ORIGINAL ARTICLE
ORIGINALNI RAD

DOI: 10.2478/sdj-2019-0012
UDC: 615.46:616.314-7

Antibacterial activity of newly synthesized endodontic nanomaterial based on calcium aluminate

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SUMMARY

Introduction Materials used for root canal obturation and root perforation are expected to have, in addition to preventing apical, lateral and coronary leakage, antimicrobial effects on microorganisms that are not accessible to chemomechanical instrumentation and intra-canal medications. The aim of this study was to evaluate the antimicrobial effect of a novel calcium aluminate-based endodontic nanostructured biomaterial (ALBO-MCCA) using agar diffusion test.

Material and methods The two materials were tested in the study. The nanostructured calcium aluminate was synthesized by the hydrothermal sol-gel method from individual components of calcium aluminate ($CaO\mathbf{x}Al_2O_3$), calcite ($CaCO_3$) and barium sulfate ($BaSO_4$) as radiocontrast agent in the ratio of 2:2:1 according to V. Jokanovic's recipe. The other used material was calcium silicate MTA Angelus (Londrina, Brazil). The antimicrobial effect was assessed using agar diffusion test. Standard strains of *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, cultured on blood Mueller-Hinton agar and *Candida albicans* cultivated on Sabouraud Dextrose Agar, were used as test microorganisms.

Results The best antimicrobial effect after 24 h both materials showed against *S. aureus.* The mean values of the growth inhibition zone for ALBO MCCA were 5.7 ± 0.25 mm and MTA 6.2 ± 0.4 mm. The ALBO MCCA material showed slightly stronger antibacterial effect against *E. coli* compared to MTA ($p < 0.05$), whereas none of the materials showed antibacterial effect against *E. faecalis*.

Conclusion The ALBO MCCA material showed certain antibacterial effect on *Escherichia coli, Staphylococcus aureus and Candida albicans* that was comparable to antibacterial effect of MTA.

Keywords: mineral trioxide aggregate; calcium aluminate; antimicrobial effect

INTRODUCTION

The main goal of endodontic treatment is removal of infective agents (microorganisms) from the root canal. Therapeutic procedures involving mechanical canal debridement with the use of antiseptics and topical administration of intracanal medicaments greatly reduce the number of bacteria in the root canal. However, due to complexity of root canal system, it is very difficult to remove all microorganisms with this procedure, especially from inaccessible parts and lateral canals. Infected canals are characterized by the dominance of strict anaerobic species with some facultative anaerobes and rare aerobic species [1]. Microorganisms such as *Enterococcus faecalis* and *Streptococcus* species are commonly considered responsible for the failure of endodontic treatment [2]. Due to the fact that leakage and consequent inflow of nutrient-rich fluid cannot be completely prevented by any of the materials available today, antibacterial properties of materials used in final stages of endodontic therapy are important. Therefore, materials used for permanent root canal obturation and root perforation are expected to have, in addition to preventing apical, lateral and coronary leakage, antimicrobial effects as well.

Mineral trioxide aggregate (MTA) is the material of choice in numerous endodontic indications (retrograde root canal filling, direct pulp capping and pulpotomy, apexification and apexogenesis, lateral and interr-radicular perforation treatment). This material is characterized with optimal biocompatibility and bioactivity [3]. High pH value achieved during the set-up demonstrates potential antibacterial use of the material [4]. MTA also releases some of its components during hydration, stimulating biomineralization processes and exerting an antibacterial effect [5, 6]. However, certain disadvantages such as long setting time, difficult manipulation, tooth discoloration, release of certain toxic elements, and high market prices limit its clinical application and demand development of new materials that retain good MTA properties and overcome its existing deficiencies [3].

Use of nanoparticles has become a significant segment of material research in dentistry, with particular focus on improving mechanical properties and their antibacterial effect. In the recent years, a new nanostructured material

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based on the calcium aluminate system has been synthesized at the Vinca Nuclear Research Institute. The material was obtained by hydrothermal sol-gel method and self-expanding combustion reaction. This mode of synthesis provides high particle activity, faster hydration and short setting time [7]. Calcium aluminate cements have been specifically studied for use in dentistry [8, 9, 10]. Up to date in *in vitro* studies, nanostructured calcium aluminate did not show cytotoxic and genotoxic effects in the culture of human fibroblasts MRC-5, while in an *in vivo* study in experimental animals it showed satisfactory biocompatibility [11, 12].

