Comparative study of pulpal responses to pulpotomy with ProRoot MTA®, RetroMTA®, and TheraCal® in dogs' teeth

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Sincere gratitude and appreciation to all my professors,

friends and family

for their love, support and guidance

이 논문이 나오기 까지 지도해 주신 모든 스승님들과

저를 아껴주신 친구들과 선후배님들

그리고 사랑하고 존경하는 부모님 감사드립니다.

기대에 어긋나지 않는 모습으로 베풀어주신 은혜에 보답하겠습니다.

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This study was conducted to evaluate and compare pulpal responses to ProRoot MTA®, RetroMTA®, and TheraCal® in dog partial pulpotomy models.

Partial pulpotomies were performed on 60 beagle teeth. The exposed pulp tissues were randomly capped with either ProRoot MTA® (n=15), RetroMTA® (n=15),TheraCal® (n=15), or interim restorative material as a negative control (n=15). After 4 weeks, the teeth were extracted and processed for histologic and

immunohistochemical(IHC) examinations using osteocalcin(OC) and dentin sialoprotein(DSP). Calcific barrier formation, inflammatory reaction, and the odontoblastic layer were evaluated and scored in a blind manner. The areas of newly formed calcific barriers were measured for each group.

In most of the ProRoot MTA® and RetroMTA® specimens, continuous calcific barriers were formed, and the pulps contained palisading patterns in the odontoblastic layer that were free of inflammation. However, the TheraCal® specimens had lower quality calcific barrier formation, extensive inflammation, and less favorable odontoblastic layer formation. Overall, areas of newly formed calcific barrier were higher in the ProRoot MTA® and RetroMTA® specimens than in the TheraCal® specimens. Also, IHC revealed that OC and DSP were more clearly visible in the ProRootMTA® and RetroMTA® specimens than in the TheraCal® specimens.

RetroMTA® could provide an alternative to ProRoot MTA®. Both materials produced favorable pulpal responses that were similar in nature, whereas TheraCal® produced less favorable pulpal responses.

Key words : Mineral trioxide aggregate, partial pulpotomy, pulpal response, pulpal inflammation, calcific barrier, odontoblastic layer

Comparative study of pulpal responses to pulpotomy with ProRoot MTA®, RetroMTA®, and TheraCal® in dogs' teeth

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I. Introduction

The key factor in vital pulp therapy, such as pulp capping and pulpotomy, is to maintain the pulp vitality by protecting the exposed pulp with a biocompatible material. Ideally, the exposed pulpal surface under the capping agent is enclosed by the formation of a calcific barrier, leaving the apical portion of the pulp free of inflammation.

The classical method of vital pulp therapy is performed with calcium hydroxide [$Ca(OH)_2$]. However, using $Ca(OH)_2$ has several limitations, including its high solubility and unpredictable treatment outcomes (Cox et al., 1996; Mente et al., 2014). With the introduction of mineral trioxide aggregate (MTA), a novel pulp treatment agent, the short-term and long-term success rates in vital pulp therapy has increased up to 93% (Leye Benoist et al., 2012) and 85% (Mente et al., 2014), respectively. MTA has been recognized as a biocompatible material that produces excellent induction of hard-tissue formation (Parirokh and Torabinejad, 2010b; Torabinejad and Parirokh, 2010). However, conventional MTA (ProRoot MTA®) has disadvantages, such as a long setting time (4 hours) and tooth discoloration (Felman and Parashos, 2013;Parirokh and Torabinejad, 2010b), that have led to the development of MTA-like materials with improved physical properties.

Biodentine (Septodont, Saint Maur des Fosses, France) has been introduced as an alternative MTA-like material with a reduced setting time, better physical properties, and ease of handling. Biodentine is compatible with dental pulp cells and stimulated the formation of tertiary dentin *in vitro* (Perard et al., 2013; Zanini et al., 2012). It also induced differentiation of cultured pulp cells into odontoblast-like cells (Laurent et al., 2012). An *in vivo* study demonstrated that Biodentine is tissue compatible and promotes mineralized tissue bridge formation with comparable morphology and integrity to those produced by ProRoot MTA® (De Rossi et al., 2014). Similar results are being achieved with other MTA materials such as Angelus MTA (Angelus, Londrina, PR, Brazil), Bioaggregate (Innovative BioCeramix, Vancouver, Canada) and MM-MTA (Micromega, Besançon, France).

