Histological evaluation of direct pulp capping with DSP-derived synthetic peptide in beagle dog

Jae-Hoon Kim[1] · Jun-Bae Hong[1] · Bum-Soon Lim[2] · Byeong-Hoon Cho[1]*
[1]*Department of Conservative Dentistry, School of Dentistry, Seoul National University*
[2]*Department of Dental Biomaterials Science, School of Dentistry, Seoul National University*

ABSTRACT

The purpose of this study was to investigate the pulpal response to direct pulp capping with dentin sialoprotein (DSP) -derived synthetic peptide in teeth of dogs, and to compare its efficacy to capping substances $Ca(OH)_2$ and white mineral trioxide aggregate (WMTA). A total of 72 teeth of 6 healthy male beagle dogs were used. The mechanically exposed pulps were capped with one of the following: (1) DSP-derived synthetic peptide (PEP group); (2) $Ca(OH)_2$ (CH group); (3) a mixture paste of peptide and $Ca(OH)_2$ (PEP+CH group); or (4) white MTA (WMTA group). The access cavity was restored with a reinforced glass ionomer cement. Two dogs were sacrificed at each pre-determined intervals (2 weeks, 1 month, and 3 months). After the specimens were prepared for standard histological processing, sections were stained with hematoxylin and eosin. Under a light microscope, inflammatory response and hard tissue formation were evaluated in a blind manner by 2 observers. In the PEP group, only 3 of 17 specimens showed hard tissue formation, indication that the DSP-derived synthetic peptide did not induce proper healing of the pulp. Compared with the CH group, the PEP group demonstrated an increased inflammatory response and poor hard tissue formation. The CH and WMTA groups showed similar results for direct pulp capping in mechanically exposed teeth of dogs [J Kor Acad Cons Dent 34(2):120-128, 2009]

Key words: Pulp capping, Dentin sialoprotein, Reparative dentin, Histological evaluation, Calcium hydroxide, Beagle dog

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I. Introduction

Preserving the vitality of pulp is important to maintaining physiologic integrity of teeth. Dental pulp plays a crucial role in reparative dentin formation. For decades, dentists have attempted to preserve pulp exposed by mechanical factors or carious lesions[1-4].

Pulp capping is the application of a dental material over an exposed pulp to promote the dentinogenic potential of pulp cells. Many materials and drugs have been used as pulp capping agents. Since first introduced by Herman[1], calcium hydroxide has been widely used and is still the gold standard as a pulp capping agent. However, some have questioned the suitability of calcium hydroxide, because of its poor sealing properties, degradation over time, and association with the formation of tunnel defects in dentinal bridges[5].

Mineral trioxide aggregate (MTA) was proposed for direct pulp capping procedures. In 1993, it was advocated as root-end filling and lateral perforation repair material by Lee, Monsef, and Torabinejad[6]. MTA has demonstrated favorable results in pulp capping procedures when compared with $Ca(OH)_2$[7-9]. However, there are no long-term studies on the effectiveness of MTA as an agent for vital pulp therapy.

Recently, there were attempts to use extracellular

*Corresponding Author: **Byeong-Hoon Cho***
Department of Conservative Dentistry,
School of Dentistry, Seoul National University
28 Yeongeon-dong, Jongro-gu, Seoul, 110-749, Korea
Tel: +82-2-2072-3514 Fax: +82-2-2072-3859
E-mail: chobh@snu.ac.kr

matrix components found in dentin, such as tumor growth factors (TGFs) and bone sialoproteins (BSP) for pulp capping[4]. Goldberg *et al.*[10] examined the effects of bone morphogenic protein-7 (BMP-7) on reparative dentin formation in the pulp of rat molars, providing the basis for the development of bioactive molecules as pulp capping agents. His group also observed the effects of BSP under the same conditions[11,12]. BSP is an extracellular matrix protein, found in bone and dentin. It is a phosphorylated protein and stimulates an osteoblastic activity[13].

Other more abundant non-collagenous proteins of the extracellular matrix of dentin include dentin phosphoprotein (DPP) and dentin sialoprotein (DSP)4), which are produced when the dentin sialophosphoprotein (DSPP) gene product is immediately cleaved into the 2 proteins. The most unusual feature of DPP is the high proportion of aspartic acid (Asp) and phosphoserine (Pse)[14,15]. This feature fits well with the purported function of DPP in the nucleation and modulation of apatite crystal formation. DPP is clearly associated with dentin mineralization. DSP is a special sialic acid-rich glycoprotein that was first described by Butler[16]. DSP, which is also rich in phosphoserine, shares overall characteristics with other sialoproteins such as osteopontin and bone sialoprotein. In the past, DPP and DSP were believed to be odontoblast-specific. However, it was recently revealed that DSP is also expressed by osteoblasts[15]. The exact function of DSP is unknown, and there are currently no studies in the literature about the effect of DSP as a pulp capping agent.

