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Dental pulp responses to pulp capping materials and bioactive molecules

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Abstracts

An ideal treatment outcome of pulpal exposure during restorative procedures is to regain the primary structure of tubular dentin as well as maintain the vitality and healthiness of the dental pulp. Presumably this gold standard result requires pulp capping materials with antibacterial, anti-inflammatory, and dentin-pulp tissue regenerative properties. Various capping materials have been used in dentistry but none have been able to predictably induce the regeneration of underlying tubular dentin. Recently, potentially applicable tissue engineering strategies using scaffolds containing growth factors were introduced with promising results for dentin regeneration in animals, and similar approaches have been shown to be successful in non-dental clinical problems such as bone regeneration. This article presents a review of dental pulp responses to commercially available pulp capping materials and discusses candidate bioactive molecules investigated in animals for dentin-pulp regeneration.

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Introduction

Dental pulp is a loose connective tissue that derives from cranial neural crest. The dental pulp resides in the innermost part of the tooth, called the pulp cavity. This unique tissue consists of a heterogeneous cell population including fibroblasts, odontoblasts, residential immunocompetent cells, undifferentiated mesenchymal cells, and cellular components from sensory nerves and blood vessels including pericytes, endothelial cells, and vascular smooth muscle cells.^{1,2}

When injuries invade close enough to affect the pulp cavity, the dental pulp forms reparative dentin. This response is generated by odontoblasts or odontoblast-like cells, depending on environmental factors, nature and severity of injuries.³ Newly differentiated odontoblasts may originate from various cell types in the pulp such as undifferentiated mesenchymal cells, endothelial cells and/or pericytes which migrate to the injured site from the central regions of pulp tissues.⁴⁻⁷ A subpopulation of human dental pulp cells, termed dental pulp stem cells (DPSCs), has been characterized *in vivo* to present the ability for self-renewal and odontoblastic differentiation.^{8,9} This cell population has stem cell-like properties, and is claimed to be a major source of odontoblast progenitor cells and play an important role in dentin/pulp repair.

Applications of pulp capping materials are the standard treatment for accidentally injured pulp with no other symptoms. Clinical guideline indication and objective of various pulp therapy techniques for both primary and young permanent teeth was published by American Academy of Pediatric Dentistry.¹⁰ An ideal treatment outcome of pulpal exposure during restorative procedures is to regain the primary structure of tubular dentin as well as maintain the vitality and healthiness of the dental pulp. To enhance this repair process, ideally, the capping agents should have a capacity to induce the dentinogenic potential of DPSCs. In this review, the responses of DPSCs and other pulp cells to

pulp capping materials, and bioactive molecules are discussed.

Dental pulp responses to pulp capping materials Calcium hydroxide

Calcium hydroxide has been used as a pulp capping agent for decades. The alkaline environment created by calcium hydroxide was suggested to be a major mechanism for the induction of dentin repair. Although this alkali pH damages primary odontoblasts and induces local tissue necrosis, it subsequently promotes the odontoblastic differentiation and formation of calcified dentin bridge.¹¹⁻¹³ From such relatively reliable mechanism to induce dentin regeneration, calcium hydroxide has been widely used in the clinic as a pulp capping agent with successful induction of reparative dentin. However, several disadvantages were reported. Firstly, calcium hydroxide produces inflammation within the underlying pulp, which can last for up to 3 months in human teeth.^{14,15} Secondly, the tissue responses to calcium hydroxide are not always predictable.¹⁵ Lastly, the reparative dentin formed beneath calcium hydroxide in monkey teeth and *ex vivo* human teeth model is irregular and exhibits tunnel defect due to multiple tissue inclusions in the dentin bridge.¹⁶⁻¹⁸ This tunnel defect may increase permeability of the dentin bridge and allow bacterial invasion through the dentin bridge. However, there is currently no direct evidence available to support the clinical relevance of tunnel defects.

Molecular mechanisms of calcium hydroxide:

Though, several publications indicated that calcium hydroxide had an antimicrobial effect that may prevent pulpal infection when used for pulp capping treatment.¹⁹⁻²¹ *In vitro* studies showed that calcium hydroxide reduced viability of odontoblast-like cells,^{22,23} which may result from strong alkalinity of material. Several hypotheses have been proposed as dentin/pulp repair mechanism by calcium hydroxide. First, calcium hydroxide may promote dentin/pulp repair by inducing

