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Clinical and molecular perspectives of reparative dentin formation: Lessons learned from pulp-capping materials and the emerging roles of calcium

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Synopsis

The long-term use of calcium hydroxide and the recent increase in the use of hydraulic calcium-silicate cements as direct pulp-capping materials provide important clues in terms of how reparative dentin may be induced to form a “biological seal” to protect the underlying pulp tissues. In this review article, we will discuss clinical and molecular perspectives of reparative dentin formation based on evidence learned from the use of these pulp-capping materials. We will also discuss the emerging role of calcium as an odontoinductive component in these pulp-capping materials.

Keywords

Reparative dentin; calcium hydroxide; hydraulic calcium-silicate cements; calcium; odontoinductive; odontoconductive; ORAI1

I. INTRODUCTION

Dental caries is the most prevalent infectious oral diseases experienced by more than 90% of adults in the United States [1,2]. A quarter of U.S. populations do not have dental insurance [3], and more than 60% of underserved areas are still in need of dentists [4]. Considering these

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potentially unidentified individuals, it is expected that almost all individuals may have experienced dental caries at least once in their lifetime.

Due to its high prevalence, removing dental caries is one of the most common procedures performed in routine dental practices. During caries removal, deep caries penetrating through the enamel and dentin frequently leads to either indirect or direct pulp-capping procedures in order to induce tertiary (reactionary or reparative, respectively) dentin formation [5]. Several pulp capping materials, including calcium hydroxide [$Ca(OH)_2$ or CH] and hydraulic calcium silicate cements (HCSCs) such as mineral trioxide aggregate (MTA), are used for this purpose. For indirect pulp capping, these materials are placed on the "unexposed" pulp to enhance reactionary dentin formation from the existing odontoblasts at the dentino-pulpal complex (DPC). In contrast, direct pulp capping refers to placing the pulp-capping materials on the "exposed" pulp – where odontoblast layers are breached – to enhance reparative dentin formation mediated by odontoblast-like cells differentiated from dental pulp stem cells (DPSCs) at the materio-pulpal complex (MPC).

Unlike indirect pulp capping which usually has predictable clinical outcomes, direct pulp capping has outcomes that are often variable depending on the operator technique, the material properties, and the host pulpal responses. In direct pulp capping the ultimate goal is to preserve the underlying pulp and maintain pulp vitality by regenerating reparative dentin at the MPC, which functions as a "biological seal" to protect the underlying pulp tissues, to increase the life expectancy of the tooth, and to improve the overall oral health. A successful pulp capping procedure can avoid more invasive and expensive dental treatment such as root canal therapy. Therefore, it is important to optimize direct pulp-capping techniques, improve biocompatibility of the materials, and enhance biological responses of the pulp tissues in order to maximize regeneration of reparative dentin.

Here, we will discuss the current status of different types of direct pulp-capping materials with specific focuses on CH and HCSCs due to their extensive clinical utilization and substantial amounts of available studies. We will then attempt to delineate molecular mechanisms by which reparative dentin forms based on the common properties of these pulp-capping materials, as well as known bone-grafting materials. Finally, we will suggest possible roles of calcium ions (Ca^{2+}) in the formation of mineralized tissues including reparative dentin and bone.

II. CLINCAL PERSPECTIVES: PULP-CAPPING MATERIALS IN PULPAL THERAPY

1) Calcium hydroxide (CH)

CH was first introduced to the dental profession in 1920s, and has long been recognized as the gold standard pulp-capping material [6–8]. Early clinical studies including over 2,300 cases of direct pulp capping showed 80–90% success rate [6]. Recent review of literature revealed that the overall success rates, as mostly determined by an asymptomatic tooth without radiographic lesions, fall between 68.5%–80.1% within a two-year follow-up period but

drops to 58.7–76.3% after ten years [9–12]. As such, the use of CH as a direct pulp-capping material is frequently performed (Figure 1).

One of the most clinically relevant functions of CH is the facilitation of reparative dentin formation. Histological studies of pulp-capped teeth revealed a thin layer of coagulation necrosis in the pulp due to the irriation from high pH [13,14]. Mild inflammation, cellular debris and calcium-protein granules were observed below the superficial necrotic layer onto which the eventual dentin bridge formed. While detailed mechanisms remain elusive, the superficial necrosis was believed to be vital for initiation of dentin-bridge formation [7,14–16]. It was also suggested that CH facilitates reparative dentin formation through the creation of an alkaline environment and an increased availability of calcium ions [7,14,17,18].