In this study, a synthetic peptide derived from DSP was used as a pulp capping agent. The purpose of this study was to investigate the pulpal response and hard tissue formation resulting from direct pulp capping with DSP-derived synthetic peptide *in vivo*, and to compare its efficacy to that of $Ca(OH)_2$ and WMTA.

Ⅱ. Materials and methods

A total of 72 teeth of 6 healthy male beagle dogs (each dog weighed approximately 10 kg) were used. All experimental procedures were carried out according to the protocols approved by the Institute of Laboratory Animal Resources, Seoul National University. The operation field and dog's teeth were disinfected with 0.5% chlorhexidine and isolated by sterilized cotton rolls and gauze swabs, under general anesthesia with intravascular injection of 10 mg/kg tiletamine HCl (Zoletil 50; Virbac, Carros, France) and 0.2 mg/kg xylazine HCl (Rumpen; Bayer Korea, Seoul, Korea). Using a No. 330 bur in a high-speed handpiece with copious water spray, a box-shaped cavity was prepared on the buccal surface of the tooth to the depth at which the floor of the cavity looked pinkish due to reflction from the pulp. Thereafter, by using a No. 1/2 round bur in a low-speed handpiece, the pulp was exposed by approximately 1 mm in diameter. Hemostasis was achieved by using cotton pellets soaked in saline. The assigned pulp capping material was then applied without pressure. The access cavity was restored with a reinforced glass ionomer cement (Ketac Molar; 3M ESPE, St Paul, MN, USA).

The jaw quadrant of each dog was randomly assigned to one of 4 experimental groups, and 3 teeth were used from each quadrant. The experimental materials used were as follows: (1) DSP-derived synthetic peptide (PEP group); (2) $Ca(OH)_2$ (CH group); (3) a mixture paste of peptide and $Ca(OH)_2$ (PEP+CH group); and (4) WMTA (WMTA group) (ProRoot® MTA, Dentsply, Tulsa, USA).

DSP-derived synthetic peptide was synthesized chemically using an automatic peptide synthesizer (APEC 396; Advanced Chem Tech, Louisville, KY, USA) by co-workers. Propylene glycol was used as a carrier. $Ca(OH)_2$ was applied as a paste (the powder was mixed with distilled water). WMTA was used according to the manufacturer's instructions.

Two dogs were sacrificed at each pre-determined

Table 1. Number of teeth and the experimental periods

Group	Experimental period		
	2 weeks	1 month	3 months
PEP	6	6	6
CH	6	6	6
PEP+CH	6	6	6
WMTA	6	6	6

interval (2 weeks, 1 month, and 3 months). The numbers of teeth and the experimental periods are summarized in Table 1.

After vital perfusion, the teeth and their surrounding tissue were removed as block sections and fixed in 10% formalin for 1 week. In the next step, specimens were decalcified in 20% formic acid for 1 month, after which the specimens were prepared for standard histological processing. Sections which were 5 ㎛ thick, were obtained in the buccolingual direction and stained with hematoxylin and eosin. Under a light microscope, inflammatory response and hard tissue formation were graded by 2 observers in a blind manner, according to the criteria listed in Tables 2 and 3. The data were statistically analyzed using the Kruskal-Wallis test (SigmaStat 3.11; Systat Software, Inc., San Jose, CA, USA).

Ⅲ. Results

The results for the experimental groups are presented in Table 4.

At 2 weeks experimental period

PEP group. In 2 of 6 specimens, a thin layer of partially calcified dentin matrix was observed (Figure 1). All specimens showed disruption of the pulp tissue at the exposed site. Four specimens showed slight to moderate inflammation characterized by the infiltration of inflammatory cells at the exposed site. Two specimens showed severe inflammatory reactions in which the entire pulp was infiltrated with inflammatory cells.

CH group. In this group, 5 teeth were available for histological evaluation. All specimens showed hard tissue formation at the interface between $Ca(OH)_2$ and the pulp tissue (Figure 2). Odontoblast-like cells were arranged beneath the newly formed hard tissue. In most specimens, a few scattered inflammatory cells were observed around the exposed site and the remaining pulp tissue showed normal histological characteristics.

PEP+CH group. Two specimens showed a thin layer of scattered hard tissue (Figure 3). A few or moderate inflammatory reaction was presented in 4 teeth and degeneration of the pulp was observed in 2 teeth.

