# Tertiary Dentin Formation after Indirect Pulp Capping Using Protein CPNE7

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**Abstract**

If there is a partial loss of dentin, the exposed dentinal surface should be protected by an indirect pulp capping (IPC) procedure to preserve pulp vitality and prevent symptoms of dentin hypersensitivity. In our previous study, copine7 (CPNE7) induced odontoblast differentiation in vitro and promoted dentin formation in vivo. The aim of this study was to investigate the possibility of IPC therapy using the CPNE7 protein at the exposed dentinal surface and the resulting effects on tertiary dentin formation in a beagle model. CPNE7 promoted mineralization of odontoblasts and had high calcium ion-binding capacity. The in vivo IPC model with canine teeth showed that regeneration of physiologic reactionary dentin with dentinal tubule structures was clearly observed beneath the remaining dentin in the CPNE7 group, whereas irregular features of reparative dentin were generated in the mineral trioxide aggregate (MTA) group. The CPNE7+MTA group also showed typical reactionary dentin without reparative dentin, showing synergistic effects of CPNE7 with MTA. A scanning electron microscopy analysis showed that dentinal tubules beneath the original dentin were occluded by the deposition of peritubular dentin in the CPNE7 and CPNE7+MTA groups, whereas those in the control group were opened. Therefore, CPNE7 may be able to serve as a novel IPC material and improve symptoms of dentin hypersensitivity.

**Keywords:** dentinogenesis, regeneration, biomaterial(s), epithelial-mesenchymal interaction, mineralized tissue, development

## Introduction

Dental pulp serves several important functions, including nutritive, formative, sensory, and defense reactions for maintaining tooth vitality and homeostasis. Dentin protects the underlying dental pulp against various harmful stimuli. If there is partial loss of the overlying dentin in response to mechanical trauma, such as tooth preparation, removal of dentinal decay, or tooth attrition, dentinal tubules and odontoblast processes will be exposed to the external environment. In this case, the exposed dentinal surface should be protected, because bacteria can invade dentinal tubules, and bacterial products can diffuse across the dentin to provoke pulpal reactions (Opal et al. 2014). Vital pulp therapy using an indirect pulp capping (IPC) procedure is needed to preserve pulp vitality and further prevent the possible painful symptoms of dentin hypersensitivity. It is well known that dental pulp potentially regenerates new dentin under various traumatic conditions.

Several IPC studies have shown varying success rates of 73%–97%, but the question of which capping material is optimal is still debatable (Petrou et al. 2014). The American Academy of Pediatric Dentistry guidelines report available biocompatible liners for IPC, including dentin-bonding agents, calcium hydroxide, zinc oxide/eugenol, and glass ionomer (GI) cement. Among these materials, calcium hydroxide is preferred by most clinicians as the gold standard for indirectly accelerating the formation of tertiary dentin (Hayashi et al. 2011). Recently, it was suggested that the success rates for mineral trioxide aggregate (MTA) appear better for pulp capping compared with calcium hydroxide (Aeinehchi et al. 2003). However, there are some limitations of conventional IPC using calcium hydroxide or MTA, such as the formation of a reparative dentin bridge that shows irregular features and structures with an atubular appearance and entrapment of cells resembling osteocytes (Liu et al. 2015).

Previously, we reported that dental epithelium-derived factors induce odontoblastic differentiation of dental pulp stem

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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cells in vitro and promote pulp/dentin regeneration in vivo via epithelial-mesenchymal interactions (Lee et al. 2011; Choung et al. 2013). Among the secreted dental epithelium-derived factors, we identified the protein copine7 (CPNE7) as a candidate signaling molecule for odontoblastic differentiation (Oh et al. 2015). CPNE7 is a member of the calcium-dependent phospholipid-binding protein family that contains 2 C2 domains in the N terminus and a von Willebrand factor–type A domain in the C terminus (Creutz et al. 1998). Our recent studies showed that CPNE7 is a soluble, secreted protein from dental epithelial cells that could be transmitted to odontoblastic cells by a paracrine mechanism (Thesleff 2003). CPNE7 was shown to promote the expression of odontoblastic markers such as dentin sialophosphoprotein (*Dspp*) mRNA and Dsp protein in the MDPC-23 odontoblastic cell line in vitro (Oh et al. 2015). Furthermore, CPNE7 induced odontoblast differentiation and promoted dentin-like mineralized tissue formation in human dental pulp cells (hDPCs) in vivo. These positive effects of CPNE7 on dentin formation by induction of odontoblast differentiation suggest the possibility of its clinical application in vital pulp therapy.

Given these findings, we hypothesize that if CPNE7 protein is used to treat exposed dentinal surfaces, it may be inserted into dentinal tubules, diffuse across the dentin, and induce tertiary dentinogenesis in vivo. This means that CPNE7 protein might serve as a bioactive molecule for IPC. Therefore, the aim of this study was to investigate the possible use of CPNE7 for IPC therapy at the exposed dentinal surface and its effects on tertiary dentin formation using a canine model.