However, there are several disadvantages. CH dressings dissolve clinically within 1–2 years [19,20]. The susceptibility to dissolution by acid and tissue fluid presented a problem during subsequent acid-etch resin restoration [21]. CH lacks inherent adhesion to dentin, but in newer formulations, may bind to dentin via urethane dimethacrylate, which also partially adds resistance to acid dissolution [22]. The most prominent issue with CH is that 89% of dentin bridges formed contained tunnel defects [20,23]. Considering that the dissolution of the dressing leaving a void beneath the restoration, these “tunnel defects” present a high risk for microleakage, leading to bacterial reinfection, persisting pulpal inflammation and necrosis. These disadvantages may potentially be linked to notable variation in outcomes and a decrease in clinical success rate over follow-up time [9,10]. Consistent with such notions, numerous studies reported that the success rate of pulp capping with CH varies significantly, ranging from 13 to 95% [24–26].

In summary, CH remains favored by practitioners for pulpal therapy due to its excellent antimicrobial activity and capacity to form reparative dentin. However, tunnel defects and progressive dissolution compromise the integrity of reparative dentin, and these shortfalls call into questions about the use of CH as a long-term pulp therapeutic agent.

2) Hydraulic calcium-silicate cements (HCSCs)

Mineral trioxide aggregate (MTA), a prototype HCSC, was first introduced in early 1990s by Torabinejad and his coworkers [27,28]. It was initially recommended as a root-end filling material but subsequently used in various clinical applications such as pulp capping, pulpotomy, apexogenesis, apical barrier formation, and repair of root perforations [29]. The main composition of MTA is tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide and calcium sulfate dehydrate. MTA is hygroscopic; its setting requires and is not adversely affected by the presence of water [30], which is a central and unique advantage that contrasts with existing dental materials.

Increasing lines of evidence support a notion that MTA confers superior clinical outcomes. Although some reports showed insignificant clinical outcomes with MTA when compared to CH [31], other clinical studies showed more favorable success rates with MTA than CH [12,32–34]. In some of these studies, the success rates of direct pulp capping using MTA have been reported to exceed more than 90% [35–37]. In addition, success rates seem to be maintained over long follow-up periods with MTA [33,37,38], which was different from CH’s

showing time-dependent decline in success rates [39,40]. Therefore, more pulp capping procedures are being performed using HCSCs (Figure 2).

The superior clinical outcomes of MTA are attributed to its physical properties: flexural strength, compressive strength, and push-out strength as well as antimicrobial effect, radiopacity, dimensional stability, and tolerance to moisture [41]. In addition, MTA has shown to have better sealing properties [30,42], biocompatibility [43,44] and osteogenic/odontogenic differentiation capacity [45,46]. As the type of pulp capping material was shown to be the single most important factor influencing the pulpal survival rate among others [34], these properties contribute to its favorable clinical outcomes.

Similar to other biomaterials, however, MTA displays some limitations: discoloration, long setting time, difficulty in manipulation, and high cost [29]. A long setting-time (e.g., 2 h 45min) is one of the major drawbacks of MTA, which may delay the completion of the treatment in a single appointment in the clinic. Indeed, the prototype MTA requires temporization of the tooth to achieve the proper hardness before final restoration, which creates another potential problem of microleakage or reinfection while the temporary is in place [47]. Discoloration is another major drawback especially when it is used on the anterior teeth, requiring additional treatment such as bleaching [48]. A tooth-colored white MTA was developed to overcome this limitation; however, unexpected tooth discoloration has also been reported by this white MTA [49,50]. Furthermore, handling of MTA is another challenge for clinicians, which potentially discourages its routine use in clinical applications.

Since the expiration of the MTA patent in 2013, a number of different HCSCs were introduced. Similar to MTA, these materials share common physicochemical and biocompatible properties but are claimed to have improved characteristics [51]. For example, Biodentine (Septodont, Saint-Maur-des-Fosses, France) and Endocem (Maruchi, Wonju, Korea) are fast-setting HCSCs with the setting time of 12 min and 4.5 min, respectively. Biodentine has a shortened setting time with the addition of a setting accelerator ($CaCl_2$) and the removal of the liquid component [52], whereas Endocem has small particle sizes that increase surface contact with water, resulting in fast setting and ease of manipulation [53]. Another improvement is the replacement of the opacifier with bismuth oxide to zirconium oxide [54,55]. Because bismuth oxide causes MTA discoloration after light irradiation in an oxygen-free environment [56], the zirconium oxide-containing HCSCs, such as Endocem, RetroMTA (BioMTA, Seoul, Korea), Biodentine and Endosequence (Brasseler USA, Savannah, Ga.) are suggested as better choices for esthetic reasons. Additional improvement includes better handling properties; Endosequence (Brasseler USA, Savannah, Ga.) is a premixed, ready-to-use syringeable paste that is condensable, which make them user-friendly with an ease of handling and application. It also has a demonstrated strength and biologic effect similar to MTA [57].