RetroMTA® (Meta Biomed Co., LTD, Seoul, Korea) consists of a hydraulic calcium zirconia complex that has a setting time of 150 seconds. According to the manufacturer, RetroMTA® consists of calcium carbonate [60–80 percent by weight (wt%)], silicon dioxide (5–15 wt%), aluminum oxide (5–10 wt%), and calcium zirconia complex (20–30 wt%) (http://www.biomta.com). Despite the increase in use of RetroMTA® as a vital pulp therapy agent, there is limited information about RetroMTA® in the literature.

TheraCal® (Bisco Inc, Schamburg, IL, USA) is a light-cured, resin-modified calcium silicate filled liner designed for use in various vital pulp therapies (Gandolfi et al., 2012). TheraCal® consists of type III Portland cement (45 wt%), radiopacific material (10 wt%), fumed silica (5 wt%) and resin (40 wt%) (Suh et al., 2008). According to an *in vitro* study on resin-based liners, TheraCal® has been introduced as a low cytopathic light-cured liner (Hebling et al., 2009). Moreover, TheraCal® has been reported to have higher calcium release, a less alkaline pH, and lower solubility when compared to ProRoot MTA or Dycal (Gandolfi et al., 2012). Although TheraCal® has already been in use as an effective protective liner, to the best of our knowledge, the biological effect of TheraCal® *in vivo* has not yet been investigated.

This study was conducted to evaluate and compare calcific barrier formation, inflammation, and odontoblastic layer formation of ProRoot MTA®, RetroMTA® and TheraCal® in dog pulpotomy models.

II. Materials and Methods

1. Animal Model

Six male beagle dogs were chosen for this study. Each animal weighed 12 kg and was 18 months old. The animals had intact dentition and a healthy periodontium. Animal selection, management, surgical protocol, and preparation were carried out according to routine procedures approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (certification #2013-0153).

2. Surgical Protocol

The surgical procedures were performed under general anesthesia in a sterile operating room. The animals received a preanesthetic intravascular injection of Tramadol (1 mg/kg; Kwangmyung Pharmaceutical Co., Seoul, Korea) and an intramuscular injection of xylazine (0.2 mg/kg; Rompun, Bayer Korea, Seoul, Korea) and Zoletil (5 mg/kg; Ketalar, Yuhan, Seoul, Korea). Isoflurane (Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea) was administered as inhalation anesthesia. In order to prevent infection, a subcutaneous injection of Enrofloxacin (5 mg/kg) was given just before and after treatment and intraoral amoxicillin clavulanate (12.5 mg/kg) was given for 5-7 days post-op.

3. Partial Pulpotomy Procedure

After disinfection of the surgical site, infiltration anesthesia was administered using lidocaine (2% lidocaine hydrochloride with epinephrine 1:100,000; Kwangmyung Pharmaceutical Co., Seoul, Korea). Sixty teeth, including incisors, canines, and premolars, of each animal were allocated randomly to four pulpotomy treatment groups (n=15 per group): ProRoot MTA®, RetroMTA®, TheraCal®, and interim restorative material (IRM). Initially, the coronal pulp was removed after occlusal reduction in each root canal system using a high-speed carbide bur No. 330 (H7 314 008, Brasseler, Germany) and distilled water spray. The exposure was then rinsed with sterile saline, and hemostasis was achieved by placing a cotton pellet moistened with normal saline over the exposure site for 2 minutes. ProRoot MTA®, RetroMTA®, and IRM were each mixed according to the manufacturer's recommendations and placed over the exposure. TheraCal® was placed over the exposure in 0.5–1 mm thickness and light-cured for 20 seconds. The remainder of the cavity preparation was restored with Ketac Molar (3M ESPE, St. Paul, MN), a self-curing glass ionomer cement. The dogs were sacrificed 4 weeks after surgery.

4. Histological Analysis

The teeth were removed using extraction forceps and the apical third of each root was sectioned with a high-speed bur to facilitate fixation in 10% buffered formalin (Sigma,

MO, USA) for 1 day. After fixation, the teeth were decalcified with 10% EDTA (pH 7.4; Fisher Scientific, TX, USA) for 6 weeks and embedded in paraffin. Sagittal sections were cut at a thickness of 3μm. Sections were deparaffinized and stained with hematoxylin and eosin (HE). They were imaged with an optical microscope (Olympus BX40, Olympus Co., Tokyo, Japan). Images of the HE stained sections were captured with a CCD digital camera (Infinity 2.0, Lumenera Co., Ottawa, Ontario, Canada) and digitized using image analyzer software (InnerView 2.0, iNNERViEW Co., Seongnam-Si, Gyeonggi-do, Korea).

