치의학석사 학위논문

Antifungal efficacy of a synthetic human β-defensin-3-C15 against *Candida albicans*-infected dentin infection model

Candida albicans 상아질 감염 모델을 이용한 human β-denensin-3-C15 합성 펩타이드의 항진균효과

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-ABSTRACT-

Antifungal efficacy of a synthetic human β-defensin-3-C15 against *Candida albicans*-infected dentin infection model

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Objectives

The purposes of this *ex vivo* study were (1) to assess the anti-fungal efficacy of a synthetic human β-defensin-3-C15 (HBD3-C15, 15 amino acids in length) peptide against *C. albicans* biofilms and (2) to evaluate the drug potential of synthetic HBD3-C15 peptide as a substitute of calcium hydroxide drug in the fungi-infected root canal. In addition, (3) a recent issue regarding the 'Human Root Dentin Block' approach that proposed the usefulness as *ex vivo* intra-canal fungal infection model was also discussed.

Materials and methods

Six millimeter height of human dentin blocks (internal diameter: 0.5 mm) were infected with *C. albicans* for three weeks. Non-medicament gel (n = 8), saturated calcium hydroxide mixed with distilled water (n = 8), non-functional HBD3 peptide gel (n = 8), and synthetic HBD3-C15 peptide gel (n = 8), were tested. After one week of

medicament, the debris of dentinal tubules at the depth of 200 and 400 μm were collected using Gates-Glidden drills from root canal lumen. Efficacy of medicaments was assessed by measuring Colony Forming Unit (CFU) of *C. albicans* after 72 hours of 37°C, 5% CO_2 incubation. Data were statistical analyzed with Kruskal-Wallis and Wilcoxon test using SPSS version 23.

Results

The synthetic HBD3-C15 peptide group demonstrated significantly lower CFU values than the non-medicament gel group at both depths ($p < 0.05$). The synthetic HBD3-C15 peptide group also shows significantly lower CFU values than the non-functional HBD3 peptide group at both depths ($p < 0.05$). Nonetheless, there was no significant difference in the CFU values between synthetic HBD3-C15 and calcium hydroxide at both depths ($p > 0.05$).

There was no significant difference in the CFU values of the inner layer and outer dentinal layer for any group ($p > 0.05$).

Conclusions

The synthetic HBD3-C15 peptide has an anti-fungal efficacy against *C. albicans* biofilms. The synthetic HBD3-C15 peptide showed comparable anti-fungal activity to calcium hydroxide group in *C. albicans*-infected human root dentin model. The dentin block model effectively reproduced *ex vivo* situation to evaluate anti-fungal activity of intra-canal medicaments.

Keywords

Anti-fungal efficacy, human dentin block, *C. albicans* biofilms, synthetic human β-defensin-3-C15 peptide, colony forming unit.

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I. INTRODUCTION

(Background of C. albicans)

Among the approximately 50,000 species of fungi, more than 200 species are common to cause diseases in vertebrate animals and humans.[1] *Candida albicans* is a well-known fungis. *Candida* species are pathogenic fungi and they can be appeared in the oral cavity, gastrointestinal tract, anus and vaginal canal of healthy people.[2] In the oral cavity of both medically and healthy compromised human beings, *C. albicans* species is detected easily. In the oral cavity, the percentage *C. albicans* has been annonced to be 30% to 45% in healthy adults.[3,4] *C. albicans* may duplicate by budding, which results in the forming of yeast cells.[5]

(Infection of C. albicans into dentinal tubules of root canal)

Biofilm can be described an irreversibly attached surface which is a community of microorganisms.[6] *C. albicans* has the ability to form biofilms on diverse surfaces. Forming biofilms easily than other *Candida* species can be one of the reasons why *C. albicans* is considered more pathogenic.[7] *C. albicans* has ability to readjust to a spectum of extreme environments, such as alkali pH, which allows *C. albicans* to produce and withstand in the neutral pH in most tissues including oral environment.[8]

(Antifungal endodontic treatment of C. albicans)

It has been well-known that *Candida* species are resistant to some intra-canal medicaments commonly used in endodontics. *C. albicans* were highly against to calcium hydroxide among endodontic medicaments.[9] Also *Candida* species showed higher resistance to aqueous calcium hydroxide solution than *E. faecalis.*[10] The alkali pH of calcium hydroxide solution could not kill *C. albicans* because *C. albicans* endures in alkali pH values.[8] Furthermore, calcium hydroxide solution can supply the Ca^{2+} ions which are crucial to keep the production and morphogenesis of *Candida* species.[11] Previous study shows that aqueous calcium hydroxide was ineffective for *candidal* growth with colonies that were too numerous to count.[12]

(Background of intra-canal medicaments)

Intra-canal medicaments is a drug for treating root canal infections. There are several intra-canal medicaments commonly used such as ledermix, formocresol, calcium hydroxide, chlorhexidine et al. Ledermix is a paste that contains tetracycline antibiotic and corticosteroid agent. Its combination might have a chance to decrease flare-ups. Nonetheless, the suitability of the corticosteroid has been questioned as regard to the infection.[13]

Formocresol had been also widely used before. It is a represented agent with usefulness against the bacteria found in an infected root canal.[14] However, it has toxicity to vital tissue. When it is applied to the periapical tissue, it has a potential of causing a painful reaction.[15,16]

Calcium hydroxide has become a more popular intra-canal medicament. It is a potent anti-bacterial agent against the bacteria within an infected root canals by consisting of a highly alkaline environment.[17] Calcium hydroxide has a beneficial influence on periapical healing and inflammation.[18]

