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Research Article

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The Evaluation of Possibility for Future Root Canal Irrigant Candidate by Catechin Solution

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Abstract

To treatment for periapical periodontitis, root canal irrigation is one of most important process to obtain success of healing Sodium hypochlorite (NaOCl) is the most widely used as root canal irrigant. However using NaOCl on endodntics treatment should be considered its problems such as allergy, serious tissue damage when inadvertently injected into the maxillary sinus or oral mucosa. Therefore another root canal irrigant that is safety and having high antimicrobial effect is desirable.

In this study, catechin was to evaluated microbicidal efficacy to the Enterococcus. faecali (E.faecalis) and considered the possibility to be the future root canal irrigant candidate.

10%(100mg/ml) to 30%(300mg/ml) of catechin solution were prepared and used for this study. The anti-microbiologic effect against E.faecalis was evaluated with different concentrations (low: $5x10^7$ CFU/ml, high: $5x10^9$ CFU/ml), and different reaction time like as from 5minutes to 30 minutes respectively. Then they were placed onto BHI agar plates and incubated for 1 week at 37°C. Then the number of colony formation was counted.

Prevent colony formation by 30% catechin solution was caused within 5minutes in low density of E.faecalis, and within 20 minutes in high density of it. 20% catechin solution leaded to no colony formation at 10 min (low density) and 30 min (high density), respectively. For 10% catechin solution, it was necessary to attain no colony formation should be 30 minutes (low density), however high density of E.faecalis was not killed completely within 30minutes.

These results suggested that Catechin has a benefit to anti-microbiologicl effect against E.faecalis. However to determine Satisfactory anti-microbiological effect, high concentration of catechin solution and long reaction time should necessary. Therefore usage of catechin in future root canal treatment might be recommended not for candidate of root canal irrigant but for candidate of intracanal medication.

Keywords: Catechin, *Enterococcus. faecali*, Root canal irrigant, Sodium hypochlorite

Introduction

Although periapical periodontitis is known high frequency in the dental disease, complete method to recover it has not been established. Many researchers has been reported success rate of endodntic treatment and they insisted success rate was varied from 40 to 90% depend on the state of periapical periodontitis [1,2].

After retreatment of endodntics, success rate was decrease compared with primary endodntic treatment and lowest was 59.5% [3]. These results demonstrated that the treatment of endodntics to periapical periodontitis seems not so easy and the possibility of periapical periodontitis recurrent even after retreatment. To avoid future retreatment occurring, removing inflammatory factors as

microorganisms, infected organic matter and debris as possible are utmost important factor to success root canal treatment. To attain removing such undesirable inflammatory objects, chemo-mechanical root canal preparation is necessary.

Chemo-mechanical root canal preparation is known to the combination of mechanical preparation and chemical root canal irrigation.

Generally mechanical preparation has been performed with several length and thickness of hand files. Recently nickel-titanium rotary file was produced and has been spread to mechanical preparation addition with hand file preparation and improve removing infected tooth structure and microorganisms [4,5]. However even using those several hand and nickel-titanium rotary files, only mechanical preparation to the root canal might hard to gain a bacteria-free canal, especially in cases with complex root canal morphology [6]. Furthermore Peters OA et al also insisted that complete removal of the bacteria by this mechanical procedure alone is unlikely to be seen [7].

Thus root canal irrigation is required process to achieve bacteria free environment. Irrigating solutions play a main role in the successful biomechanical preparation of root canals [8].

Sodium hypochlorite (NaOCl) is the most widely used irrigating solution that has been used for endodontic treatment since 1920 [9]. The benefit using NaOCl to endodntic treatment is a low-cost solution and shows a very effective antimicrobial activity against microbiota of infected root canals.

NaOCl has capacity to dissolve necrotic dissolving tissues [10,11]. The organic components of the smear layer and inactivation of endotoxin by hypochlorite has also been reported [12-17].

However using NaOCl on endodntics treatment should be considered some problems.

First, several cases of allergic reactions to NaOCl from a root canal irrigant have been reported [18,19]. Second, the complications causing severe tissue reactions associated with the accidental extrusion of NaOCl into periapical tissues have been reported.

Some authors have mentioned clinical situations where NaOCl was inadvertently injected into the maxillary sinus [20,21], was unintentionally injected into the oral mucosa [22] or irrigant injected into a patient's eye during endodontic therapy [23].