The HE sections were evaluated by three experienced examiners (Y. Shin, H. Lee, and JS. Song) in a blind manner. The calcific barrier formation, pulp inflammation, and odontoblastic layer were graded according to criteria that were based on a modified scoring system adapted from Nowicka et al. as described in Table 1. A final score was decided by the three examiners. Also, the areas of newly formed hard-tissue were measured using Image J (ver.1.48, National Institute of Health, Bethesda, Maryland, USA).

Table 1. Scores used during histological analysis of calcific barriers and dental pulp

Scores	Calcific barrier continuity
1	Complete dentin bridge formation
2	Partial/incomplete dentin bridge formation extending to more than one-half of the exposure site but not completely closing the exposure site
3	Initial dentin bridge formation extending to not more than one-half of the exposure site
4	No dentin bridge formation
Scores	**Calcific barrier morphology**
1	Dentin or dentin associated with irregular hard tissue
2	Only irregular hard tissue deposition
3	Only a thin layer of hard tissue deposition
4	No hard tissue deposition
Scores	**Tubules in calcific barrier**
1	No tubules present
2	Mild (tubules present in less than 30% of calcific barrier)
3	Moderate to severe (tubules present in more than 30% of calcific barrier)
Scores	**Inflammation intensity**
1	Absent or very few inflammatory cells
2	Mild (an average of <10 inflammatory cells)
3	Moderate (an average of 10–25 inflammatory cells)
4	Severe (an average >25 inflammatory cells)
Scores	**Inflammation extensity**
1	Absent
2	Mild (inflammatory cells next to dentin bridge or area of pulp exposure only)
3	Moderate (inflammatory cells observed in one-third or more of the coronal pulp or in the midpulp)
4	Severe (all of the coronal pulp is infiltrated or necrotic)
Scores	**Inflammation type**
1	No inflammation
2	Chronic inflammation
3	Acute and chronic inflammation
4	Acute inflammation
Scores	**Dental pulp congestion**
1	No congestion
2	Mild (enlarged blood vessels next to dentin bridge or area of pulp exposure only)
3	Moderate (enlarged blood vessels observed in one-third or more of the coronal pulp or in the midpulp)
4	Severe (all of the coronal pulp is infiltrated with blood cells)
Scores	**Odontoblastic cell layer**
1	Palisade pattern of cells
2	Presence of odontoblast cells and odontoblast-like cells
3	Presence of odontoblast-like cells only
4	Absent

5. Immunohistochemistry

For immunohistochemistry (IHC), 3 μm sections were deparaffinized in xylene, rehydrated, and rinsed with distilled water. For antigen retrieval, protease K (Dako, Carpinteria, CA, USA) was used in osteocalcin (OC) and dentin sialoprotein (DSP) staining. Endogenous peroxidase activity was quenched by the addition of 3% hydrogen peroxide. Sections were incubated in 5% bovine serum albumin (Sigma, MO, USA) to block nonspecific binding, and incubated with primary antibody overnight. For OC staining, a 1:10,000 dilution of the anti-OC antibody (rabbit polyclonal, Ab109112, Abcam, Cambridge, UK) was used. For DSP staining, a 1:500 dilution of the anti-DSP antibody (rabbit polyclonal, sc-33586; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used. After incubation, EnVision+ System-HRP Labelled Polymer anti-rabbit (K4003, Dako North America Inc., CA, USA; ready to use) was applied for 20 minutes. Color development was performed using labeled streptavidin biotin kits (Dako) according to the manufacturer's instructions. The sections were counterstained with Gill's hematoxylin (Sigma).

6. Statistical Analysis

Statistical analysis was performed with SPSS (19.0, Chicago, IL, USA). One-way ANOVA ($p<0.05$) and the post-hoc Scheffé test (Bonferroni correction; $p<0.017$) were applied to analyze the area of the newly formed calcific barrier.

III. Results

We evaluated 13 ProRoot MTA®, 12 RetroMTA®, 12 TheraCal®, and 15 IRM specimens histopathologically. The other teeth were excluded from the study because of failures during extraction and histopathological processing. The percentage of scores for each group is shown in Tables 2, 3, and 4. During sectioning, the specimens with amputated pulp tissues were excluded from pulpal inflammation and odontoblastic layer evaluation. As shown in Figures 1 and 2, all groups showed better results than IRM in terms of calcific barrier formation, inflammatory response, and odontoblastic layer formation. However, as can be seen in Figure 3, the TheraCal® group had inferior results compared to those of the ProRoot MTA® and RetroMTA® groups. The TheraCal® group had relatively incomplete calcific barriers and an unfavorable inflammatory response.