However, one of the previous studies shows that yeasts were equally or more resistant to alkali pH than *E. faecalis* on calcium hydroxide.[10] In the plasma membrane of the yeast cells, proton pumps are dependent on the resistance of the high pH by oral yeasts.[19,20] Moreover, hydroxyl ions of calcium hydroxide do not immediately scatter through dentin because hydroxyapatite has a buffering capacity. Buffering capacity of the dentin leads that bacteria may be escaped from exposure to hydroxyl ions at the beginning stage.[21]

Chlorhexidine is a broad-spectrum antimicrobial agent. Its positively charged molecules absorb onto dentin[22] and allow microbial colonization prevention.[23] However, the dentin must be open to chlorhexidine for a longer time than that afforded by irrigation to achieve a substantive antimicrobial activity.[24] Chlorhexidine was also reported that it was not effective to kill microbes in mature multispecies biofilms.[25]

(Model for experiments of intra-canal medicament)

One of the main goals is to establish a model that could standardize in doing the experiments on the various root canal medicaments. Root dentin powder model can be made from crushed extracted teeth into powder.[27] It can be separated in quantities because dentin powder consists of small particle size[27] Microorganisms and root canal medicaments can be added on dentin powder[26,28] However, if

autoclaving was done after powdering, there is a possibility that the physical properties of the dentin could be changed.[28] Moreover, small mineral particles from the disk used to grind dentin can be add up to grind dentin could be included within the sample matrix.[27]

Root dentin blocks are cylindrical dentin specimens which is 4 mm in height, 6 mm in diameter, and a widen canal.[28] The model proved quite precise and looks convenient for *ex vivo* experiment on root canal medicaments. On the other hand, the model is hard on technique and time consuming, which can be the study of only those microbes that penetrate the dentin block in a limited period.[29]

(Background of synthetic HBD3 peptide)

The human β-defensin-3 (HBD3) is a cationic peptide. The HBD3 has strong antibacterial and immuno-regulatory activities.[29] The HBD3 is showed in epithelial tissues, which contributes to the basic of defense between the environment and the microorganism.[30] The HBD3 is induced from heat and lipopolysaccharide of human dental pulp cell. As a whole HBD-3 is a peptide of 67 amino acids in length, it is unstable and thus has a short-life.[31] Synthetic peptide of 15-22 amino acids in length has been used as an antibacterial agent. Synthetic derivatives have been demonstrated that has significant antimicrobial activity comparable to the natural antimicrobial peptides.[20]

(Antimicrobial mechanism of HBD3 peptide)

The cationic property allows the HBD3 to cooperate with the membrane of penetrating microorganism which are negative due to lipopolysaccharides (LPS) and lipoteichoic acid (LTA) existing in the cell membrane. The HBD3 has affinity to the binding site of bacterial membrane compared to cationic ions Ca^{2+} or $Mg^{2+,}$ which give as bridges between LPS molecules. The HBD3 can exchange with those ions and it can displace cations. As affecting the stability of the membrane, the HBD3 can change the electric potential of membrane. The HBD3 can pass across the membrane, form pore complex, and thus cause membrane depolarization and cell lysis of bacteria.[32]

(Purposes)

This study begins with recent reports of calcium hydroxide treatment failure to treat *C. albicans*, and interest of synthetic human β-defensin-3-C15 has been proposed as an antifungal medicament. Therefore, the specific aims of this *ex vivo* study included (1) to assess the anti-fungal efficacy of a synthetic human β-defensin-3 (HBD3-C15, 15 amino acids in length) peptide against *C. albicans* biofilms and (2) to evaluate the drug potential of synthetic HBD3-C15 peptide as a substitute of calcium hydroxide drug in the fungi-infected root canal. In addition, (3) a recent issue regarding the 'Human Root Dentin Block' approach that proposed the usefulness *ex vivo* intra-canal fungal infection model was also discussed.

II. MATERIALS AND METHODS

1. Human root dentin block model

This study was carried out under approval of the Institutional Review Board of Seoul National University Dental Hospital, Seoul, Korea (IRB Number : CRI15007).

First of all, human mandibular premolars with single root and a straight canal with fully formed apex were selected through orthodontic treatment extractions Root surfaces were cleaned of calculus by using ultrasonic scaler (EMS) or curette. The middle one-third of each root was sliced in height of 6 mm root block. The cementum was removed from the root surfaces. Root canal was enlarged by using #2 Gates-Glidden bur which is 0.5 mm in diameter (ISO size). Smear layer was removed by using 17% EDTA and 2.5% NaOCl in ultrasonic bath each for 5 mins. Dentin blocks were washed three times with distilled water three times and sterilized by autoclaving for 15 mins at 121°C.

2. Infection of *C. albicans* biofilms into human dentin blocks

Twenty microliter of *C. albicans* stock was used as streaking source on Yeast Mold agar plate to get a single colony. A single colony of *C. albicans* was incubated with 10 ml Yeast Mold broth for 12 hours at 37°C, 5% CO_2. 100 µl volume was taken after the initial incubation and further incubated again with new 10 ml Yeast Mold broth for 6 hours to reach the mid log phase. The prepared sample was diluted to adjust to optical density (O.D.) less than 1. After adjusting of *C. albicans* and Yeast Mold broth to total volume 10 ml into conical tubes, and the dentin blocks were putting to the conical tubes together. Conical tubes were incubated for three weeks at 37°C, 5% CO_2. The Yeast Mold broth was replaced every two days.

After three weeks for dentinal tubule infection, the Scanning Electron Microscope (SEM) was used to confirm dentinal tubule infection of *C. albicans*. Magnification was x500, x2000, x5000.