expression of bioactive molecules that significantly involve in dentin formation. Yasuda et al. showed that calcium hydroxide induced mRNA expression of bone morphogenic protein-2 (BMP-2) in rat dental pulp cells.²³ BMP-2 may further regulate calcified matrix formation and result in dentin bridging. Moreover, the mechanism of calcium hydroxide promoting dentin repair may involve in the regulation of Notch signaling. The study in rat molar showed that Notch 1 was observed in subodontoblastic zone beneath lesions treated with calcium hydroxide, where as Notch 2 expressed in coronal pulp tissues. Notch 1 and 3 were detected in perivascular cells.²⁴ The specific localization of Notch signal expression after treating with calcium hydroxide suggests that Notch signaling may control dental pulp cell fate in response to calcium hydroxide treatment, which may further turn on different differentiation pathway and promote dentin regeneration. Second, calcium hydroxide may promote dentin/pulp repair by inducing bioactive molecules release from dentin matrix. In this regard, calcium hydroxide induced releasing of adrenomedullin and transforming growth factor-beta (TGF- β) from human dentin matrix.^{25,26} Both TGF- β and adrenomedullin are pluripotent growth factors,²⁶ which possibly involve in the dentin repair mechanism. Moreover, Graham et al. reported that bioactive molecules releasing from calcium hydroxide treated dentin matrix enhanced expression of TGF- β mRNA in mouse odontoblast culture,²⁵ implying the role of matrix dissolution in dentin repair. Third, calcium ions release from calcium hydroxide may act as signaling molecules to promote dentin/pulp repair. Upon incubating calcium hydroxide in culture medium, the release of calcium ions was significantly increased.²³ Mizuno and Bansai showed that calcium ions, possible active molecules from calcium hydroxide, enhanced fibronectin expression, which was shown to induce differentiation of human dental pulp cells.²⁷ Moreover, calcium ions induced osteopontin (OPN) and BMP-2 mRNA expressions in

human dental pulp cells.²⁸ Together these data suggest that calcium hydroxide, calcium hydroxide solubilized bioactive molecules from dentin matrix, and calcium ions play significant roles in dentin repair by inducing expressions of gene regulating mineralized tissue formation.

Mineral trioxide aggregate (MTA)

Recently, MTA has been introduced as an alternative pulp capping material. MTA is a non-toxic material and hypothesized to stimulate reparative dentin formation by a normal defensive mechanism of an early pulpal wound healing.²⁹ Nair et al. reported that exposed human pulp treated with MTA exhibited minimal inflammation at early healing stage and reparative dentin with evidences of odontoblast-like cell lining was observed at 1-3 months.¹⁵ In contrast to calcium hydroxide, no necrotic tissue was found underneath MTA.^{15,30} Dentin bridge formation was significantly thicker in MTA treated exposed pulp than those treated with calcium hydroxide in canine tooth model (Fig. 1; "Reprinted from Journal of Endodontics, Vol. 34, Min K, Park H, Lee S, et al. Effect of mineral trioxide aggregate on dentin bridge formation and expression of dentin sialoprotein and heme oxygenase-1 in human dental pulp, pages 666-70, Copyright @ 2008, with permission from Elsevier.").³¹ Consistent with these observations, Briso et al. showed that MTA pulp capping in canine teeth has higher success rate to promote dentin bridge formation, lower level of tissue necrosis and infection when compared to calcium hydroxide.³² Chronic inflammation, macrophage and giant cells associated with capping materials were presented in great extent in calcium hydroxide treated pulp but rarely observed in MTA treated human pulp.³³ Conversely, Iwamoto et al. reported no significant difference in inflammatory cell response, dentin bridge formation and pulp vitality between MTA and calcium hydroxide treated human pulp.³⁴ In

human immature teeth, no significant difference was observed between MTA and calcium hydroxide when used for direct pulp capping, although, the authors suggested superior performance of MTA compared to calcium hydroxide.³⁵ Together, the accumulating

data suggest the promising potential use of MTA in direct pulp exposure, and possibly result in better clinical outcome. Although, the direct clinical evidence for the superiority of MTA pulp capping has not yet been well established.