Table 2. Score percentages for calcific barrier continuity

Groups	Calcific barrier continuity (%)			
	1	2	3	4
ProRoot MTA®	69.23 (9/13)*	15.38 (2/13)	7.69 (1/13)	7.69 (1/13)
RetroMTA®	50 (6/12)	16.67 (2/12)	25 (3/12)	8.33 (1/12)
TheraCal®	33.33 (4/12)	50 (6/12)	16.67 (2/12)	—
IRM	—	6.67 (1/15)	20 (3/15)	73.33(11/15)

Table 3. Score percentages for calcific barrier morphology

Groups	Calcific barrier morphology (%)			
	1	2	3	4
ProRoot MTA®	53.85 (7/13)	38.46 (5/13)	—	7.69 (1/13)
RetroMTA®	41.67 (5/12)	33.33 (4/12)	16.67 (2/12)	8.33 (1/12)
TheraCal®	25 (3/12)	50 (6/12)	25 (3/12)	—
IRM	—	20 (3/15)	6.67 (1/15)	73.33 (11/15)

Table 4. Score percentages for tubules in calcific barrier

Groups	Tubules in calcific barrier (%)		
	1	2	3
ProRoot MTA®	44.44 (4/9)	44.44 (4/9)	11.11 (1/9)
RetroMTA®	16.67 (1/6)	50 (3/6)	33.33 (2/6)
TheraCal®	—	75 (3/4)	25 (1/4)
IRM	—	—	—

*(number of teeth receiving the score/total number of teeth evaluated)

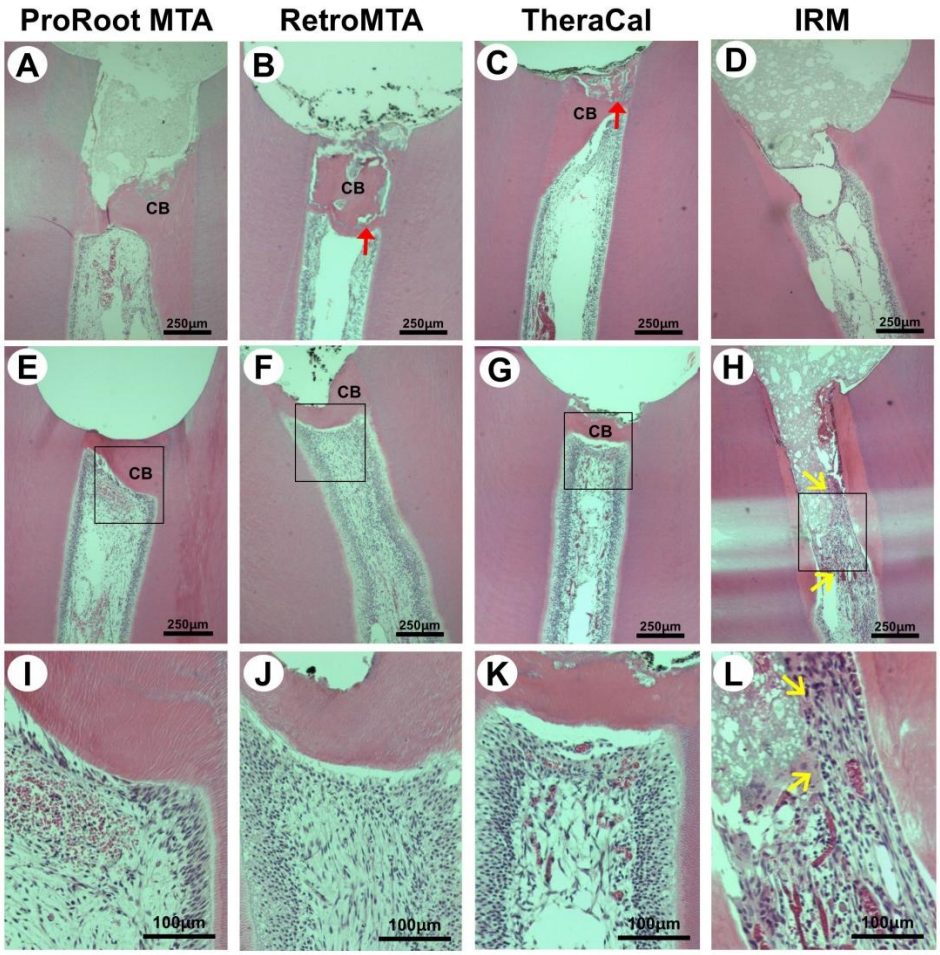

Figure 1. Histomorphologic characteristics of the newly formed calcific barrier (CB) after 4 weeks. A–H shows the characteristics of the CB for each test material in HE staining (scale bars=250 μm). (I–L) Dentinal tubules can be seen in higher-magnification views (scale bars=100 μm). Red arrows indicate tubules present within newly formed CBs, and yellow arrows indicate inflammatory cells.