3. Assessment of anti-fungal activity

Four groups of canal medicaments were as follows;

Synthetic HBD3-C15 peptide gel 50 µg/ml, non-medicament gel group, non-functional HBD3 peptide

gel 50 μg/ml, saturated calcium hydroxide group.
20% polyvinylpyrrolidone was used as a solvent of synthetic HBD3-C15 peptide gel, non-functional HBD3 peptide gel. In case of calcium hydroxide, distilled water was used as a mixing vehicle.

Synthetic HBD3-C15 peptide (NIBEC, Seoul, Korea) was prepared by F-moc-based chemical solid phase synthesis and were composed of 15 amino acids. As a control peptide, the mismatched sequence of the HBD3 peptide was prepared using a same method.

After three weeks of infection, the dentin blocks were fixed with paraffin wax in the petri dishes, side faces of dentin blocks were varnished with manicure to prevent leakage of root canal medicaments through side faces. 1.5 μl of intra-canal medicaments was injected into root canal lumen and incubated for one week at 37°C, 5% CO_2. After one week of medicament, all root canals of dentin blocks were washed with sterile saline, and dried with paper point. Calcium hydroxide groups were treated with 5% sodium thiosulfate to remove residual drug effect. Debris of inner dentin layer from root canal lumen was harvested by using #3 Gates-Glidden bur (0.7mm in diameter, ISO size). Debris of outer dentin layer from root canal lumen was collected by using #4 Gates-Glidden bur (0.9mm in diameter, ISO size) **(Figure 1)**. Collected debris with Yeast Mold broth were incubated for 12 hours at 37°C, 5% CO_2. Then, the values of optical density (O.D) and Colony Forming Unit (CFU) of four groups were measured.

4. Statistical analysis

After checking a basic assumption for the normality and the equality of variance by measuring O.D and CFU values, the Kruskal-Wallis and Wilcoxon test were performed to compare the difference between the groups using SPSS version 23. A *p*-value of less than 0.05 was considered statistically significant.

III. RESULTS

1. Confirmation of biofilm formation and dentinal tubule infection

Biofilm formation of *C. albicans* was confirmed after three weeks incubation by using Scanning Electron Microscope (SEM). Tubular penetration depth of *C. albicans* was approximately 300 μm (range 0~500 μm) from root canal lumen (**Figure 2**).

2. Measurement of Colony Forming Unit

The mean and standard deviation of CFU values from the inner and outer depths of the dentinal tubules of all groups were below **(Figure 3)**.

At the depth of 200 μm, the synthetic HBD3-C15 peptide had a significant anti-fungal effect compared to non-medicament gel group ($p < 0.05$) and to non-functional HBD3 peptide ($p < 0.05$).

At the depth of 400μm, the synthetic HBD3-C15 peptide had a significant anti-fungal effect compared to non-medicament gel group ($p < 0.05$) and to non-functional HBD3 peptide ($p < 0.05$).

At the depth of 200 μm, calcium hydroxide had a significant anti-fungal effect compared to non-medicament gel group ($p < 0.05$). However, at the depth of 400 μm, calcium hydroxide had no significant anti-fungal effect compared to non-medicament gel group ($p > 0.05$).

Nonetheless, there was no significant anti-fungal effect between synthetic HBD3-C15 peptide and calcium hydroxide at both depths for each ($p > 0.05$).

There was no significant difference in the CFU values between the inner and outer dentinal samples for any group ($p > 0.05$).

IV. DISCUSSION

(Infection of C. albicans into dentinal tubules of root canal)

C. albicans has ability to form biofilms on diverse surfaces. *C. albicans* is pathogenic due to the ability of forming biofilms easily.[7,8] In the present study, *C. albicans* penetrated into the dentinal tubules after three weeks of infection of dentin blocks. Tubular penetration depth of *C. albicans* was approximately 300μm from root canal lumen. EDTA solution has a potent effect of demineralizing, it makes dentin soft and keep dentin tubules to get enlargement and denaturation of the collagen fibers.[33] These effects may easily cause infection of *C. albicans* into dentin blocks which were previously treated with 17% EDTA. Furthermore, *C. albicans* has ability to adapt to an extreme environment field.[8] Such qualification also allows *C. albicnas* to grow into the dentinal tubule.

(Penetration of C. albicans into dentinal tubules of root canal)

C. albicans can be colonized at the dentinal surface and the shape of *C. albicans* is usually spherical or oval. However, the chance of infection was low and no infection into dentinal tubules was detected.[34] In this study, the lumen of root canal walls were covered with *C. albicans* with formation of densed colonies. *C. albicans* were also detected moving into dentinal tubules with variable depths. The previous study shows the difference of growth between *C. albicans* and *E. faecalis*. *C. albicans* penetrate slightly by hyphae and yeast cell, whereas *E. faecalis* pass through deeply. In present study the penetration of dentin proves a possible infection by *C. albicans*.[35] However, the size of root canal lumen was accounted as a limitation.

(Difficulty of penetration of C. albicans into dentinal tubules of root canal)

An *in vitro* study reports the penetration of *C albicans* cells into the human dentinal tubules lightly, that the number of tubules penetrated is low.[35] Nonetheless, other studies demonstrate the *in vivo* penetration of *C albicans* into dentinal tubules is forceful.[36] [37] The extracellular matrix proteins, type I collagen and fibronectin is necessary for *C.albicans* to adhere to dentin.[38] Mainly, collagen and Ca^{2+} of dentin content

decrease as using agent such as NaOCl and EDTA in order to remove smear layer of human dentin block. *C. albicans* show less attachment and penetration ability to dentin due to altered state of composition.