## Materials and Methods

### Cell Culture

We collected human impacted third molars at the Seoul National University Dental Hospital. The experimental protocol was approved by the Institutional Review Board of the Seoul National University. Informed consent was acquired from the patients (S-D20140006). All experiments using animals followed protocols approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-111013-2). hDPCs were isolated and used in an in vitro experiment as described previously (Lee et al. 2011). MDPC-23 cells were provided by Dr. J.E. Nör (University of Michigan, Ann Arbor, MI, USA). See the Appendix for further details.

### Luciferase Assay

MDPC-23 cells were seeded on 24-well plates at a density of $5 \times 10^4$ cells/well. After 24 h, the cells were transfected with Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's instructions. See the Appendix for further details.

### Alizarin Red S Staining

MDPC-23 cells were cultured in differentiation medium for 10 d with or without recombinant copine7 (rCPNE7), and the formation of mineralized nodules was evaluated by alizarin red S staining. See the Appendix for further details.

### Noncollagenous Dentin Matrix Proteins and Western Blot Analysis

Noncollagenous dentin matrix proteins (NCDMPs), which were isolated and purified from powdered human teeth, were obtained as described in previous reports (Kim et al. 2009). See the Appendix for further details.

### Calcium-Binding Assay

The $^{45}Ca^{2+}$-binding assay was based on a procedure described by He et al. (2003). See the Appendix for further details.

### Cell Migration and Proliferation Assay

A wound-healing assay and transwell assay were performed to evaluate the effects of CPNE7 on hDPC migration. The effect of CPNE7 on hDPC proliferation was evaluated with an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Sigma-Aldrich). See the Appendix for further details.

### Real-Time Polymerase Chain Reaction Analysis

hDPCs were cultured with rCPNE7 or Dulbecco's modified Eagle's medium/10% fetal bovine serum (control) for 3 d. RNA was extracted from hDPCs with TRIzol reagent according to the manufacturer's instructions (Invitrogen). See the Appendix for further details.

### IPC Model with Canine Teeth

Three beagle dogs (aged 2 y) were used for this experiment. Cervical parts of the maxillary and mandibular premolars were cleaned with 0.5% chlorhexidine. Then, dish-shaped class V cavities were prepared using a round bur (head diameter, 1 mm) with several gentle strokes, which prevent the exposure of the underlying pulp. The drilling was stopped when the color of the remaining dentin looked reddish gray. Teeth with exposed pulp were not used for the experiment. After sufficient irrigation, surgical sites were gently dried with a cotton pellet. The cavities were divided into 4 groups for the experiment. Group 1 received GI cement (GC Fuji II LC; GC America Inc.) only (control group), group 2 received GI cement after MTA (ProRoot MTA; Dentsply Tulsa Dental) treatment (MTA group), group 3 received GI cement after the topical treatment with the rCPNE7 protein (CPNE7 group), and group 4 received GI cement after the topical treatment with the rCPNE7 protein under MTA (CPNE7+MTA group). In groups 3 and 4, we repeated the topical application of rCPNE7 protein (total 1 μg of rCPNE7 per tooth in a buffer containing 25 mM Tris-HCl, 100 mM glycine, and 10% glycerol) and waited for diffusion. Each group had 2 premolars per animal, totaling 24 premolars. Teeth with exposed pulp or remaining dentin thickness greater than 500 μm were not used for the experimental analysis.