Table 5. Score percentages for inflammation intensity and extensity

Groups	Inflammation intensity (%)				Inflammation extensity (%)			
	1	2	3	4	1	2	3	4
ProRoot MTA®	81.82 (9/11) *	18.18 (2/11)	—	—	81.82 (9/11)	18.18 (2/11)	—	—
RetroMTA®	72.73 (8/11)	27.27 (3/11)	—	—	72.73 (8/11)	27.27 (3/11)	—	—
TheraCal®	36.36 (4/11)	45.45 (5/11)	18.18 (2/11)	—	36.36 (4/11)	45.45 (5/11)	18.18 (2/11)	—
IRM	—	6.67 (1/15)	46.67 (7/15)	46.67 (7/15)	—	13.33 (2/15)	40 (6/15)	46.67 (7/15)

Table 6. Score percentages for inflammation type and dental pulp congestion

Groups	Inflammation type (%)				Dental pulp congestion (%)			
	1	2	3	4	1	2	3	4
ProRoot MTA®	81.82 (9/11)	18.18 (2/11)	—	—	18.18 (2/11)	45.45 (5/11)	36.36 (4/11)	—
RetroMTA®	72.73 (8/11)	27.27 (3/11)	—	—	27.27 (3/11)	36.36 (4/11)	36.36 (4/11)	—
TheraCal®	36.36 (4/11)	63.64 (7/11)	—	—	—	90 (9/10)	10 (1/10)	—
IRM	—	100 (15/15)	—	—	—	22.22 (2/9)	66.67 (6/9)	11.11 (1/9)

Table 7. Score percentages for odontoblastic cell layer

Groups	Odontoblastic cell layer (%)			
	1	2	3	4
ProRoot MTA®	33.33 (4/12) *	33.33 (4/12)	25 (3/12)	8.33 (1/12)
RetroMTA®	9.09 (1/11)	54.55 (6/11)	27.27 (3/11)	9.09 (1/11)
TheraCal®	9.09 (1/11)	36.36 (4/11)	45.45 (5/11)	9.09 (1/11)
IRM	—	—	27.27 (3/11)	72.73 (8/11)

*(number of teeth receiving the score/total number of teeth evaluated)

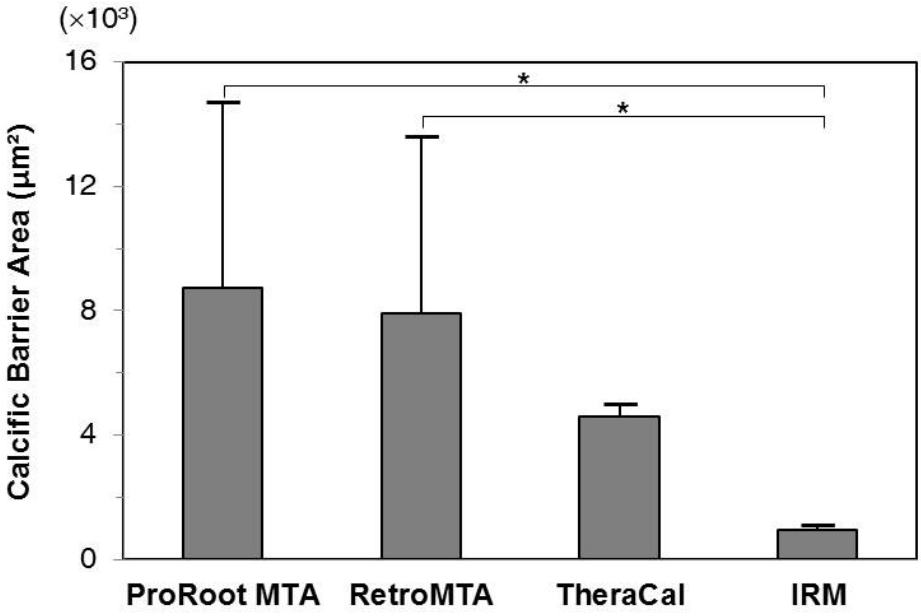

Figure 2. Area of newly formed calcific barrier for each material after 4 weeks. The y-axis represents the area of calcific barrier (1×10^3 μm^2). The bars represent the mean ± standard deviation.