The aim of this study was to evaluate the antimicrobial effect of a novel calcium aluminate-based endodontic nanostructured biomaterial (ALBO-MCCA) by an agar diffusion test.

MATERIAL AND METHODS

Two materials were used in the study. The nanostructured calcium aluminate was synthesized by the hydrothermal sol-gel method from the individual components of calcium aluminate ($CaO \times Al_2O_3$), calcite ($CaCO_3$) and barium sulfate ($BaSO_4$) as an radiocontrast agent in a ratio of 2:2:1 according to V. Jokanovic's recipe. The other used material was calcium silicate MTA Angelus (Londrina, Brazil).

Antimicrobial effect was investigated by the agar diffusion test. Standard strains of *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) were used as the test microorganisms. Cultures of microorganisms aged 24 h were adjusted to a McFarland density standard that corresponded to 10^6 per milliliter of microorganisms, and then seeded on appropriate substrates. *E. faecalis*, *E.coli* and *S. aureus* were cultured on Mueller-Hinton blood agar, while *C. albicans* was cultured on Sabouraud Dextrose Agar. Prior to placing the test materials, freshly seeded cultures of the microorganisms were incubated under aerobic or anaerobic conditions, respectively. The materials were mixed according to the appropriate formulations and applied on sterile paper disks in a diameter of 5 mm (6 for each material) and then placed in substrates with seeded microorganisms. The seeded plates were then left at the room temperature for 2 h to allow diffusion of the material through agar and then incubated aerobically and anaerobically, respectively, for 24 h at 37°C in a GAS-PAK (CO_2-H_2) system, after which the results were read. Negative controls were paper disks with no material.

Antimicrobial effect of the material was interpreted based on the growth inhibition zone diameter of the tested microorganisms expressed in millimeters. Measurements were performed on all 6 fields (3 measurements for each field). The data were processed using statistical software SPSS 20 (IBM Corp., Armonk, NY, USA). Data analysis was performed using Student's t-test.

RESULTS

The mean diameters of the microbial growth inhibition zone are shown in Figure 1. The inhibition zones were the highest for *S. aureus* after 24 h with ALBO MCCA (5.7 ± 0.25 mm) and MTA (6.2 ± 0.4 mm). The mean values of the *C. albicans* inhibition zone were measured for ALBO MCCA (4.8 ± 0.18 mm) and MTA (5.0 ± 0.35 mm), respectively. No statistically significant difference in the values between the tested materials was observed in respect to these two microorganisms. ALBO MCCA showed slightly higher antibacterial effect against *E. coli* compared to MTA ($p < 0.05$), while none of the tested materials exhibited any antibacterial effect against *E. faecalis* (Figure 1).

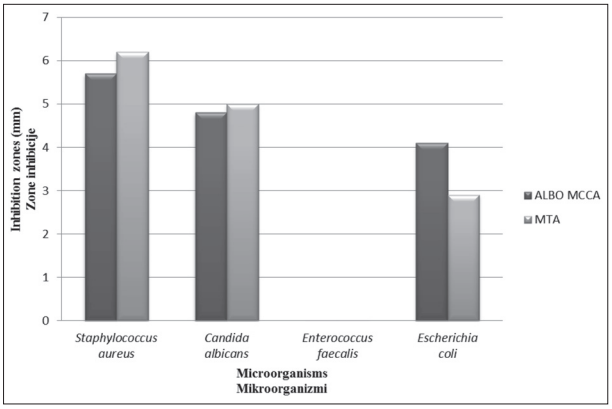

Figure 1. Mean values of microbial growth inhibition zone
Grafikon 1. Prosečne vrednosti zone inhibicije rasta mikroorganizama

DISCUSSION

Antimicrobial properties of dental materials are mainly tested in *in vitro* studies by agar diffusion test, agar dilution test and direct contact test. Agar diffusion test is the most commonly used experimental model for the evaluation of antimicrobial activity, while bacteria that are part of the infected root canal flora and present in cases of failed endodontic treatment are mainly used as a test microorganisms in endodontic research. Agar diffusion test was used to evaluate the antimicrobial activity of the tested materials, ALBO MCCA and MTA. The results obtained by this method *in vitro* in the environment can depend on a number of factors: pH, type and viscosity of the agar itself, used microorganisms and their number, incubation time, solubility of the test materials and their ability to diffuse or expand through the agar. The agar diffusion test expresses certain limitations. By this method, it is not possible to determine whether the test material exhibits bactericidal or bacteriostatic activity alone as well as the duration of the antibacterial effect. For this research, microorganisms that present integral part of the infected canal flora were selected. *Enterococcus faecalis* and *Candida albicans* are considered the most resistant microorganisms in the oral cavity and are often associated with root canal treatment failure [13, 14], while