WMTA group. Two of the 6 teeth were not suitable for histological evaluation. Three of the 4 available specimens showed modest hard tissue formation. Newly formed hard tissue appeared mainly among the dentin debris, but did not grow into a complete bridge (Figure 4). A few inflammatory cells were present around the exposed site; however, one of the specimens exhibited partial disorganization of the pulp.

For this 2 week period, the differences in inflammatory response (P=0.764) and hard tissue forma-

Table 2. Criteria for grading inflammatory cell response

Grade	Characterization
0	None or a few scattered inflammatory cells present in the pulpal area corresponding to pulp exposure, characteristic of normal tissue
1	Slight inflammatory cell infiltration with polymorphonuclear or mononuclear leukocytes
2	Moderate inflammatory cell infiltration involving the coronal pulp
3	Severe inflammatory cell infiltration involving the coronal pulp or abscess present

Table 3. Criteria for grading hard tissue formation

Grade	Characterization
0	Absent
1	Modest hard tissue deposition beneath the exposed area
2	Moderate hard tissue deposition beneath the exposed area
3	Heavy hard tissue deposition beneath the exposed area, appearing as a complete dentin bridge

tion (P=0.114) among the groups were not statistically significant.

At 1 month experimental period

PEP group. None of the specimens showed hard tissue formation, and no hard tissue formation was observed even around the fragments of dentin that were pushed into the pulp during the procedure (Figure 5). Inflammatory cells was present around the exposed site, and pulp necrosis was also observed in 2 specimens.

CH group. Five specimens showed hard tissue formation. Compared to 2 weeks group, the hard tissue increased in thickness, In two of the specimens, the newly formed hard tissue matured into a complete dentin bridge (Figure 6). One tooth presented moderate inflammatory response around the exposed site but no hard tissue formation.

PEP+CH group. None of the specimens showed a complete hard tissue bridge. In 5 specimens, the thickness of hard tissue increased; however, it grew mainly around the fragments of dentin that were pushed into the pulp during cavity preparation (Figure 7). Slight inflammatory responses persisted around the exposed site and one tooth exhibited partial disorganization of pulp.

WMTA group. Five of 6 specimens showed hard tissue formation, and the thickness of hard tissue increased (Figure 8). One tooth showed severe inflammatory cell infiltration and degeneration of the pulp. Another tooth exhibited fibrosis of the pulp. The other two specimens showed slight inflammatory cell infiltration, which was restricted to the exposed site.

For this period, the differences in inflammatory response among the groups were not significant (P=0.056). However, the differences in hard tissue formation (P=0.011) among the groups were significant.

At 3 months experimental period

PEP group. In this group, 5 teeth were available for histological evaluation. Inflammatory response persisted at the exposed site. No dentin bridge was observed. Modest hard tissue formation was observed around the dentin debris in only 1 specimen (Figure 9). However, the hard tissue formation did not seem to be related to the capping agent.

CH group. Four of the 6 specimens showed hard tissue formation; among them, 2 specimens exhibited a complete dentin bridge (Figure 10). In all cases, the pulp was vital. A few or slight inflammatory reaction was observed, which was restricted to the exposed sites, except in the case of one tooth that showed a microabscess at the exposed site.

PEP+CH group. Newly formed hard tissue mainly around the dentin debris was observed in only one of the 6 specimens. All specimens showed an infiltra-

Table 4. Inflammatory responses and hard tissue formation with 4 experimental pulp capping materials at 3 observation intervals

Group	Experimental period	Number of specimens	Inflammatory response				Hard tissue formation			
			0	1	2	3	0	1	2	3
PEP	2 weeks	6	0	3	1	2	4	1	1	0
	1 month	6	0	2	2	2	6	0	0	0
	3 months	5	0	3	2	0	4	1	0	0
CH	2 weeks	5	1	2	2	0	0	2	2	1
	1 month	6	4	1	1	0	1	1	1	3
	3 months	6	2	2	2	0	2	0	2	2
PEP+CH	2 weeks	6	2	1	1	2	4	0	2	0
	1 month	6	1	4	1	0	2	3	1	0
	3 months	6	1	1	1	3	5	1	0	0
WMTA	2 weeks	4	0	3	1	0	1	3	0	0
	1 month	6	3	2	0	1	1	1	2	2
	3 months	5	4	1	0	0	2	1	0	2

tion of inflammatory cells at the exposed site without a dentin bridge (Figure 11).

WMTA group. In this group, 5 teeth were available for histological evaluation. In two of the specimens, a thick dentin bridge was observed (Figure 12). The mature dentin bridge included the dentin debris, although it did not have a tubular structure. The other specimen showed partial degeneration of the pulp and no hard tissue formation.

