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Literature Review

Combination calcium hydroxide and epigallocatechin-3-gallate in dentistry: A narrative review

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ABSTRACT

Background: Pulp capping and pulpotomy are treatments for exposed pulp due to injury. Calcium hydroxide is the gold standard material in pulp capping and pulpotomy because it stimulates reparative dentin formation in exposed pulp. Calcium hydroxide has the disadvantage of causing chronic inflammation, cell necrosis, the formation of tunnel defects, and weak antibacterial properties against certain bacteria. Epigallocatechin-3-gallate (EGCG) is the main catechin component of green tea and has potent anti-inflammatory and antibacterial properties against both gram-negative and positive bacteria. The addition of EGCG to calcium hydroxide has the potential to prevent chronic inflammation and improve the antibacterial properties of calcium hydroxide. **Purpose:** This review aims to explain the potential of the combination of calcium hydroxide and EGCG in dentistry. **Review:** EGCG has potent anti-inflammatory and antibacterial effects against Enterococcus faecalis. The addition of EGCG to calcium hydroxide has the potential to reduce the inflammatory effect and improve the antibacterial properties of calcium hydroxide. **Conclusion:** This review concludes that the combination of calcium hydroxide and EGCG has the potential to reduce the inflammatory effect and promote the antibacterial effect of calcium hydroxide. Further research is needed to prove the potential of the combination of calcium hydroxide and EGCG in dentistry as a material for pulp capping and pulpotomy.

Keywords: EGCG; calcium hydroxide; dentistry; pulp capping; pulpotomy.

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INTRODUCTION

The pulp that undergoes an inflammatory process in response to injury is not capable of self-healing like other organs of the body. Pulp self-healing ability is limited due to minimal collateral blood supply to the pulp.[1] Damaged pulp can heal if assisted with adequate care. Pulp capping and pulpotomy are treatments for pulp exposed due to injury.[2,3] Pulp capping serves as a barrier that prevents bacteria from entering the pulp, promotes soft tissue healing, and promotes hard tissue repair by promoting reparative dentin formation. Calcium hydroxide is a commonly used material in pulp capping procedures because it is able to stimulate the formation of reparative dentin in exposed pulp.[3] Calcium hydroxide has the disadvantage of causing chronic inflammation, cell necrosis, and the formation of tunnel defects in reparative dentin, as well as weak antibacterial properties against certain bacteria.[4,5] A tunnel defect is a cavity that forms on the surface of reparative dentin so as to provide entry for bacteria to the pulp and increase the pulp's inflammatory process.[6]

Calcium hydroxide has weak antibacterial ability against pulpitis-causing bacteria, such as *Enterococcus faecalis, and* can cause pulp inflammation; therefore, it's important to find a way to make up for this deficit by incorporating something such as *epigallocatechin-3-gallate* (EGCG). EGCG is the main catechin component extracted from green tea.[7] Many studies with EGCG ingredients in dentistry have been carried out due to its anti-inflammatory effect on neutrophil cells. EGCG compounds can reduce the inflammatory response of traumatized tissues.[8] EGCG has shown the ability to prevent enamel demineralization.[9] EGCG also has potent antibacterial properties against both gram-negative and gram-positive bacteria.[10] The addition of EGCG to calcium hydroxide has the potential to treat chronic inflammation caused by calcium hydroxide. Inflammation is the body's defense process when it is damaged by bacteria, trauma, or chemicals.[11] EGCG with a concentration of 90 ppm is effective in increasing the number of fibroblast cells proliferating in the dental pulp perforation of Wistar rats.[12] The combination of calcium

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hydroxide and green tea extract has been proven to activate more p38 and form a wider reparative dentin.[13] A review of the calcium hydroxide-EGCG mixture has never been done before. Thus, the aim of this narrative review is to describe the potency of the combination of calcium hydroxide and EGCG in dentistry.