Staphylococcus aureus is also isolated from primary and persistent secondary infection. The results of the current study showed for both tested materials that the greatest growth inhibition zones were recorded for *S. aureus*, while they showed no antimicrobial property against *E. faecalis*. Factors that could affect the antibacterial activity of both materials are baseline pH and diffusion of components and ions released into the medium [6]. High alkalinity creates an unfavourable environment for the growth of microorganisms [15], and during the hydration of MTA, released hydroxyl ions are highly reactive free radicals that together with increased pH values can cause damage to the cytoplasmic membrane and DNA of bacterial microorganisms [16].

When it comes to calcium aluminate cement, the antimicrobial effect could be due to both Ca^{2+} ions and Al^{3+} oxide nanoparticles. These nanoparticles, as positively charged ions, can bind to negatively charged bacterial cell walls and reduce cell viability. Mukherjee et al. pointed out that aluminum oxide nanoparticles could exert an inhibitory effect against *E. coli*. The same authors state that SEM analyzes have shown after the interaction of *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* cells with aluminum nanoparticles, there is a change in cell morphology, and its distortion [17]. In a study by Sadiq et al. aluminum oxide nanoparticles exhibited mild antimicrobial activity against *E. coli* [18]. In a recent study, Manyasree et al. investigated the antibacterial effect of alumina nanoparticles on *E. coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Streptococcus mutans* with a minimum inhibitory concentration test. It was observed that with successful increase in the concentration of nanoparticles (10-50mg / ml), the antibacterial effect against all tested microorganisms increased as well [19]. However, the influence of calcium aluminate cement components on antimicrobial activity needs to be investigated in further studies. On the other hand, one of the factors that could limit the antibacterial activity of the tested materials is that they are cements in composition; consequently their binding makes their diffusion through the medium difficult. Absence of antibacterial effect of both materials against *E. faecalis* in our study may be explained by the fact that this microorganism is highly resistant to high pH and its elimination requires pH greater than 11.5. The main *E. faecalis* defense mechanism is a proton pump located in the cell membrane. By acidification of the cytoplasm, the proton pump maintains the pH in the physiological range and thus allows the enzymes and proteins to function normally in the cell [14]. Similar to our study, the findings of Torabinejad et al. and Estrela et al. also confirmed the absence of the antibacterial action of MTA against *E. faecalis* [3, 20]. Contrary to these claims, Lovato and Sedgley concluded that MTA exerted an antimicrobial effect against the clinical isolate of *E. faecalis* [21]. Miyagak et al. have shown that MTA and Portland cement did not exert an antimicrobial effect against *C. albicans, S. aureus*, and *E. coli*, which contradicts the results of our study [22]. In the study by Ribeiro et al. MTA also showed no inhibitory activity against *E. faecalis* and *E. coli* [23].

Numbers of other studies have shown contradictory results when it comes to MTA antimicrobial effect. Tanomaru-Filho et al. have shown that MTA-based materials exhibit antimicrobial activity against *S. aureus* and *E. faecalis* [4]. Similarly, in the study of Demiryürek et al. MTA Angelus exerted antimicrobial effect against *E. faecalis* and *C. albicans* [24]. MTA and novel endodontic nanostructured nanomaterials based on highly active calcium silicates exerted an antibacterial effect against *E. faecalis* and *S. aureus* [25]. Al-Hezaimi et al. examined the antifungal effect of different concentrations of two MTA commercial variants (white and gray) on *C. albicans*. The results of the agar diffusion assay showed that the concentration of the material significantly contributed to MTA antifungal activity. At 25 mg/mL and 50 mg / mL concentrations, both MTA variants exhibited antifungal activity, which was not the case when the material concentration was below 25 mg/mL [26]. In the study by Kim et al. MTA Angelus exerted antimicrobial activity on *S. mutans, Lactobacillus rhamnosus, Lactobacillus paracasei* and *Porphyromonas gingivalis*, with no inhibitory effect on *E. faecalis* [15]. Özyürek and Demiryürek, by examining the antimicrobial activity of MTA Angelus against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Enterococcus faecium*, concluded that this material exhibits antimicrobial activity against all tested microorganisms [27]. Different results about MTA antibacterial property in the studies are attributed to different strains of tested microorganisms, the concentration and type of used MTA, as well as the methodological protocol itself. Some authors recommend the use of chlorhexidine instead of distilled water to improve the antimicrobial properties of MTA [28]. In the study of Holt et al., mixing MTA powder with 2% chlorhexidine gluconate contributed to an increase in the antibacterial effect against *E. faecalis* [29].