(Anti-fungal endodontic treatment of C. albicans)

The previous studies show that composing a highly alkaline environment is an effective anti-bacterial agent against the bacteria.[17,18] In this study, the alkalinity of calcium hydroxide solution could reduce *C. albicans* comparable to synthetic HBD3-C15 peptide at both depths. However, *Candida* species are resistant to some of intra-canal medicaments commonly used in endodontics[9-12] *C. albicans* cells extremely withstand to calcium hydroxide among endodontic medicaments.[9,10] Also, *Candida* species show more resistance to aqueous calcium hydroxide solution than *E. faecalis.*[10] Similarly, in present study, calcium hydroxide showed no significant anti-fungal effect compared to non-medicament group at the depth of 400μm, which is consistent with the previous findings.

(Anti-fungal efficacy of a synthetic HBD3-C15 peptide)

HBD3 is known to have strong antimicrobial activity.[12] Nonetheless, few studies have performed to access anti-fungal efficacy of HBD3 on *C. albicans* using dentin blocks. Among a huge family of antimicrobial peptides, defensins are broad-spectrum members, consisting of antimicrobial effect on epithelial host defense in the skin and elsewhere.[39] It is consistent in this study that synthetic HBD3-C15 peptide had significant anti-fungal effect compared to non-medicament gel group and non-functional HBD3 peptide at both depths. Calcium hydroxide showed comparable effectiveness to synthetic HBD3-C15 in eliminating *C. albicans* at both depths in dentinal tubule. This is consistent with previous studies of microorganisms such as *E. faecalis* or *Candida* species' persistency despite the use of antimicrobial agents.[40] However, the difference between that synthetic HBD3-C15 peptide and calcium hydroxide was not significant at both of depths.

(Drug potential of synthetic HBD3-C15 peptide as a substitute of calcium hydroxide)

Calcium hydroxide was not effective in treating *C. alibcans* in the previous studies.[9-12,19,20] Using filter paper discs immersed in yeast were tested to compare the efficacy of disinfectant solutions including

calcium hydroxide. The results showed that treating calcium hydroxide was not effective than other medicaments.[16] In the present study, the anti-fungal efficacy of synthetic HBD3-C15 peptide and saturated calcium hydroxide were comparable at inner and outer dentin layers. This indicated that a synthetic HBD3-C15 peptide had a drug potential as a substitute of saturated calcium hydroxide.

(Advantages of synthetic HBD3-C15 peptide as an intra-canal medicament)

Synthetic HBD3-C15 peptide was used as a gel state in this study. The peptide solvent used was 20% polyvinylpyrrolidone solution. The viscosity of the gel kept the synthetic HBD3-C15 peptide within root canal lumen and to function as an intra-canal medicament. The synthetic HBD3-C15 peptide analogs can be modified in regard to which microbes are targeted. Because of the significant anti-fungal activity of synthetic HBD3-C15 peptide, synergistic efficacy could be further studied with calcium hydroxide or chlorhexidine or other intra-canal medicaments.

(Usefulness of human root dentin model ex vivo intra-canal fungal infection)

In this *ex vivo* study, 6mm height of human root dentin block models were designed to resemble intraoral environment. In this study, tubular penetration of *C .albicans* was observed using SEM on experimental human dentin block. In evaluating anti-fungal activity of intra-canal medicaments, *ex vivo* dentin block situation proved its usefulness. This means human root dentin block may efficiently supply the environment as same as the oral cavity. Alternatively, root dentin powder model can be an option.[27,28] However, there is a possibility that physical properties of the dentin could be modified via sterilization techniques and a possible disc debris contamination through grinding process to make dentin power.[28]

(Needs for further specific materials and methods)

It was difficult to collect pure debris of each layer separately. Because dentin debris form two depths was obtained using sequential drilling from the inner dentin layer to outer dentin layer. This means that the debris of inner layer and outer layer may have mixed up to each other and it should have influenced the CFU values. Moreover, the chance of penetration of *C. albicans* is low compared to bacteria such as *E. feacalis*. It means there is possibility that some of colony from inner depth and outer depth may have

come from human source. Also, the diameter of root canal lumen of dentin blocks was not identical. This is because all the mandibular premolars varied in size as it was not possible to get multiple mandibular premolars from a single patient. Because of the difference of root canal lumen size, the debris collected should have been standardized by the weight.

(Needs for further research of anti-fungal mechanism of the non-functional HBD3 peptide)

In this study, synthetic HBD3-C15 peptide has a significant anti-fungal activity compared to non-medicament gel group and non-functional HBD3 peptide at both depths. However, non-functional HBD3 peptide also shows lower CFU values compare to non-medicament gel group at both depths, which is not consistent with previous studies of that HBD3 peptide has very low cytotoxicity against host cells.[41] There is a chance of variations regarding infection into dentinal tubules among groups. Although three weeks incubation period allowed the fungus to thoroughly penetrate the root canal lumen, the present dentin infection methodology could not be same, deep and homogenous suspension in all specimens. Further study is need for comparison between non-medicament gel group and non-functional HBD3 peptide.

(Needs for further research of anti-fungal mechanism of the synthetic HBD3 peptide at molecular level)

Future studies for enhanced experiments will be standardize in regard to premolar, debris and drilling. Mandibular premolars should be gathered from a single sex, similar ages and under selected condition as possible. Collecting pure debris should be standardized by using a micro weighing protocol. Sequential drilling may be improved by using automatic gear. Synergy effect between synthetic HBD3-C15 peptide and present intra-canal medicaments could be a good option in the future as the synthetic HBD3-C15 peptide has anti-fungal efficacy.