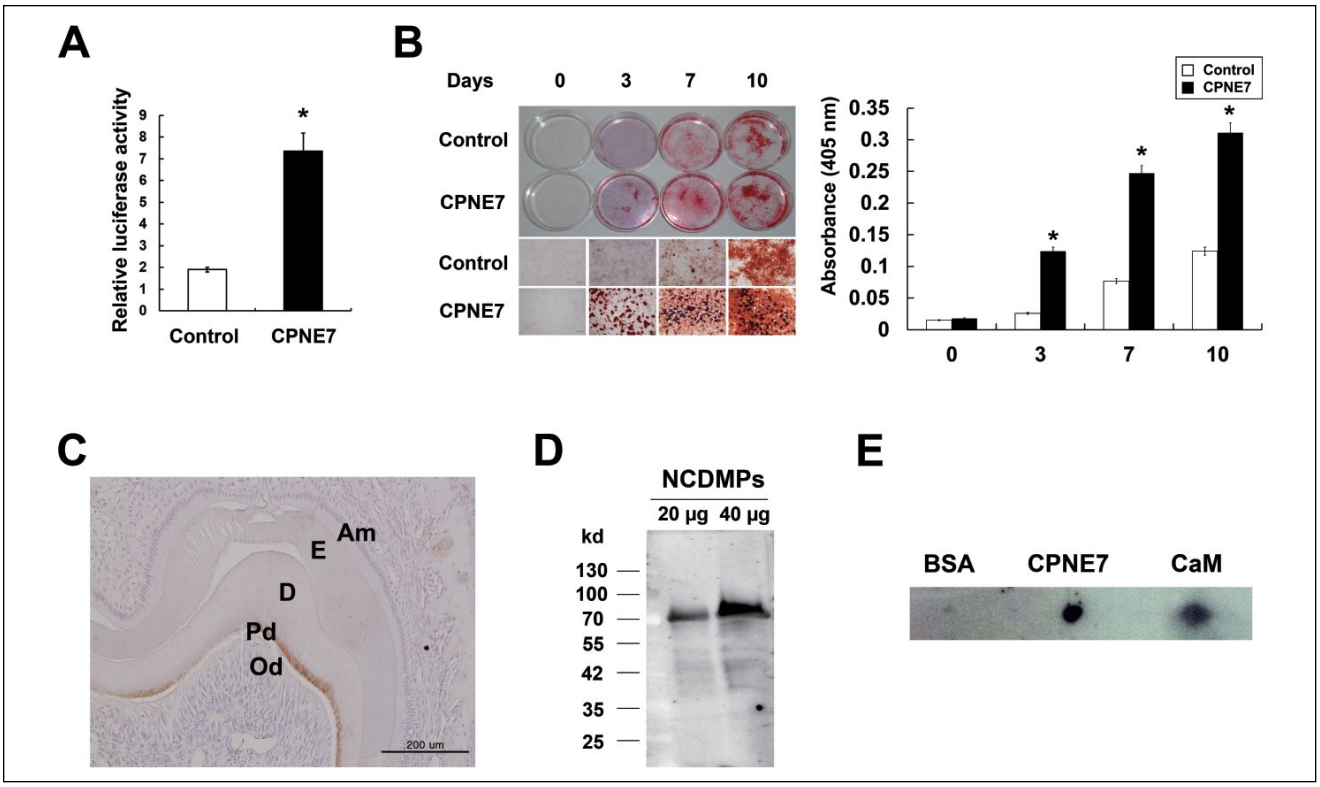


**Figure 1.** Mineralization capacity of copine7 (CPNE7) in odontoblasts in vitro. (**A**) Alkaline phosphatase (ALP) promoter activity assessed in MDPC-23 cells for 48 h. (**B**) Effects of recombinant copine7 (rCPNE7) on the mineralized nodule formation of MDPC-23 cells in vitro analyzed by alizarin red S staining. (**C**) CPNE7 expression by immunohistochemistry during mouse tooth development on postnatal day 10. Scale bars, 200 µm. (**D**) CPNE7 expression in noncollagenous dentin matrix proteins by Western blot analysis. (**E**) Calcium-binding assay. Bovine serum albumin (BSA) and calmodulin (CaM) were used as negative and positive controls in equivalent amounts, respectively. All values represent the mean ± standard deviation of triplicate experiments. *$P < 0.05$ compared with control. Am, ameloblast; D, dentin; E, enamel; NCDMPs, noncollagenous dentin matrix proteins; Od, odontoblast; Pd, predentin.

### *Statistical Analysis*

The data were analyzed for statistical significance using a non-parametric Mann-Whitney *U* test.

## Results

### *Mineralization Capacity of CPNE7 on Odontoblasts In Vitro*

It has been reported that CPNE7 promotes differentiation of odontoblastic MDPC-23 cells in vitro (Oh et al. 2015). Alkaline phosphatase (ALP) is a characteristic molecule in mineralized tissue-forming cells, such as osteoblasts and odontoblasts (Lee et al. 2012). To investigate the effects of CPNE7 on odontoblast mineralization, ALP promoter activity in MDPC-23 cells was evaluated. ALP promoter activity was significantly increased in the rCPNE7-treated group compared with controls (Fig. 1A). According to the results of alizarin red S staining, the rCPNE7-treated group began to demonstrate mineralized nodules on day 3, which significantly increased until day 10, whereas mineralized nodules were detected after day 7 in the control group and showed fewer mineralized nodules than the rCPNE7-treated group microscopically (Fig. 1B).

During mouse tooth development, CPNE7 protein was clearly detected in the odontoblasts, especially the predentin area, at postnatal day 10 by immunohistochemistry (Fig. 1C). These findings suggest that the expression of CPNE7 coincides with initial dentin calcification. Reactionary dentinogenesis is mediated by the sequestered bioactive molecules in the dentin matrix under injurious stimuli, and ethylenediamine tetraacetic acid (EDTA)–soluble dentin matrix proteins stimulate tertiary dentinogenesis in vivo (Duque et al. 2006). To investigate whether dentin matrix proteins contain CPNE7 protein, expression of CPNE7 in extracted dentin matrix proteins (NCDMPs) from human teeth was evaluated by Western blot analysis (Fig. 1D). As a result, CPNE7 was identified in NCDMPs. To investigate the binding affinity of calcium to CPNE7, a calcium-binding assay was performed. The strong calcium-binding protein calmodulin (CaM) was used as a positive control; bovine serum albumin (BSA) was the negative control. As a result, rCPNE7 had high calcium ion-binding capacity relative to either BSA or CaM (Fig. 1E).

### *Effects of CPNE7 on Migration, Proliferation, and Odontoblastic Differentiation of hDPCs*

Results of wound-healing and transwell assays showed that there were no significant differences in migratory activity of hDPCs between the control and rCPNE7-treated groups (Fig. 2A). The effect of CPNE7 on hDPC proliferation was evaluated.