Calcium aluminate belongs to the new group of endodontic materials and available literature provides little information on its antimicrobial activity. In the study by Silva et al. calcium aluminate cement (EndoBinder) and MTA exhibited antimicrobial activity against all three tested microorganisms (*Staphylococcus aureus, Enterococcus faecalis and Candida albicans*) by agar diffusion test after 24 h and 48 h [10]. In a similar study, Pires-de-Souza et al. investigated antimicrobial effect of MTA and calcium aluminate cement EndoBinder with different radiocontrast agents (bismuth oxide, zirconium oxide and zinc oxide). Their results showed that MTA exerted better antibacterial effect against *S. aureus* over all calcium aluminate formulations. The zones of inhibition against *C. albicans, E. coli* and *E. faecalis* were not statistically significant between MTA and calcium aluminate powder or bismuth oxide formulation. Calcium aluminate with zirconium oxide and zinc oxide did not exert an antimicrobial effect on the three mentioned microorganisms. The authors of the study attributed the antimicrobial activity of EndoBinder to calcium aluminate hydrate breakdown formed during hydration and release of calcium and hydroxyl ions. The absence of antimicrobial effect in some calcium aluminate formulations has been attributed to radiocontrast

components that are not inert but can modify hydration processes, reducing the release of calcium ions and affecting the individual physicochemical properties of the material [30].

CONCLUSION

ALBO MCCA material showed certain antibacterial effect against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 that is comparable to the antibacterial effect of MTA.

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Received: 13.03.2019 • Accepted: 10.07.2019

Antimikrobna aktivnost novosintetisanog endodontskog nanomaterijala na bazi kalcijum-aluminata

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KRATAK SADRŽAJ

Uvod Od materijala koji se koriste za opturaciju kanala korena i perforacija na korenu se očekuje da pored sprečavanja apikalnog, lateralnog i koronarnog curenja, poseduju i antimikrobno delovanje na mikroorganizme, koji nisu dostupni hemomehaničkoj obradi i intrakanalnim medikamentima. Cilj ovog istraživanja je bio da se testom difuzije u agaru proceni antimikrobni efekat novog endodontskog nanostrukturnog biomaterijala na bazi kalcijum-aluminata (ALBO-MCCA).

Materijal i metode U istraživanju su korišćena dva materijala. Nanostrukturni kalcijum-aluminat je sintetisan hidrotermalnom sol-gel metodom od pojedinačnih komponenata kalcijum-aluminata ($CaO{\cdot}Al_2O_3$), kalcita ($CaCO_3$) i barijum-sulfata ($BaSO_4$) kao rendgen-kontrastnog sredstva u odnosu 2 : 2 : 1 prema recepturi V. Jokanovića. Drugi korišćeni materijal je kalcijum-silikatni MTA Angelus (Londrina, Brazil). Antimikrobni efekat je ispitivan testom difuzije u agaru. Kao test mikroorganizmi korišćeni su standardni sojevi *Enterococcus faecalis, Escherichia coli, Staphylococcus aureus,* koji su kultivisani na krvnom agaru MuellerHinton, dok je *C. albicans* kultivisana na agaru Sabouraud Dextrose.

Rezultati Najbolji antimikrobni efekat nakon 24 h oba materijala su pokazala prema bakteriji *S. aureus*. Izmerene prosečne vrednosti zone inhibicije rasta iznosile su kod ALBO-MCCA (5,7 ± 0,25 mm), odnosno kod MTA (6,2 ± 0,4 mm). Materijal ALBO-MCCA je pokazao nešto veći antibakterijski efekat prema bakteriji *E. coli* u odnosu na MTA ($p < 0,05$), dok prema bakteriji *E. faecalis* nijedan materijal nije ispoljio antibakterijski efekat.

Zaključak Materijal ALBO-MCCA je ispoljio određeni antibakterijski efekat na bakterije *Escherichia coli, Staphylococcus aureus* i *Candida albicans* komparabilan sa antibakterijskim efektom MTA.