Currently, several chemical agents like as an acidic acid, EDTA, MTAD and CHX have been using in root canal treatment to antibacterial effect [24-28]. However there are no root canal irrigant that was exceeded NaOCl has been established yet.

Catechin is famous for the main ingredients of green tea and known that it has anti-microbial effect to several bacteria either [29].

Therefore it is possible that catechin also has an anti-microbial effectiveness to the periapical periodontitis.

Enterococcus faecalis (*E. faecalis*) is a nonspore-forming, fermentative, facultatively anaerobic, Gram-positive coccus and the most commonly isolated or detected species from oral infections, including marginal periodontitis, infected root canals, and periradicular abscesses and they are survive even in a highly alkaline environment [30-32].

Moreover existence of *E. faecalis* thought to be one of the reasons the failure of root canal treatments, hence killing of *E. faecalis* is extremely important factor to lead endodontic treatment successful, and usage of the antimicrobial agents against *E. faecalis* are necessary.

The object of this study was to evaluated microbicidal efficacy of catechin against the *E. faecali* and considered the possibility to be the future root canal irrigant candidate.

Materials and Methods

In this study, the mixture solution of four types of catechins (epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) were used as a catechin solution. A catechin mixture powder (Fujifilm Wako Pure Chemical Corporation) was diluted each 10%(100mg/ml), 20%(200mg/ml), 30%(300mg/ml) with purified water. Subject to *E. faecalis* was prepared 2 different kinds of density ($5x10^7$ CFU/ml, $5x10^9$ CFU/ml). In addition, phosphate-buffered saline (PBS) was used as control solution.

The procedure of this experiment process shown in Figure 1. *E. faecalis* (4532D) was incubated overnight at 37ºC in brain-heart infusion (BHI) broth. 10 µl of planktonic bacterial cells was transferred into each test tube. These tubes were centrifuged (10000 g x 5 min) and supernatants were discarded. 100 µl of test solution (each percentage of catechin solution and PBS solution) was added to the each tube and mixed adequately. Then those tubes were stored at the room temperature for 5 minutes, 10 minutes, 20 minutes, and 30 minutes respectively. Thereafter each 100 µl of test solution was placed onto BHI agar plates and incubated for 1 week at 37ºC. After incubation, the number of colony formation was counted to determine of antimicrobial effect by catechin solution.

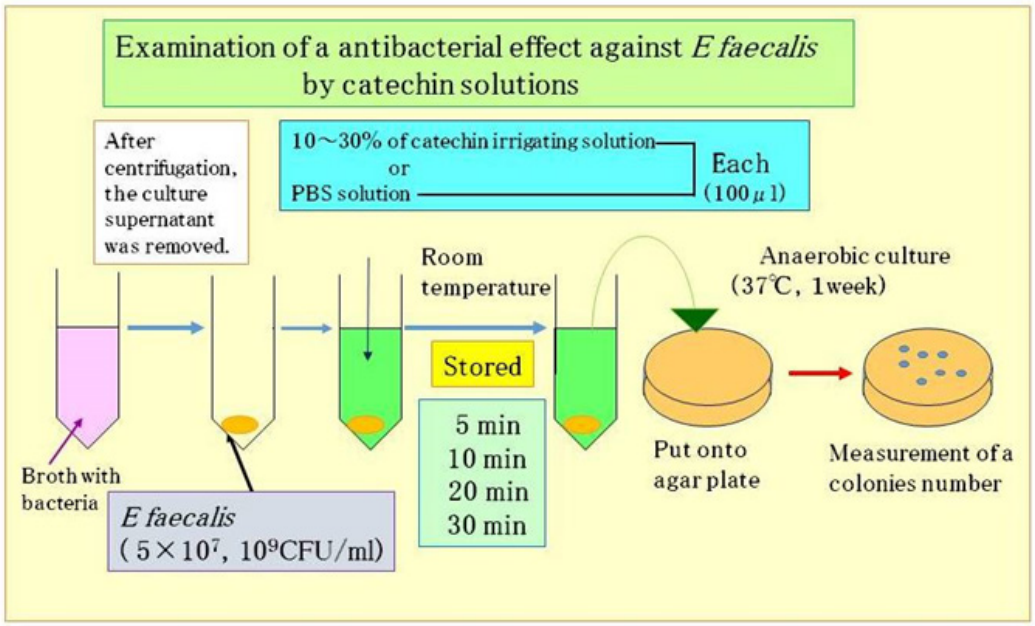

Figure 1: Experimental procedure

Results

The results of this study are shown in the Figure.2 and Figure.3. With regard to 5x10^7 CFU/ml density of *E.faecalis*, colony formation on the plate under 30% catechin usage was not observed after 5 minutes application. Whereas, few colony formation were seen after 5 minute applying plate, however no colony was confirmed on the plate of 10 minutes application with 20% catechin. Then the cases of 10% catechin, no colony was observed after 30 minutes application.