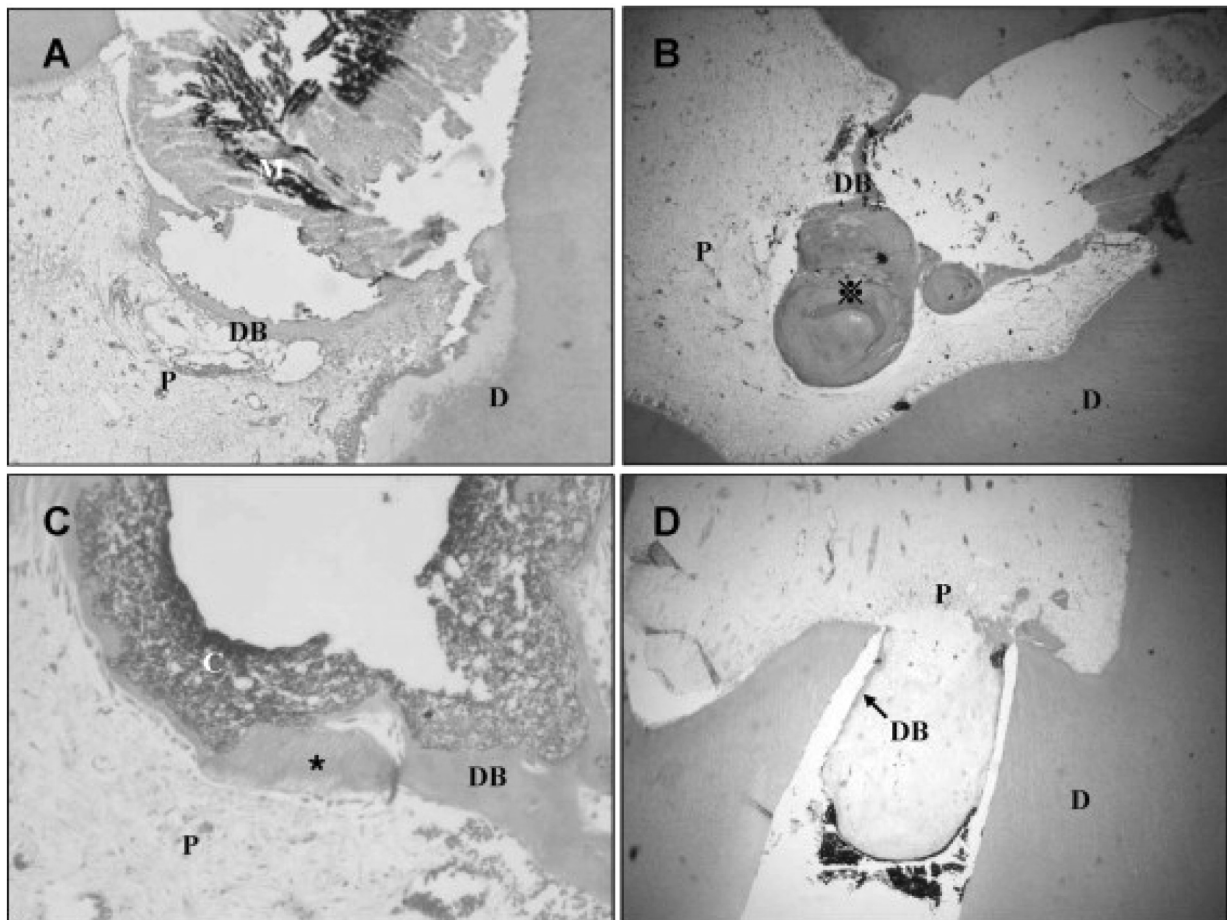


Figure 1. Human pulp capped with MTA (A and B) and CH (C and D). (A) Hematoxylin-eosin; original magnification, X40: hard tissue formation at the pulp exposure site. (B) Hematoxylin-eosin; original magnification, X20: an example of an excellent dentin bridge incorporating pulp stones (⊗). (C) Hematoxylin-eosin; original magnification, X40: dentin bridge incorporating dentin chips (*). (D) Hematoxylin-eosin; original magnification, X20: pulpal exposure led to a proliferative response similar to that of a “pulp polyp.” The hard tissue formation started at the borders of the polyp. (D) Dentin, (P) pulp; (DB) dentin bridge, (C) calcium hydroxide, (M) MTA. “Reprinted from Journal of Endodontics, Vol. 34, Min K, Park H, Lee S, et al. Effect of mineral trioxide aggregate on dentin bridge formation and expression of dentin sialoprotein and heme oxygenase-1 in human dental pulp, pages 666-70, Copyright @ 2008, with permission from Elsevier.”

Molecular mechanism of MTA: Upon treating exposed rat pulp with MTA, cell proliferation was noted in the pulp regenerative area and around blood vessels.³⁶ OPN, protein involved in mineralization process, was observed at the interface between very thin necrotic layer and the underlying pulp tissue.³⁶ In addition, dentin sialoprotein (DSP), and heme oxygenase-1, protein involved in cellular regulatory and protective mechanism, were expressed in greater extent in odontoblast-like cells and pulp fibroblasts beneath the dentin bridge of human dental pulp.³¹ Morphological evaluation using transmission electron microscope revealed the homogeneous organized crystalline structure at the pulpal front of MTA. Crystalline structure was noted at apical pole and cytoplasmic process of pulp cell. Further, dense collagen fibers with predentin-like pattern were noted between crystalline-like zone and columnar cell layer.³⁷ Reparative dentin was irregular and exhibited small tubular-like structure.¹⁶

MTA promoted mineralization in rat dental pulp cells *in vitro* and enhanced expression of BMP-2.²³ Moreover, MTA reduced chemokine ligand 5 (CCL5), interferon-gamma (IFN- γ), interleukin-1 alpha (IL-1 α) mRNA expressions in mouse dental pulp tissues *in vivo*, suggesting the regulatory role in inflammation.³⁸ Interestingly, MTA-based cement was also shown to have an antimicrobial activity^{39,40}, possibly due to the high alkali pH.⁴¹

Huang et al. reported that MTA induced ERKs activity, which decreased in dose and time-dependent manner in human osteoblast cells.⁴² The ERK/MAPK pathway was shown to be involved in human dental pulp cell proliferation and differentiation.^{43,44} Similar to calcium hydroxide, after dentin matrix was exposed to MTA, TGF- β 1 and adrenomedullin were released. TGF- β 1 provides anti-inflammatory signals⁴⁵ and promotes cell proliferation and differentiation⁴⁶ while adrenomedullin acts as a vasodilator and increases

survival of cells from oxidative stress and hypoxic injury.⁴⁷

Taken together, these data imply that the mechanism of MTA promoting dentin repair may occur through growth factor released from the dentin matrix, antibacterial effect, anti-inflammatory property, and induction of morphogen expression as well as signaling pathway associated with dental pulp cell differentiation.