V. CONCLUSIONS

This was an experimental study of human root dentin blocks from extracted premolars as a purpose of orthodontic treatment. In this study, the Kruskal-Wallis test and Wilcoxon test were used to statistically analyze the efficacy of a synthetic HBD3-C15 peptide over experimetal groups. This might allow some insights into the spectrum and management of a synthetic HBD3-C15 peptide as an intra-canal medicament. The major results were as follows.

1. The synthetic HBD3-C15 peptide has an anti-fungal effiect against *C. albicans* biofilms.
2. The synthetic HBD3-C15 peptide can be an useful option as a substitute of calcium hydroxide in intra-canal medicament treating intra-fungal infection.
3. The human root dentin block model effectively reproduced the usefulness *ex vivo* situation to evaluate anti-fungal activity of intra-canal medicaments.
4. Further reserch of using the synthetic HBD3-C15 peptide should be needed as an intra-canal medicament as it has anti-fungal effect on *C. albicans.*
5. Further research of anti-fungal mechanism of the synthetic HBD3-C15 peptide should be needed at molecular level.

VI. REFERENCES

1 Moore-Landecker E. Fundamentals of fungi. 4th ed. Upper Saddle River (NJ): Prentice Hall; 1996.

2 Soll DR. *Candida* commensalism and virulence: the evolution of phenotypic plasticity. Acta Trop 2002;81:101-110.

3 Lucas VS. Association of psychotropic drugs, prevalence of denture-related stomatitis and oral candidosis. Community Dent Oral Epidemiol 1993;21:313-316.

4 Arendorf TM, Walker DM. The prevalence and intra-oral distribution of *Candida albicans* in man. Arch Oral Biol 1980;25:1-10.

5 Jose´ F. Siqueira Jr. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-641.

6 Bilge Hakan Şen. Colonization of *Candida albicans* on cleaned human dental hard tissues. Arch Oral Biol 1997;42:513–520.

7 Haynes K. Virulence in *Candida* species. Trends Microbiol 2001;9:591-596.

8 Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol 2001;9:327-335.

9 Waltimo TM, Ørstavik D, Sire´n EK, Haapasalo MP. In vitro susceptibility of *Candida albicans* to four disinfectants and their combinations. Int Endod J 1999;32:421-429.

10 Waltimo TM, Sire´n EK, Ørstavik D, Haapasalo MP. Susceptibility of oral *Candida* species to calcium hydroxide in vitro. Int Endod J 1999;32:94-98.

11 Holmes AR, Cannon RD, Shepherd MG. Effect of calcium uptake on *Candida* morphology. FEMS Microbiol Lett 1991;61:187-193.

12 J.W. Ferguson, J.F. Hatton, M. Jane Gillespie. Effectiveness of Intracanal Irrigants and Medications against the Yeast *Candida albicans*. J Endod 2002;28:68–71.

13 Abbott PV, Heithersay GS, Hume WR. Release and diffusion through human tooth roots *in vitro* of corticosteroid and tetracyclic trace molecules from Ledermix R paste. Endod Dent Traumatol 1988;4:55-62.

14 Mahmoud T, Robert H et al. Clinical implications of the smear layer in endodontics: A review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:658-666.

15 Powell DL, Marshall FJ, Melf RL. A histopathologic evaluation of tissue reactions to the minimum effective doses of some endodontic drugs. Oral Surg 1973; 36: 261-272.

16 Harrison JW, Bellizzi R, Osetek EM. The clinical toxicity of endodontic medicaments. J Endod 1979;5:42-47.

17 Bystrom A, Claesson R. Antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol, 1985;1:170-175.

18. P. C. Foreman, I. E. Barnes. A review of calcium hydroxide Int Endod J, 1990;23:283–297.

19. Monk BC, Perlin DS. Fungal plasma membrane proton pumps as promising new antifungal targets. Crit Rev Microbiol 1994;20:209 –223.

20 Monk BC, Mason AB, Abramochkin G, Haber JE, Seto young D, Perlin DS. The yeast plasma membrane proton pumping ATPase is a viable antifungal target. I. Effects of the cysteine-modifying reagent omeprazole. Biochim Biophys Acta 1995;1239:81–90.

