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Antibakterijski učinak endodontskog uloška na Enterococcus faecalis u dentinu zubnog korijena

Antibacterial Effects of Endodontic Dressings on Enterococcus Faecalis in Human Root Dentine

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Sažetak
Svrha istraživanja bila je procijeniti antimikrobnu aktivnost kalcijeva hidroksida (Calasept) i klorheksidina (CHX-a) u različitim koncentracijama u odnosu prema bakteriji *Enterococcus *faecalis u dentinu ljudskog korijena u dubini do 100 µm. **Materijal i metode:** Do standardne veličine (ISO 40) prošireno je 48 ljudskih korijenskih kanala te inokulirano 21 dan bakterijom *Enterococcus faecalis*. Nakon toga kanali su bili tretirani jednim od sljedećih preparata: 2-postotnim, 1-postotnim ili 0,2-postotnim klorheksidinskim gelom, gutaperkom koja otpušta CHX (aktivni štapić) i kalcijevim hidroksidom, a destilirana voda služila je kao kontrola. Na kraju jednotjednoga dezinfekcijskog razdoblja uzeti su uzorci dentina pomoću proširivača i H-pilice (ISO 45 i 50) te su nasađeni na ploče s Columbia agarom. Rast bakterija procjenjivao se brojenjem jedinica koje stvaraju kolonije (CFU-om; engl. colony forming units) i to nakon inkubacije od 24 i 48 sati. **Rezultati:** CHX gelovi penetrirali su u dentin čak do 100 µm. Dvopostotni CHX gel bio je malo jači od jednopostotnoga (p-vrijednost 0,0925)samo u uzorku perifernog dentina 48 sati nakon inkubacije, a 0,2 postotni je imao manji učinak na *E. faecalis* od 2-postotnoga (p-vrijednost 0,0191). U uzorku centralnog dentina nije bilo razlike u djelovanju CHX gelova. Općenito, CHX gelovi bili su učinkovitiji od drugih ispitivanih medikamenata. Nije bilo statistički veće razlike između Ca(OH)2 i destilirane vode. **Zaključak:** Za djelotvornu eliminaciju E. faecalis, posebice u dubljim slojevima dentina, potrebno je koristiti se CHX-om u koncentraciji od 1 posto i većoj. Između posjeta pacijenata liječniku, intrakanalni uložak 2-postotnoga CHX gela mogao bi uništiti E. faecalis in vivo.

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Ključne riječi
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Uvod

Bakterije i njihovi metaboliti najčešći su uzroci pulpalnih i periapikalnih bolesti i glavni razlog za neuspjelo endodontsko liječenje (1-7). Obavlja li se takav tretman u aseptičnim uvjetima, stope uspjeha su obično visoke - od 85 do 90 posto (8,9 - Sjögren i njegovi suradnici, 1990. te Kerekes i Tronstad, 1979.). Mnogo neuspješnih slučajeva uzrokovali su tehnički problemi tijekom liječenja, a ponekad po-

Introduction

Bacteria and their degradatory byproducts are the most common causes of pulpal and periapical diseases and the dominant reason for a failure of root canal treatment (1-7). When teeth are treated by root canal therapy in aseptic conditions, the success rate is generally high with 85% – 90% (8, 9). Many failure cases are caused by technical problems during treatment, some cases fail even when apparently

stupak nije uspio iako se sve tretiralo kako treba. Identificirano je mnogo čimbenika koji mogu uzrokovati neuspjeh endodontske terapije - ekstraradikularna infekcija, reakcije na strana tijela te prave ciste (3,4,5,10,11 - Sjögren i suradnici 1988., Nair i suradnici 1990, 1990a, 1993, 1999). No, najčešći su za to krivi mikroorganizmimi koji prežive liječenje i ostaju u apikalnim dijelovima punjenih korijenskih kanala (12,13 - Sjögren i suradnici 1997., te Sundqvist 1998.). Osim u glavnom kanalu, bakterije mogu živjeti u kriptama u cementu te u sekundarnim kanalima ili dentinskim tubulima (14). Na tim mjestima one uopće ne moraju biti zahvaćene kemijsko-mehaničkom preparacijom korijenskog kanala te se pokazalo da antibakterijski uložak u kanalu, između posjeta pacijenata liječniku, može djelovati baš na te bakterije (15,16 - Byström i suradnici 1985., Holland i suradnici 1992). Kalcijev hidroksid je snažna lužina i ima jak antibakterijski učinak na većinu bakterijskih vrsta u slučaju endodontskih infekcija (17). No, loše djeluje na **E. faecalis** (17-20 - Byström i Sundqvist 1985., Haapasalo i Ørstavik 1987. te Ørstavik i Haapasalo 1990.). Iako uvjeti u neliječenim korijenskim kanalima ne pogoduju rastu **E. faecalis**, tu se bakteriju često može naći u neuspješno liječenim zubima (21,22). Zato je potrebno pronaći intrakanalni uložak s jakim antibakterijskim djelovanjem na **E. faecalis** i visokom stopom penetracije u dentin. Posljednjih se godina klorheksidinski gel (CHX) koristi kao snažno dezinfekcijsko sredstvo u endodontskoj terapiji. Može se rabiti i kao sredstvo za ispiranje (20). Testovi difuzije na agaru dokazali su da snažno djeluje na **E. faecalis** (23 - Siqueira i suradnici 1998.). U tim istraživanjima koristila se CHX otopina s kratkim antimikrobnim djelovanjem u korijenskim kanalima (24,25 - Jung i suradnici 1999., White i suradnici 1997.). Za duže djelovanje u korijenskom kanalu dentin mora biti izložen CHX-u dulje nego što je to kod irigacije (26,27). Zato su se ispitivali CHX gelovi različitih koncentracija i gutaperke koje sadržavaju CHX, budući da se navodi kako imaju dugotrajno djelovanje (28).