Portland cement (PC)

PC was non-toxic and biocompatible.⁴⁸ It was able to induce reparative dentin formation in short-term evaluation. After pulp capping in human teeth with PC for 1 day, inflammation and disarrangement of odontoblasts were observed in the underlying pulp tissue. Dentin bridge formation was noted at material-pulp tissue interface around 14-21 days.⁴⁹

PC slightly decreased human dental pulp cell viability at early time point.⁵⁰ However, these cells were able to attach, spread and form cytoplasmic extension on the PC.⁴⁸ Cells also increased expressions of inducible nitric oxide synthases (iNOS), heme oxygenase-1 (HO-1) and osteonectin when exposed to PC.^{48,50} PC had comparable antimicrobial activity to MTA-based cement.^{39,40} Even though, there were only few publications studying PC for pulp capping treatment, the results were intriguing and promising for the use of PC in pulp therapy.

Dentin adhesive system

During the last decade, several types of dentin adhesive resin have been studied for pulp capping treatment. Human pulp tissues were found congested with dilated blood vessels and inflammation when exposed to dentin adhesive resin as pulp capping materials.⁵¹⁻⁵⁴ Recruitment of macrophages and giant cells was observed at the pulp exposure site and also associated with resinous materials.^{53,54,55} Importantly,

dentin bridge formation was significantly less than those treated with calcium hydroxide in both human and canine teeth (Fig. 2; "Reprinted from Dental Materials, Vol 21(9), Accorinte M, Loguercio A, Reis A, et al.

Adverse effects of human pulps after direct pulp capping with the different components from a total-etch, three-step adhesive system, page 599-607, Copyright @ 2005, with permission from Elsevier.").^{53,56-63}