REVIEW

Pulp capping is a treatment for pulp that is still vital but has lesions. Pulp capping treatment aims to maintain the vitality of the pulp. Pulp capping consists of direct pulp capping and indirect pulp capping. The ideal material for pulp capping must meet several requirements, including being able to maintain pulp vitality, stimulate reparative dentin formation, and have bactericidal or bacteriostatic properties.[3] Indirect pulp capping is a pulp capping procedure that is performed when caries has reached the dentin and has a high potential to expose the pulp. Direct pulp capping is a pulp capping procedure performed when the pulp is exposed due to mechanical trauma during tooth preparation. Direct or indirect pulp capping is required to trigger the formation of reparative dentin so that pulp vitality can be maintained.[1]

Pulpotomy is a treatment to maintain pulp vitality, similar to direct pulp capping, but by removing infected or inflamed coronal pulp. The vital pulp of the root canal is preserved.[3] Pulpotomy treatment is a treatment that facilitates root development in immature permanent teeth by maintaining the vitality of the radicular pulp.[14] Calcium hydroxide is the material of choice for pulpotomy treatment.

Table 1. EGCG research in dentistry

No	Researcher/ Year	Sample	Control Group & Intervention Group	Result	Conclusion
1	Li Y *et al* (2021)[18]	Human third molar without caries extracted due to impaction (n=5). Mice with pulpitis (n=12) were randomly divided into 3 groups pulpitis model (model=4) saline control(control=4) EGCG experimental(n=4)	Control Group: Cell culture in medium containing no EGCG Alkaline phosphatase activity, alizarin red staining, PCR test: Cell culture in normal osteogenic medium (control group) Intervention Group: Cell culture in medium containing EGCG at concentrations of 10, 25, 50, and 75 µg/mL Alkaline phosphatase activity, alizarin red staining, PCR test: Cell culture in EGCG-containing osteogenic medium (EGCG Group)	CCK-8 assay revealed that 10 µg/mL EGCG had a significant anti-inflammatory effect on hDPSCs without affecting cell proliferation or differentiation. EGCG inhibits expression of IL-1β, IL-6, and TNF-α. EGCG rescued cell proliferation ability, increased SOD activity and reduced ROS expression under hypoxia. Reduced inflammatory cell accumulation was observed in the coronal pulp in the EGCG group. Diffuse inflammatory cells were observed in the radicular pulp in the control group.	EGCG had no obvious effects on calcified nodule formation but significantly inhibited the inflammatory response of hDPSCs and inhibited apoptosis of hDPSCs caused by hypoxia injury. In vivo, EGCG exerts inhibitory effects on pulp tissue inflammation.
2	Schneider-Rayman *et al* (2021)[20]	EGCG 70 mg/mL, *S.mutans* mono species	Control Group: Control bacteria received the same incubation conditions without EGCG. *S.mutans* cultures were diluted 1:10 in BHI in the absence of EGCG. Intervention Group: *S.mutans* cultures were diluted 1:10 in BHI in the presence of various concentrations of EGCG (0.55, 1.1, 2.2, 4.4 mg/ml)	EGCG inhibits in a dose-dependent manner both the planktonic growth and the biofilm formation of *S.mutans*. Significant reduction of *S.mutans* biofilm formation, DNA content, and EPS production was observed at 2.2–4.4 mg/ml EGCG. EGCG reduced the expression of gtfB, gtfC and ftf genes involved in EPS production, and the nox and sodA genes involved in the protection against oxidative stress. Moreover, EGCG caused an immediate change in membrane potential.	EGCG has a significant inhibitory effect on *S.mutans* dental biofilm formation and EPS production.

