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Short title: Antibacterial effects of ALBO-MPCA
Abstract
Aim: The main purpose of this study was to evaluate antimicrobial activity of new endodontic nano-structured highly active calcium silicates based materials (ALBO-MPCA and CS) in comparison to MTA+ and CH. Material and methods: The antimicrobial activity of materials was tested against Staphylococcus aureus (ATCC 25923) and Enterococcus faecalis (ATCC 14506) strains, and following clinical isolates: Rothia dentocariosa, Enterococcus faecalis, Staphylococcus aureus, Streptococcus anginosus and Streptococcus vestibularis using a double layer agar diffusion test. The pH measurements were performed using pH meter. Total amount of released ions was determined by ICP-OES. Results: All tested materials showed the best antibacterial potential after 1h incubation. After 3h and 24h incubation period antibacterial potential of all tested materials are similar. Agar diffusion test showed that ALBO-MPCA, CS and MTA+ have similar inhibition zones (p>0.05) except in activity against Staphylococcus aureus where ALBO-MPCA showed better antimicrobial properties than MTA+ in 3h and 24h incubation period (p<0.05). Following 24h of incubation, inhibition zones were the strongest with CH against Staphylococcus aureus, (16.67±2.34 mm) followed by ALBO-MPCA (14.67±1.21 mm), and the weakest with CS against Enterococcus faecalis (6.50±1.76 mm). CH showed the highest pH, followed by ALBO-MPCA, CS and MTA+.
Conclusion: Expressed antibacterial effects indicate that materials based on nano-structured highly active calcium silicates represent effective therapeutic agents for root canal obturation in one-visit apexification treatment, therefore they are recommend for further examination and clinical trials as they are proposed for MTA substitution.

Key words: Calcium silicates, Calcium hydroxide, New material

Сажетак
Циљ: Основни циљ овог истраживања је био да се процени антибактеријска активност нових ендодонтских нано-структурираних материјала на бази високо активних калијум силиката (ALBO-MPCA и CS) у односу на MTA+ и CH. Материјал и методе: Тестирана је антибактеријска активност материјала против Staphylococcus aureus (ATCC 25923) и Enterococcus faecalis (ATCC 14506), као и
клинчих изолата: *Rothia dentocariosa, Enterococcus faecalis, Staphylococcus aureus, Streptococcus anginosus* и *Streptococcus vestibularis* помоћу агар дифузионог теста. Мерења рН вредности обављена су коришћењем рН метра. Укупан износ ослобођених јона одређиван је помоћу ICP-OES. Резултати: Сви тестиране материјале показали су најбољи антибактеријски ефекат након 1h од инкубације. Након 3h и 24h од инкубације антибактеријски ефекат свих тестираних материјала био је сличан. Агар дифузиони тест показао је да материјали ALBO-MPCA, CS и МТА⁺ испољавају сличне зоне инхибиције расти (p>0.05) осим у случају *Staphylococcus aureus*, где је материјал ALBO-MPCA показао боље антибактеријско дејство него МТА⁺ након 3h и 24h од инкубације (p<0.05). Након 24h од инкубације, зоне инхибиције су биле најизраженије у случају материјала CS против *Staphylococcus aureus* (16.67±2.34 mm) затим ALBO-MPCA (14.67±1.21 mm), а најслабије у случају CS против *Enterococcus faecalis* (6.50±1.76 mm). Материјал СН испољио је највећу рН вредност, затим ALBO-MPCA, CS и МТА⁺. Закључак: Испољени антибактеријски ефекти указују на то да материјали на бази високо активних калијум силикати могу да представљају ефикасну замену за МТА у терапији зуба са незавршеним растом корена у једној посети, те се стога препоручују за даља клиничка испитивања.