There were also no significant differences in MTT activity from days 0 to 5 between the control and rCPNE7-treated groups (Fig. 2B). To identify the effects of CPNE7 on odontoblastic differentiation of dental pulp stem cells, hDPCs were collected from human dental pulp and cultured in differentiation medium for 3 d with or without rCPNE7. Recombinant CPNE7 upregulated the levels of *DSPP*, dentin matrix protein 1 (*DMP1*), osterix (*OSX*), osteocalcin (*OC*), bone sialoprotein (*BSP*), and nestin (*NESTIN*) mRNAs in hDPCs on day 3 (Fig. 2C). *DSPP* expression increased approximately 20-fold, and *DMP1* increased approximately 10-fold in the rCPNE7-treated group compared with controls. These results indicate that CPNE7 does not affect migration but induces odontoblastic differentiation of hDPCs in vitro.

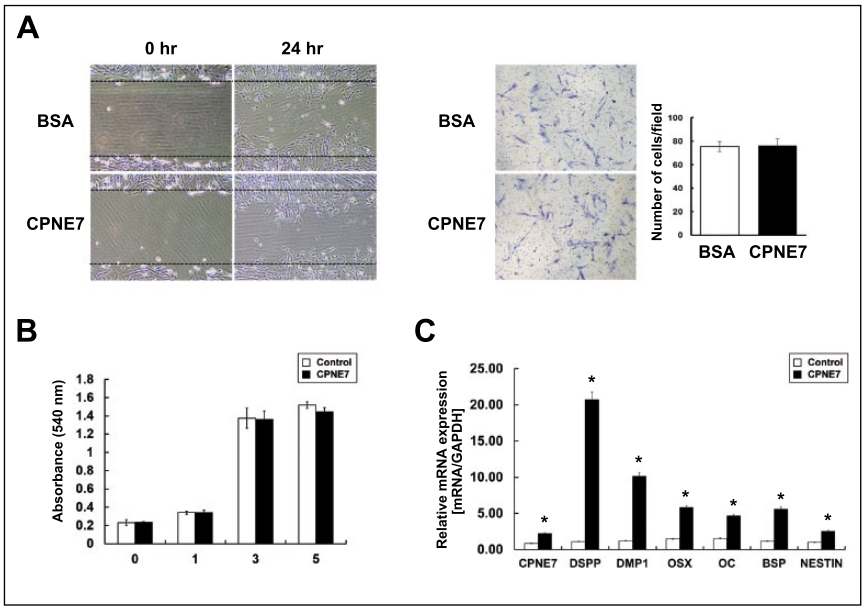


**Figure 2.** Effects of copine7 (CPNE7) on migration, proliferation, and odontoblast differentiation of human dental pulp cells (hDPCs). (**A**) Effect of recombinant copine7 (rCPNE7) protein on migration of hDPCs was determined using a wound-healing assay and a transwell assay. (**B**) The effect of rCPNE7 protein on the proliferation of hDPCs was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. (**C**) Levels of copine7 (*CPNE7*), dentin sialophosphoprotein (*DSPP*), dentin matrix protein 1 (*DMP1*), osterix (*OSX*), osteocalcin (*OC*), bone sialoprotein (*BSP*), and nestin (*NESTIN*) mRNAs in hDPCs were evaluated by quantitative real-time polymerase chain reaction after 3 d of culture. All values represent the mean ± standard deviation of triplicate experiments. *$P < 0.05$ compared with control. BSA, bovine serum albumin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

## *IPC Model with Canine Teeth*

Prepared cavities were divided into 4 groups (Fig. 3A): GI cement only (control group), GI cement after MTA sealing (MTA group), GI cement after topical treatment of rCPNE7 (CPNE7 group), and GI cement after topical treatment of rCPNE7 under MTA sealing (CPNE7+MTA group). In the control group, newly formed mineralized tissue was rarely detected in the pulp cavity (Fig. 3B, F, and J), whereas in the MTA group, irregular features of tertiary dentin were generated beneath the remaining dentin at cavity-prepared sites (Fig. 3C, G and K). This newly generated dentin was considered reparative dentin, which has atubular structures with cellular components in the calcified matrix (arrows in Fig. 3K). In the CPNE7 group, the regeneration of physiologic tertiary dentin was clearly observed beneath the remaining dentin at cavity-prepared sites (Fig. 3D, H, and L). The regenerated dentin showed typical characteristics of reactionary dentin, such as odontoblast processes, and was continuous with remaining original dentin structures. Tertiary dentin was also regenerated in the CPNE7+MTA group and showed typical reactionary dentinal structures as in the CPNE7 group (Fig. 3E, I, and M). Unlike the MTA group, characteristics of reparative dentin were hardly detected. The overall thickness of the regenerated tertiary dentin in the CPNE7+MTA group was thicker than in the CPNE7 group. Furthermore, odontoblasts formed a typical layer beneath the regenerated dentin.