Despite the rapid increase in newly developed HCSCs with multiple modifications in their compositions, there are insufficient studies to experimentally support whether they are superior or even comparable alternatives to MTA. Although HCSCs are evidently promising for pulp-capping materials, more clinical, pre-clinical, and molecular studies are needed to define the clinical efficacy for regenerating the pulp for reparative dentin formation.

3) Emdogain

Emdogain is an enamel matrix derivative (EMD) product extracted from the Hertwig's root sheath during porcine tooth development. Originally developed to promote regeneration of periodontium [58], Emdogain has been proposed in several recent studies as a potential pulp capping material [59,60]. Amelogenins, the major components of EMD, can mimic epithelial-mesenchymal interactions by triggering the release of various growth factors and cytokines such as BMP and TGF-β, which in turn promote differentiation of mesenchymal stem cells in the pulp into odontoblast-like cells [61,62]. In addition, EMD may also enhance dentin mineralization since osteoblast-like cells reportedly upregulate mineralization-inducing genes upon treatment with Emdogain [63]. In the context of pulp capping, EMD may facilitate formation of thicker reparative dentin barrier compared to CH, as was indeed proven in several animal studies [64,65]. However, with the limited number of human studies available, the efficacy of Emdogain is debatable in comparison to CH [66–68]. A blinded randomized clinical study on experimentally exposed human pulp showed ineffective hard tissue barrier formation by Emdogain, although post-operative symptoms were less frequent compared to CH [67]. More pulpal inflammation was also associated with Emdogain treatment. Two other studies on primary teeth [68], and partial pulpotomy in permanent teeth [66] failed to establish superiority of reparative dentin formation by Emdogain. Of note, these studies consisted of a limited sample size (<45 subjects) and short follow-up period (3–12 months). Hence, the clinical efficacy and safety of Emdogain as a pulp capping material is inconclusive at best and requires further investigation.

4) Growth factors and matrix-derived proteins

Since the turn of the century, several bioactive materials for pulp capping alternatives have been proposed and have been reviewed elsewhere [69]. Growth factors (BMPs, IGF-1, EGF, FGF, TGF, PDGF-BB) and matrix-derived proteins (BSP) may stimulate reparative dentin formation that is comparable or even superior to CH, but appropriate delivery carrier and dosage need to be considered for controlled reparative dentin regeneration. The limited half-life also necessitates the need for multiple applications, which may incur high cost. As such, studies for efficacies of these bioactive molecules in pulp capping remain to be determined in the *in vitro* and animal study stages.

III. MOLECULAR PERSPECTIVES: COMMON PROPERTIES OF PULP-CAPPING MATERIALS

Pulpal wound healing including reparative dentin formation is a multi-factorial, complex process that is orchestrated by discrete but overlapping steps of migration, proliferation, and mineralization of pulp cells [70]. Unlike reactionary dentin, which is formed by existing odontoblasts, reparative dentin is formed by odontoblast-like cells presumably differentiated from DPSCs when the pulp becomes exposed and the existing odontoblastic layers are breached. At the cellular level, these DPSCs are expected to migrate to and proliferate on the MPC, ultimately undergoing odontogenic differentiation to form reparative dentin.

Although both CH and HCSCs are thought to be odontoconductive by functioning as scaffolds for proper execution of these processes by DPSCs, clinical and molecular studies

suggests evidently that they are also odontoinductive such that these materials can stimulate DPSCs to undergo odontogenic differentiation and mineralization. Therefore, identifying the factors released from pulp-capping agents that regulate DPSCs to form reparative dentin and defining the fundamental molecular mechanisms by which DPSCs respond to these pulp-capping agents are critically important for regenerating reparative dentin and creating a "biological seal."

As was discussed previously, there exist a number of pulp-capping materials that are clinically proven to induce reparative dentin formation in human. Based on this historical evidence, some of the properties are suggested to play key roles in regenerating reparative dentin. These properties include:

1. high pH,
2. anti-bacterial activity, and
3. calcium ion release.

1) High pH

Alkaline environment (high pH) is known to promote osteogenic differentiation and bone formation [71,72]. Conversely, acidic environment is demonstrated to inhibit bone formation [73]. Alkaline phosphatase, an important enzyme in initiating calcification, allows for the increase in local concentration of inorganic phosphate at an alkaline pH [74]. Because CH is a water-soluble compound that dissolves upon contact with tissue fluid and releases hydroxyl anions (OH^-) to increase pH to 12–13, it was suggested that such alkaline pH attributes to reparative dentin formation by CH [75–77]. Similarly, HCSCs were also demonstrated to create a high-pH environment as the end product of the chemical reaction is CH [78–80].