Кључне речи: Калијум силикати, Калијум хидроксид, Нови материјал

Introduction

The main task of endodontic treatment is to eliminate pathologically altered tissue, to disinfect root canal space and to obtain its three-dimensional hermetic obturation, as residual microorganisms are usually present in apical ramifications and isthmuses that are never completely filled. More than 99.5 % of Gram-positive bacteria, is eliminated by a proper chemo-mechanical root canal treatment. Residual microorganisms, particularly *Enterococcus* and *Streptococcus* species are considered to be responsible for the treatment failure. Moreover, *Enterococcus faecalis* has the ability to bind with the collagen fibers and survive up to 12 months in the environment without the substrate. Facultative anaerobes and Gram-positive species, revealing a heterogeneous profile of polymicrobial
infection are frequently isolated from the root canals following an unsuccessful endodontic treatment. An ideal material for root canal obturation must prevent both, apical and coronal leakage. It has to be biocompatible, noncancerous and nongenotoxic, dimensionally stable and insoluble in tissue fluids. Considering residual microorganisms' ability to provoke periapical irritations, it is preferable for sealing materials to possess antibacterial activity. So far, sealers based on calcium hydroxide proved to be the most efficient against a range of pathogenic microorganisms. Their major advantage is high pH which is toxic to bacterial cells, leading most likely to protein denaturation and damages of cytoplasmic membrane or DNA. However, it is also proved that calcium hydroxide based sealers have limited antimicrobial effect on *Enterococcus faecalis*. In the early 1990, different commercial products of mineral trioxide aggregate (ProRoot MTA, WMTA Angelus, GMTA Angelus) have been synthesized. Initially, MTA was recommended as a root-end filling material, while today it is used in a number of endodontic procedures, particularly as an apical barrier in teeth with incomplete root development. MTA is composed of hydrophilic particles which, in the presence of water, form a colloidal gel that is transformed into solid cement. When mechanically mixed, MTA based materials achieve better marginal adaptation, and consequently possess better sealing property. The high pH value achieved during the setting suggests potential antibacterial activity of the material. Due to variations in the chemical composition of MTA based materials, and the grain size, differences in hydration rate, flowability, consistency and setting time can be expected. Incorporation of the hydrosoluble polymer can reduce dry consistency of MTA based materials and thus to improve the material handling. Several attempts were made to improve MTA manipulation characteristics which complicate its use during orthograde canal filling procedures. Similar to MTA, new nano-structured materials, calcium silicates (CS) and albo-mineral polyoxide carbonate aggregate (ALBO-MPCA), with the reduce setting time and morphology which provides a distinct activity after their placing into vital tissues, has been introduced. The aim of this study was to evaluate pH, ion release and the antimicrobial effects of two new endodontic materials based on nano-structured highly active calcium silicates (ALBO-MPCA and CS) in comparison to MTA and UltraCal XS (CH).
Materials and Methods

The study was carried out at the University of Belgrade: Faculty of Dental Medicine, Institute for Nuclear Sciences “Vinca”, Faculty of Veterinary Medicine and Institute of Chemistry, Technology and Metallurgy. Prior to conducting this study, informed written consent was obtained from the patients. The study was designed in accordance to the guidelines of the Declaration of Helsinki and approved by Ethics Committee.

The isolation of microorganisms

All clinical isolates used in the experiment were obtained at the University Clinic, from patients’ infected root canals, using endodontic needles. Endodontic needle samples have been taken in pairs (for aerobic and anaerobic cultivation) and collected in thioglycollate broth (Institute of Virology, Vaccines and Sera-Torlak, Belgrade, Serbia) and brain heart infusion agar (BHI, Becton, Dickinson and Company, Sparks, USA) and left for 24 h at 37°C. Overnight cultures were streaked on appropriate media for cultivation; aerobic cultures on Columbia agar with 5% sheep blood (COS, bioMérieux, Marcy-l'Étoile, France) and MacConkey agar (Becton, Dickinson and Company, Sparks, USA) and incubated in aerobic atmosphere overnight, while anaerobic ones on Columbia agar with 5% sheep blood and incubated in a jar under anaerobic conditions using GasPack (GasPak™ EZ Gas Generating Container Systems, Becton, Dickinson and Company, USA), at 37°C for 2 to 5 days. Grown bacterial colonies from anaerobic conditions were put on Columbia agar with 5% sheep blood at 37°C overnight to determine demand for obligatory anaerobiosis in such bacteria. Preliminary identification of clinical isolates has been done by Gram stain, hemolysis on COS, catalase, oxidase (Oxidase Reagent Droppers Becton, Dickinson and Company, USA) and coagulase tests (Rabbit plasma, Veterinary Medicine Institute Inc., Zemun, Serbia). In order to confirm identification of the Gram positive bacteria, BD BBL Crystal™ Identification Systems Gram–Positive ID (Becton, Dickinson and Company, Sparks, USA) has been conducted.
Materials