21 Wang, J.D. Hume, W.R. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. Int

Endod J 1988;21:17-26.
22 Emilson CG. Susceptibility of various microorganisms to chlorhexidine. Scand J Dent Res 1977;85:255-265.
23 Parsons GJ, Patterson SS, Miller CH, Katz S, Kafrawy AH,Newton CW. Uptake and release of chlorhexidine by bovine pulp and dentin specimens and their subsequent acquisition of antibacterial properties. Oral Surg Oral Med Oral Pathol 1980;49:455-459.
24 Heling I, Sommer M, Steinberg D, Friedman M, Sela MN. Microbiological evaluation of efficacy of chlorhexidine in sustained release device for dentine sterilization. Int Endod J 1992;25:15-19.
25 Y Lee, SH Han, SH Hong, JK Lee, H Ji, KY Kum. Antimicrobial efficacy of a polymeric chlorhexidine release device using in vitro model of *Enterococcus faecalis* dentinal tubule infection. J Endod 2008;34:855– 858.
26 Isabelle Portenier et al. Inactivation of the Antibacterial Activity of Iodine Potassium Iodide and Chlorhexidine Digluconate Against *Enterococcus faecalis* by Dentin, Dentin Matrix, Type-I Collagen, and Heat-Killed Microbial Whole Cells. J Endod 2002;28:634–637.
27 M. Haapaslo et al. Inactivation of local root canal medicaments by dentine: an *in vitro* study. Int Endod J 2000;33:126-131.
28 M. Haapasalo, D. Ø´rstavik. *In vitro* infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375–1379.
29 F.L. Brancatisano, G. Maisetta. Reduc.ed Human Beta Defensin 3 in Individuals with Periodontal Disease. J Dent Res 2011;90:241-245.
30 Weinberg A, Krisanaprakornkit S, Dale BA Epithelial antimicrobial peptides: review and significance for oral applications. Crit Rev Oral Biol Med 1998;9:399–414.
31 Josef Johann Schneider,Angela Unholzer et al. Human defensins, J Mol Med 2005;83:587-595.
32 Hancock RE. Peptide antibiotics. Lancet 1997;249:418-422.
33 R Garberoglio, C Becce. Smear layer removal by root canal irrigants: a comparative scanning electron microscopic study. Oral Surg 1994;78:359–367.
34 José F. Siqueira, Bilge H. Sen . Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Endod 2004:97;632–641.
35 T. M. T. Waltimo, D. Orstavik, E. K. Siren, Haapasalo. In Vitro Yeast Infection of Human Dentin. J of Endod 2000;26:207–209.
36 M.J. Kinirons. Candidal invasion of dentine complicating hypodontia. Br Dent J 1983;154: 400–401.
37 D.D. Damm, B.W. Neville, R.H. Geissler Jr., D.K. White, J.F. Drummond, G.A. Ferretti. Dentinal candidiasis in cancer patients. Oral Surg Oral Med Oral Pathol 1988;65:56–60.
38 SA Klotz, MJ Rutten, RL Smith, SR Babcock, MD Cunningham. Adherence of *Candida albicans* to immobilized extracellular matrix proteins is mediated by calcium-dependent surface glycoproteins. Microb Pathog 1993;14:133–147.
39 Tomas Ganz. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003;3:710-720.
40 I. Heling, M. Sommer, D. Steinberg, M. Friedman, M.N. Sela. Microbiological evaluation of the efficacy of chlorhexidine in a sustained-release device for dentine sterilization. Int Endod J 1992;25:15–19.
41 Batoni, G.; Maisetta, G.; Esin, S.; Campa, M. Human Beta-Defensin-3: A Promising Antimicrobial Peptide. Mini Rev. Med. Chem. 2006;6:1063-1073.

FIGURES

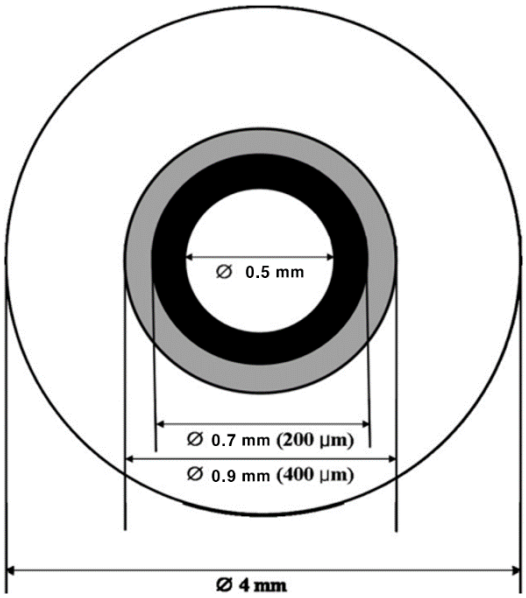
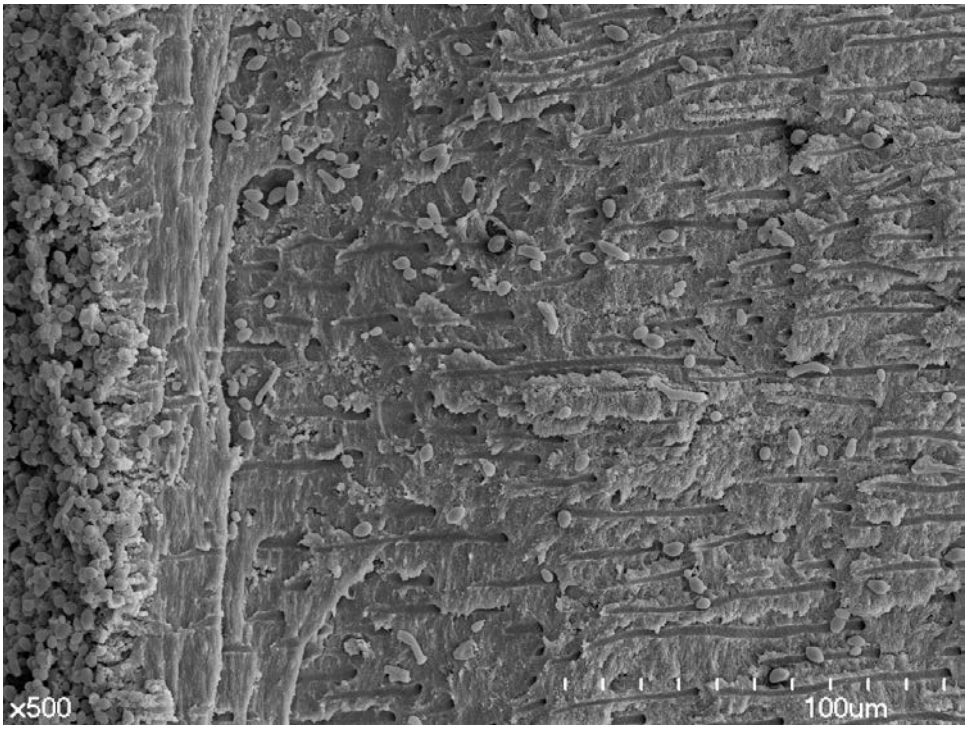


Figure 1.

#3 GG bur which is 0.7 mm in diameter, inner dentin layer of depth 200 µm from root canal lumen.

#4 GG bur which is 0.9 mm in diameter, outer dentin layer of depth 400 µm from root canal lumen.