Regarding to 5x10^9 CFU/ml density of *E.faecalis*, colony formation on the plate under 30% catechin usage was not observed after 20 minutes application. Whereas, no colony was confirmed on the plate with 20% catechin at 30 minutes applications. However several numbers of colonies still remained on the plate with 10% concentration of catechin solution application at 30 minutes.

The plates of using PBS solution as a control were shown enormous number of colonies and no decrease even after 30 minutes application.

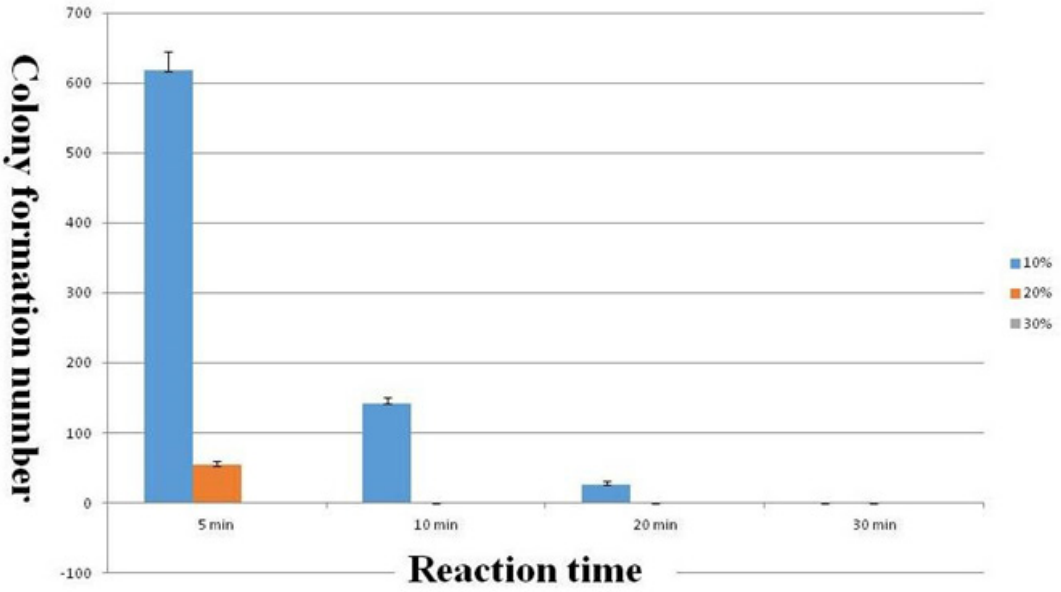

Figure 2: Examination of an antibacterial effect to *E faecalis* by catechin solution (low density of *E faecalis*: 5x10^7 CFU/ml)