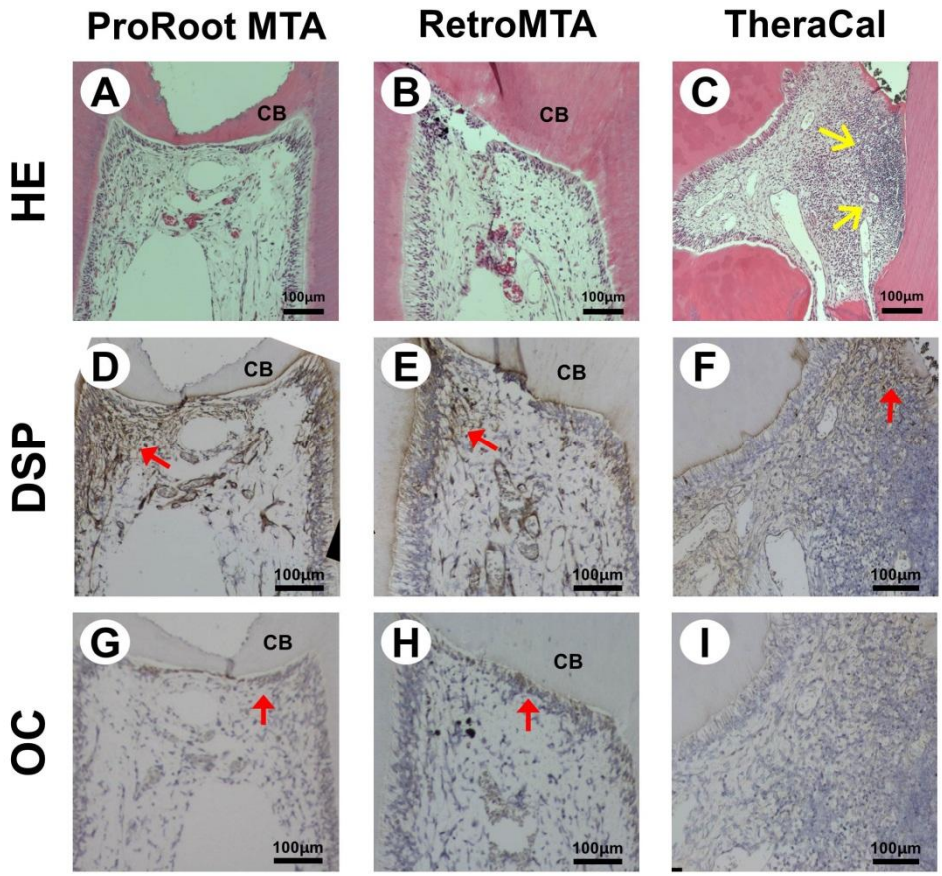

Figure 3. Immunohistochemical staining of DSP and OC. Red arrows indicate cells with a positive signal, and yellow arrows indicate inflammatory cells (scale bars=100 μm).

ProRoot MTA®

Over 69% of the ProRoot MTA® specimens exhibited complete calcific barrier formation. These calcific barriers exhibited a lower incidence of tunnel defects (44% mild

and 11% moderate) as compared to other groups. The dental pulp in 82% of the ProRoot MTA® specimens was found to be free of inflammation. The palisading pattern of the odontoblastic cell layer was visible in 33% of the specimens.

RetroMTA®

Complete calcific barrier formation was observed in 50% of the RetroMTA® specimens, among which mild (50%) to moderate (33%) tubules were present. The dental pulp in 73% of the RetroMTA® specimens was found to be free of inflammation. The palisading pattern of the odontoblastic cell layer was visible in only 9% of the specimens, with 55% showing odontoblasts and odontoblast-like cells.

TheraCal®

Complete calcific barrier formation was observed in 33% of the TheraCal® specimens. Mild tubule formation was observed in 75% of the specimens. The TheraCal® dental pulp exhibited mild (45%) to moderate (18%) inflammation, with 90% showing mild dental pulp congestion. The palisading pattern of odontoblastic cell layer was visible in only 9% of the specimens, with 36% showing odontoblasts and odontoblast-like cells.