(a)



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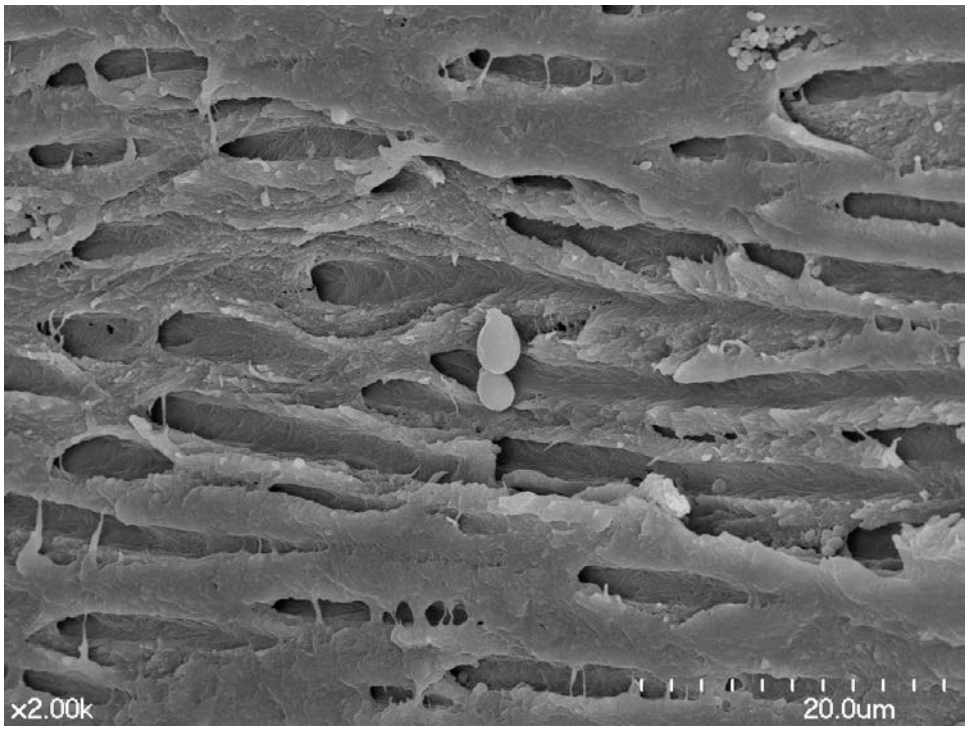


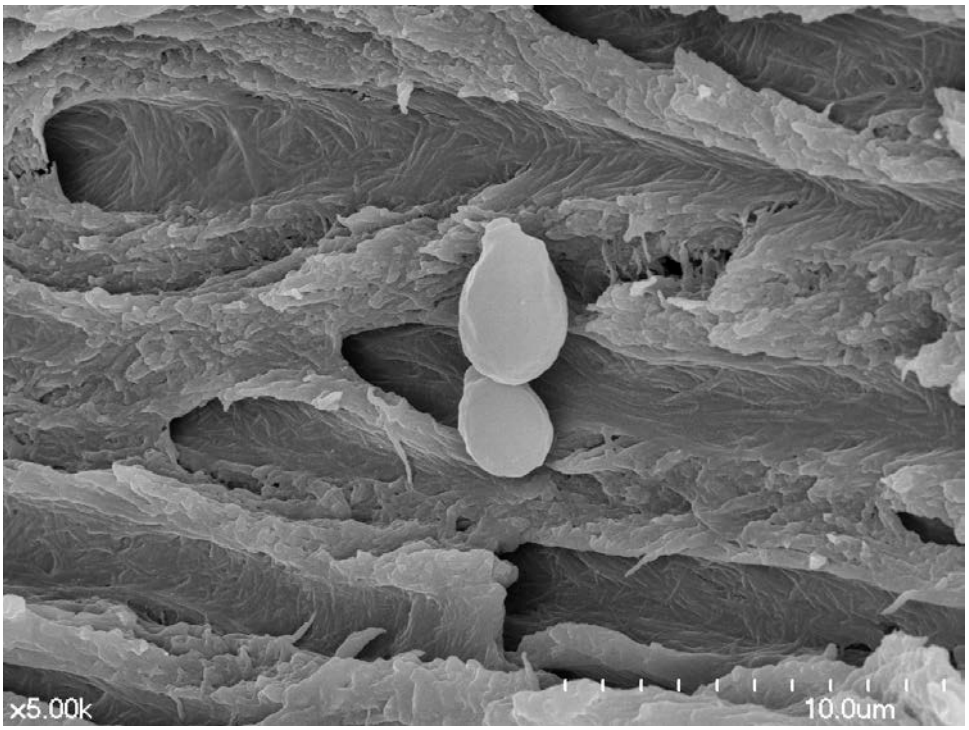
(c) 

Figure 2.