## *Scanning Electron Microscopy of Pulp-Side Surfaces of the Remaining Dentin and Dentinal Tubules*

To examine the morphological features of pulp-side surfaces of the remaining original dentin and dentinal tubules, scanning electron microscopy (SEM) was performed at the original dentin/pulp (control group) or original dentin/tertiary dentin (MTA, CPNE7, and CPNE7+MTA groups) interfaces of cavity-prepared sites via the gap of the tertiary dentin matrix after removal of the dental pulp tissues (Fig. 4A–E). A large portion of the dentinal tubules of the original dentin was occluded with mineral deposition of peritubular dentin in the CPNE7 and CPNE7+MTA groups, whereas those in the control group were open and no precipitate was observed (Fig. 4F, H, and I). In the MTA group, some of the dentinal tubules were slightly occluded, although most of the dentinal tubules remained open (Fig. 4G).

# Discussion

Dental pulp capping, a vital pulp therapy, is subdivided into direct and indirect pulp capping, depending on pulp exposure. Several previous studies have reported direct pulp capping using rodent or beagle dog models; however, there are few studies with respect to IPC animal models (Filipović et al. 1989; Pavlica et al. 2000; Duque et al. 2006; De Rossi et al. 2014; Gu et al. 2015). Specifically, an IPC model of canine teeth with an application of a bioactive molecule has not yet been documented. To the best of our knowledge, in the present study, we report for the first time a canine IPC model using a protein, CPNE7, as a bioactive IPC material. Moreover, we demonstrate that CPNE7 could be used in IPC therapy because of its capacity to induce physiologic tertiary dentin formation in vivo.

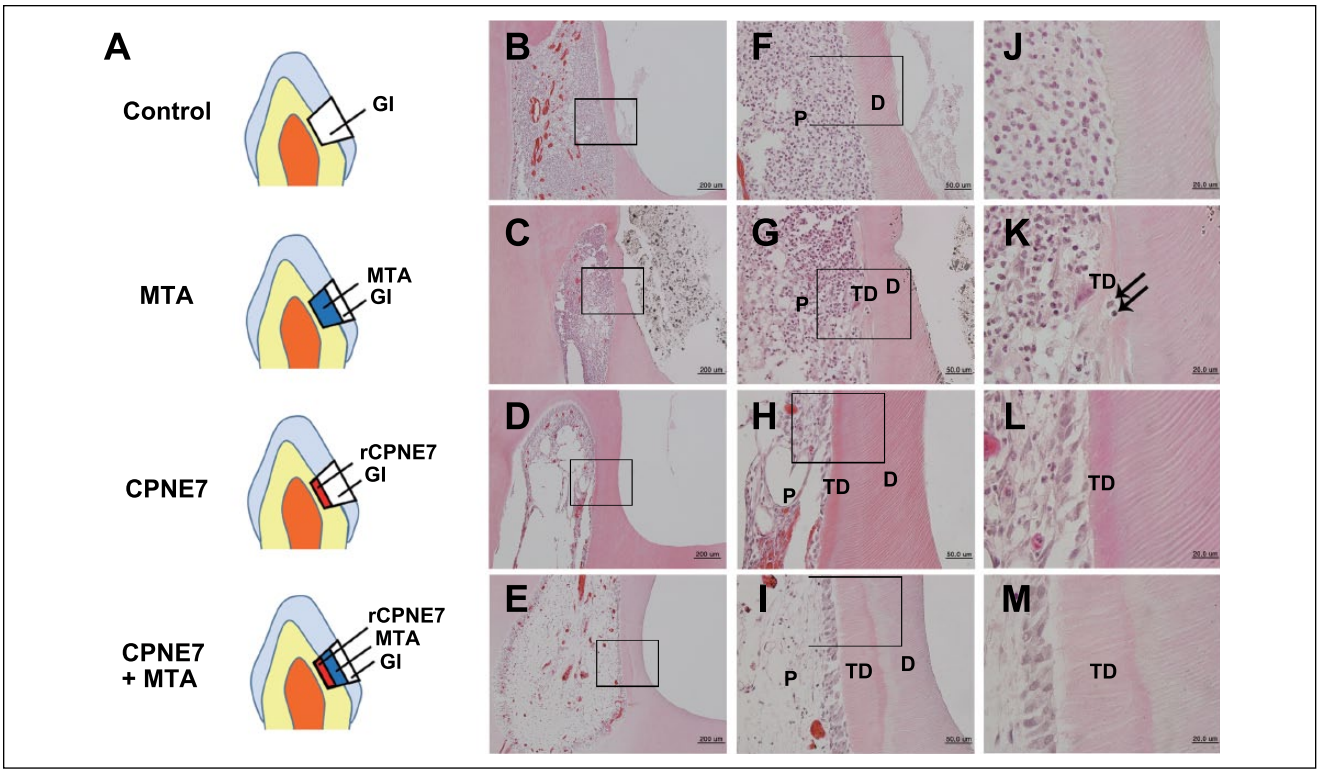


**Figure 3.** Indirect pulp capping (IPC) canine model. (**A**) Schematic diagrams of the IPC model. Defect areas were covered with glass ionomer (GI) cement only (control group), GI cement after mineral trioxide aggregate (MTA) sealing (MTA group), GI cement after the topical treatment with recombinant copine7 (CPNE7 group), and GI cement after the topical treatment with recombinant copine7 (rCPNE7) under MTA sealing (CPNE7+MTA group). (**B–M**) Histological analysis of dental pulp responses at the IPC area after 3 wk by hematoxylin/eosin staining (4 of 6 samples from each group). (B, F, and J) Control group. (C, G, and K) MTA group. (D, H, and L) CPNE7 group. (E, I, and M) CPNE7+MTA group. (F–I) Boxed areas in B–E are shown at higher magnification in F–I. (J–M) Boxed areas in F–I are shown at higher magnification in J–M. Arrows in K indicate the cellular components in the calcified matrix. Scale bars, 200 μm (B–E), 50 μm (F–I), and 20 μm (J–M). D, remaining dentin; P, pulp, TD, newly formed tertiary dentin.