For the synthesis of two new nano-structured materials based on active silicate systems (CS and ALBO-MPCA), mixture components have been prepared\textsuperscript{14,15}. Briefly, calcium silicate phases, $\text{2}\beta\text{-CaSiO}_4$ ($\text{C}_2\text{S}$) and $\text{C}_3\text{SiO}_5$ ($\text{C}_3\text{S}$), were synthesized using stoichiometric quantities of $\text{CaCl}_2\times\text{5H}_2\text{O}$ and silica sol by hydrothermal treatment, in a following ratio: $\text{C}_3\text{S}:\text{C}_2\text{S}=2:1$. $\text{Al(C}_2\text{H}_3\text{O}_2)$ was added to allow production of an active $\text{C}_3\text{A}$ phase. Calcium chloride tetrahydrate was used as the precursor for production of $\text{CaCO}_3$, while sulfonyl dodecyl sulfate was added as an antiagglomeration agent. The final mixture was made by mixing $\text{CaCO}_3$ with calcium silicate phases ($\text{C}_3\text{S}$ and $\beta\text{-C}_2\text{S}$) in the case of CS, while the monoclinic $\text{Bi}_2\text{O}_3$ was added in case of as ALBO-MPCA as a radiocontrast agent.

As control materials, mineral trioxide aggregate (MTA\textsuperscript{+}, Cercamed, Stalowa Wola-Poland), consisting of calcium hydroxide and silicon, iron, aluminium, sodium, potassium, bismuth, magnesium oxides and calcium phosphate; as well as calcium hydroxide based paste (UltraCal XS, UltraDent, South Jordan, USA), were used.

Agar diffusion test

Antimicrobial activities were examined against following bacterial strains: $\text{Staphylococcus aureus}$ ATCC 25923, $\text{Enterococcus faecalis}$ ATCC 14506 and clinical isolates: $\text{Enterococcus faecalis}$, $\text{Staphylococcus aureus}$, $\text{Rothia dentocariosa}$, $\text{Streptococcus anginosus}$ and $\text{Streptococcus vestibularis}$. After activation from stock culture, microorganisms were maintained as overnight cultures on Cation Adjusted Mueller-Hinton Broth (CAMHB, Becton, Dickinson and Company, Sparks, USA) and seeded on Cation Adjusted Mueller-Hinton agar (CAMHA, Becton, Dickinson and Company, USA) and COS at 37°C for 24 h before use.

Examination of antimicrobial activity of endodontic materials was conducted by double layer agar diffusion test (ADT) on 90 mm sterile Petri plates. The base layer was made of 10 ml sterilized CAMHA. After 24 h, four uniform wells (5 mm in diameter), each one corresponding to a single tested sealer, were made by sterile plastic tubes and filled with freshly mixed materials. The seeding layer that was put over the base, consisted of 10 ml sterile CAMHA inoculated to achieve $10^8$ CFU/ml of tested bacteria, which corresponds to
0.5 McFarland scale. The plates were left at room temperature for 2 h, in order to allow prediffusion of materials, and after that they were incubated for 1 h, 3 h and 24 h, at 37°C. Aliquots of 5 ml of triphenyltetrazolium chloride (TTC) prepared with 0.05% of TTC and 1% CAMHA were added for optimization. After solidification of CAMHA+TTC, plates were incubated for 30 min at 37°C. Negative control was conducted using the same method without placing the materials. Diameters of inhibition zones of bacterial growth were measured in above mentioned time intervals. All tests were done in sixtriplicate, except the positive controls which were done in triplicate.