Svrha ovoga istraživanja in vitro bila je procijeniti antimikrobnu aktivnost Ca(OH)2 i različite koncentracije CHX gelova u odnosu prema bakteriji **Enterococcus faecalis** u dentinu ljudskog korijena. Postavljena su dva glavna pitanja: najprije smo pokušali provjeriti prodire li **E. faecalis** u dentin. To smo ispitivanje ograničili na dentinske slojeve do dubine od 100 μm. Ako se pojavila infekcija, ispitivali smo antimikrobno djelovanje Ca(OH)2 i CHX-a u tim slojevima.

well treated. A number of factors have been identified as agents associated with failure of endodontic therapy including extraradicular infection, foreign body reactions and true cysts (3, 4, 5, 10, 11). However, most treatment failures are caused by microorganisms surviving the treatment procedure and persisting in the apical parts of root canals of obturated teeth (12, 13). Besides the main root canal, bacteria can also lodge in cementum crypts, secondary canals or dentine tubules (14). In these locations, bacteria may be unaffected by the chemomechanical preparation of the root canal. Thus, the use of an intracanal antibacterial dressing between appointments has been proved to eliminate surviving bacteria (15, 16). Calcium hydroxide, a powerful alkaline substance, has a pronounced antibacterial effect on most of the bacterial species found in endodontic infections (17). Nevertheless, it is poorly effective against *E. faecalis* (17, 18, 19, 20). Although the conditions found in the untreated canal generally do not favor the development of *E. faecalis*, it is often found in canals of teeth in which the previous treatment has failed (21, 22). Therefore it is necessary to find intracanal dressings with a high antibacterial effect on *E. faecalis* and with a high penetration rate in dentine. In recent years, chlorhexine gluconate (CHX) has emerged as an effective disinfection agent in endodontic therapy. It can be used effectively as an irrigant (20). Agar diffusion tests have also demonstrated a high antibacterial effect on *E. faecalis* (23). In these studies CHX was used as a solution, which has only a short antimicrobial effect in root canals (24, 25). For long-term activity of CHX in the root canal the dentine must be exposed to CHX for a longer time than that afforded by irrigation (26, 27). CHX gels in different concentrations and CHX containing gutta-percha points were therefore tested in this study, since they are said to have a long-term effect (28).

The aim of the present in vitro study was to assess the antimicrobial activity of $Ca(OH)_2$ and CHX in various concentrations with respect to *Enterococcus faecalis* in human root dentine. Two major questions were investigated: First, we tried to find out whether *E. faecalis* penetrates dentine. We restricted our investigation to dentine layers up to a depth of 100 μm. In the case of infection, we then analyzed the question of antimicrobial activity of $Ca(OH)_2$ and CHX in these layers.

Materijal i postupci

Za pokus se koristilo 48 nedavno ekstrahiranih ljudskih zuba. Bili su uronjeni 24 sata u 3-postotnu otopinu H2O2, kako bi se uklonile organske tvari i dezinficirala površina. Korijenski kanali bili su prošireni okruglim svrdlima i pilicama Hedström (VDW-Antaeos, Vereinigte Dentalwerke GmbH & Co KG) ISO veličine 015-040, do ISO 40. U skladu s tehnikom koju su opisali Haapasalo i Ørstavik (19), apeksi i krune bili su prerezani rotirajućom dijamantnom pilicom, te ohlađeni vodom (Horico, Hopf/Ringleb & Co. GmbH, Berlin, Njemačka) kako bi se stvorili usklađeni uzorci od 7 mm. Cement je uklonjen cilindričnim dijamantnim svrdlom na malom broju okretaja. Kako bi se spriječila dehidracija, uzorci su stalno bili u vodi. Zaostali sloj uklonjen je ispiranjem svih kanala s 10 ml Tubulicida (Dental Terapeutics AB, Saltsjö-Boo, Švedska) i to 30 sekundi i s 10 ml 3-postotnog NaOCl-a. Zatim su bili stavljeni 20 minuta u autoklav na 121°C. Nakon toga su osušeni sterilnim papirom, presvučeni izvana dvama slojevima laka za nokte te postavljeni u dno sterilne Petrijeve posude uz pomoć voska Kerr (Impression Compound, Kerr Italija).

Inokulacija

Inokulat **E. faecalis** (DSM 2570) koji je narastao preko noći na TSB-u i bio prilagođen na vrijednost od 0,5 prema McFarlandovoj ljestvici (1,5 x 108 bakterija/ml), injiciran je u svaki kanal. Inokulirani uzorci inkubirani su tri tjedna na 37 °C. Svaki su im dan bile dodane bakterije kako bi kanali bili puni.