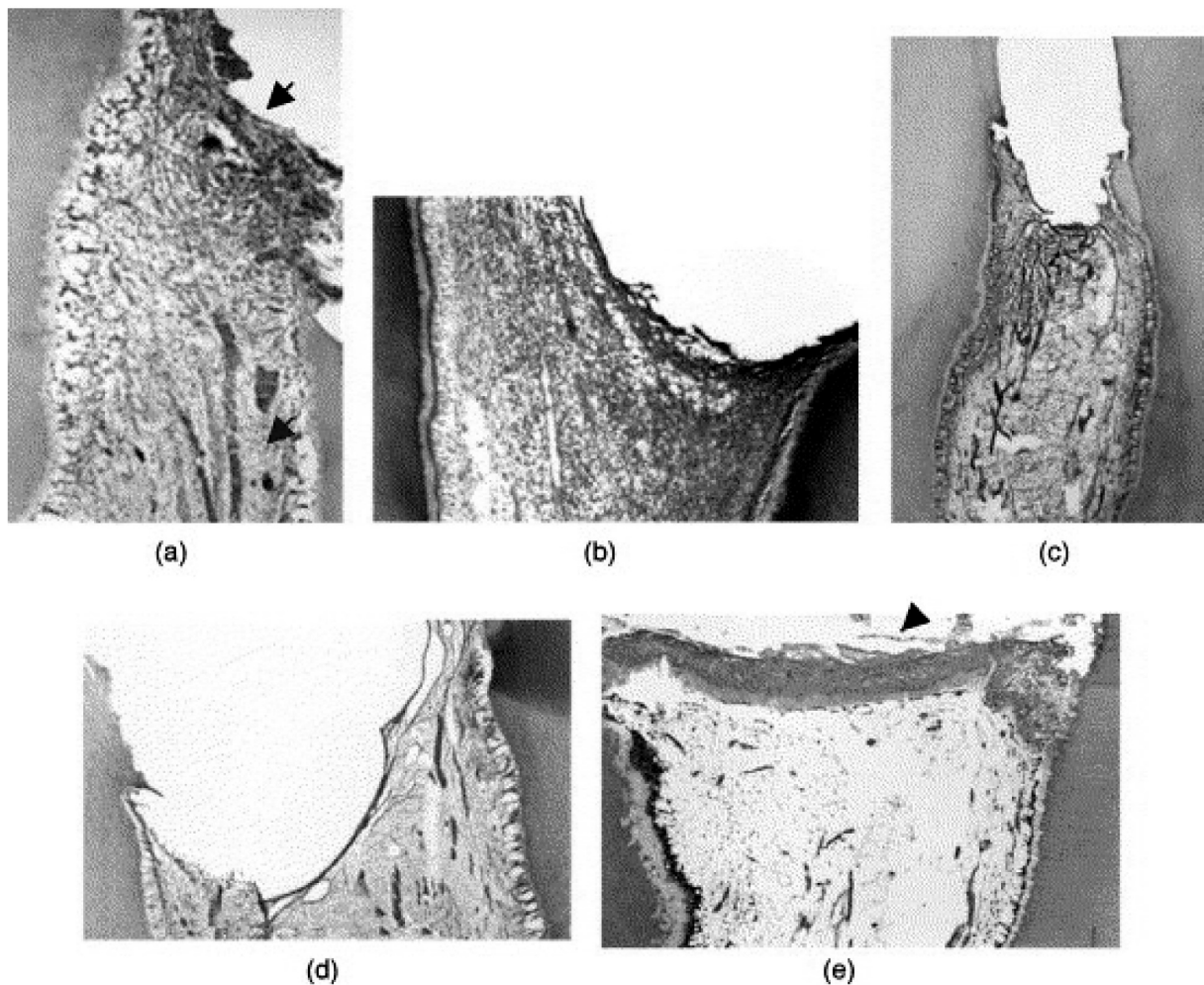


Figure 2. (a) Adhesive systems capping after 60 days (group 1). Observe the chronic inflammatory response adjacent the exposure site (black arrow). Below this site, the pulp morphology is normal (blue arrow) (HE, original magnification-25.6X). (b) Primer+composite resin after 60 days (group 2). There is a chronic inflammatory infiltrate subjacent to the exposure site (HE, original magnification-100X). (c) Adhesive+composite resin after 60 days (group 3). Normal pulp tissue around the exposure site. No dentin bridge formation (HE, original magnification-25.6X). (d) Composite resin after 60 days (group 4). Normal pulp tissue around the exposure site. No dentin bridge formation (HE, original magnification-25.6X). (e) Calcium hydroxide after 60 days. Observe the formation of a thick dentin bridge (black arrow) and the normal features of the tissue below it (HE, original magnification-25.6X). "Reprinted from Dental Materials, Vol 21(9), Accorinte M, Loguercio A, Reis A, et al. Adverse effects of human pulps after direct pulp capping with the different components from a total-etch, three-step adhesive system, page 599-607, Copyright @ 2005, with permission from Elsevier."

Adequate moisture control to obtain a relatively dry field of operation is a strict requirement for the use of adhesive resin and is a major disadvantage of using adhesive resin for pulp capping. Severe inflammation with chronic abscesses in coronal pulp was observed in human teeth treated with adhesive resin without rubber dam isolation, whereas, normal dentin bridge formation was observed in calcium hydroxide treated group.⁶⁴

Various negative effects of dentin adhesive on dentin regeneration were reported. Exposed human dental pulp treated with dentin adhesive system exhibited significant disorganization of odontoblast and predentin layers.⁵⁵ Moreover, Hebling et al. reported the death of adjacent odontoblast cells associated with dentin adhesive capping materials.⁵⁴ No evidence of odontoblast-like cell differentiation and dentin bridge formation were observed at 30 days after treatment.⁵⁴ Fibronectin and type III collagen, proteins related to reparative dentin, were significantly decreased in the odontoblast layer, predentin layer and pulp tissue when treated with dentin adhesive system.⁶⁵ Lanza et al. reported that dentin adhesive materials (Clearfill SE bond, Clearfil Protect Bond, Adper Prompt L-Pop, Xeno III, and Adper Single Bond) significantly reduced cell metabolic activity using transdental diffusion,⁶⁶ however, Demirci et al. reported no cytotoxicity of adhesive system using the same testing model.⁶⁷ This may be due to the cell types and source of dentin slides used in the experiment. Partially polymerized adhesive resin resulted in apoptosis of mouse odontoblast-like cell and cell cycle arrest.⁶⁸ The apoptotic induction occurred via cysteine protease Caspase-3 mechanism.⁶⁸ Lanza et al. showed that HEMA was a major component eluted from dentin adhesive materials, which may be responsible for the toxic effect of test materials.⁶⁶ Potentially, cytotoxicity of substances released from dentin adhesive materials may limit their abilities to promote dentin repair.⁶⁹

However, the possible mechanisms for dentin repair underneath the dentin adhesive pulp capping were

also discussed. Kitasako et al. reported the superior barrier of adhesive resin system compared to calcium hydroxide, regarding the prevention of bacterial invasion to dental pulp following dentin bridge formation beneath capping materials in monkey teeth.¹⁸ Although, adhesive resin system failed to induce dentin bridging, the local blood vessels were increased.⁷⁰ This may be due to the mechanism that adhesive resin system (Single Bond and HEMA) induced VEGF protein expression as observed in mouse odontoblast-like cells and macrophages.⁷⁰

Taken together, dentin adhesive resins may be beneficial in promoting angiogenesis and preventing bacterial invasion. However the promotion of dentin/pulp healing is impaired when the adhesive resin is used as pulp capping material. Therefore, the adhesive resin may not be material of choice for pulp capping treatment.