Although an alkaline environment is essential for creating an osteoinductive environment, mineralization is highly sensitive to pH change; alkaline phosphatase activity peaks at pH 7.37 and significantly diminished above this physiologic level [73]. Furthermore, pH above 8.0 was shown to inhibit mineralization process both *in vitro* and *in vivo* [81,82]. Because measuring the precise pH at or around the interfaces between pulp-capping materials and pulp tissue is technically challenging, further investigation is needed to clarify the actual effects of high pH on reparative dentin formation *in vivo*.

2) Anti-microbial activity

One of the clinical advantages of CH as a pulp medicament largely derives from its antimicrobial activity due to OH^- release (pH up to 12.5). The highly reactive hydroxyl radicals along with the raised pH can cause damage to the cytoplasmic membrane and DNA of bacterial microorganisms [83,84]. In addition, the high pH may also provide anti-inflammatory effect, via denaturation of proinflammatory cytokines [85] and stimulation of regulatory IL-10 [86]. Sustained attenuation of bacterial irritation and inflammatory response in turn may provide a conductive environment for reparative dentin formation. However, such effects are indirect; the removal of bacterial organisms does not actively contribute to reparative dentin formation. Therefore, it remains to be elucidated whether anti-microbial

activity of the dental pulp-capping materials is a molecular determinant in reparative dentin formation.

3) Calcium ions (Ca^{2+})

Although Ca^{2+} is one of the major constituents released by both CH and HCSCs [78,87], the role of Ca^{2+} in reparative dentin formation is largely underexplored. An earlier study demonstrated that, when CH with radiolabeled Ca^{2+} was used as a direct pulp-capping material on pulp-exposed teeth in dogs, radiocalcium was not found in the reparative dentin area, suggesting that Ca^{2+} necessary for formation of reparative dentin matrix itself is not derived from CH but from the pulp [88]. However, emerging evidence supports a notion that Ca^{2+} plays indispensable roles not only in formation of mineralized matrixes but also in transduction of the intracellular signaling pathway that are involved in maintaining and regulating normal biological processes [89,90]. Indeed, recent studies suggested that Ca^{2+} released from biomaterials is one of the key factors mediating mineralization process [7,87] and that Ca^{2+} released from pulp-capping materials may be an active component in reparative dentin formation.

Unlike its role in dentin formation, Ca^{2+} in bone formation is well documented. High amounts of extracellular Ca^{2+} induced expression of alkaline phosphatase (ALP), osteocalcin (OC) and osteopontin (OP) in pre-osteoblast cells [91,92]. Furthermore, extracellular Ca^{2+} also induced osteoblast differentiation and mineralization both *in vitro* and *in vivo* [93–95], indicating that Ca^{2+} alone has a *de novo* characteristic to induce osteogenic differentiation.

To enhance bone formation *in vivo*, biomaterials such as hydroxyapatite (HA), tricalcium phosphates (TCP), or biphasic calcium phosphates (BCP), a mixture of HA and TCP, are frequently used as scaffolds for bone grafting [96]. Although these materials are known to be osteoconductive in nature by serving as scaffold for bone growth, they are also suggested to be osteoinductive; these materials by themselves actively stimulate differentiation of pre-osteoblastic cells to osteoblasts and formation of new bone [97,98]. Interestingly, all of these materials are highly enriched with Ca^{2+} [99], and their capacity to induce bone formation seems to differ depending on the amount of Ca^{2+}. Indeed, Ca/P ratio of TCP is 1.67 when that of HA is 1.5, and TCP is capable of releasing more Ca^{2+} than HA [100]. Furthermore, Barradas *et al.* demonstrated that TCP induces more bone formation than HA both *in vitro* and *in vivo*, and such difference is primarily attributed to high solubility of TCP to release Ca^{2+} [101].

Similar to osteoblasts, extracellular Ca^{2+} also induce odontogenic differentiation of dental mesenchymal cells. Ca^{2+} treatment alone induced osteogenic gene expression, such as osteopontin and BMP2 in dental pulp cells [102,103]. Mizuno *et al.* also showed that Ca^{2+} released from CH stimulated fibronectin gene expression in dental pulp cells, a mechanism that may induce differentiation of these cells to become mineralized tissue forming cells [104]. Elevated Ca^{2+} is also known to stimulate differentiation and mineralization of other dental mesenchymal cells such as cementoblasts by increasing fgf-2 expression [105].