For this period, the difference in inflammatory response among the groups was significantly different (P=0.041), while the difference in hard tissue formation among the groups was not statistically significant (P=0.100).

In summary, the differences in inflammatory response (P=0.001) and hard tissue formation (P<0.001) among the groups were statistically significant.

Ⅳ. Discussion

Recently, new materials such as MTA and bioactive molecules are being advocated for vital pulp therapy[4,7]. Favorable results have been reported with MTA, BSP, and BMP-7 as a pulp capping agents[8,11,12,17-19]. Decup *et al.*[11] reported that BSP implanted in the pulp of rat teeth induced formation of an atubular dentin-like structure. BSP is an extracellular matrix protein of bone that is also found in dentin but is absent in the pulp. Decup et al. attributed the major biological properties of BSP to the recruitment of osteogenic cells, its interaction with collagen fibrillation, and the potential role of glutamic acid-rich sequences in the nucleation of hydroxyapatite. Six *et al.*[12] reported that BMP-7, a member of TGFβ family, induced mineralization of rat pulp and compared it to BSP induced mineralization. According to Six et al., BSP induced an atubular dentin-like structure, whereas BMP-7 induced a porous and inhomogeneous osteodentin. In addition, BSP induced mineralization in the crown part, while BMP-7 induced mineralization in the root part of the pulp. Considering those studies, bioactive molecules such as BSP and BMP-7 may induce a different pulp response from that induced by the traditional pulp capping agent $Ca(OH)_2$. It seems that DSP, a specific extracellular matrix of dentin, might be more suitable for inducing reparative dentinogenesis. Although the practical differences between BSP and DSP have not yet been specifically elucidated, our study was worth while in order to investigate DSP as a bioactive molecule for reparative dentinogenesis.

Our research found that DSP-derived peptide is not efficacious as a direct capping material. DSP-derived synthetic peptide induced an inflammatory response in the pulp and was not successful in promoting hard tissue formation during any experimental period. In a pilot study using odontoblasts in vitro, we confirmed that DSP-derived peptide induced hard tissue formation. Although pulp reaction in vivo is different from that of a single cell in a culture medium, we had expected that the peptide would be efficacious in an animal model. However, only 3 out of 17 teeth in the PEP group showed hard tissue formation. Most of the newly formed hard tissue was related to the dentin fragments that were pushed into the pulp during cavity preparation. Regardless of the capping agents, dentin debris was encapsulated by newly formed hard tissue. Therefore, dentin debris might be an endogenous biomaterial that could contribute to the initiation of hard tissue formation[20].

At 2 weeks, there was no significant difference in inflammatory response among the groups. However, compared to the CH group, the inflammatory cell infiltration at the exposed site persisted longer in the PEP group, remaining evident at 1 month and 3 months. The PEP+CH group also showed persistent infiltration of inflammatory cells at the exposed site until 3 months. Although the PEP+CH group exhibited similar results to the PEP group, the PEP+CH group had a tendency to display more hard tissue formation than the PEP group. This could be attributed to $Ca(OH)_2$, which was included in the PEP+CH group. This result suggests that the DSP-derived peptide may have provoked a foreign body reaction in the pulp.

We observed more hard tissue formation in the CH group than in the PEP group. At 2 weeks, although there was no significant difference among the groups, the CH group showed hard tissue formation in all specimens that were available for evaluation. At 1 month and 3 months, the thickness of newly formed

hard tissue increased in the CH group; however, the exact mechanisms of the effect of $Ca(OH)_2$ was not been clear. $Ca(OH)_2$ has been shown to stimulate a rapid differentiation of odontoblast and odontoblast-like cells[21] and $Ca(OH)_2$ is known to have a direct effect on the precapillary sphincters. This results in reduced plasma outflow, which favors a calcific response in the involved tissue[22]. The alkaline nature of $Ca(OH)_2$ contributes to the mineralization process[23]. $Ca(OH)_2$ therefore may act as a local buffer against the acidic reactions produced by the inflammatory process, neutralizing lactic acid produced by the osteoclasts and preventing further destruction of the mineralized tissue. It also has good antibacterial properties due to its alkaline pH.