Dental pulp responses to bioactive molecules

The understanding of molecular mechanisms for dentin formation leads to new strategies to treat injured pulp. Various applications have been introduced in order to replace the conventional pulp capping materials. Examples of these approaches include the use of combination of dental pulp progenitor/stem cells and a variety of scaffolds, the use of scaffolds containing growth factors, the use of scaffolds containing plasmid DNA, and the combination of progenitor/stem cells with scaffolds and growth factors. These strategies may enhance the healing process after dental pulp injuries by mimicking the natural dentin/pulp repair process or resembling tooth developmental process.

The common approach for dentin/pulp repair experiment is the use of scaffolds releasing growth factors. Various bioactive molecules have been anticipated to have ability to promote dentin/pulp healing. Thorough understanding for the mechanisms of dental pulp response to bioactive molecules is

required to design a new clinical application using these bioactive molecules. The current knowledge for the use of bioactive molecules in treatment of injured pulp is reviewed and discussed below.

Transforming growth factor-betas (TGF- β s) are cytokines in TGF superfamily. TGF- β s are secreted during early tooth development by epithelial cells to induce mesenchymal differentiation.⁷¹ In human and rabbit mature teeth, TGF- β s are expressed and secreted by odontoblasts.⁷² Zhao et al. reported that TGF- β s are released during dentin repair and act as a stimulating factor for dentin/pulp regeneration.⁷³ TGF- β 1 has been studied *in vitro* and *in vivo* regarding the capacity of inducing odontoblast differentiation and dentin bridge formation.⁷⁴⁻⁷⁷ TGF- β 1 upregulated type I collagen expression in odontoblasts. This upregulation occurred via c-jun, a nuclear proto-oncogene, and further regulated an activator protein-1 (AP-1).⁷⁸ AP-1 is also shown as a crucial regulator of cellular proliferation, migration, and differentiation.⁷⁹ It has been reported that AP-1 regulated the production of dentin matrix.⁷⁹

TGF- β 1 also participates in the regulation of Notch signaling.^{80,81} Delta/Notch signaling plays a fundamental role in tissue fate determination during embryonic tooth development,⁸² directs pulp cell differentiation into odontoblastic lineage⁸³ and regulates proliferation and migration of progenitor/stem cells in pulp of both developing and injured teeth.⁸³ Further, TGF- β 1 controls inflammatory reactions in the human dental pulp in response to carious bacteria by reducing the expressions of toll-like receptor 2 and 4 and consequent production of pro-inflammatory cytokines through these receptors.⁴⁵

Investigations of potential use of TGF- β 1 for dentin repair were reported. Collagen membrane containing TGF- β 1 significantly promoted dentin/pulp healing in injured rat teeth.⁸⁴ In addition, Zhang et al. reported significant dentin formation in goat incisor treated with

combination of poly(lactic-co-glycolic acid) containing TGF- β 1 and calcium phosphate cement.⁸⁵ Taken together, these results suggest the potential use of TGF- β 1 to enhance dentin/pulp repair.

Bone morphogenic proteins (BMPs) are also members in TGF superfamily, which play critical roles in embryonic tooth development and odontoblast differentiation.⁸⁶ BMP-2, BMP-4, and BMP-6 were identified in human primary culture of dental pulp cells.⁸⁷ Several studies have reported the potential use of BMPs in dentin/pulp repair. BMP-2 stimulated odontoblastic differentiation in murine and canine pulp cells.⁸⁸⁻⁹⁰ Moreover, transfection of BMP-2 in Stro-1 positive rat dental pulp stem cells resulted in induction of odontoblast differentiation *in vitro*.^{91,92} Recombinant human BMP-2, BMP-4, and BMP-7, were employed as bioactive molecules for dental tissue engineering *in vitro* and *in vivo*.⁹³⁻⁹⁹ Several studies reported significant osteodentin formation in injured pulp when treated with BMP-7.¹⁰⁰⁻¹⁰⁴ In contrast, da Silva et al. reported no beneficial effect of BMP-7 as pulp capping agent in dogs.¹⁰⁵⁻¹⁰⁶ Nakashima reported similar effects from BMP-7, BMP-2, and BMP-4 on the dentin/pulp repair in canine teeth with amputated pulp.¹⁰⁷ Due to these conflicting evidences, the beneficial application of BMPs in dentin repair and vital pulp treatment remains questionable. Nonetheless, the use of high dose BMP-2 in pulp capping may result in excessive osteodentin formation which may lead to complete pulp canal obliteration.