There are 2 types of tertiary dentin, as defined by the survival and death of remaining original odontoblasts reacting to external stimulation: reactionary and reparative (Smith et al. 1995). Reactionary dentinogenesis occurs in response to a mild stimulus, which is not lethal to odontoblasts. However, severe stimuli may result in the death of underlying odontoblasts, leading to reparative dentinogenesis. This includes the migration of endogenous stem cells to the injury site, differentiation of the cells into odontoblast-like cells that can replace necrotic odontoblasts, and secretion of the dentin matrix by the newly differentiated odontoblast-like cells to form reparative dentin (About et al. 2000). The mechanisms of such processes have not been clearly identified. The mechanism of reactionary dentinogenesis appears to be related to the solubilization of certain growth factors and bioactive molecules sequestered within the remaining dentin matrix (Murray et al. 2000). Under injurious stimuli, these factors diffuse through the dentinal tubules to surviving odontoblasts. The cells, then, respond to the factors and begin to increase the rate of secretion of the extracellular matrix, resulting in the appearance of reactionary dentin (Murray et al. 2002). Indeed, EDTA-soluble dentin matrix proteins have been shown to stimulate tertiary dentinogenesis in vivo (Duque et al. 2006). Thus, we hypothesized that CPNE7 may be one of those sequestered bioactive molecules in the dentin matrix during reactionary dentinogenesis. There are several reasons for this hypothesis. First, many aspects of reactionary dentinogenesis are similar to those of primary dentinogenesis, including complex inducible signaling molecules and the resulting final dentin structures. In our previous study, dental epithelium-derived factors were proposed to play crucial roles in odontoblast differentiation via epithelial-mesenchymal interactions. Among those epithelial factors, CPNE7 was regarded as a key signaling molecule for odontoblast differentiation and dentin formation during primary dentinogenesis. Second, CPNE7 can also be detected in the predentin and dentin matrix areas (Fig. 1C) during tooth development (Oh et al. 2015). Third, CPNE7 was detected in NCDMPs, which were isolated and purified from powdered human dentin (Kim et al. 2009). In addition, CPNE7 is a soluble, secreted protein from conditioned medium of dental epithelial cells and can be translocated to odontoblasts. This high cytopermeability of CPNE7 is one of the great advantages for its direct clinical application.

Various IPC materials have been proposed for good treatment outcomes (Reston and de Souza Costa 2009). In the present study, the regenerated dentin in the CPNE7 group showed typical physiologic reactionary dentin with dentinal tubular

structures, rather than reparative dentin, and was continuous with remaining original dentin, whereas the MTA group showed irregular features of reparative dentin whose quality is lower than reactionary dentin (Tziafas et al. 2000). Furthermore, regenerated dentin in the CPNE7+MTA group showed typical reactionary dentin without reparative dentin, with a greater thickness than that in the CPNE7 group. These synergistic effects of CPNE7 and MTA suggest the possible clinical application of CPNE7 with MTA. Thus, CPNE7 may serve as a novel IPC material, but the clinical availability of CPNE7 with MTA is still unknown, representing an interesting direction for further study.

It has been documented that the depth of the prepared cavity and the thickness of the remaining dentin beneath the cavities are important for the quantity of newly formed tertiary dentin. The diffusion distance of CPNE7 along the dentinal tubules will be a consideration with regard to its stimulatory effect on the underlying odontoblasts. Filipović et al. (1989) concluded that remaining dentin with a thickness between 501 and 700 µm showed the greatest rate of reparative dentin, whereas that less than 500 µm or more than 900 µm resulted in the least amounts of tertiary dentin under conventional liners. According to Murray et al. (2002), remaining dentin ranging from 250 to 500 µm provided maximal reactionary dentin. In the present study, reactionary dentin was well formed in the CPNE7 and CPNE7+MTA groups beneath the 100- to 200-µm–thick remaining dentin, which was in poorer condition than those in previous reports (Filipović et al. 1989; Murray et al. 2002). These results may be attributable to the potent mineralization capacity of CPNE7, which suggests its clinical application as an IPC material.

