted by Nair et al. Teeth treated with Dycal® revealed distinctly less consistent formation of a hard tissue barrier that had

numerous tunnel defects. Further, the presence of acute and chronic inflammation of the pulp until the longest observation period (3 months) after capping, was a common feature in Dycal specimens (Nair et al. 2008).

In the control group or the group which received only composite as the pulp capping agent no reparative dentin formation was noted. Twenty percent of the specimens showed notable bone loss and PARL formation. It was observed that in some of the specimens, reactionary dentin was visible at pulp canal walls, however the reactionary dentin formation was not in close proximity to the capping material. Composites have been shown to cause chronic pulpal inflammation and prevent reparative dentin formation when applied directly to pulp exposures (Accorinte et al.,2005, Modena KC et al.,2009).

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. Conclusion

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 UltraCal® XS (Dycal).

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