At the molecular level, extracellular Ca^{2+} level is detected by the calcium sensing receptor (CaSR), a seven-transmembrane homodimer receptor that belongs to the C family of the G-protein-coupled receptor superfamily. An activated CaSR elicits intracellular signaling pathways that ultimately lead to migration, proliferation and differentiation of cells [106]. Recently, it was shown that CaSR mediates osteogenic differentiation and mineralization of bone marrow mesenchymal stromal cells [107]. However, the presence of CaSR in bone-forming cells is controversial [108], and osteoblasts derived from CaSR-null mice remained to possess osteogenic differentiation potential [109,110]. Further, another study demonstrated that inhibition of CaSR further induced, rather than suppressed, Ca^{2+}-mediated osteogenic differentiation [111], suggesting that CaSR is dispensable in osteogenic differentiation and mineralization.

Ca^{2+} itself is an important intracellular signaling molecule, and there exist different types of Ca^{2+} channels that regulate intracellular Ca^{2+} level [112]. Among them, L-type voltage-gated calcium channel was shown to be associated with Ca^{2+}-mediated osteogenic differentiation and mineralization [113–116]. Similarly, recent studies showed that L-type calcium channel plays a key role in differentiation of dental pulp stem cells and periodontal ligament cells [111,117]. However, L-type voltage-gated calcium channel is a large transmembrane multi-protein complex that mediates Ca^{2+} influx in response to membrane depolarization via voltage differences [112]. As such, it remains to be elucidated as to how membrane depolarization links to differentiation and mineralization of osteoblasts and odontoblasts.

Recent studies identified another class of calcium channel, Orai1, that regulates intracellular Ca^{2+} level and Ca^{2+}-mediated signaling pathway in most non-excitable cells [118]. Orai1 is an essential subunit of Ca^{2+} release-activated Ca^{2+} (CRAC) channel that mediates Ca^{2+} influx via the store-operated Ca^{2+} entry (SOCE) mechanism. Although Orai1 is extensively studied and characterized in immune cells [119], recent studies showed that it plays a critical role in mediating bone formation. In particular, Orai1-null mice exhibited osteoporotic phenotypes, and disruption of Orai1 function in osteoblasts suppressed osteogenic differentiation and mineralization [120–122]. Similarly, Sohn *et al.* recently demonstrated that Orai1 also plays an indispensable role in odontogenic differentiation and mineralization [123]. When Orai1 was knocked down in dental pulp stem cells (DPSCs), these cells exhibited not only incompetent Ca^{2+} influx (Fig. 3) but also inability to undergo odontogenic differentiation and mineralization as demonstrated by alkaline phosphatase staining and activity as well as alizarin red staining (Fig. 3). More importantly, transplantation of DPSCs harboring Orai1/E106Q, a dominant negative form of Orai1, caused no formation of mineralized nodules *in vivo*, indicating that Orai1 is required for odontogenic differentiation and mineralization both *in vitro* and *in vivo*. Further studies on the role of Orai1 in reparative dentin formation warrant closer examination.

IV. FUTURE PERSPECTIVES AND CONCLUSIONS

Although a substantial numbers of clinical and molecular studies support the use of CH and HCSCs for direct pulp capping, achieving clinically successful outcomes in a reproducible and reliable manner still requires more investigations. Such shortfalls may be due, in part, to the lack of thorough understanding in the fundamental mechanisms of pulpal wound healing

and reparative dentin formation. Many pulp-capping animal models were previously used to better understand the mechanisms of reparative dentin formation [124–126]; however, pulp-capping studies in large animals are usually observational in nature. For this reason, transgenic or knockout mice that have an overexpressed or deleted gene of interest in an inducible and cell-type specific manner would help in expediting our understanding in reparative dentin formation at the molecular level (Figure 4) [127,128].

Due to the favorable clinical outcomes with the prototype MTA, its derivative products are widely available with modifications to their compositions. Nonetheless, their relative efficacy, or even their toxicity, is still far from complete understanding. Further comparative studies on validating and standardizing the effects of different HCSCs also warrant closer examination.

CH and HCSCs are odontoconductive by functioning as scaffolds onto which DPSCs migrate, proliferate, and differentiate to form reparative dentin. Clinical and preclinical studies support a notion that they are also odontoinductive as they also stimulate DPSCs to form reparative dentin. It would be beneficial and effective to incorporate bioactive materials as one of their constituents to further potentiate odontoconductive and odontoinductive properties of the pulp-capping materials.