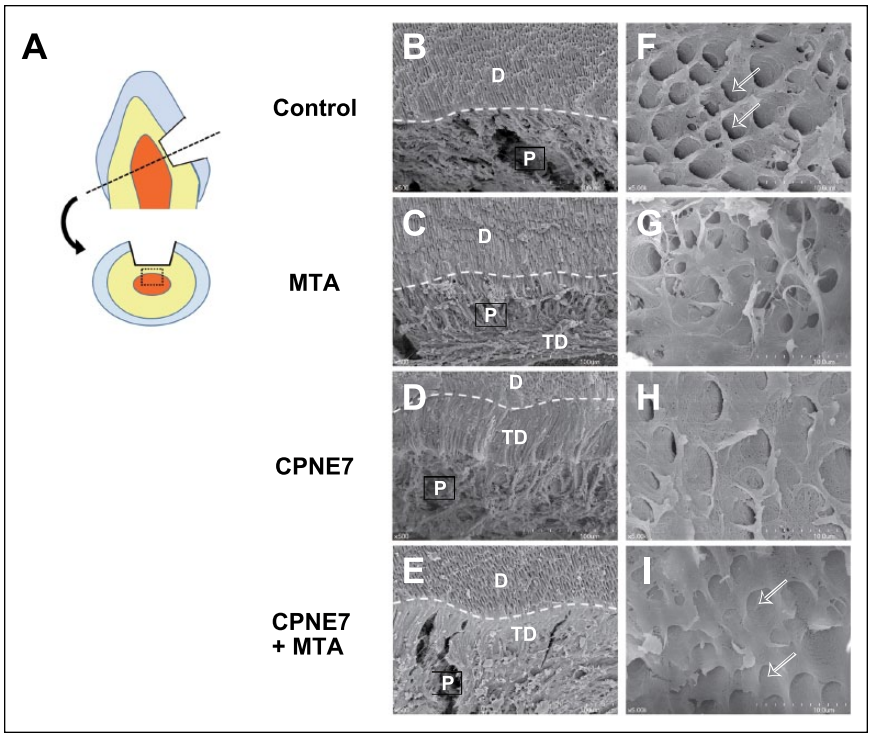


**Figure 4.** Scanning electron microscopy (SEM) of pulp-side surfaces of the remaining dentin and dentinal tubules. (**A**) Schematic diagrams of the tissue samples for SEM analysis (2 of 6 samples from each group). (**B–E**) Corresponding SEM images of the dashed rectangle of A in each group. Dashed lines indicate the remaining original dentin/newly formed dentin interfaces. (**F–I**) Boxed areas in B–E are shown at higher magnification in F–I, which show the dentinal tubules. Arrows in F and I indicate the dentinal tubules of pulp-side dentinal surfaces. CPNE7, copine7; D, remaining dentin; MTA, mineral trioxide aggregate; P, pulp-side dentinal surface; TD, newly formed tertiary dentin. Scale bars, 100 µm (B–E) and 10 µm (F–I).

Dentin hypersensitivity is a common dental disease characterized by pain derived from exposed dentin in response to thermal, chemical, or osmotic stimuli. In general, 2 types of clinical treatments have been suggested to relieve such pain, including occlusion of dentinal tubules and interruption of the transmission of nerve impulses (Dababneh et al. 1999). To occlude dentinal tubules, fluorides, varnishes, adhesive resins, lasers, or restorative materials have been used for treatment; however, these are neither sufficient nor very successful (Davari et al. 2013). In the current study, CPNE7 or CPNE7 under an MTA sealing treatment of the exposed canine dentin showed that dentinal tubules of the remaining dentin were almost occluded as observed under SEM. This result suggests that CPNE7 stimulates odontoblasts to produce intratubular dentin, as well as reactionary dentin, for the reduction of dentin permeability and protection against painful stimuli through dentinal tubules. Moreover, the high calcium ion-binding affinity of CPNE7 may contribute to this obstruction of dentinal tubules via the initiation of mineral deposition, followed by nucleation of hydroxyapatite formation, similar to that of dentin matrix protein 1 (He et al. 2003). The data showing CPNE7 expression in the predentin area, which is known as an initial mineralization site of dentin, also support these results. This means that CPNE7 can prevent the painful symptoms of dentin hypersensitivity by the formation of both tertiary dentin and intratubular dentin. Further investigations should elucidate the most efficient amounts of the protein for tertiary dentinogenesis and intratubular dentin formation, as well as the most effective delivery methods for human patients requiring IPC and/or dentin hypersensitivity treatments.

In conclusion, CPNE7 has a potent mineralizing capacity in odontoblasts and high calcium ion-binding affinity in vitro; it stimulates reactionary dentin formation and peritubular dentin formation in vivo. These results suggest that CPNE7 could serve as both a novel IPC material with MTA and a new treatment for dentin hypersensitivity.

## Author Contributions

H.W. Choung, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; D.S. Lee, Ji-Hyun Lee, contributed to conception and data acquisition, critically revised the manuscript; W.J. Shon, contributed to design and data interpretation, critically revised the manuscript; Jong-Ho Lee, Y. Ku, contributed to data interpretation, critically revised the manuscript; J.C. Park, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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## References

About I, Bottero MJ, de Denato P, Camps J, Franquin JC, Mitsiadis TA. 2000. Human dentin production in vitro. Exp Cell Res. 258(1):33–41.

Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS. 2003. Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod J. 36(3):225–231.

Choung HW, Lee JH, Lee DS, Choung PH, Park JC. 2013. The role of preameloblast-conditioned medium in dental pulp regeneration. J Mol Histol. 44(6):715–721.