IRM

Complete calcific barrier formation was not observed in any of the IRM specimens, with 75% of the specimens having no calcific barriers formed. All IRM specimens exhibited chronic inflammation and 50% showed severe intensity and extensity. The odontoblastic cell layer was absent in 73% of the specimens.

IV. Discussion

This study used a dog model to evaluate and compare various pulpal responses of different MTA and MTA-like materials using ProRoot MTA® as the gold standard. Overall, our study showed favorable results when either ProRoot MTA® or RetroMTA® was used. These MTAs performed better than TheraCal® when used as partial pulpotomy agents. Both ProRoot MTA® and RetroMTA® induced the formation of a complete dentinal bridge at the interface with the pulp tissue and controlled the level of inflammation underneath.

The high success rates observed with ProRoot MTA® and RetroMTA® in forming a calcific barrier could be attributed to their calcium oxide content, which can form calcium hydroxide in the presence of water (Faraco and Holland, 2001). Calcium hydroxide has been described as a compound that directly affects the microvasculature to reduce the plasma outflow, which in turn favors a calcific response in the adjacent pulp tissue (Heithersay, 1975). Calcite crystal-like structures have been found close to dentinal tubules that were filled with MTA in animal models (Holland et al., 1999). These calcite crystals have been known to attract fibronectin, which is responsible for cellular adhesion and differentiation (Seux et al., 1991). However, the role of calcific barrier formation is a controversial issue, as it does not always equate to healthy pulp tissue. Rather, calcific barrier formation should be considered as both a healing process and as a reaction to

irritation (Dominguez et al., 2003; Schroder, 1985). The release of calcium from the set material stimulates dentin bridge formation and its alkaline pH is known to cause necrosis by producing coagulation in contact pulp tissues (Soares, 1996). This reaction may occur due to MTA's high alkalinity, which rises to 12.5 at 3 hours after mixing (Parirokh and Torabinejad, 2010a; Torabinejad et al., 1995). The alkaline pH (11.0–12.0) remains high for at least 8 weeks after setting in aqueous environment (Fridland and Rosado, 2005), and is known to have roles in both inflammation and the induction of a hard-tissue barrier by inducing a favorable environment for cell division, matrix formation, and antimicrobial activity (Accorinte Mde et al., 2008; Fridland and Rosado, 2005).

The rate of the calcification reaction could also be a significant factor in vital pulp therapy agents. The formation of a calcific bridge does not mean that the pulp will be sealed completely from the environment because the bridges are permeable initially. Previous studies have reported that connective tissue is present in bridges formed after pulpotomy treatment due to the initial disorganized formation of reparative dentin that engulfs cellular inclusions (Dominguez et al., 2003). With progression of time and mineralization, the permeability decreases and eventually forms a tight seal between the pulp and the cavity.

According to the results of this study, RetroMTA® has similar biological features to ProRoot MTA® without extensive disadvantages. We found that RetroMTA® resulted in a slightly lower pulpal response and smaller mean area of calcific barrier when compared with ProRoot MTA®; however, the differences were not statistically significant.

RetroMTA® was developed as a bioceramic for root end repair and vital pulp therapy. The main advantage of RetroMTA® over ProRoot MTA® includes its reduced setting time and absence of heavy metals. The MTA setting time could be a factor that is directly related to pulpotomy success. Longer setting times could become a major limitation because the MTA material becomes more prone to tissue fluids, leading to material wash out and leaching of cytotoxic substances (Camilleri et al., 2005). Despite the advantage of a fast setting time, the lower pulpal response of RetroMTA® could be due to differences in the manufacturing process and its components. According to the manufacturers, ProRoot MTA® is produced through various refining processes for Portland cement, whereas RetroMTA® is synthesized by mixing the essential chemical components (http://www.biomta.com). A more precise evaluation of each component and their effects should be performed.