\textit{pH measurements}

All pH values were repeatedly measured (three times), using pH-meter (pH-vision Microcomputer 6071, JENCO Electronics Ltd., Linkou Shiang, Taiwan) combined with the HI-type electrode 1131 (Hanna Instruments WTW GmbH, Woonsockets, RI-USA). The calibration of pH-meter was performed using biftalato (pH=4.01) and phosphate buffer (pH=7.00) (Carlo Erba Reagents SpA, Rodano, Italy). Suspensions of 50 mg/mL of each tested material into deionized water were prepared, then shaken on vortex for 30 min and centrifuged for 15 min at 4000 rpm. Readouts of pH measurements were carried out after 1 h, 3 h and 24 h. The solutions of deionized water were used as controls (5.76±0.51).

\textit{Inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis}

Investigated materials were prepared according to the manufacturers’ instruction and placed into plastic molds (5 mm in diameter and 5 mm high) to set. After the setting, discs of each investigated materials were placed into 20 ml of deionized water (n=3). Deionized water was changed after 1 h, 3 h and 24 h and the concentrations of ions were measured using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK) spectrometer equipped with RACID86 Charge Injector Device detector, concentric PTFE nebulizer, quartz torch, and alumina injector. ICP-OES measurements for each sample were carried out three times. Quantifications of released ions into deionized water were performed at the adequate emission wavelength of light.
Statistical analysis

Data analysis was performed using ANOVA Repeated Measures test, post hoc Tukeys’ test. The level of significance was set at p<0.05 and the data were processed using statistical software IBM SPSS 20.

Results

Data obtained in the ADT for each of the investigated materials are presented in Fig1, Fig 2, Fig 3, Fig 4, Fig 5, Fig 6 and Fig 7. The CH had the largest inhibitions zones against all bacterial strains (Fig 8). Inhibition zones of tested materials, 24 h following the incubation, were the largest with the CH against *Staphylococcus aureus* (16.67±2.34 mm) followed by the ALBO-MPCA (14.67±1.21 mm), and the weakest with the CS against *Enterococcus faecalis* (6.50±1.76 mm). *Streptococcus anginosus* didn’t exhibit any growth after 1h. Statistically significant differences were observed between the CH and other investigated materials with respect to: *Streptococcus anginosus* and *Enterococcus faecalis*; *Enterococcus faecalis* ATCC and *Streptococcus vestibularis*, except between CH (24h following the incubation) and ALBO-MPCA (1h following the incubation). Statistically significant differences concerning antibacterial activity against *Staphylococcus aureus* were also registered between: the CH and MTA⁺, in all observation periods; the CH and CS (3h and 24h following the incubation); the MTA⁺ (3h and 24h following the incubation) and ALBO-MPCA (1h and 3h following the incubation).

The values of inhibitions zones decreased over time in most tested bacterial strains and incubation periods, but increased or remained in size in the certain cases: ALBO-MPCA against *E. faecalis* ATCC 14506 (8.17±1.47); CH (14.83±2.64) and MTA⁺ (8.17±1.94) against *Rothia dentocariosa*; and CH (16.67±2.94) against *S. aureus*. Although without observed statistical differences, the investigated materials in our study seem to show better antibacterial activity against clinical isolates in comparison to *S. aureus* ATCC 25923 strain, with an exception in case of the CH and the MTA⁺ 1h following the incubation. Contrary, smaller inhibition zones concerning clinical isolates of *E. faecalis* were observed, then referent strain.
The mean pH values of investigated materials are presented in Table 1. All of them acquired pH values above 11, with an increasing trend during time, except in the case of the MTA\(^+\). The pH values for the MTA\(^+\) were the lowest (8.23±0.01), but still alkaline.

Table 2. represent ion releases by investigated materials into deionized water. The calcium ion release increased over time with regard to all tested materials, except the MTA\(^+\), where the release kept declining. Unlike the cumulative aluminium ion release (MTA\(^+\)>ALBO-MPCA>CS>CH), the values for the cumulative release of calcium were as follows: CH>ALBO-MPCA>CS>MTA\(^+\). Although weaker antibacterial performance, AMBO-MPCA have multiple larger potassium, magnesium and sodium ion release compared to CH.