Dezinfekcija

Uzorci su bili podijeljeni u pet eksperimentalnih skupina i jednu kontrolnu. U aseptičnim uvjetima korijenski su kanali tretirani jednim od sljedećih preparata: 2-postotnim CHX gelom, 1-postotnim CHX gelom, 0,2-postotnim CHX gelom, sredstvom koje otpušta CHX, kalcijevim hidroksidom Ca(OH)2 – Calaseptom (Calasept-Speiko -R - dr. Speier GmbH, Münster, Njemačka) te destiliranom vodom. Preparirani kanali svakog uzorka uneseni su uloškom do kraja (0,1 ml). Nakon što su postavljeni ulošci ili kontrola u kontrolnu otopinu, uzorci su sedam dana bili koronalno zabrtvljeni voskom i inkubirani na 37 °C. Ulošci nisu obnavljani.

Material and Methods

Forty-eight freshly extracted human teeth were used for the current experiments. They were stored in a 3% H_2O_2 solution for 24 hours to remove organic debris and disinfect the surface. The root canal of each specimen was enlarged throughout with round burs (reamer burs) and Hedström-files (VDW-Antaeos, Vereinigte Dentalwerke GmbH & Co KG) – ISO sizes 015-040 – to ISO 40. In accordance with the technique described by Haapasalo & Ørstavik (19), the apices and the crowns of the teeth were sectioned with the use of a rotating diamond saw and water irrigation (Horico, Hopf/Ringleb & Co GmbH, Berlin, Germany) to produce uniform specimens of 7 mm in length. Cementum was removed from the surface of the specimens by using a cylindrical diamond at low speed. To prevent dehydration, specimens were kept in water during all procedures. The smear layer was removed by rinsing each canal with 10 ml Tubulicid (Dental Terapeutics AB, Saltsjö-Boo, Sweden) for 30 s and 10 ml 3% NaOCl. The root specimens were autoclaved for 20 min at 121° C. They were blotted dry with sterile paper, coated externally with 2 layers of nail varnish and mounted in the bottom of a sterile Petri dish with Kerr wax (Impression Compound, Kerr Italia).

Inoculation

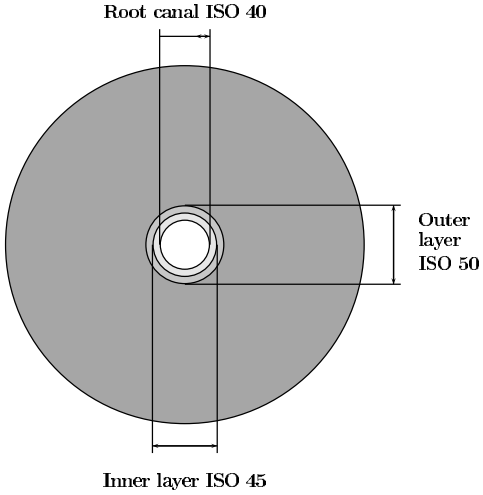
An inoculum of *E. faecalis* (DSM 2570), grown overnight in TSB and adjusted to a 0.5 turbidity reading on the McFarland scale (1.5 x 10^8 bacteria/ml), was injected into each canal. The inoculated specimens were incubated at 37° C for 21 days. Fresh bacteria were added daily to keep the canals full.

Disinfection

The specimens were divided into 5 experimental groups and 1 control group. Under aseptic conditions, the root canals were medicated with one of the following: 2% chlorhexidin gel, 1% chlorhexidin gel, 0.2% chlorhexidin gel, CHX-containing controlled-release device, calcium hydroxide $Ca(OH)_2$ as Calasept (Calasept-Speiko®, Dr. Speier GmbH, Münster, Germany) and aqua dest. The prepared canal of each specimen was injected with the intracanal dressing until full (0.1 ml). After placement of the dressings or control solution, the specimens were coronally sealed with wax and incubated at 37° C for 7 days. The medicaments were not replenished.

Uzorkovanje dentina korijenskog kanala

Na kraju svakoga dezinfekcijskog razdoblja svi su kanali bili temeljito isprani s 10 ml sterilne fiziološke otopine i osušeni suhim papirnatim štapićima. Zatim su uzeta dva uzorka dentina sterilnim svrdlima ISO 045 i 050 i Hedströmovim pilicama (VDW-Antaeos, Vereinigte Dentalwerke GmbH & Co KG) i to na sljedeći način: središnji uzorak uzet je svrdlom ili pilicom ISO 045 do dubine od 50 μm s površine korijenskog kanala, a periferni svrdlom ili pilicom ISO 050 s dubine od 100 μm s površine korijenskog kanala (Slika 1.). Budući da su svi kanali bili iste duljine i promjera (7 mm, ISO 040), iz svakoga njihova sloja izvađena je ista količina dentina. Uzorci su zatim preneseni na ploče s Columbia agarom te su ravnomjerno raspoređeni kako bi se osigurali isti uvjeti za rast bakterija. Ploče su inkubirane na 37 °C tijekom 48 sati. Nakon 24 i 48 sati izbrojene su jedinice koje stvaraju bakterije (CFU; engl. colony-forming units).



Slika 1. Shematski prikaz cirkumferentnog uzorkovanja dentina
Figure 1 Schematic representation of the circumferential sampling of the dentine

Statistička metoda

Statistička analiza obavljena je generaliziranim linearnim modelima (GLM-om), budući da je opažena pojava - **broj kolonija**, imala samo negativne vrijednosti te se smatralo da se podaci neće normalno raspodijeliti. Osim toga, podaci nisu bili homoscedastični, što je također uvjet za primjenu analize varijance (ANOVA). Srednji μ broja kolonija, nakon rasta, oblikovan je na sljedeći način:

$$*g*(\mu) = \mu^{0.5} = b$$

(*b* je učinak liječenja *B*, a g vezna funkcija koja opisuje vezu između linearnog prediktora i srednje vrijednosti).