The absence of complete bridging in TheraCal® group could be related to the lower biocompatibility of the material, which causes a higher degree of inflammation. The lower biocompatibility of TheraCal® could be attributed to the acrylic monomers in the material. Bis-Glycidyl methacrylate (BisGMA) is an acrylic monomer in TheraCal® that is cytopathic to cultured cells (de Souza Costa et al., 2006; de Souza Costa et al., 2007;Hanks et al., 1991). BisGMA inhibits glutathione synthesis, one of the major intracellular antioxidants, and interferes with the expression of some fundamental proteins for pulpal repair such as collagen type I and dentin sialoprotein (Mantellini et al., 2003). Moreover, the manufacturer has recommended a depth-of-cure of about 1

mm (Griffin Jr, 2012). Despite these guidelines, complete curing of the pulpotomy material is difficult to achieve *in vivo*. The uncured monomer contents leach into the pulp and dentinal tubules and have cytotoxic effects on pulpal cells (de Souza Costa et al., 2006; Hanks et al., 1994; Pashley et al., 2000). In our study, the prominent tubules that were present within the newly formed calcific barriers in teeth treated with TheraCal® could have also been the result of exposure to leaching of the uncured monomer contents. Consequently, it is not surprising that TheraCal® exhibited less favorable pulpal responses in terms of inflammation and calcific barrier formation in the pulpotomy models.

In our study, treatment with ProRoot MTA® and RetroMTA® resulted in the up-regulation of differentiation markers and produced the palisading pattern of odontoblast cells. These results indicate that ProRoot MTA® and RetroMTA® have a higher odontogenic differentiation potential than TheraCal®. OC is a specific and relatively late-stage marker of osteoblastic differentiation (Malaval et al., 1994), and DSP is a specific marker for odontoblasts and is believed to play a regulatory role in the mineralization of reparative dentin (Papagerakis et al., 2002). Many *in vitro* studies have reported that MTAs stimulate dental pulp stem cells to undergo odontogenic differentiation (Hakki et al., 2009; Min et al., 2009;Seo et al., 2013). Calcium ions released from MTAs are known to play an important role in odontoblastic differentiation, although the precise mechanisms underlying MTA-induced odontoblastic differentiation are not completely understood (Woo et al., 2013).

In the present study, a 4-week period was used to evaluate the effects of various MTAs on pulp tissues. However, further research may be required because 4 weeks may not be a sufficient time period for evaluating the long-term effects of the pulpotomy agents. Moreover, the findings of the current study in dogs may not directly correspond to those in humans. Therefore, clinical trials using human teeth are necessary for a more accurate understanding of the materials

V. Conclusion

In conclusion, this study demonstrated that RetroMTA® may be an alternative to ProRoot MTA® because both materials produced similar pulpal responses, whereas TheraCal® produced lower calcific barrier formation, higher inflammatory reactions, and less favorable odontoblastic layer formation.

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Abstract (in Korean)

개의 치아에서 ProRoot MTA®, RetroMTA® 및 TheraCal®을 이용한 부분치수절단술에 따른 치수반응에 대한 비교연구

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지도교수: 송제선

본 연구는 개를 이용한 *in vivo*상의 치아에서 MTA기반의 치수치료재인 ProRoot MTA®, RetroMTA® 및 TheraCal®을 이용한 부분치수절단술 시행 후, 각 재료에 의한 치수 반응을 비교 분석하고자 하였다.

60개의 비글 치아에서 부분치수절단술을 시행하였다. 절단된 치수조직의 상부는 각각 ProRoot MTA®, RetroMTA® 및 Theracal®을 사용하여 복조되었다. 4주 후 발치된 치아들을 사용하여 조직학적 검사 및 osteocalcin (OC)과 dentin sialoprotein (DSP)을 이용한 면역조직화학염색법을 통해, 각 군별로 새롭게 형

성된 경조직의 양과 질, 치수염증반응의 정도 및 상아모세포층에 대한 분석 및 평가를 시행하였다.

ProRoot MTA®와 RetroMTA®군의 경우 대부분 연속성의 경조직층이 형성되었으며 치수는 염증반응 없이 잘 배열된 상아모세포층이 관찰되었다. 하지만 TheraCal®군에서 형성된 경조직층은 양과 질적인 면에서 모두 비교적 떨어졌으며 치수는 심한 염증반응과 잘 배열되지 않은 상아모세포층이 관찰되었다. 면역조직화학염색결과 역시 OC와 DSP는 ProRoot MTA®와 RetroMTA®군에서 TheraCal®군에서보다 높은 발현율을 보였다.

결론적으로 TheraCal®는 앞선 두 재료에 비해 치수반응에 있어 열등한 결과를 나타내었지만, RetroMTA®의 경우 *in vivo* 상에서 ProRoot MTA®와 유사하게 양호한 치수반응을 나타내어 ProRoot MTA®의 대체재료로 사용될 수 있을 것으로 기대된다.

핵심되는 말: Mineral trioxide aggregate (MTA), 부분치수절단술, 치수반응, 치수염증, 경조직형성, 상아모세포층