Historically, CH has been used as a pulp-capping material, and its efficacy has proven to protect the exposed pulp based on clinical experiences for many decades. Recently introduced HCSCs have increasingly gained popularity due to improved physical and chemical properties to enhance reparative dentin formation. Nonetheless, many practitioners still prefer complete removal of the pulp once exposed rather than pulp-capping placement, primarily due to apprehension that they would be perceived as an “incompetent dentist” for the unsuccessful outcomes and multiple subsequent visits should the pulp capping fails. Because successful direct pulp capping is largely dependent on operator technique, material properties, and the host pulpal responses, it is important to recognize the importance of, and to optimally maximize the merits of each component so that reparative dentin can be regenerated in a predictable and reproducible manner. In this regard, more studies are needed at the clinical, preclinical, and molecular levels to improve each component.

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KEY POINTS

- Direct pulp capping is often performed on the exposed pulp after deep caries removal in order to induce reparative dentin, a physical barrier that functions as a “biological seal” to protect the underlying pulp tissues and maintain pup vitality.
- Although calcium hydroxide (CH) has been used as the “gold standard” pulp-capping material for many decades, recently introduced hydraulic calcium-silicate cements (HCSCs) such as mineral trioxide aggregate (MTA) have increasingly gained popularity due to their superior material properties that are biocompatible, odontoconductive, and to certain degree, odontoinductive.
- These pulp-capping materials confer capacity to induce reparative dentin by providing an alkaline environment and anti-bacterial activity; however, increasing lines of evidence support a notion that the release of calcium ions (Ca^{2+}) actively induces in reparative dentin formation by eliciting intracellular Ca^{2+} signaling pathways.
- Among the intracellular Ca^{2+} regulators, ORAI1 protein was recently shown to have an indispensable role in odontogenic differentiation and mineralization in dental pulp stem cells by regulating Ca^{2+} influx.
- Successful clinical outcomes of direct pulp capping depend on the operator technique, the material properties, and the host pulpal responses. Therefore, it is important to develop strategies that maximize the efficacy of each component for regenerating reparative dentin in a predictable and reproducible manner.

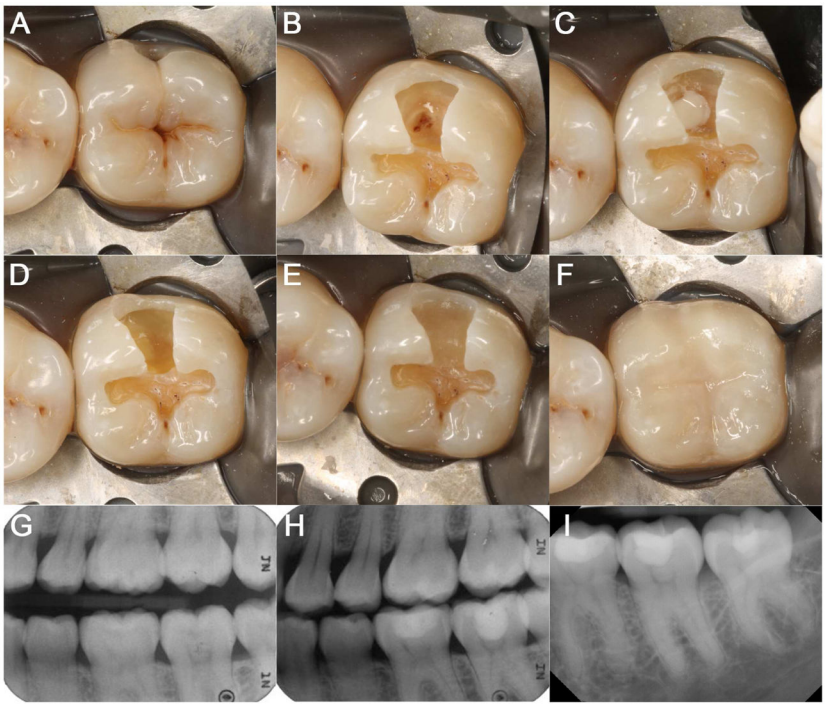

Figure 1. Direct pulp capping on #18 using CH

(A) Pre-operative clinical photograph of #18. (B) OB preparation with exposed MB pulp horn. (C) Dycal placement on the exposed pulp. (D) Fuji Lining LC placement as a liner directly on the Dycal. (E) Fuji II LC placed as a base on #18. (F) Composite restoration on #18. (G) Pre-operative radiograph of #18. (H) Post-operative radiograph of #18. (I) Periapical radiograph of #18 at follow-up after 2½ years.