Fibroblast growth factor-2 (FGF-2) is detected in dentin matrix and released upon matrix degradation. FGF-2 involves in enamel and dentin formations.¹⁰⁸⁻¹¹⁰ It has been shown that FGF-2 released from biodegradable gelatin hydrogel induces neo-vessel formation and regenerates bone and periodontal tissues.¹¹¹⁻¹¹³ This protein was incorporated into the gelatin hydrogel and placed over the exposed pulp to stimulate the formation of reparative dentin in rat molar.^{114,115}

However, the formation of dentin bridge was observed only when 0.5 mg/ml of FGF-2 was used.¹¹⁵ Higher doses of FGF-2 releasing from gelatin hydrogel (1 and 5 mg/ml) resulted in scattered calcified nodules in pulp tissue beneath the pulp exposure area.¹¹⁵ This result emphasizes the importance of optimized biomolecule doses in promotion of dentin repair.

Insulin-like growth factor-I (IGF-I) has high sequence similarity to insulin.¹¹⁶ IGF-I induces mineralization of canine dental pulp cells *in vitro*.¹¹⁷ Addition of IGF-I with heparin increases BMP-4 mRNA expression in murine dental papillae¹¹⁸ and promotes odontoblast-like cell differentiation.⁷⁴ Lovschall et al. reported that recombinant IGF-I enhanced reparative dentin formation when used as pulp capping in rat molars.¹¹⁶ The complete dentin bridge was more frequently observed in the teeth treated with recombinant human IGF-I compared to control.¹¹⁶

Bone Sialoprotein (BSP) is a phosphorylated protein secreted from soluble dentin matrix. Recently, this protein was shown to play an important role in pulp regeneration.¹¹⁹ BSP has a hydroxyapatite-binding site, which enhances initial nucleation of hydroxyapatite crystals necessary for mineralization process. BSP is only expressed in odontoblast cells but not in pulp cells.¹²⁰ MacDougall et al. reported that implantation of BSP into the exposed pulp of rat molars activated the cellular changes of pulp cells and induced homogeneous and well-mineralized reparative dentin.¹²⁰

Matrix extracellular phosphoglycoprotein (MEPE), another type of phosphorylated proteins, is identified as a non-collagenous protein in human bone and dental tissues.¹²¹ The role of MEPE in dentin mineralization has been proposed.¹²¹ Dentonin, a bioactive fragment of MEPE, was shown to have a capability to enhance human DPSCs proliferation. This ability is beneficial for dental pulp regeneration.¹²¹ However, the reparative dentin generated after treating the injured rat teeth with dentonin was occurred

incompletely,¹²² suggesting that dentonin is useful for the initial repair process in term of pulp cell proliferation before terminal mineralization.¹²²

Amelogenin, a main component of enamel matrix proteins, is produced by several types of cells such as ameloblasts, odontoblasts, and brain cells.¹²³ The implantation of splicing products of amelogenin including A+4 and A-4 into the pulp resulted in recruitment and enhancement of pulpal osteo/odontoblast progenitor cell proliferation.¹²⁴ Amelogenin peptide A+4 induced significant osteodentin formation, which almost occluded the whole pulp cavity^{120,125}, in mouse incisor, owing to the variable results from different experiments, there still be a need to further study the effect of this bioactive molecule for future use in pulp repair.

Enamel matrix proteins are products derived from ameloblasts and their secreted products during crown formation.¹²⁶ These enamel matrix proteins include amelogenin, ameloblastin, amelotin, tuftlein, and enamelin and most likely other morphogens/growth factors/proteins associated with crown formation.¹²⁶ The form used clinically for regenerating periodontal tissues is marketed under the trade name Emdogain® (EMD) which is composed mainly of amelogenin and other ameloblast secreted proteins. Nakamura et al. showed that EMD significantly increased reparative dentin formation over the exposed pulp in swine teeth compared to those treated with calcium hydroxide.^{127,128} However, the discrepancies of EMD treated injured human pulp are noted. Sabbarini et al. showed the promising application of EMD in primary human teeth.¹²⁹ On the contrary, Olsson et al. reported no benefit of EMD in dentin/pulp repair of human permanent premolars. EMD-treated human pulp resulted in greater inflammatory response compared to calcium hydroxide.¹³⁰ In addition, Garrocho-Rangel et al. reported the failure in randomized controlled trial of EMD in direct pulp capping of primary molars.¹³¹ Due

to the discrepancy of clinical outcomes, additional well-controlled trials are needed to verify the clinical benefit of these proteins.

EphB/ephrin-B molecules, tyrosine kinase receptors and their ligands in the Eph family, play essential roles in neural crest cell migration during development and maintaining stem cell niche.¹³² EphB/ephrin-B molecules participated in restricting human DPSC attachment by the mitogen-activated protein kinases (MAPK) pathway.¹³³ The study validated that EphB/ephrin-B signaling was an essential mechanism to maintain and recruit DPSCs within their stem cell niche under steady state conditions in dental pulp.¹³³ The migration of DPSCs after injury to the damaged site may be induced by the interaction of Eph/ephrin and explains the importance of such molecules in aspect of dentin regeneration.¹³³ These results suggest that the application of the Eph/ephrin molecules would be advantageous for dental pulp development and regeneration.