Despite its applicability, some questioned the suitability of $Ca(OH)_2$ because of its poor sealing properties, degradation over time, and assocication with the formation of tunnel defects in dentinal bridges[5]. In the present study, we also found tunnel defects in dentin bridge (Figure 10). Cox *et al.*[24] reported multiple tunnel defects of the dentin bridge in the presence of hard setting $Ca(OH)_2$. They demonstrated that these morphologic defects in the dentin bridge failed to provide a permanent barrier. However, Stanley *et al.*[25] refuted this assumption and attributed the tunnel defects to blood vessel trauma caused by mechanical pulp exposure. It has been stated that the most critical factor for the success of vital pulp therapy is a seal against bacterial ingress[26]. A seal against bacterial microleakage depends not only on a capping material but on a definitive restorative material as well. It is therefore not justified to assume that the reason for recurrent pulpal inflammation after pulp capping with $Ca(OH)_2$ is attributable only to tunnel defects. Pulpal reactions depend on many factors, such as status of pulp, operative procedure, mechanical trauma, or bacterial contamination.

We could not evaluate all of the teeth in the experimental groups. Although we used teeth of dogs, which are easier to manipulate than teeth of rats, it was not always possible to expose the pulp without damage or to perfectly section along the perpendicular axis of the tooth in histological processing. Therefore, we failed to examine specimens of all teeth due to these inherent difficulties. We also found impaction of the pulp capping agents into the pulp in many specimens in all experimental groups. Although we tried to minimize damage to the pulp, pulp tissue was displaced to some degree, probably due to the bur used for pulp exposure. In addition, because the reinforced glass ionomer (Ketac Molar; 3M ESPE, St Paul, MN, USA) used for definite sealing had high viscosity, there may have been pressure applied to the pulp space when packing the material. However, the impaction of pulp capping agents was not a discriminating factor among the experimental groups, because the factor was similar in all experimental groups.

In the present study, we observed similar responses of the pulp in the CH and WMTA groups. These results do not correspond with other studies. In many studies, when compared to $Ca(OH)_2$, MTA produced more dentinal bridge with less inflammation[7,17-19]. Holland *et al.*[27] suggested that hard tissue formation might be induced by virtue of the CaO in hardened MTA, which might have a mechanism similar to that of $Ca(OH)^2$. MTA also has superior sealing ability[28-30] and biocompatibility[31-33]. Nair *et al.*[19] reported that WMTA should be the material of choice for direct pulp capping procedures instead of hard setting $Ca(OH)_2$ cements. On the other hand, Myers *et al.*[34] found no significant differences in pulpal status or bridge formation between MTA and $Ca(OH)_2$ in a dog study. Additionally, in a study on human subjects, Iwamoto *et al*[9]. reported that there were no significant differences in inflammatory cell response and dentin bridge formation between WMTA and hard setting $Ca(OH)_2$. In the present study, WMTA induced a slightly lower level of hard tissue than $Ca(OH)_2$, but the difference was not statistically significant. Commercial MTA is available in grey and white forms. Except in a few studies, grey MTA (GMTA) is primarily used as a direct pulp capping agent. The composition of the 2 products differs slightly in that WMTA is free from iron and is composed mainly of tricalcium silicate and bismuth oxide, while GMTA is composed of dicalcium phosphate and bismuth oxide, which contains a relatively higher amount of iron[35]. Few studies have directly compared GMTA and WMTA in vital pulp therapy[36-38].

In a clinical study of pulpotomy on human primary molars by using MTA, the pulp tissue of WMTA group presented more inflammatory cells and less favorable pulpal responses than that of GMTA group[36]. The reason for the difference was attributed to the absence of tetracalcium aluminoferrite in WMTA. Another clinical study using human primary molars also showed that WMTA produced fewer radiographic dentin bridges than GMTA[37]. On the other hand, Parirokh *et al.*[38] found no difference in formation of hard tissue and inflammation with the use of GMTA and WMTA as pulp capping agents in a dog study. Although we did not compare GMTA to WMTA, the difference between GMTA and WMTA warrants further investigation in order to understand the reason for the relatively less favorable result of the WMTA group observed in the present study.

The present study failed to demonstrate the efficacy of DSP-derived peptide as a pulp capping agent. However, in the future, vital pulp therapy will be based on biological strategies. Further studies with other types of peptides and more efficient carriers for novel bioactive molecules in reparative dentinogenesis are necessary.

Ⅴ. Conclusions

DSP-derived synthetic peptide failed to induce proper healing of the pulp. Overall, higher inflammatory response and lesser hard tissue formation was observed in the PEP group than in the CH and WMTA groups. $Ca(OH)_2$ and WMTA showed similar results for direct pulp capping in mechanically exposed teeth of dogs.