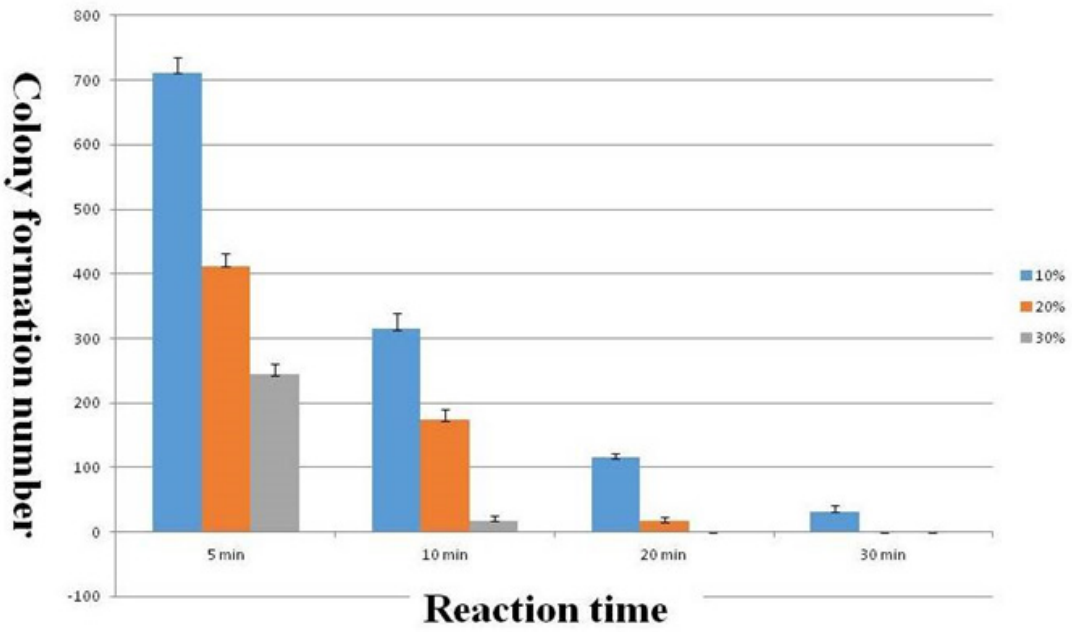

Figure 3: Examination of an antibacterial effect to *E faecalis* by catechin solution (high density of *E faecalis*: $5x10^9$ CFU/ml)

Discussion

Tea is one of the most commonly consumed beverage in worldwide, and it derive from the leaves and bids of the plant *Camellia sinensis*. Catechins are main components of green tea, black tea, and oolong tea. Green tea is rich in catechins compared with other teas [33]. They are also included into dark chocolate and cocoa, grape, apple, strawberries, blueberries [34-38].

Catechin is one of the major flavonoid components and it divided into four separate forms. The main forms of tea catechin are epicatechin (EC) and its hydroxy form (EGC). Others are their gallate types, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Catechins in green tea, the most abundant catechin is EGCG (~60%), and the next most abundant is EGC (~20%), then ECG (~14%), and EC (~6%), and EGCG is presence only in green tea and the most studied in association with health, In those catechins, EGCG has been analyzed by numerous studies as highly bioactive catechin [39].

To date catechin has been researched their beneficial effects on extensive diseases such as brain disorder, neurodegenerative diseases, cancer, reduce body weight, obesity diabetes, treatment to nonalcoholic fatty liver disease and anti-oxidative stress [40-50]. Other important activities of catechin are the antibacterial, antifungal and antiviral effects. Especially the anti-biological effect to *Staphylococcus aureus*, *Escherichia Coli* (*E.coli*), anti-virus include influenza A, B virus and the hepatitis B, C and development newly drugs against these bacteria, fugal and virus using catechin are highly expected [51-56].

Against oral field, several researchers reported that catechins have possessed controlling harmful oral diseases like as dental caries and chronic periodontitis.

As influence of dental caries, catechin causing antimicrobial effects against oral streptococci [57-62]. Especially EGCG has inhibit the growth and glucosyltransferases activity of *S. mutans* [63]. In addition, animal studies have demonstrated that various tea extracts prevented or reduced caries formation [64-67].

The role of catechin to periodontal disease was demonstrated that EGCG attacked *P. gingivalis* which is important factor of periodontitis, and inhibits biofilm formation of them. Therefore EGCG may represent a novel anti-biofilm agent that prevents infections involving bacterial biofilms such as periodontitis [68].

While various studies that anti-caries and anti-periodontal effect by catechin were investigated, there are not so many on endodontics field. Therefore it is important to examine the effectiveness of catechins on the periapical periodontitis.

E. faecalis is one groups of *Enterococci*, and they are present in human female genital tracts and the oral cavity in lesser numbers [69]. *E. faecalis* are known as multidrug resistant organisms and are a particular hazard to medical practice [70,71]. The number of *E. faecalis* is increased in the patients who have experience of endodontic treatment compared with no endodontic history, and tendency of increasing of *E. faecalis* is related with treated frequency of retreatment [72]. In fact, failed root canal treatment cases are approximately nine times more likely to harbor *E. faecalis* than cases of primary endodontic infections [30].

Thence it is suggested that the presence of *E.faecalis* is greatly concerned refractory endodontic infections.

To date, antimicrobial effect of catechins on *E. faecalis* has been reported by several researchers, and most of them have been showed favorable results on *E.faecalis*, a gram-positive cocci [73,74].