Creutz CE, Tomsig JL, Snyder SL, Gautier MC, Skouri F, Beisson J, Cohen J. 1998. The copines, a novel class of C2 domain-containing, calcium-dependent, phospholipid-binding proteins conserved from paramecium to humans. J Biol Chem. 273(3):1393–1402.

Dababneh RH, Khouri AT, Addy M. 1999. Dentine hypersensitivity - an enigma? A review of terminology, mechanisms, aetiology and management. Br Dent J. 187(11):606–611; discussion 603.

Davari A, Ataei E, Assarzadeh H. 2013. Dentin hypersensitivity: etiology, diagnosis and treatment; a literature review. J Dent (Shiraz). 14(3):136–145.

De Rossi A, Silva LA, Gaton-Hernandez P, Sousa-Neto MD, Nelson-Filho P, Silva RA, de Queiroz AM. 2014. Comparison of pulpal responses to pulpotomy and pulp capping with biodentine and mineral trioxide aggregate in dogs. J Endod. 40(9):1362–1369.

Duque C, Hebling J, Smith AJ, Giro EM, Oliveira MF, de Souza Costa CA. 2006. Reactionary dentinogenesis after applying restorative materials and bioactive dentin matrix molecules as liners in deep cavities prepared in non-human primate teeth. J Oral Rehabil. 33(6):452–461.

Filipović V, Ivanović V, Pajić M. 1989. [Effect of remaining dentine thickness beneath deep cavities on the rate of reparative dentine formation–experimental study]. Stomatol Glas Srb. 36(5):393–399.

Gu J, Ikeda H, Suda H. 2015. Sympathetic regulation of tertiary dentinogenesis via beta-2 adrenergic receptor on rat odontoblasts. J Endod. 41(7):1056–1060.

Hayashi M, Fujitani M, Yamaki C, Momoi Y. 2011. Ways of enhancing pulp preservation by stepwise excavation–a systematic review. J Dent. 39(2):95–107.

He G, Dahl T, Veis A, George A. 2003. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. Nat Mater. 2(8):552–558.

Kim HS, Lee DS, Lee JH, Kang MS, Lee NR, Kim HJ, Ko JS, Cho MI, Park JC. 2009. The effect of odontoblast conditioned media and dentin non-collagenous proteins on the differentiation and mineralization of cementoblasts in vitro. Arch Oral Biol. 54(1):71–79.

Lee JH, Lee DS, Choung HW, Shon WJ, Seo BM, Lee EH, Cho JY, Park JC. 2011. Odontogenic differentiation of human dental pulp stem cells induced by preameloblast-derived factors. Biomaterials. 32(36):9696–9706.

Lee W, Oh JH, Park JC, Shin HI, Baek JH, Ryoo HM, Woo KM. 2012. Performance of electrospun poly(epsilon-caprolactone) fiber meshes used with mineral trioxide aggregates in a pulp capping procedure. Acta Biomater. 8(8):2986–2995.

Liu S, Wang S, Dong Y. 2015. Evaluation of a bioceramic as a pulp capping agent in vitro and in vivo. J Endod. 41(5):652–657.

Murray PE, About I, Lumley PJ, Smith G, Franquin JC, Smith AJ. 2000. Postoperative pulpal and repair responses. J Am Dent Assoc. 131(3):321–329.

Murray PE, Lumley PJ, Smith AJ. 2002. Preserving the vital pulp in operative dentistry: 3. Thickness of remaining cavity dentine as a key mediator of pulpal injury and repair responses. Dent Update. 29(4):172–178.

Oh HJ, Choung HW, Lee HK, Park SJ, Lee JH, Lee DS, Seo BM, Park JC. 2015. CPNE7, a preameloblast-derived factor, regulates odontoblastic differentiation of mesenchymal stem cells. Biomaterials. 37:208–217.

Opal S, Garg S, Dhindsa A, Taluja T. 2014. Minimally invasive clinical approach in indirect pulp therapy and healing of deep carious lesions. J Clin Pediatr Dent. 38(3):185–192.

Pavlica Z, Juntes P, Pogacnik M. 2000. Defence reaction in dental pulp after pulp capping and partial pulpectomy in dogs. Acta Vet Hung. 48(1):23–34.

Petrou MA, Alhamoui FA, Welk A, Altarabulsi MB, Alkilzy M, Splieth CH. 2014. A randomized clinical trial on the use of medical Portland cement, MTA and calcium hydroxide in indirect pulp treatment. Clin Oral Investig. 18(5):1383–1389.

Reston EG, de Souza Costa CA. 2009. Scanning electron microscopy evaluation of the hard tissue barrier after pulp capping with calcium hydroxide, mineral trioxide aggregate (MTA) or ProRoot MTA. Aus Endod J. 35(2):78–84.

Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H. 1995. Reactionary dentinogenesis. Int J Dev Biol. 39(1):273–280.

Thesleff I. 2003. Epithelial-mesenchymal signalling regulating tooth morphogenesis. J Cell Sci. 116(Pt 9):1647–1648.

Tziafas D, Smith AJ, Lesot H. 2000. Designing new treatment strategies in vital pulp therapy. J Dent. 28(2):77–92.