Ključne reči: mineral-trioksidni agregat, kalcijum-aluminat, antimikrobni efekat

UVOD

Osnovni cilj endodontske terapije jeste uklanjanje uzročnika infekcije, odnosno mikroorganizama iz kanala korena. Terapijski postupci koji uključuju mehaničku obradu kanala uz primenu antiseptika i lokalnu primenu interseansnih medikamenata u velikoj meri smanjuju broj bakterija u kanalu korena. Međutim, zbog kompleksne anatomije kanalnog sistema ovim postupkom je veoma teško ukloniti sve mikroorganizme, pogotovo iz nepristupačnih delova i lateralnih kanala. Inficirane kanale odlikuje dominacija striktnih anaerobnih vrsta sa ponekim fakultativnim anaerobima i retkim aerobnim vrstama [1]. Mikroorganizmi, kao što su vrste *Enterococcus faecalis* i *Streptococcus,* najčešće se smatraju odgovornima za neuspeh endodontskog lečenja [2]. S obzirom na to da se mikrocurenje i posledični dotok fluida bogatog hranljivim sastojcima ne mogu apsolutno sprečiti nijednim danas dostupnim materijalom, antibakterijsko svojstvo materijala koji se koriste u pojedinim završnim fazama endodontske terapije bi donekle moglo kompenzovati ovaj nedostatak. Tako se od materijala koji se koriste za trajnu opturaciju kanala korena i perforacija na korenu očekuje da pored sprečavanja apikalnog, lateralnog i koronarnog curenja, poseduju i antimikrobno delovanje na preostale mikroorganizme, koji nisu bili dostupni hemomehaničkoj obradi i intrakanalnim medikamentima.

Mineral-trioksidni agregat (MTA) danas je materijal izbora u brojnim endodontskim indikacijama (retrogradno punjenje kanala korena, direktno prekrivanje pulpe i pulpotomija, apeksifikacija i apeksogeneza zuba, terapija lateralnih i interradiksnih perforacija korena). Ovaj materijal odlikuje optimalna biokompatibilnost i bioaktivnost [3]. Visoka pH vrednost postignuta tokom postavljanja ukazuje na moguće antibakterijsko delovanje materijala [4]. MTA takođe otpušta neke od svojih komponenata tokom hidratacije, čime podstiče procese biomineralizacije i ispoljava antibakterijski efekat [5, 6]. Međutim, određeni nedostaci, kao što su dugo vreme vezivanja, otežana manipulacija, prebojavanje zuba, oslobađanje pojedinih toksičnih elemenata i visoka cena na tržištu, ograničavaju njegovu kliničku primenu i uslovljavaju potrebu za razvojem novih materijala kojima bi se sačuvale dobre osobine MTA, a prevazišli postojeći nedostaci [3].

Upotreba nanočestica je postala značajan segment istraživanja materijala u stomatologiji, sa posebnim akcentom na poboljšanju mehaničkih osobina i njihovog antibakterijskog efekta. Poslednjih godina na Institutu za nuklearna istraživanja u Vinči je sintetisan novi nanostrukturni materijal na bazi kalcijum-aluminatnog sistema dobijen hidrotermalnom sol-gel metodom i samoširećom reakcijom sagorevanja. Ovakav način sinteze obezbeđuje visoku aktivnost čestica, bržu hidrataciju i kratko vreme vezivanja [7]. Kalcijum-aluminatni cementi su posebno proučavani za upotrebu u stomatologiji [8, 9, 10]. U dosadašnjim ispitivanjima u *in vitro* uslovima nanostrukturni kalcijum-aluminat nije ispoljio citotoksični i genotoksični efekat u kulturi humanih fibroblasta pluća MRC-5, dok je u *in vivo* studiji na eksperimentalnim životinjama pokazao dobru biokompatibilnost [11, 12].

Cilj ovog istraživanja je bio da se testom difuzije u agaru proceni antimikrobni efekat novog endodontskog nanostrukturnog biomaterijala na bazi kalcijum-aluminata (ALBO-MCCA).

MATERIJAL I METODE

U istraživanju su korišćena dva materijala. Nanostrukturni kalcijum-aluminat je sintetisan hidrotermalnom sol-gel metodom

od pojedinačnih komponenata kalcijum-aluminata ($CaO{\cdot}Al_2O_3$), kalcita ($CaCO_3$) i barijum-sulfata ($BaSO_4$) kao rendgen-kontrastnog sredstva u odnosu 2 : 2 : 1 prema recepturi V. Jokanovića. Drugi korišćeni materijal je kalcijum-silikatni MTA Angelus (Londrina, Brazil).