**Discussion**

ADT is widely used method for determination of antibacterial activity of soluble materials. Results obtained by this method may depend on solubility of tested materials, their ability to diffuse in agar and cell medium\(^{16}\). The materials' diffusion ability may be influenced by numerous factors, such as: agar type, contact between material and agar, molecular mass, size and form of antibacterial agent, load and concentration of tested material, agar viscosity, ion concentration in relation to medium, used microorganisms, agar quantity, incubation time, etc.\(^{16}\). One of the major limitations of ADT method is that it is not capable to determine whether material possesses bacteriostatic or bactericidal effect\(^{17}\).

So far, many researchers reported conflicting results on antibacterial effects of a range of sealers and their different forms, weather they were freshly mixed or completely set\(^{16,18}\). Nevertheless, sealers may have the ability to release constituents with antibacterial effects even after their complete setting\(^{19}\). Since in everyday clinical practice the sealing materials are commonly applied freshly mixed, in our study we have investigated antibacterial effects of materials in such a form. We left Petri dishes for 2 hours at the room temperature to rest in order to achieve prediffusion of the tested materials, which is an important step in demonstrating the antibacterial effects, as previously observed\(^{20}\). Optimization with 0.05\% TTC was performed in order to differentiate the exact growth of bacterial colonies\(^{20}\). Special attention was paid to prediffusion and optimization with TTC procedures, which
allowed us to precisely determine zones of bacterial growth inhibition, and avoid possible misinterpretation with a diffusion capacity of the materials.

Up to now, little attention was devoted to investigation of antibacterial efficacy of materials similar to MTA against bacterial clinical isolates. With regard to that, a major part of our experiment was conducted using clinical isolates collected from patients' infected teeth. Since similar materials have previously shown the highest and the lowest antibacterial effects against *E. faecalis* and *S. aureus*, in our experiment we compared results of ADT using clinical and ATCC strains of the same two important bacteria. Though without statistical differences and with exceptions in cases of the CH and MTA+ following 1h of incubation, the investigated materials in our study appear to possess better antibacterial activity against clinical isolates in comparison to *S. aureus* ATCC strain(s).

Antimicrobial activity of calcium hydroxide based sealers is linked to the release of hydroxyl ions, as strong free radicals, and the capacity to absorb carbon dioxide. The similar mechanism may be proposed for MTA based materials, taking into account their setting process. It is quite familiar that pH values above 12 inhibit the growth of many microorganisms, including *E. faecalis*. Despite the high pH, even 7-day period time appears to be insufficient for CH pastes to kill bacterial cells in biofilm. Limited antibacterial efficacy against *E. faecalis* for calcium hydroxide based sealers which have pH beyond 12, put this particular bacteria in closer scope. Evens et al. suggest that the main reason for *E. faecalis* resistance lies in proton pumps that exist in its cell membrane. Our results are in agreement that the materials' solo property extremely high pH value is not sufficient and that apart from it, some other factors also interfere with bacterial growth.

All materials tested in our study had the highest antibacterial effects against *S. aureus* and the lowest against *E. faecalis*, which is in accordance with results of some previous studies. In addition, MTA based materials may also fail to inhibit the growth of *E. faecalis*, but inhibited the growth of caries-associated bacteria. An increase in inhibition zones exceeding 10%, between 1 h and 24 h, was observed in case of the CH and MTA+ against *E. faecalis* ATCC 14506, and in case of the CH, MTA+ and CS against *Rothia dentocariosa*. Our results obviously do not support previously reported ones which state that an increase in duration of incubation leads to a decrease in effectiveness of tested materials, which is probably due to differences in methodology applied (the authors of this study compared 24 hours and 7 days samples), chemical composition of tested
materials and bacterial strains. It’s known that the CH is formed during hydration reaction of MTA based materials, but for the complete maturation of different phases the time should be sufficient. This might be a possible reason for acquiring conflicting results, in addition to the fact that measured pH values may not necessarily match the ones achieved during the complex process of MTA setting and thus do not depict in vivo conditions.