The role of inflammation in tissue regeneration has been proposed; immune cells and inflammatory cytokines may function to enhance dental pulp repair after applying such bioactive molecules. Similar to the response of dental pulp to carious lesion, the inflammatory process is generated after implantation with bioactive molecules into the pulp cavity.¹³⁴ An initial inflammation process is considered as one of important steps in wound healing.¹³⁵ Progenitor/stem cells in dental pulp are quiescent in the absence of pulpal injury.^{8,9} During the damage, the inflammation is produced and the amount of pulp cells is simultaneously increased. Inflammation induced by implanted bioactive molecules may be necessary for the proliferation and differentiation of DPSCs to promote dentin and pulpal repair. In short-term *in vivo* study, inflammation alone can initiate wound healing, but the healing will not be completed unless the bioactive molecule is added into the exposed pulp.¹³⁶

The bioactive agents may directly induce DPSC differentiation into odontoblast-like cells that form the dentin bridge. Alternatively, these molecules may induce the de-differentiation or trans-differentiation of differentiated cells in dental pulp to participate in wound healing process. Another possibility is that the bioactive molecules function indirectly to cause the inflammation, which induces the migration of inflammatory immune cells and secretion of proinflammatory cytokines. These inflammatory cells and cytokines may be involved in the reparative process, de-differentiation or trans-differentiation of dental pulp cells.

Conclusion

The inflammation induced in the dental pulp in response to injury plays an important role in tissue regeneration. The migration of immune cells into the injured site leads to the collection of secreted inflammatory cytokines, which may enhance dentin/pulp repair by activating the differentiation of odontoblast and/or odontoblast-like cells. These cells are further responsible for the reparative dentin formation. Numbers of conventional and experimental pulp capping materials are recently introduced but none has a consistent capacity to regenerate a primary structure of tubular dentin-pulp complex. The mechanism of such materials is to mimic process of normal dentin/pulp regeneration. Although, calcium hydroxide seems to be the standard material for direct pulp capping treatment, the mechanism of action is still unclear. Other new materials provide such a promising result to promote dentin/pulp regeneration but need a rigorous clinical verification for cost and benefit. Understanding the mechanisms of dental pulp responses to pulp capping materials and bioactive molecules will assist the dental personnel to select appropriate materials and treatment approaches for pulp injury.

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การตอบสนองของเนื้อเยื่อในฟันต่อวัสดุ ปิดเนื้อเยื่อในฟันและไบโอแอคทีฟโมเลกุล

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บทคัดย่อ

ผลในอุดมคติของการรักษาภาวะการผุของเนื้อเยื่อในฟัน คือ การเกิดใหม่ของเนื้อฟันที่มีรูปร่างแบบปฐมภูมิ และการคงสภาพความมีชีวิตและความสมบูรณ์ของเนื้อเยื่อในฟัน ดังนั้นวัสดุปิดเนื้อเยื่อในฟันที่ใช้ในการรักษาควรมีคุณสมบัติ คือ มีฤทธิ์ด้านแบคทีเรีย มีฤทธิ์ด้านการอักเสบ และสามารถกระตุ้นการงอกใหม่ของเนื้อฟันและเนื้อเยื่อในฟัน วัสดุปิดเนื้อเยื่อในฟันที่ใช้ในการรักษาอยู่ในปัจจุบันนั้นมีหลายชนิดแต่ยังไม่มีวัสดุชนิดใดที่ให้ผลกระตุ้นการงอกใหม่ของเนื้อฟันที่ดีและสมบูรณ์ ในปัจจุบันแนวความคิดของวิทยาการทางด้านวิศวกรรมเนื้อเยื่อเข้ามามีบทบาทและให้ผลการงอกใหม่ของเนื้อฟันในสัตว์ทดลอง เป็นที่น่าพอใจ แนวความคิดเดียวกันนี้ยังถูกนำไปใช้ในการรักษาทางคลินิกด้านอื่นๆ แล้ว เช่น การงอกใหม่ของกระดูก บทความปริทัศน์นี้ได้ทบทวนวรรณกรรมที่เกี่ยวข้องกับการตอบสนองของเนื้อเยื่อในฟันต่อวัสดุปิดเนื้อเยื่อในฟันที่มีในท้องตลาด รวมทั้งยังทบทวนและวิจารณ์วรรณกรรมที่เกี่ยวข้องกับการตอบสนองของเนื้อเยื่อในฟันกับไบโอแอคทีฟโมเลกุลที่ใช้ในการทดลองให้เกิดการงอกใหม่ของเนื้อฟันในสัตว์ทดลอง

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คำสำคัญ: การงอกใหม่; เนื้อเยื่อในฟัน; วัสดุปิดทับเนื้อเยื่อในฟัน