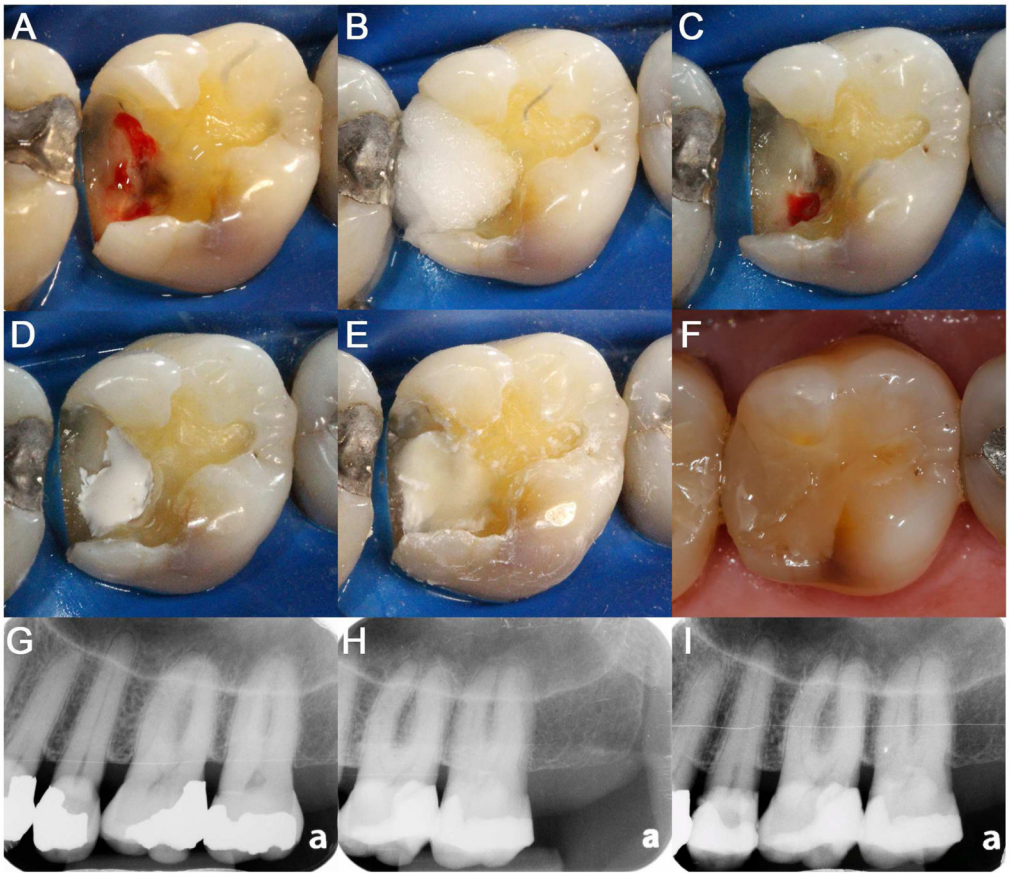

Figure 2. Direct pulp capping on #14 using HCSCs

(A) DO preparation with exposed pulp. (B) Cotton pellet soaked with 3.5% NaOCl. (C) Hemostasis achieved at the pulp-exposed area. (D) Placement of bioceramics on the exposed pulp. (E) Fuji Plus placement as a base directly on the HCSCs. (F) Composite restoration on #14. (G) Pre-operative radiograph of #14. (H) Post-operative radiograph of #14. (I) Periapical radiograph of #14 at the follow-up after 1½ years.