Tanomaru-Filho et al. showed that MTA based materials possess antimicrobial activity against S. aureus and E. faecalis, although the sealers based on zinc oxide and eugenol made larger inhibition halos. Asgary and Kamrani also tested antibacterial activity of gray GMTA and WMTA, CH and a new endodontic cement (NEC) on the same bacteria species and confirmed antibacterial activity of all tested materials, with significant differences observed between the CH and NEC in comparison to the MTAs. The conclusions reported by Holt et al. and Sipert et al. were similar, in addition that antibacterial activity may be increased by aerobic conditions (created by inducing reactive oxygen species). In contrast to the previous studies, Yasuda et al. and Miyagak et al. concluded that the ProRoot MTA had no antimicrobial activity against any investigated species (S. aureus, E. faecalis, C. albicans, S. mutans and S. sanguinis), while the AH plus exhibited the highest antimicrobial activity out of all tested materials.

Previous studies have shown aluminium ions possess antibacterial effects. Investigated material MTA$^+$ showed largest aluminum cumulative ion release. Regarding the correlation between aluminium ion release and antibacterial effects, results of our study seem to be not enough conclusive, meaning that the individual impact of other factors has to be further investigated. The CH showed highest cumulative calcium ion release after 24 h (145610 ppb), and though an initial calcium release was high with respect to the MTA$^+$ (44570 ppb), it declined over time, but only in case of this material. The CH also exhibited the smallest sodium cumulative ion release. The above stated information contributes to understanding of their antibacterial efficacy and longevity. While sodium is a vital nutrient for many oral Streptococci, calcium is alkaline metal with relatively high atomic mass which diffuses slowly.
Conclusions

Calcium hydroxide pastes have been considered for decades as a “golden standard” for the treatment of immature teeth, but the risk of tooth fractures, potential reinfections, incomplete calcifications difficulties and consequently therapy duration remains. Considering the fact that materials based on nano-structured highly active calcium silicates possess favourable physicochemical properties, biocompatibility and as shown in this study express satisfactory antibacterial effects, they are effective therapeutic agents for root canal obturation in one-visit apexification treatment and thus significantly decrease duration of therapy. Microbiological properties of new-age nano-structured highly active materials CS and ALBO-MPCA suggest further investigations in clinical aspect and they may substitute MTA materials in dental medicine of the future.

Acknowledgements

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REFERENCES


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Different small letters indicate absence of statistically significant differences between tested materials (p>0.05)
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<td>92820</td>
<td>1074</td>
<td>383</td>
<td>2620</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>0</td>
<td>145610</td>
<td>1333</td>
<td>618</td>
<td>4437</td>
<td>0</td>
</tr>
</tbody>
</table>

MV- mean value, ppb- parts per billion
**Fig. 1** Inhibition zones of *E. faecalis* ATCC (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃

**Fig. 2** Inhibition zones of *E. faecalis* (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃

**Fig. 3** Inhibition zones of *Rothia dentocariosa* (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃. Different small letters indicate statistically significant differences between tested materials (p<0.05)

**Fig. 4** Inhibition zones of *Streptococcus anginosus* (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃

**Fig. 5** Inhibition zones of *Streptococcus vestibularis* (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃
Fig. 6 Inhibition zones of *Staphylococcus aureus* ATCC 25923 (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃. Different small letters indicate statistically significant differences between tested materials (p<0.05)

Fig. 7 Inhibition zones of *Staphylococcus aureus* (in mm) determined by double layer agar diffusion test in different time periods

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃

Fig. 8 Representative inhibitions zones of a clinical isolate *S. aureus* determined by double layer agar diffusion test, after 1 hour.

CH- UltraCal XS; MTA- mineral trioxide aggregate, MTA⁺; ALBO-MPCA- calcium silicate based material with Bi₂O₃; CS- calcium silicate based material without Bi₂O₃
The image contains two bar charts comparing bacterial inhibition zones in mm for different materials over time.

**Top Chart:**
- **X-axis:** Time (1h, 3h, 24h)
- **Y-axis:** Bacterial inhibition zones in mm
- Materials compared: CH, ALBO-MPCA, CS, MTA+

**Bottom Chart:**
- **X-axis:** Time (1h, 3h, 24h)
- **Y-axis:** Bacterial inhibition zones in mm
- Materials compared: CH, ALBO-MPCA, CS, MTA+
Bacterial (E. faecalis, ATCC) inhibitory zones in mm

Time

- CH
- ALBO-MPCA
- CS
- MTA+