3	Wang *et al* (2018)[9]	Forty enamel and dentine specimens were prepared from human teeth (third molars and premolars)	Control Group: Tooth specimens were randomly divided into 4 groups, according to the following treatments: Group A: distilled water (DW) as control; Intervention Group: Group B: 0.5 M NaF solution (NF); Group C: 400 mM EGCG (EG); Group D: NaF (0.5 M) and EGCG (400 mM) (FG / combination of Sodium fluoride and EGCG)	More substance loss was found in the dentin specimens than in the enamel specimens for each group. The dimensions of the tubular orifices were wider in the DW group than in the NF (sodium fluoride group) and EG (EGCG group groups ($p < 0.05$). Regarding the dentin specimens, less erosive destruction was detected in the intertubular dentin than in the peritubular dentin. Surface roughness increases in the enamel specimens were more prominent in the DW (226.4 36.1%) and NF (230.6 25.3%) groups than in the EG (158.4 15.6%) and FG (155.7 13.6%) groups ($p < 0.05$). No difference in surface roughness increase was found in the dentin specimens. The microhardness after erosive treatment decreased in all specimens. The DW group showed the greatest decrease in microhardness in the enamel and dentin specimens. Chelating of metal ions by EGCG may play an important role in prevention of tooth structure from erosive damage.	Fluoride and EGCG can reduce the erosive damage caused by the beverage and reduce the risk of developing tooth hypersensitivity.
4	Han *et al* (2021)[16]	EGCG was then added to the TYG culture at final concentrations of 0.5, 1, and 2 mg/L	Control Group: contained *S.mutans* grown in the absence of EGCG. Intervention Group: Four milliliters of TYG medium were inoculated with 0.1 mL of overnight culture of *S.mutans*. EGCG was then added to the TYG culture at final concentrations of 0.5, 1, and 2 mg/L	In the presence of 2 mg/mL EGCG,*S.mutans* could not grow in TYG medium. In the presence of 1 or 0.5 mg/mL EGCG, the growth of *S.mutans* was limited and slowed down, respectively. EGCG (up to 2 mg/mL) had no significant effect on the viability of *S.mutans* over a 2-h co incubation period (Fig. 1b). However, co incubating *S.mutans* with 2 mg/mL EGCG for 4 h reduced the number of viable bacteria by 40%.	EGCG might have short-term antibacterial effects on caries-associated streptococci in the oral cavity.

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5	Yu *et al* (2017)[21]	Two hundred and twenty-four human premolars with single and straight root canal of similar length were used in this study. The roots were accepted regular root canal treatments and post space preparations, and further divided into eight groups according to the four post space pretreatments and two dentin adhesives [Single Bond 2 (SB2) and Clearfil SE Bond (CSB)] used.	Control Group: Black control group (referred as BC group): post spaces were irrigated with 10mL distilled water for 1 minute (min); Intervention group; (ii) NA group: post spaces were irrigated with 10 mL 5.25% NaOCl for 1 min, then stop with 10 mL distilled water for 1 min; (iii) NEG group: post spaces were irrigated with 10 ml 5.25% NaOCl for 1 min, followed with 10 mL distilled water, and 10ml EGCG irrigant for 1min as final irrigation; (iv) NE group: post spaces were irrigated with 10ml 5.25% NaOCl for 1min, followed with 10mL distilled water, and 10 ml anhydrous ethanol solution for 1min as final irrigation	NaOCl+EGCG groups showed the highest push-out strength regardless of the adhesive type, root region and time ($P<0.05$). NaOCl pretreatment significantly decreased the push-out strengths and DC (degree of conversion) of CSB ($P<0.05$). EGCG could improve the bonding properties of both SB2 and CSB to NaOCl treated intraradicular dentin. The effect of NaOCl on bonding of a fiber post depended on the type of the adhesive	EGCG as final irrigation increased the push-out strength and bond stability of fiber post to NaOCl treated intraradicular dentin for both self-etching adhesive CSB and etch-and-rinse adhesive SB2 regardless of root region
6	Widjiastuti *et al* (2020)[12]	24 male Wistar rats which were divided into four groups, namely the negative control group and the treatment group were given EGCG 60 ppm, 90 ppm, and 120 ppm and were decapitated on the 7th day after treatment.	Control group: the maxillary first molar teeth of the experimental animals were prepared and perforated without hydrogel EGCG application Control (given injury without being treated); Intervention group: three treatment groups which received cavity preparation and hydrogel EGCG application in 60 ppm (T1), 90 ppm (T2), and 120 ppm (T3) group.	The higher the concentration of EGCG, the higher the average number of fibroblast cells present in the pulp tissue on the 7th day after treatment.	The effective concentration of hydrogel EGCG for increasing the number of fibroblast cell proliferation is 90 ppm

Calcium hydroxide is not indicated for pulpotomy in primary teeth.[3]