Root dentine sampling

At the end of the disinfection period, each canal was thoroughly rinsed with 10 ml of sterile saline solution and blotted dry with sterile paper points. Two types of samples of dentine were then obtained by enlarging the canal with sterile ISO 045 and 050 burs and Hedström-files (VDW-Antaeos, Vereinigte Dentalwerke GmbH & Co KG) as follows: the central sample was obtained with bur or file ISO 045 to a depth of 50 μm from the root canal surface, and the peripheral sample was then obtained with bur or file ISO 050 to a depth of 100 μm from the root canal surface (Figure 1). Since all canals were standardized in length and diameter (7 mm in length, ISO 040 in diameter) before infection, the same amount of dentine was collected for each root and each layer. The dentine samples were transferred to Columbia agar plates, whereby the dentin showings were uniformly distributed to guarantee equal conditions for bacterial growth on the agar plates. The dentin samples were then incubated at 37° C for 48 hours. After 24 hours and 48 hours the colony-forming units (CFU) were counted.

Statistical Method

The statistical analysis was carried out using generalized linear models (GLM), because the observed feature, *number of colonies*, only took non-negative values. Thus the data was not supposed to be normally distributed. In addition, the data did not mirror homoscedasticy, which is also a condition for the applicability of an analysis of variances (ANOVA). The mean μ of the number of colonies, growing after a treatment, was modelled by

$$g(\mu) = \mu^{0.5} = b,$$

where *b* indicates the effect of treatment *B* and *g* is the link-function describing the connection between the linear predictor and the mean.

Funkcija varijance oblikovana je tako da je $V(\mu) = \mu\theta$. Parametar θ koristio se kao mjera snage varijacije. U tom modelu parametar θ iznosio je 0,6. Ta je vrijednost određena eksplorativnom statistikom.

Testiranje je obavljeno analizom devijacija i Waldovim testom, a odnosio se na to razlikuje li se broj kolonija sistematično ili slučajno na prvom i drugom očitavanju za središnje i periferne uzorke.

Rezultati

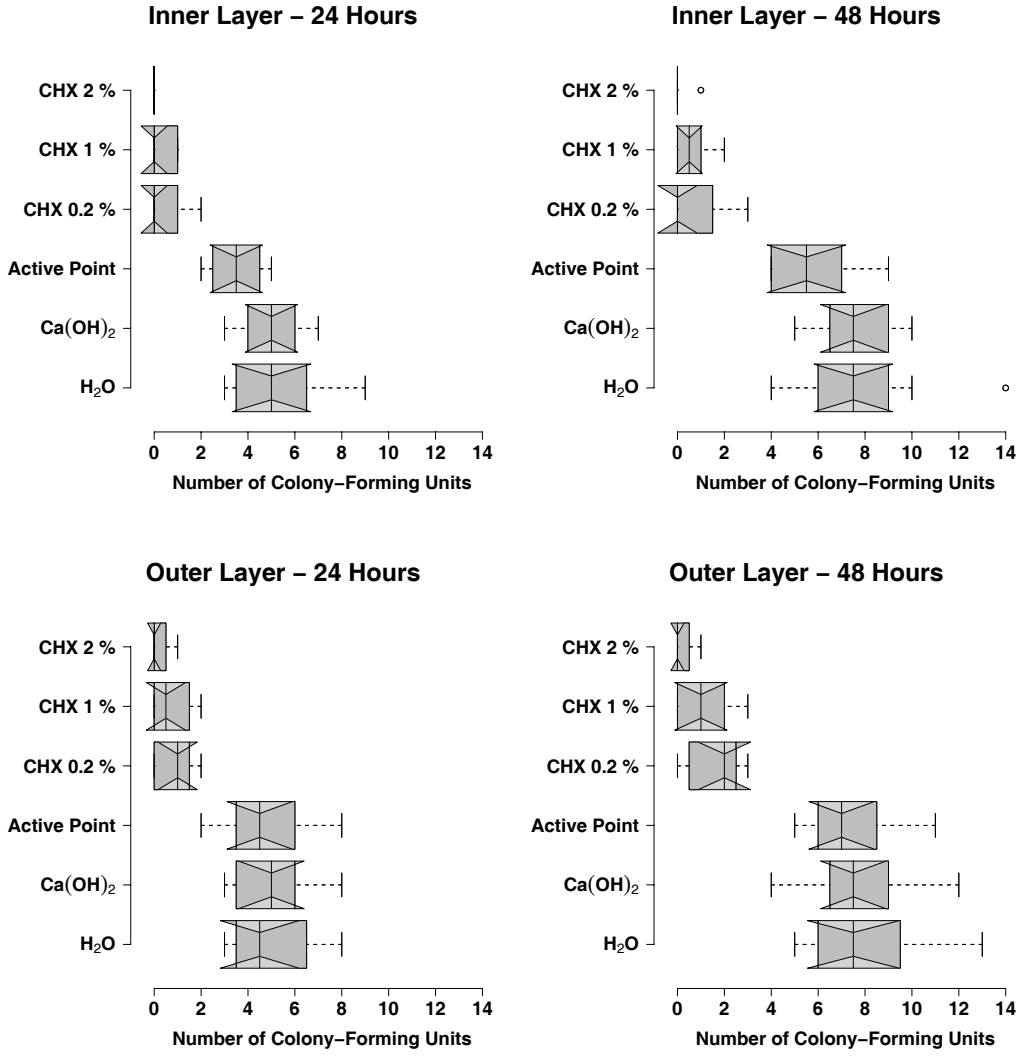
Slika 2. i Tablica 1. sažeto pokazuju rezultate. Istraživanje podupire tvrdnju da je **E. faecalis** u stanju prodrijeti u dentin. Točnije, kontrolna terapija vodom pokazala je da ta bakterija prodire u dentin do dubine od 100 μm. Prema procijenjenom modelu, za središnji se uzorak može očekivati 5,25 kolonija nakon 24 sata i 7,88 kolonija nakon 48 sati. Te

The variance function was modelled in such a way that $V(\mu) = \mu^{\theta}$. The parameter θ serves as a measure of the strength of variation. In this model the parameter $\theta = 0.6$. This value was determined using exploratory statistics.