Antimikrobni efekat je ispitivan testom difuzije u agaru. Kao test-mikroorganizmi korišćeni su standardni sojevi *Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923* i *Candida albicans ATCC 10231*. Kulture mikroorganizama stare 24 h su podešavane na gustinu Mekfarlandovog (McFarland) standarda koji je odgovarao broju od 10^6 po mililitru mikroorganizama, a zatim su zasejane na odgovarajuće podloge. *E. faecalis, E. coli* i *S. aureus* su kultivisani na krvnom MuellerHinton agaru, dok je *C. albicans* kultivisana na agaru Sabouraud Dextrose. Pre postavljanja ispitivanih materijala sveže zasejane kulture mikroorganizama su inkubirane pod aerobnim odnosno anaerobnim uslovima. Materijali su zamešani po odgovarajućim recepturama i naneseni na sterilne papirne diskove prečnika 5 mm (po šest za svaki materijal), a zatim postavljeni u podloge sa zasejanim mikroorganizmima. Zasejane ploče su potom ostavljene na sobnoj temperaturi u trajanju od 2 h kako bi se omogućila difuzija materijala kroz agar, a zatim inkubirane aerobno, odnosno anaerobno tokom 24 h na 37°C u GASPAK(CO_2H_2)sistemu, nakon čega su očitavani rezultati. Negativnu kontrolu činili su papirni diskovi bez materijala.

Antimikrobni efekat materijala je tumačen na osnovu prečnika zone inhibicije rasta testiranih mikroorganizama, izraženog u milimetrima. Merenja su obavljena na svih šest polja (po tri merenja za svako polje). Za antimikrobni efekat testiranih materijala uzimana je srednja vrednost izmerenih zona inhibicije rasta testiranih mikroorganizama. Statistička obrada podataka urađena je u programu SPSS 20.0 (IBM Corp., Armonk, NY, USA), a dobijeni rezultati su obrađeni Studentovim t-testom.

REZULTATI

Rezultati ispitivanja antibakterijskog efekta materijala, odnosno srednje vrednosti prečnika zone inhibicije rasta mikroorganizama, prikazani su na Grafikonu 1. Zone inhibicije su bile najveće za *S. aureus* nakon 24 h i kod ALBO-MCCA (5,7 ± 0,25 mm) i kod MTA (6,2 ± 0,4 mm). Izmerene srednje vrednosti zone inhibicije prema bakteriji *C. albicans* iznosile su 4,8 ± 0,18 mm za ALBO-MCCA, odnosno 5 ± 0,35 mm za MTA. Nije uočena statistički značajna razlika u vrednostima između testiranih materijala u odnosu na ova dva mikroorganizma.

Materijal ALBO-MCCA je pokazao nešto veći antibakterijski efekat prema *E. coli* u odnosu na MTA ($p < 0,05$), dok prema bakteriji *E. faecalis* nijedan materijal nije ispoljio antibakterijski efekat (Grafikon 1).

DISKUSIJA

Antimikrobna svojstva stomatoloških materijala uglavnom se ispituju u *in vitro* studijama, testom difuzije u agaru, testom dilucije agara i direktnim kontakt-testom. Test difuzije u agaru je najčešće korišćeni eksperimentalni model za procenu antimikrobne aktivnosti, a kao test mikroorganizmi u endodonciji uglavnom se koriste bakterije koje su sastavni deo flore inficiranog kanala korena zuba i koji su prisutni u slučajevima neuspelog endodontskog lečenja. Za ispitivanje antimikrobne aktivnosti testiranih materijala ALBO-MCCA i MTA korišćen je test difuzije u agaru. Rezultati dobijeni ovim metodom u *in vitro* uslovima mogu zavisiti od niza faktora: pH, tipa i viskoznosti samog agara, korišćenih mikroorganizama i njihovog broja, vremena inkubacije, zatim rastvorljivosti ispitivanih materijala i mogućnosti difuzije odnosno širenja istih kroz agar. Test difuzije u agaru ima i određena ograničenja. Ovom metodom nije moguće utvrditi da li ispitivani materijal ispoljava baktericidno ili samo bakteriostatsko delovanje, kao i kolika je dužina trajanja antibakterijskog efekta. Za ovo istraživanje odabrani su mikroorganizmi koji su sastavni deo flore inficiranog kanala. *Enterococcus faecalis* i *Candida albicans* se smatraju najotpornijim mikroorganizmima u usnoj duplji i često su povezani sa neuspehom lečenja kanala korena [13, 14], a *Staphylococcus aureus* je takođe izolovan iz primarne i uporne sekundarne infekcije. Rezultati ovog istraživanja su pokazali da su kod oba testirana materijala najveće zone inhibicije rasta zabeležene prema bakteriji *S. aureus*, dok prema bakteriji *E. faecalis* nisu ispoljili antimikrobno svojstvo. Faktori koji bi mogli uticati na antibakterijsku aktivnost oba materijala su bazne vrednosti pH i difuzija komponenata i jona koji se oslobađaju u medijum [6]. Visoka alkalnost stvara nepovoljnu sredinu za rast mikroorganizama [15], a u toku hidratacije MTA dolazi do oslobađanja hidroksilnih jona, koji kao visoko reaktivni slobodni radikali zajedno sa povećanim pH vrednostima mogu prouzrokovati oštećenje citoplazmatske membrane i DNK bakterijskih mikroorganizama [16].