Calcium hydroxide is a dental material that is available in powder and paste preparations.[5] Calcium hydroxide is the material of choice in endodontic treatments such as pulp capping and pulpotomy.[2] The disadvantages of calcium hydroxide include tunnel defects formed in reparative dentin and weak antibacterial properties against certain bacteria.[1,5] Tunnel defects are cavities that form on the surface of reparative dentin, giving bacteria access to the pulp and increasing the inflammatory process of the pulp.[6] Calcium hydroxide has weak antibacterial properties against *Enterococcus faecalis* bacteria.[1] Calcium hydroxide also has a high pH, which can cause chronic inflammation and cell necrosis in vivo.[4]

EGCG is the main polyphenol catechin in green tea that has antioxidant abilities. EGCG has been widely studied in the medical field because of its ability to inhibit cell proliferation and trigger cancer cell apoptosis, as well as its antibacterial and anti-inflammatory properties.[15] Research on EGCG in dentistry is currently being carried out, such as the potential antibacterial properties of EGCG against pathogenic bacteria in the oral cavity.[16] Research conducted by Lee and Tan on the effect of EGCG on biofilms and the virulence of *Enterococcus faecalis* showed that EGCG was able to inhibit growth at a concentration of 5µg/mL. EGCG was able to kill *Enterococcus faecalis* bacteria at a minimum concentration of 20µg/mL.[17] EGCG at a concentration of 10 g/mL had a significant anti-inflammatory effect without affecting cell proliferation or differentiation.[18] EGCG has anticancer, antioxidant, anti-inflammatory, anti-collagenase, anti-fibrosis, and osteogenesis properties. EGCG prevents diethyl nitrosamine-induced obesity-associated liver tumorigenesis by inhibiting the IGF/IGF-1R axis, promoting hyperinsulinemia, and attenuating chronic inflammation. EGCG can restrain tumor proliferation by acting against angiogenesis. The phenol ring in the EGCG structure acts as an electron trap, functions to capture free radicals, prevents the formation of reactive oxygen species, and prevents oxidative stress. As an antioxidant, EGCG can improve mitochondrial function.[19]

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DISCUSSION

Exposed pulp due to injury requires pulp capping and pulpotomy treatment to form reparative dentin that protects the vitality of the pulp.[3,5] Calcium hydroxide is the gold standard for pulp capping treatment, which can trigger the mineralization process. Calcium and hydroxyl ions in calcium hydroxide undergo dissociation. The dissociated calcium ions will decrease capillary permeability, so the serum flow rate will decrease. The hydroxyl ion neutralizes the acid produced by the osteoclasts, thereby creating the optimum pH for pyrophosphate activity. This raises the level of calcium ion-dependent pyrophosphate. A decrease in serum rate and an increase in calcium ion-dependent pyrophosphate levels reduce inhibitory pyrophosphate levels, resulting in mineralization.[3] Alkaline pH conditions produced by calcium hydroxide can trigger chronic inflammation of the pulp.[2]

Calcium hydroxide has antibacterial properties through the hydroxyl ion. The hydroxyl ion creates an alkaline pH environment so that it can kill bacteria by damaging the cytoplasmic membrane, denaturing proteins, and damaging DNA. Bacterial cytoplasmic membranes have essential functions, including metabolism, cell division, and development. Damage to the cytoplasmic membrane due to calcium hydroxide and hydroxyl ions causes disruption of essential cell functions.[22] Antibacterial properties of callcium hydroxide are less effective against *Enterococcus faecalis*.[7] *Enterococcus faecalis* has the ability to maintain the internal pH of the cytoplasm through ion pumps when exposed to an alkaline pH environment created by calcium hydroxide.[23]

Epigallocatechin-3-gallate is capable of being an anticariogenic compound because it has antibacterial properties against *Streptococcus mutants*.[10] Anticariogenic EGCG occurs through inhibition of the production of lactic acid, formic acid, and acetic acid by *Streptococcus mutans*. Acid produced by bacteria is the main cariogenic factor of a bacterium.[16] EGCG also has potent antibacterial properties against *Enterococcus faecalis* bacteria through hydroxyl radicals. Antibacterial EGCG through hydroxyl radicals capable of damaging lipids, proteins, and bacterial DNA.[17] The potent antibacterial properties of EGCG against *Enterococcus faecalis* indicate the potential for the combination of calcium hydroxide and EGCG in dentistry as a pulp capping and pulpotomy material.