The test, whether the number of colonies between the treatment groups differ systematically or just randomly at first and second reading and for central and peripheral sample, was carried out using an analysis of deviances and Wald's test.

Results

Figure 2 and Table 1 summarize the results. The study supports the first question of whether *E. faecalis* is able to penetrate dentine. More precisely, the control treatment H_2O showed that *E. faecalis* was able to penetrate dentine up to 100 μm uniformly. According to the model estimated, for the central sample 5.25 colonies are expected after 24 hours



Slika 2. Grafikoni CFU-a za središnji i periferni sloj te mjerenja
Figure 2 Boxplots of CFUs for the central layer and peripheral layer and various readings

su vrijednosti znatno različite od nule (p-vrijednost 0,0000). Zato je bilo opravdano ispitati antibakterijsko djelovanje različitih uložaka u dentinu oko korijenskog kanala.

Drugo se pitanje odnosilo na antibakterijski učinak nekoliko različitih uložaka na dubini do 100 μm, u slučaju infekcije. Ne djeluju svi isto na **E. faecalis** u dentinu. Pokazalo se da Ca(OH)2 nema nikakav antibakterijski učinak na **E. faecalis**, ni u središnjem, ni u perifernom dentinu. Nije bilo znatne razlike između Ca(OH)2 i destilirane vode (p-vrijednost > 0.5).

CHX iz gutaperke prodirao je u središnji sloj korijenskoga dentina, ali nedovoljno u periferni. Nakon 24 i 48 sati bile su zabilježene statistički znatne razlike u odnosu prema vodi (p-vrijednost <0,05), ali samo kad je riječ o uzorcima središnjeg dentina.

CHX gelovi prodirali su u dentin do dubine od 100 μm. Dvopostotni CHX gel bio je jači od jednopostotnoga s p-vrijednosti od 0,0925, ali samo u perifernom dentinu nakon 48 sati inkubacije, a 0,2-postotni imao je manji učinak na bakteriju od 2-postotnoga (p-vrijednost 0,0191). U uzorku središnjeg dentina nije bilo razlike između različitih koncentracija CHX gelova, ali oni su bili djelotvorniji u objema vrstama uzoraka nakon 24 i 48 sati (p-vrijednost 0,0000).

Tablica 1. sažeto prikazuje rezultate antibakterijskog djelovanja ispitivanih sredstava. X označava znatno antimikrobno djelovanje, (X) predstavlja slabije djelovanje, 0 označava izostanak antimikrobnog djelovanja, a X* djelovanje između X i (X).

and 7.88 colonies after 48 hours. These values are significantly different from zero (p-value 0.0000). For that reason the investigation of the antibacterial effects of various treatments against bacteria penetrating the adjacent dentine makes sense.

The second question concerned the antibacterial effect of several dressings in dentine layers up to a depth of 100 μm in the case of infection. Not all dressings had the same effect on *E. faecalis* in dentine. $Ca(OH)_2$ turned out to have no antibacterial effect on *E. faecalis* in central as well as in peripheral dentine layers. There was no significant difference between $Ca(OH)_2$ and aqua dest (p-value > 0.5).

CHX of active points were able to penetrate the central layer but not sufficiently the peripheral layer of root dentine. Thus, after 24 hours and after 48 hours significant differences to H_2O were observed (p-value < 0.05). However, this was not the case for the peripheral layer (p-value > 0.5).

CHX gels were able to penetrate dentine up to 100 μm. Two% CHX gel was stronger than 1% CHX gel with a p-value of 0.0925 only in the peripheral dentine sample after 48 hours of incubation, and 0.2% CHX gel had less effect on *Enterococcus faecalis* than 2% CHX with a p-value of 0.0191. In the central dentine sample no difference between the CHX gels were observed, but CHX gels were more effective in the central and peripheral dentine sample after 24 and 48 hours of inoculation than the other tested medicaments (p- value 0.0000).

Table 1 summarizes the antibacterial effects of medicaments tested. X indicates that the corresponding medicament has a significantly antimicrobial effect on *E. faecalis*. (X) stands for a slightly less significant effect. 0 means that the medicament did not inhibit bacterial growth. X* indicates that the effect is between (X) and X.