Scanning electron microscopic shows the penetration of C. albicans into the dentinal tubules after three weeks of infection of dentin blocks. (a: x500, b: x2000, c: x5000)

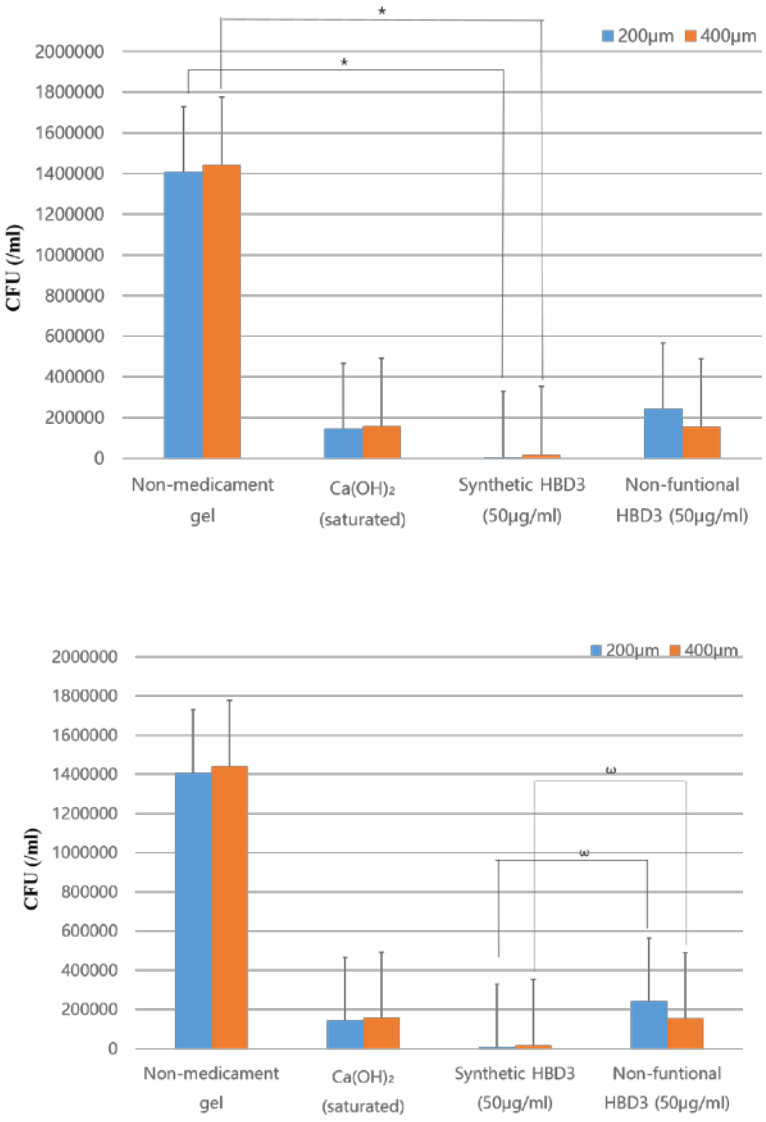


Figure 3.

Colony forming unit values of the inner layer (200μm) and outer layer (400μm) dentinal samples obtained from four groups. An asterisk (*) indicates synthetic HBD3 peptide has a statistically significant difference from non-medicament gel at both depths for each ($p < 0.05$). An omega (ω) points out synthetic HBD3 peptide has a statistically significant difference from non-functional HBD3 peptide at both depths for each ($p < 0.05$).

국문초록

Candida albicans 상아질 감염 모델을 이용한 human β-denensin-3-C15 합성 펩타이드의 항진균효과

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1. 연구목적

이번 *ex vivo* 연구의 목적은 첫째, *C. albicans* biofilm에 대한 synthetic human β-defensin-3 (HBD3, 15개의 아미노산)에 대하여 anti-fungal 효과를 측정한다. 둘째, 진균이 감염된 근관에서 현재 사용되는 대표적인 intra-canal fungal drug인 calcium hydroxide와 synthetic HBD3-C15의 첨약제로서의 효능을 비교한다. 셋째, 최근의 Human Root Dentin Model에 관한 주제로 *ex vivo*에서 intra-canal fungal infection model로의 유효성을 알아본다.

2. 연구방법

6mm 길이의 상아질 감염 모델(내부 직경 0.5mm)에 *C. albicans*를 3주간 접종한다. Non-medicament gel (n=8), 증류수에 포화시킨 calcium hydroxide (n=8), non-functional HBD3 peptide gel (n=8), 그리고 synthetic HBD3-C15 peptide gel (n=8)의 첨약제를 사용한다. 약제 처리 일주일 후에, Gates-Glidden drill을 사용하여 치근 중앙으로부터 깊이 200㎛와 400㎛의 상아세관의 잔사를 얻는다. 잔사는 37℃, 5% CO_2에서 24시간 배양한 후 첨약제의 효능을 Colony Forming Unit으로 측정한다. 실험값은 SPSS 버전 23을 이용한 Kruskal-Wallis test와 Wilcoxon test로 분석한다.

3. 결과

Synthetic HBD3-C15 peptide 군은 깊이 200㎛와 400㎛ 모두에서 non-medicament 군보다 유의미하게 낮은 CFU 수치를 보였다 ($p < 0.05$). Synthetic HBD3-C15 peptide 군은 깊이 200㎛와 400㎛ 모두에서 non-functional HBD3 peptide 군보다 유의미하게 낮은 CFU 수치를 보였다 ($p < 0.05$). Synthetic HBD3-C15 peptide 군은 깊이 200㎛와 400㎛ 모두에서 calcium hydroxide 군과 비슷한 CFU 수치를 보였다 ($p > 0.05$). 모든 군에서 깊이 200㎛와 400㎛ 차이에 따른 CFU 수치의 차이는 없었다 ($p > 0.05$).

4. 결론

Synthetic HBD3-C15 peptide는 근관 첨약제로서 사람 상아질 모델에서 *C. albicans* 바이오필름에 대해 항진균효과가 있었다. Synthetic HBD3-C15 peptide는 사람 상아질 모델에서 calcium hydroxide와 비슷한 항진균효과를 보였다. 사람 상아질 감염 모델은 첨약제의 항진균효과를 평가하기 위한 *ex vivo* 상황을 효과적으로 재현하였다.

주요어

항진균효과, 사람 상아질 모델, *C. albicans* 바이오필름, synthetic human β-defensin-3-C15, colony forming unit.

학번

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