References

1. Herman BW. Dentin obliteration after treatment with calcium. *Zahnärztl Rundschau* 39:888-899, 1930. (In German)
2. Schrö der U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation and differentiation. *J Dent Res* 64(suppl. 1):541-548, 1985.
3. Pitt Ford TR, Torabinejad M, Albedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. *J Am Dent Assoc* 127:1491-1494, 1996.
4. Goldberg M, Six N, Decup F, Lasfargues JJ, Salih E, Tompkins K, Veis A. Bioactive molecules and the future of pulp therapy. *Am J Dent* 16:66-76, 2003.
5. Schuurs AHB, Gruythuysen RJM, Wesselink PR. Pulp capping with adhesive resin based composite versus calcium hydroxide: a review. *Endod Dent Traumatol* 16:240-250, 2000.
6. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *J Endod* 19:541-544, 1993.
7. Abedi HR, Torabinejad M, Pitt Ford TR, Bakland LK. The use of mineral trioxide aggregate cement as a direct pulp capping material. *J Am Dent Assoc* 127:1491-1494, 1996.
8. Dominguez MS, Witherspoon DE, Gutmann JL, Opperman LA. Histological and scanning electron microscopy assessment of various vital pulp-therapy materials. *J Endod* 29:324-333, 2003.
9. Iwamoto CE, Adachi E, Pameijer CH, Barnes D, Romberg EE, Jefferies S. Clinical and histological evaluation of white ProRoot MTA in direct pulp capping. *Am J Dent* 19:85-90, 2006.
10. Goldberg M, Six N, Decup F, et al. Application of bioactive molecules in pulp-capping situations. *Adv Dent Res* 15:91-95, 2001.
11. Decup F, Six N, Palmier B, Buch D. Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. *Clin Oral Invest* 4:110-119, 2000.
12. Six N, Decup F, Lasfargues JJ, Salih E, Goldberg M. Osteogenic proteins (bone sialoprotein and bone morphogenetic protein-7) and dental pulp mineralization. *J Mater Sci Mater Med* 13:225-232, 2002.
13. Wang J, Kennedy JG, Glimcher MJ, Salih E. Novel bioactive property of purified native bone sialoprotein in bone repair of calvarial defect. *Trans Orthop Res Soc* 23:1007, 1998.
14. George A, Bannon L, Sabsay B, Dillon JW, Malone J, Veis A, Jenkins NA, Gilbert DJ, Copeland NG. The carboxyl-terminal domain of phosphophoryn contains unique extended triplet amino acid repeat sequences forming ordered carboxyl-phosphate interaction ridges that may be essential in the biomineralization process. *J Biol Chem* 271:32869-32873, 1996.
15. Qin C, Brunn JC, Ridall A, *et al.* the expression of dentin sialophosphoprotein gene in bone. *J Dent Res* 81:392-394, 2002.
16. Butler WT. Sialoprotein of bone and dentin. *J Bio Buccale* 19:83-89, 1991.
17. Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trixoide aggregate as a pulp capping material. *J Am Dent Assoc* 127:1491-1494, 1996.
18. Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS. Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. *Int Endod J* 36:225-231, 2002.
19. Nair PNR, Duncan HF, Pitt Ford TR, Luder HU. Histological, ultrastructural and quantitative investigations on the response of health human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. *Int Endod J* 41:128-150, 2008.
20. Tziafas D, Kolokuris I, Alvanou A, Kaidoglou K. Short-term dentinogenic response of dog pulp tissue after its induction by demineralized or native dentine or by predentine. *Arch Oral Biol* 37:119-128, 1992.