Kada je u pitanju kalcijum-aluminatni cement, antimikrobni efekat bi mogao biti uslovljen i jonima Ca^{2+} i nanočesticama oksida Al^{3+}. Ove nanočestice, kao pozitivno naelektrisani joni, mogu da se vežu za negativno naelektrisane zidove bakterijskih ćelija i smanje vijabilnost ćelija. *Mukherjee* i sar. ističu da nanočestice aluminijum-oksida mogu ispoljiti inhibitorni efekat prema bakteriji *E. coli*. Isti autori navode da su SEM analize pokazale da nakon interakcije ćelija *E. coli*, *Pseudomonas aeruginosa* i *Bacillus subtilis* sa nanočesticama aluminijuma dolazi do promena u morfologiji ćelija, odnosno do njihove izobličenosti [17]. U studiji *Sadiqa* i sar. nanočestice aluminijum oksida su ispoljile blagu antimikrobnu aktivnost prema *E. coli* [18]. U nedavnoj studiji *Manyasree* i sar. su ispitivali antibakterijski efekat nanočestica alumina na *E. coli, Proteus vulgaris, Staphylococcus aureus* i *Streptococcus mutans,* testom minimalno inhibitorne koncentracije. Uočeno je da je sa sukcesivnim porastom koncentracije nanočestica (10–50 mg/ml) rastao i antibakterijski efekat prema svim testiranim mikroorganizmima [19]. Ipak, uticaj samih komponenata kalcijum-aluminatnog cementa na antimikrobnu aktivnost potrebno je ispitati u daljim istraživanjima. S druge strane, jedan od faktora koji bi mogao ograničiti antibakterijsku aktivnost testiranih materijala je to što su oni po sastavu cementi, pa je njihovim vezivanjem otežana difuzija kroz medijum. Izostanak antibakterijskog efekta oba materijala prema mikroorganizmu *E. faecalis* u našem istraživanju mogao bi se objasniti činjenicom da je ovaj mikroorganizam jako rezistentan na visok pH i da su za njegovu eliminaciju potrebne vrednosti pH veće od 11,5. Glavni odbrambeni mehanizam mikroorganizma *E. faecalis* je protonska pumpa bakterijske ćelije koja se nalazi u ćelijskoj membrani. Acidifikacijom ci-

toplazme protonska pumpa održava pH u fiziološkom opsegu i na taj način omogućava normalno funkcionisanje enzima i proteina u ćeliji [14]. Slično našem istraživanju, *Torabinejad* i sar. i *Estrela* i sar. takođe potvrđuju odsustvo antibakterijskog delovanja MTA prema mikroorganizmu *E. faecalis* [3, 20]. Suprotno ovim navodima, *Lovato* i *Sedgley* su zaključili da je MTA ispoljio antimikrobni efekat prema kliničkom izolatu *E. faecalis* [21]. *Miyagak* i sar. su pokazali da MTA i Portland cement ne ispoljavaju antimikrobni efekat prema bakterijama *C. albicans, S. aureus* i *E. coli*, što je u suprotnosti sa rezultatima našeg istraživanja [22]. *Ribeiro* i sar. su u studiji ukazali na to da MTA takođe nije ispoljio inhibitorno delovanje prema bakterijama *E. faecalis* i *E. coli* [23].