Calcium hydroxide causes chronic inflammation. The inflammatory response is characterized by the aggregation of immune cells in the area of injury, the production of proinflammatory cytokines, and reactive oxygen/nitrogen (ROS/RNS). Reactive oxygen and nitrogen (ROS/RNS) functions to activate transcription factors NF-κB and activator protein-1 (AP-1). Upon activation, NF-κB and AP-1 migrate from the cytoplasm to the nucleus and regulate various inflammatory expressions. This causes an inflammatory response and tissue damageAn antiti-inflammatory effect is required in the transduction process. Epigallocatechin-3-gallate (EGCG) exhibits anti-inflammatory effects by inhibiting NF-B and AP-1 transfection to reduce iNOS and COX-2 expression by destroying NO, peroxynitrite, other ROS/RNS, and reducing the production of other inflammatory factors.[19] Anti- inflammatory properties are expected to reduce inflammation formed due to the use of calcium hydroxide in pulp capping and pulpotomy procedures. It has been shown that EGCG can lower ROS such as singlet oxygen, superoxide, peroxyl, and hydroxyl radicals as well as nitrogen dioxide, nitric oxide, and peroxynitrite.[24]

EGCG has anti-inflammatory properties by inhibiting the expression of proinflammatory cytokines such as IL-1B, IL-6, and TNF-a at a concentration of 10 g/mL. Vascular Endothelial Growth Factor (VEGF) and cyclooxygenase-2 (COX-2) are also involved in the inflammatory process in the pulp. Administration of Epigallocatechin-3- gallate was able to inhibit the expression of VEGF and COX-2. This proves that EGCG is able to prevent the spread of inflammation in the pulp tissue.[18]

In recent years, research into the potential uses of EGCG in the oral field has revealed that this substance can help promote and maintain oral health by slowing the progression of periodontitis, preventing enamel and dentin erosion, protecting the oral mucosa, and showing activity against oral cancer cells in vitro. It may also help to reduce halitosis.[25] In the etiology of periodontal disease, an excess of free radicals harms gingival tissues, periodontal ligaments, and alveolar bone.[26] Green tea is thought to protect against inflammatory disorders by lowering the production of pro-inflammatory cytokines, particularly interleukin 8. Green tea contains strong antioxidant and anti-inflammatory properties (IL-8).[27] It has been discovered that green tea products have strong anti-inflammatory and antiplaque properties in addition to increasing the gingival crevicular fluid's overall antioxidant capacity.[28]

Regular green tea drinking can lessen gingival bleeding index, increase pocket depth, and accelerate periodontal repair.[29] Polyphenols may contribute to periodontal regeneration by lowering the expression of matrix metalloproteins (MMP 9), suppressing osteoclast development, and dose-dependently inducing apoptosis.[30] Green tea is a key source of polyphenol antioxidants and has the ability to guard against various oral diseases, including dental caries, gingivitis, periodontitis, halitosis, and oral cancer (protection and regression). Additionally, it can alleviate inflammation brought on by cigarette smoke, dental oxidative stress, and dentin erosion and abrasion.[31] An epidemiological study demonstrates that green tea intake and periodontal health are mutually exclusive. People who consume green tea frequently, such as at meals or work breaks, have better periodontal health.[32] Epigallocatechin-3-gallate also reduces cigarette smoke induced inflammation. Reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and hydrogen peroxide, were also present in tobacco smoke.[33] Smokers may benefit from drinking green tea. Green tea contains catechins that can neutralize superoxide oxide, NO, and ONOO.[34]

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CONCLUSION

This review concludes that the effect of EGCG in the combination of calcium hydroxide and EGCG reduce the inflammatory effect formed from calcium hydroxide and assist the antibacterial effect of calcium hydroxide to increase the potential for pulp vitality. Further research is needed to prove the potential of the combination of calcium hydroxide and EGCG in dentistry as a treatment for pulp capping and pulpotomy.

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