21. Schroder U. Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odontol Revy* 23:329-340, 1972.
22. Heithersay GS. Calcium hydroxide in the treatment of pulpless teeth with associated pathology. *J Br Endod Soc* 8:74-93, 1975.
23. Siqueira JR, Lopez HP. Mechanism of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod* 32:361-369, 1999.
24. Cox CF, Bergenholtz G, Heys DR, Syed SA, Fitzgerald M, Heys RJ. Pulp-capping of dental pulp mechanically exposed to oral microflora: a 1-to-2-year observation of wound healing in the monkey. *J Oral Pathol* 14:156-168, 1985.
25. Stanley HR, Pameijer CH. Dentistry's friend, calcium hydroxide. *Oper Dent* 22:1-3, 1997.
26. Cox CF, Bergenholtz G, Fitzgerald M, Heys DR, Heys RJ, Avery JK, Baker JA. Capping of the dental pulp mechanically exposed to the oral microflora: 5-week observation of wound healing in the monkey. *J Oral Pathol* 11:327-339, 1982.
27. Holland R, De souza V, Nevy NJ, Otobone Filho JA, Bernabe DF, Dezan Jr E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydoxide. *J Endod* 25:161-166, 1999.
28. Torabinejad M, Watson EF, Pitt Ford TR. Sealing ability of mineral trioxide aggregate when used as a root end filling material. *J Endod* 19:591-595, 1993.
29. Fischer EJ, Arens DE, Miller CH. Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. *J Endod* 24:176-179, 1998
30. Wu MK, Kontakiotis EG, Wesselink PR. Long-term seal provided by some root-end fillng materials. *J Endod* 24:557-560, 1998.
31. Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP. Histological assessment of mineral trioxide aggregate as a root-end filling in monkeys. *J Endod* 23:225-228, 1997.
32. Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Jr E. Reaction of dog's teeth to root canal filling with mineral trioxide aggregate or a glass ionomer sealer. *J Endod* 25:728-730, 1999.
33. Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fiboroblasts. *J Endod* 26:288-291, 2000.
34. Myers K, Kaminski E, Lautenschalger E, Miller D. The effects of mineral trioxide aggregate on the dog pulp (Abstract). *J Endod* 22:198, 1996.
35. Song JS, Mante FK, Romanow WJ, Kim S. Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 102:809-815, 2006.
36. Agamy HA, Bakry NS, Mounir MF, Avery DR. Comparison of mineral trioxide aggregate and formocresol as pulp-capping agents in pulpotomized primary teeth. *Pediatr Dent* 26:302-309, 2004.
37. Maroto M, Barberia E, Vera V, Garcia-Godoy F. Dentin bridge formation after white trioxide aggregate (white MTA) pulpotomies in primary molars. *Am J Dent* 19:75-79, 2006.
38. Parirokh M, Asgary S, Eghbal MJ, Stowe S, Eslami B, Eskandarizade A, Shabahang S. A comparative study of white and grey mineral trioxide aggregate as pulp capping agents in dog's teeth. *Dent Traumatol* 21:150-154, 2005.

Legends of Figures

Figure 1. Pulp capping with PEP at 2 weeks. Although the pulp capping material was impacted into the pulp, newly formed hard tissue barrier was observed. Incisal portion of the pulp from the impacted site (left portion in the figure) showed degeneration of the pulp.
Figure 2. Pulp capping with CH at 2 weeks. Newly formed hard tissue barrier was observed and odontoblast-like cells arranged beneath it. Although hemorrhage was seen, a few inflammatory cells were found.
Figure 3. Pulp capping with PEP+CH at 2 weeks. Hard tissue formation was rare. Inflammatory cells infiltrated around the impacted capping material.
Figure 4. Pulp capping with WMTA at 2 weeks. Among the dentin debris, scattered hard tissue was formed. Infiltration of inflammatory cells under odontoblast cell layer and dilatation of blood vessels were observed.
Figure 5. Pulp capping with PEP at 1 month. Many inflammatory cells infiltrated around the exposed site. There was no hard tissue formation even around the dentin debris.
Figure 6. Pulp capping with CH at 1 month. The thickness of hard tissue increased, compared with that of 2 weeks. In newly formed hard tissue, dentinal tubule-like structures were observed.
Figure 7. Pulp capping with PEP+CH at 1 month. Newly formed hard tissue grew mainly around the fragments of dentin. Dilatation of blood vessels was observed.
Figure 8. Pulp capping with WMTA at 1 month. Newly formed hard tissue grew into a complete bridge. They include the fragments of dentin that were pushed into the pulp during cavity preparation.
Figure 9. Pulp capping with PEP at 3 months. Inflammatory response persisted at the exposed site. The newly formed hard tissue were related to the dentin debris.
Figure 10. Pulp capping with CH at 3 months. There was a complete dentin bridge and normal pulp tissue organization beneath the exposed site. There were vacuole-like structure, cells and dentin chips inside a dentin bridge.
Figure 11. Pulp capping with PEP+CH at 3 months. No hard tissue formation was observed. Inflammatory response of the pulp persisted.
Figure 12. Pulp capping with WMTA at 3 months. There was a thick hard tissue barrier, that did not include a cell body and had no tubular structure, across the exposed site.

Figure 1.

Figure 2.

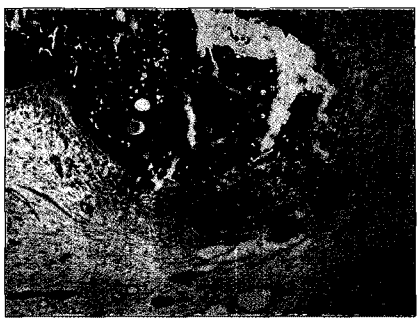


Figure 3.

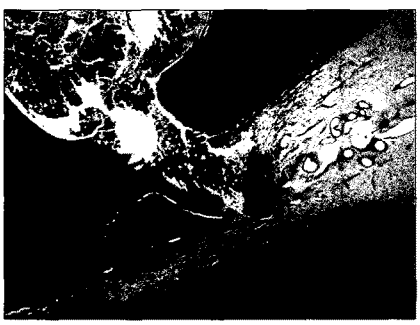


Figure 4.