Tablica 1. Antibakterijsko djelovanje različitih uložaka
Table 1 Antibacterial effects of different treatments

Inoculation period • Razdoblje inokulacije	24 sata • 24 hours		48 sati • 48 hours	
Dentine layer • Dentinski sloj	**50 μm**	**100 μm**	**50 μm**	**100 μm**
Destilirana voda • Aqua dest.	0	0	0	0
Ca(OH)2	0	0	0	0
Aktivni štapić • Active point	(X)	0	(X)	0
0.2% CHX gel	X	X	X	(X)
1% CHX gel	X	X	X	X*
2% CHX gel	X	X	X	X

Rasprava

Eksperimentalni model korišten u ovom istraživanju modifikacija je onoga koji su opisali Haapasalo i Ørstavik (19), no u ovom su se istraživanju koristili ljudski zubi, a ne goveđi. Razlika između goveđih i ljudskih zuba je velika. Standardizirani goveđi zubi, rabljeni u nekim ranijim istraživanjima, imaju promjer kanala čak i veći od 3 mm, što je čak trostruko više od ljudskih uzoraka. Volumen goveđeg kanala gotovo je deseterostruko veći od volumena ljudskog kanala iste duljine (28,29). Zna se da učinak CHX-a ovisi o količini molekula CHX-a dostupnih za interakciju s dentinom. Budući da goveđi kanal ima veću površinu od ljudskoga, može apsorbirati više molekula CHX-a.

U ranijim istraživanjima stručnjaci su se koristili visokim i niskim koncentracijama CHX-a - 2-postotnim i 0,2-postotnim (28,30.31). Basrani i suradnici (28,30) zaključili su da je, suprotno goveđim kanalima u kojima i 0,2-postotni CHX učinkovito razvija antimikrobno djelovanje, za ljudske kanale potrebna veća koncentracija CHX-a. U svrhu ispitivanja drugih koncentracija, u ovom se istraživanju koristila i koncentracija od 1 posto.

Uzorci dentina postavljani su izravno na Columbia agar. U drugim istraživanjima (32,33) dentin je najprije bio stavljen u otopinu. Zatim je bakterijska kultura položena na ploče. U ovom istraživanju rast bakterija procjenjivao se na temelju CFU-a nakon 24 i 48 sati inkubacije, a u ranijim je ispitivanjima (28,30-32) rezultat bio opažan optičkom gustoćom otopine koja sadržava dentinske uzorke. Podaci su analizirani generaliziranim linearnim modelom.

Enterococcus faecalis odabran je zato što se smatra da je rezistentan na uobičajene uloške s kalcijevim hidroksidom (17,20), a često se povezuje i s perzistentnom bolesti nakon endodontskog liječenja (13). Antimikrobna supstantivnost testirana je 21 dan, u skladu s ranijim istraživanima (28,30,34). To produženo razdoblje bilo je barem tri puta dulje od onoga u ranijim istraživanjima (25-27). Suprotno od ranijih istraživanja (28,30.34), uzorci su 21 dan bili inokulirani bakterijom. Nakon toga su primijenjeni medikamentozni ulošci.

Kalcijev hidroksid kao Calasept (Calasept-Speiko -R, dr. Speier GmbH, Münster, Njemačka) također je bio ispitan, budući da se često koristi kao intrakanalni uložak. Ovo istraživanje potvrđuje ranija - Ca(OH)2 nema antibakterijskog djelovanja na *E. faecalis* u korijenskom kanalu (19,204 Ørstavik i Haapasalo 1990., Haapasalo i Ørstavik 1987., Byström i suradnici 1985.), čak iako ta bak-

Discussion

The experimental model used for this purpose was adapted from that established by Haapasalo & Ørstavik (19). In the present report, the model was modified by adapting it to extracted human teeth rather than the previously used bovine teeth. There is a great difference in diameter between the canals of bovine and human teeth. The standardized bovine canals in some of the previous studies were more than 3 mm in diameter, which is more than 3-times that of human canals. The canal volume of the bovine root specimen is almost 10-fold larger than that of the root specimen of the same length (28, 29). The effect of CHX is known to depend on the amount of CHX molecules available for interaction with the dentine. As the dentine surface of bovine root canals exceeds the dentine surface of human root canals, the canal of bovine root specimen can absorb more CHX molecules than usual canals of human teeth.

Previous studies tested mainly CHX in high and low concentrations, such as 2% or 0.2% (28, 30, 31). Basrani et al. (28, 30) conclude that, in contrast to the large bovine canals in which 0.2% CHX effectively imparts substantive antimicrobial activity, in the small human canals a higher concentration of CHX is necessary. To investigate whether intervening concentrations are effective, a 1% CHX gel was also tested in the present study.

Dentine shaving was put directly onto the Columbia agar plates. In contrast, in other studies (32, 33) the dentine shaving was first put into a broth. Then the bacterial culture was put onto the agar plate. In the current investigation bacterial growth was assessed by counting the colony-forming units (CFU) after 24 and 48 hours of incubation, whereas in other studies (28, 30, 31, 32) the results were observed by reading the optical density of the broth containing the dentine samples. The data collected was statistically analyzed using generalized linear models.

Enterococcus faecalis was chosen because it is considered resistant to the common intracanal dressing using calcium hydroxide (17, 20) and frequently associated with persistent disease after endodontic treatment (13). Antimicrobial substantivity was tested for 21 days, in accordance with previous studies (28, 30, 34). This extended test period was at least three times longer than reported in most previous studies (25, 26, 27). Contrary to other studies (28, 30, 34), the specimens were inoculated with *E. faecalis* for 21 days. Afterwards the root canals were medicated with the antibacterial substance.

terija u epruveti brzo ugiba (317 Gomes i suradnici, 2003.).

CHX je testiran u dvama oblicima - kao gel i kao štapić gutaperke.