In particular, Lee P, et al. reported that EGCG had bactericidal effects in both planktonic and biofilm forms [73]. They also suggested that anti-microbial activity were occurred through the generation of hydroxyl radical, and it induced inhibiting bacterial growth and suppressing the expression of specific genes related to virulence and biofilm formation.

While there have been such positive reports on the bactericidal effect of catechin against *E. faecalis*, Gutiérrez-Venegas insited catechin had not possess pharmacological anti-biotic activities against *E.faecalis* [75]. In their report, they insist catechin only had antibacterial activity against *Actinomyces comitans* and *S. aureus*. The reason why the antibacterial effect did not come out is unclear, however it is possible that the concentration of catechins was less than the sufficient of killing *E. faecalis*.

In the present study, the preeminent bactericidal activity against *E. faecalis* by catechin solution was demonstrated. However, the results of obtained minimum bactericidal concentration (MBC) in this study was different compared those previous researches.

In this study, MBC against $5x10^7$ CFU/ml of *E.faecalis* was 300mg/ml at 5 minutes, 200 mg/ml at 10 minutes, 100 mg/ml at 30 minutes, while $5x10^9$ CFU/ml of *E.faecalis* was 300mg/ml at 20 minutes, 200 mg/ml at 30 minutes, and 100 mg/ml of catechin needed over 30 minutes. However previous paper reported that EGCG exhibited a MBC of 20μ/ml, and effectively eradicated *E. faecalis* biofilm [73].

The reasons for the difference in MBC results might be related with the difference of experiment system both studies like as bacterial concentration, catechin reaction time and so on.

At first, the bacterial concentrations in this study (5×10^7 CFU / ml, 5×10^9 CFU / ml) may affected this results of MBC. Because these concentrations of bacteria were higher than previous studies. Consequently more high volume of catechin might be necessary to attain antimicrobial effect against *E. faecalis* compared with previous study. However many studies have been published against *E. faecalis*, and most of them were investigated $1X10^6$ to 10^9(CFU)/ml on their researches [76-81]. Thus 5×10^7 CFU / ml and 5×10^9 CFU / ml seem to be appropriate concentrations for studying periapical periodontitis. Secondary, reaction time of catechin also should be influenced occurred these differences. Generally, reaction of catechin against *E. faecalis* over 12 to 24 hour seems suitable for determining the MIC and MBC. The greatest exponential increase in cell proliferation rate during the first 12 hours of culture, thereafter growth rate was reduced between 12 and 24 h of culture as the stationary growth phase [82]. However the purpose of this study was to determine whether catechins was able to be a candidate of new root canal irrigant. Therefore, the duration of catechin reaction against *E. faecalis* was established from 5 min to 60 minutes, because those times seem to be within general root canal treatment time.

On the side note, we verified 2.5mg/ml of mixtired catechins were able to occurrence no colony formation at 24h action even in the 10^9 CFU / ml of *E.faecalis* through preliminary experiments.

Finally, differences in several catechin form types may have influenced the differences in MBC values. Four types catechin forms mixture solution was used in this study. Thereby MBC at each action time might be higher than isolated single catechin like as EGCG.

The results of this study strongly indicated that high concentration of catechin solution should be necessary to kill *E.faecalis* on the environment of intractable periapical periodontitis within a short time as a root canal irrigant.

Although catechin has known beneficial health effects and no severe adverse effects; however, taking over dosage of them induce deleterious effects including hepatic injury in some cases [83,84].

Therefore usage high concentration of catechin solution has risk to side effect even using as root canal irrigation. The results obtained so far suggest that catechin may be difficult to use as a root canal irrigant, because a high concentration of catechin should necessary required to kill *E.faecalis* in a short time. However application as intracanal medication is not necessary of high concentration compare to root canal irrigant because intracanal medication attacks bacteria for long period, so usage of catechin is expectable new candidate not for root canal irrigant, but for intracanal medication.

Conclusion

Catechin has a benefit to anti-microbiologicl effect against *E.faecalis.*

However to determine Satisfactory anti-microbiological effect within a 60 minutes, high concentration of MIC is necessary such as 100-300mg/ml. Therefore usage of catechin in future root canal treatment might be recommended not for candidate of root canal irrigant but for candidate of intracanal medication.

Conflicts of Interest

There are no conflicts of interest.

Conflict of interest statement

The authors have declared that no competing interests exist.

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