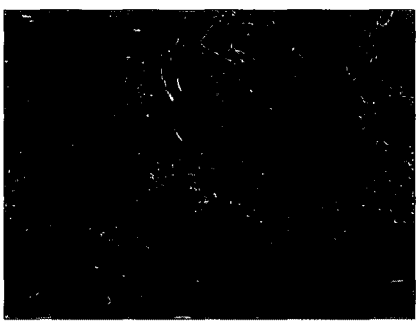
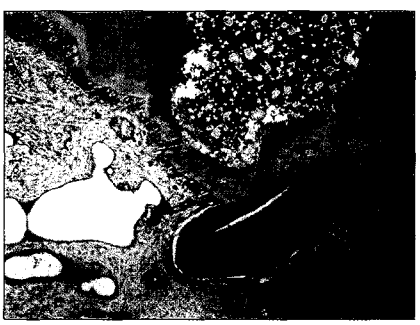


Figure 5.

Figure 6.

Figure 7.



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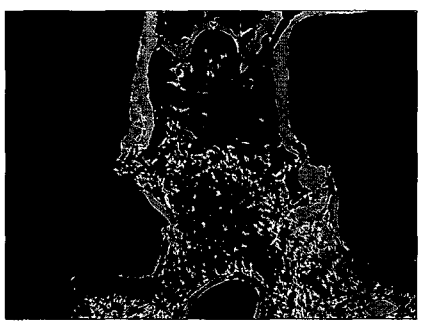

Figure 9.

Figure 10.

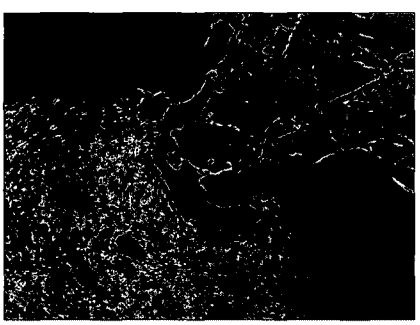

Figure 11.

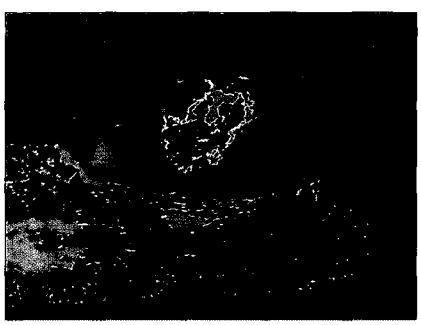

Figure 12.

국문초록

비글견에서 DSP 유도 합성 펩타이드를 이용한 직접 치수 복조술에 대한 조직학적 연구

김재훈[1] · 홍준배[1] · 임범순[2] · 조병훈[1]**
[1]서울대학교 치의학대학원 치과보존학교실, [2]서울대학교 치의학대학원 치과생체재료학교실

본 연구에서는 DSP (dentin sialoprotein)에서 유래된 합성 펩타이드를 동물실험 모델에 적용하여 치수노출 부위에서 상아질 재생을 확인하고, 기존 치수복조제와의 성능 비교를 통해 새로운 치수복조제로서의 가능성을 확인하고자 하였다. 6 마리 비글견의 72 개의 치아를 이용하여, 실험적으로 치수를 노출하고 직접 치수 복조술을 시행하였다. 사용한 치수복조제는 (1) $Ca(OH)_2$ (CH군) (2) DSP 유도 합성 펩타이드 (PEP군) (3) 합성 펩타이드와 $Ca(OH)_2$ 혼합제 (PEP+CH군) (4) White MTA (WMTA군) 이다. 노출된 치수에 치수복조제를 적용한 후 와동은 강화형 글라스 아이오노머로 충전하였다. 시술 후 2 주, 1 개월 및 3 개월에 각각 2 마리씩 비글견을 희생시키고 조직시편을 제작하였다. 시편은 H&E 염색 후 광학 현미경으로 치수 염증 반응과 경조직 형성 정도를 관찰하였다. PEP군에서는 17 개의 시편 중 3 개의 시편에서만 경조직 형성을 관찰할 수 있었으며, 대부분의 시편에서 적절한 치수 회복을 관찰할 수 없었다. PEP군은 CH군에 비해 심한 염증반응을 보이고, 경조직 형성은 불량하였다. CH군과 WMTA군은 기계적으로 노출된 치수에서의 염증반응과 경조직 형성에 있어서 유사한 결과를 보였다.

주요단어: 치수복조, dentin sialoprotein, 수복 상아질, 조직학적 평가, 칼슘하드록사이드, 비글견