Oprečnost rezultata kada je u pitanju antimikrobni efekat MTA pokazale su brojne druge studije. *Tanomaru-Filho* i sar. su pokazali da materijali bazirani na MTA ispoljavaju antimikrobnu aktivnost prema bakterijama *S. aureus* i *E. faecalis* [4]. Slično i u studiji *Demiryüreka* i sar., MTA Angelus je ispoljio antimikrobni efekat prema bakterijama *E. faecalis* i *C. albicans* [24]. MTA i novi endodontski nanostrukturni nanomaterijali na bazi visoko aktivnih kalcijum-silikata ispoljili su antibakterijski efekat prema bakterijama *E. faecalis* i *S. aureus* [25]. *Al-Hezaimi* i sar. su ispitivali antimikotični efekat različitih koncentracija dve komercijalne varijante MTA (bela i siva) na *C. albicans*. Rezultati agar difuzionog testa su pokazali da koncentracija materijala značajno doprinosi antimikotičnoj aktivnosti MTA. U koncentracijama od 25 mg/mL i 50 mg/mL obe varijante MTA su ispoljile antimikotičnu aktivnost, što nije bio slučaj kada je koncentracija materijala bila ispod 25 mg/mL [26]. U istraživanju *Kima* i sar. MTA Angelus je ispoljio antimikrobnu aktivnost na *S. mutans, Lactobacillus rhamnosus, Lactobacillus paracasei i Porphyromonas gingivalis,* dok je izostao inhibitorni efekat na *E. faecalis* [15]. *Özyürek* i *Demiryürek* su ispitujući antimikrobnu aktivnost MTA Angelusa prema mikroorganizmima *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* i *Enterococcus faecium,* zaključili da ovaj materijal ispoljava antimikrobno dejstvo prema svim testiranim mikroorganizmima [27]. Različiti rezultati antibakterijskog svojstva MTA u studijama pripisuju se različitim testiranim sojevima mikroorganizama, koncentraciji i tipu MTA koji se koristio, kao i samom metodološkom protokolu. Pojedini autori preporučuju upotrebu hlorheksidina umesto destilovane vode kako bi se poboljšala antimikrobna svojstva MTA [28]. U studiji *Holta* i sar. mešanje praha MTA sa 2% hlorheksidin-glukonatomom doprinelo je povećanju antibakterijskog efekta protiv bakterije *E. faecalis* [29].

Kalcijum-aluminat pripada novoj grupi endodontskih materijala i u dostupnoj literaturi malo je podataka o njegovom antimikrobnom delovanju. *Silva* i sar. su u svojoj studiji ukazali na to da su kalcijum-aluminatni cement (EndoBinder) i MTA ispoljili antimikrobnu aktivnost prema sva tri testirana mikroorganizma (*Staphylococcus aureus, Enterococcus faecalis* i *Candida albicans*) testom difuzije u agaru nakon 24 h i 48 h [10]. U sličnoj studiji *Pires-de-Souza* i sar. su ispitivali antimikrobni efekat MTA i kalcijum-aluminatnog cementa EndoBindera sa različitim rendgen-kontrasnim sredstvima (bizmut-oksid, cirkonijum-oksid i cink-oksid). Njihovi rezultati pokazali su da je MTA ispoljio bolji antibakterijski efekat u odnosu na sve kalcijum-aluminatne formulacije prema mikroorganizmu *S. aureus*. Zone inhibicije prema mikroorganizmima *C. albicans, E. coli* i *E. faecalis* nisu bile statistički značajne između MTA i kalcijum-aluminatnog praha, odnosno formulacije sa bizmut-oksidom. Na pomenuta tri mikroorganizma kalcijum-aluminat sa cirkonijum-oksidom i cink-oksidom nije ispoljio antimikrobni efekat. Antimikrobnu aktivnost EndoBindera autori studije pripisuju razgradnji kalcijum-aluminatnog hidrata formiranog tokom hidratacije, pri čemu dolazi do oslobađanja kalcijuma i hidroksilnih jona. Odsustvo antimikrobnog efekta kod pojedinih formulacija kalcijum-aluminata autori su pripisali rendgen-kontrastnim komponentama koje se ne ponašaju inertno, već mogu modifikovati procese hidratacije, smanjujući oslobađanje jona kalcijuma i uticati na pojedine fizičko-hemijske osobine materijala [30].

ZAKLJUČAK

Materijal ALBO-MCCA je ispoljio određeni antibakterijski efekat na bakterije *Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923* i *Candida albicans ATCC 10231* komparabilan sa antibakterijskim efektom MTA.