U ovom istraživanju gutaperkini štapići s CHX-om bili su znatno učinkovitiji od Ca(OH)2 (p-vrijednost 0,0266) u središnjem sloju nakon 24 sata. U usporedbi s gelovima, dezinfekcija štapićima imala je znatno više kolonija (p-vrijednost < 0,001). To znači da CHX kao gel prodire 50 μm dublje negoli gel iz gutaperke. Nakon 48 sati nije bilo razlike u odnosu prema vrijednosti nakon 24 sata. CHX štapići ostavljaju puno manje kolonija nego destilirana voda (p-vrijednost 0,0411). Broj kolonija nakon 48 sati bio je znatno manji od nule (p-vrijednost 0,0000). Budući da CHX štapići imaju samo blag antibakterijski učinak, možemo zaključiti da je rast bakterija poremećen, pa se može opaziti njihov odgođeni rast.

U perifernom dentinu nije bilo razlike u odnosu prema kontrolnom uzorku (p-vrijednost > 0,05) ni nakon 24, ni nakon 48 sati. CHX iz štapića nije prodirao u dublje slojeve dentina, za što možda postoje dva razloga. S jedne strane, djelokrug CHX-a iz štapića manji je zbog činjenice da se on ne može prilagoditi obliku korijenskog kanala. S druge strane, možda nije bilo dovoljno tekućine u kanalu. Kad se sredstvo postavilo, kanali su ostali zabrtvljeni tjedan dana. Proizvođači aktivnih štapića zagovaraju tekućinu kao uvjet za njihovu djelotvornost. Također preporučaju korištenje nekoliko štapića u jednom kanalu, kako bi se spriječio prazan prostor između štapića i stijenke kanala.

Nije bilo razlike između CHX gelova nakon 24 i 48 sati (p-vrijednost (0,15). Male razlike uočene su u perifernom sloju. Nakon 24 sata sve su koncentracije ostavljale mnogo manji broj kolonija od aktivnog štapića (p-vrijednost 0,0000).

CHX gelovi mogu penetrirati i do 100 μm dublje od CHX-a u aktivnim štapićima. Jedino 0,2-postotni CHX gel nije uspio uništiti sve bakterije. Broj kolonija nakon 24 i 48 sati bio je znatno različit od nule (p-vrijednost 0,0000). Nakon 48 sati, 0,2-postotni i 1-postotni gel nisu se mnogo razlikovali u perifernom sloju (p-vrijednost 0,4279). No, broj kolonija koje su rasle nakon dezinfekcije 0,2-postotnim gelom, bio je puno veći od broja kolonija nakon dezinfekcije 2-postotnim gelom. (p-vrijednost 0,0191). To znači da 0,2-postotni gel prodire do 100 μm, ali koncentracija nije dovoljna da u cijelosti eliminira *E. faecalis* u dubljim slojevima dentina. Taj rezultat objavili su i Basrani i suradnici (28,30).

Calcium hydroxide as Calasept (Calasept-Speiko®, Dr. Speier GmbH, Münster, Germany) was also tested in the present study because of its prevalent clinical application as intracanal dressing. The present study confirms the results of other reports that $Ca(OH)_2$ has no antibacterial effect on *E. faecalis* in the root canal (19, 20), even though in a test tube *E. faecalis* dies rapidly (31).

CHX was tested in two different modalities of application: as CHX gel and as CHX containing gutta-percha point.

In the current study CHX gutta-percha points (active points) had a significantly greater impact than $Ca(OH)_2$ (p-value 0.0266) in the central layer after 24 hours. Compared to CHX gels, a disinfection with active point left significantly more colonies than the CHX gels tested (p-value < 0.001). That means that CHX in gels penetrates dentine up to a distance of 50 μm from root canal to a greater extent than CHX in gutta-percha points. After 48 hours no difference compared to the results after 24 hours was observed. CHX points leave significantly fewer colonies than H_2O (p-value 0.0411). The number of colonies after 48 hours was significantly different from zero (p-value 0.000). Thus, CHX points show only a slight antibacterial effect in the central layer. All CHX gels left significantly smaller numbers of colonies than H_2O and active points (0.0000). From the fact that active points had only a slightly significant antibacterial effect we conclude that the poliferation rate of bacteria is disturbed by the CHX emmited from the gutta-percha points. Therefore, a delay in the bacterial growth can be observed.

In the peripheral layer no significant difference of active points compared to the control sample (p-value > 0.5) was noticed, neither after 24 hours nor after 48 hours. CHX in active points was not able to penetrate into deeper dentine layers. Two reasons for that may be mentioned: on the one hand CHX gutta-percha points might have a lower range of action than other CHX dressings, because the active points did not fit tightly to root canals with large diameters. On the other hand there may have been insufficient liquid in the canal. Once the medicaments were put in the canal, the canals were closed tightly for one week. The producers of active points state the necessity of liquid as a precondition for the effectiveness of this system. They also recommend using several points in one canal to avoid too great a space between the point and the canal surface.

After 24 and 48 hours there were no differences between CHX gels in various concentrations in the central layer (p-value 0.15). Slight differences of

Testovi difuzije na agaru (23,35) pokazali su da 0,2-postotna otopina CHX-a ima dobro antibakterijsko djelovanje na **E. faecalis**. Ispitivanja korijenskih kanala tretiranih otopinama CHX-a (2), pokazala su da je učinak bio dublji kada su se koristile koncentracije veće od 0,2 posto. Kad je riječ o gelovima, može se također reći da su potrebne veće koncentracije kako bi se postigao zadovoljavajući učinak u dubljim slojevima dentina (28,30).