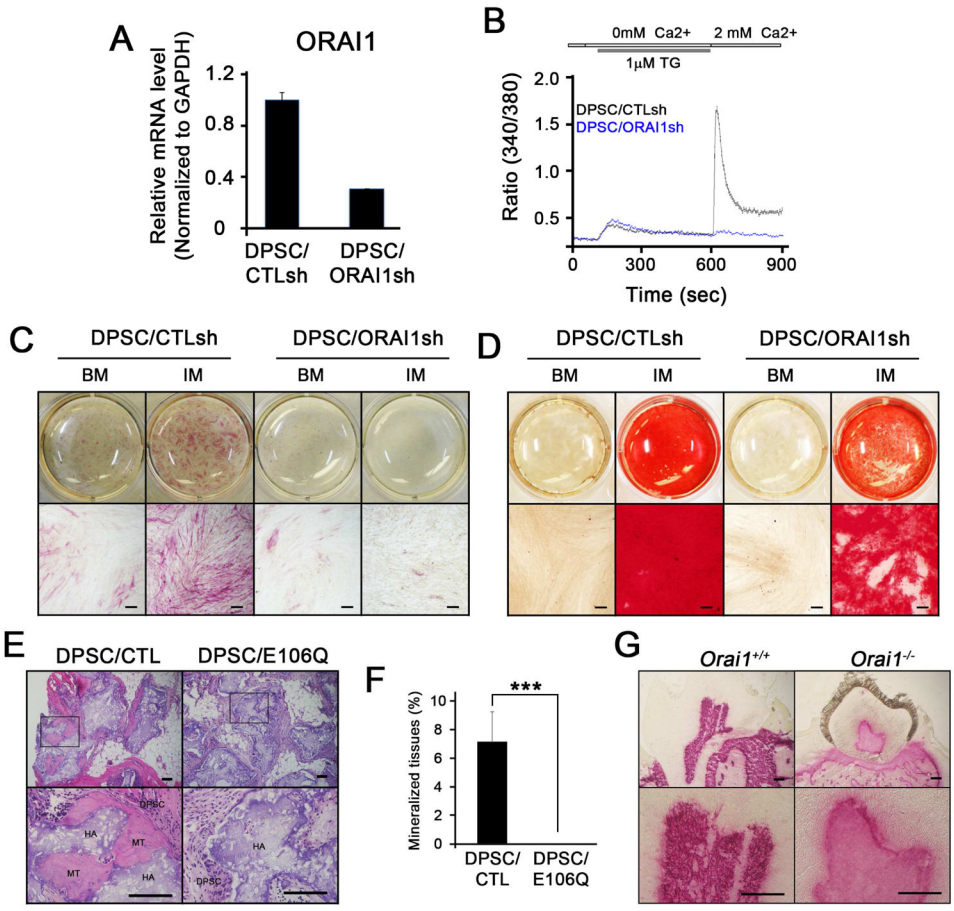

Figure 3. The calcium channel, Orai1, plays an indispensable role in odontogenic differentiation and mineralization

(A) Quantitative real-time polymerase chain reaction (qRT-PCR) of ORAI1 expression following knockdown experiment in DPSCs, showing efficient suppression of ORAI1 in DPSC/ORAI1sh cells but not in DPSC/CTLsh cells. (B) Measurement of intracellular Ca^{2+} level in DPSCs, confirming inhibition of Ca^{2+} influx when ORAI1 expression is suppressed in DPSCs. (C) Alkaline phosphatase (ALP) staining of DPSCs following treatment with basal medium (BM) and bone-forming induction medium (IM) for 5 days, demonstrating inhibition of ALP activity important for odontogenic differentiation. (D) Alizarin red staining of DPSCs following treatment with basal medium (BM) and bone-forming induction medium (IM) for 14 days, demonstrating inhibition of odontogenic mineralization. (E) Ectopic mineralized-tissue formation of DPSC/CTL cells but not DPSC/E106Q cells harboring dominant negative form of ORAI1, demonstrating indispensable role of ORAI1 in vivo. (F) Quantification of ectopic mineralized-tissue formation in vivo. (G) ALP staining of a tooth prepared from *Orai1*$^{+/+}$ and *Orai1*$^{-/-}$ mice. *From* Sohn S, Park Y, Srikanth S, et al. The Role of ORAI1 in the Odontogenic Differentiation of Human Dental Pulp Stem Cells. Journal of Dental Research 2015;94(11); with permission.

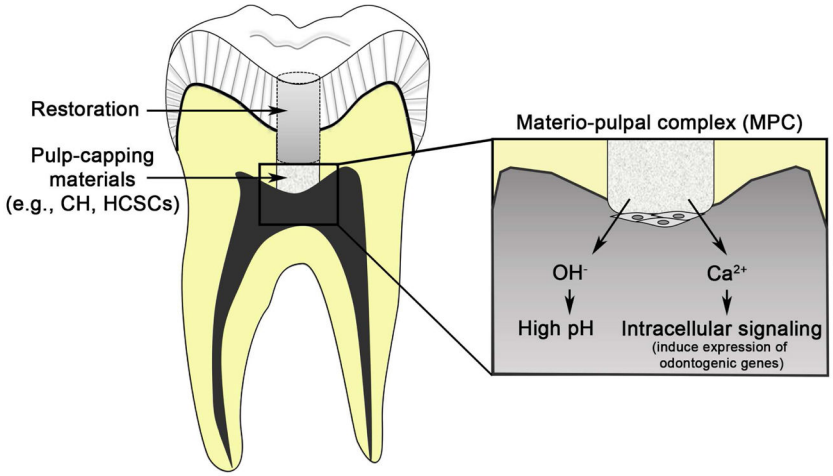

Figure 4. Schematic diagram of molecular mechanisms that govern reparative dentin formation by CH or HCSCs at the MPC

CH and HCSCs release hydroxyl group (OH^-) and increase local pH at the MPC, creating alkaline environment that induces anti-bacterial activity and potentially promotes odontogenic mineralization. CH and HCSCs also release calcium ions (Ca^{2+}), eliciting intracellular signaling pathways that ultimately promote odontogenic differentiation and mineralization.