Nakon 48 sati nije bilo velike razlike između rezultata za 1-postotni i 2-postotni gel (p-vrijednost 0,0925) za 1 posto i 0,2 posto (p-vrijednost 0,4279). To znači da je 1-postotni CHX gel u stanju uništiti **E. faecalis** gotovo kao i 2-postotni u našem eksperimentalnom okruženju.

Suprotno od drugih koncentracija CHX gelova, samo je 2-postotni CHX gel ostavio broj kolonija koji je bio procijenjen kao znatno različit od nule u perifernom sloju nakon 48 sati (p-vrijednost 0,0616, druge koncentracije 0,0000). Razlog za opažanje jedne kolonije u skupini od 8 uzoraka nakon tretiranja 2-postotnim CHX gelom, mogu biti različite anatomske varijacije i stupanj otpuštanja, ali i vrsta nosača te njegova viskoznost.

Zaključak

U ovom istraživanju 2-postotni CHX gel uspio je uništiti **E. faecalis** u dentinu. Niže koncentracije (0,2%) također su bile učinkovite, ali imale su manji radijus u dentinu. Za uspješnu eliminaciju **E. faecalis** potrebna je koncentracija od najmanje jedan posto. Intrakanalni uložak koji se primjenjuje između pacijentova posjeta, a sadržava 2 posto CHX-a, mogao bi biti djelotvoran protiv *E. faecalis *in vivo.

the CHX gels were observed in the peripheral layer. After 24 hours all concentrations of CHX gels left a significantly smaller number of colonies in peripheral layers than active point (p-value 0.0000).

CHX in gels can penetrate dentine up to 100 μm to a significantly greater extent than CHX in active points. 0.2% CHX gel was not able to eliminate all bacteria. The number of colonies observed after 24 hours and 48 hours was significantly different from zero (p-value 0.0000). After 48 hours 0.2% CHX gel and 1% CHX gel did not differ significantly in the peripheral layer (p-value 0.4279). However, the number of colonies which grew after 0.2% CHX gel disinfection was significantly higher than that after 2% CHX gel disinfection (p-value 0.0191). That means that 0.2% CHX gel was able to penetrate up to 100 μm, but the CHX concentration was too low to completely eliminate *E. faecalis* in deeper dentine layers. This result is also derived by Basrani et al. (28, 30). Agar diffusion tests (23, 35) showed that 0.2% CHX solutions provide a good antibacterial effect against *E. faecalis*. Root canal studies with CHX solutions (2) showed that the depth effect of CHX improved when higher concentrations than 0.2% CHX were used. For CHX gels higher concentrations than 0.2% are also necessary to reach a sufficient effect in deeper dentine layers (28, 30).

After 48 hours no significant difference between 1% and 2% CHX gel (p-value 0.0925) and between 1% and 0.2% CHX gel (p-value 0.4279) were observed. That means that 1% CHX gel is able to eliminate E. faecalis almost to the same extent as 2% CHX gel in our experimental setting.

Contrary to other concentrations of CHX gels, only 2% CHX gel left a number of colonies that was estimated to be significantly different from zero with a p-value of only 0.0616 (p-values for the other concentrations: 0.0000) in the peripheral layer after 48 hours. The reason for observing one colony in a sample of 8 specimens after treatment with 2% CHX gel could be various anatomical and discharge conditions, as well as the type of substance carrier and its viscosity.

Conclusion

In this study an intracanal dressing of 2% CHX gel was able to eliminate *E. faecalis* in dentine. Lower contentrations (0.2%) also eliminated *E. faecalis*, but they had a shorter radius of action in dentine. For effective elimination of *E. faecalis* in deeper dentine-layers CHX concentrations of at least 1% were necessary. An intracanal, interappointment dressing of 2% CHX may have the potential to eliminate E. faecalis in vivo.

Abstract
The aim of this in vitro study was to assess antimicrobial activity of calcium hydroxide (Calasept) and chlorhexidine (CHX) in various concentrations with respect to Enterococcus faecalis in human root dentine up to 100 µm. **Material and Methods:** Forty-eight human root canals were enlarged to standard size (ISO 40) and inoculated with Enterococcus faecalis for 21 days. After inoculation the canals were medicated with one of the following: 2 %, 1 % and 0.2 % chlorhexidin gel, Chlorhexidine releasing gutta-percha points (active point), calcium hydroxide and aqua distillate (aqua dest.). Aqua dest. served as control medium. At the end of a disinfection period of one week dentine samples were collected with reamer and H-file (ISO 45 and 50) and put onto Columbia agar plates. **Results:** Bacterial growth was assessed by counting the colony forming units (CFU) after 24 hours and 48 hours of incubation. CHX gels could penetrate dentine up to 100 µm. Two % CHX gel is slightly stronger than 1 % CHX gel (p-value 0.0925) only in the peripheral dentine sample after 48 hours of incubation, and 0.2 % CHX gel had less effects on E. faecalis than 2 % CHX (p-value 0.0191). In the central dentine sample no difference between the CHX gels could be observed. In general, CHX gels were more effective than the other medicaments tested. No significant difference between Ca(OH)2 and Aqua dest. could be observed. **Conclusion:** For effective elimination of E. faecalis, especially in deeper dentine-layers, CHX concentrations of at least 1 % are necessary. An intracanal, interappointment dressing of 2 % CHX may have the potential to eliminate E. faecalis in vivo.

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