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Djelovanje kalcijeva klorida na kemijska svojstva mineralno-trioksidnog agregata

Effect of CaCl₂ on Chemical Properties of Mineral Trioxide Aggregate

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Sažetak

Zbog visoke biokompatibilnosti mineralno-trioksidni agregat (MTA) pogodan je za različite mogućnosti liječenja (u endodonciji?). No, produljeno vrijeme stvrdnjavanja ograničava primjenu. **Svrha:** svrha istraživanja bila je procijeniti djelovanje MTA s 5-postotnim ili 10-postotnim udjelom CaCl₂ kao akceleratorom stvrdnjavanja, na pH i na količinu kalcijevih iona otpuštenih iz MTA-a (ProRoot MTA, Dentsply, Tulsa Denal, Tulsa, SAD). **Materijal i metode:** Prašak MTA-a bio je pomiješan sa sterilnom vodom u kojoj je bio kalcijev klorid ili u drugom slučaju, kada nije bio dodan. Zatim se pH-metrom (Sentron 2001, Intergrated Sensor Technology, Roden, Nizozemska) te nakon toga atomsko-apsorpcijskim spektrometrom (Perkin-Elmer 1100B, Weiterstadt, Njemačka) izmjerio pH i količina otpuštenih kalcijevih iona i to odmah nakon stvrdnjavanja, a zatim za jedan, tri te dvadeset i četiri sata. **Rezultati:** Uzorci s 10-postotnim udjelom CaCl₂ pokazali su povišeni pH odmah nakon stvrdnjavanja, a tako se nastavilo i tijekom svih triju vremenskih intervala ($p < 0,01$). Uočeno je da je najviše otpuštenih kalcijevih iona bilo u skupinama s kalcijevim kloridom (CaCl₂). **Zaključak:** Rezultati ovog istraživanja pokazuju da CaCl₂ kao akcelerator stvrdnjavanja, poboljšava biološka svojstva mineralno-trioksidnog agregata (MTA) tako da povećava pH i količinu otpuštenih kalcijevih iona.

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Uvod

U endodontskoj kirurgiji obično se za retrogradne kavitete koriste odgovarajući materijali kako bi se spriječila prohodnost između korijenskog kanala i periradikularnog tkiva ili popravili defekti korijena, kao što su resorpcija i perforacija (1). Ti se materijali ne bi smjeli resorbirati - trebali bi biti radiokontrastni, biokompatibilni i dimenzijski stabilni. Također je poželjno da mogu inducirati regeneracijsku aktivnosti (2). Materijali kao amalgam, Super EBA, cinkov oksidni eugenol (ZOE), kompozitne smole i staklenoionomerni cementi (3) dosad su se koristili za naknadno punjenje i zatvaranje perforacija. No, nijedan ne ispunjava sve zahtjeve (2,3).

Introduction

Endodontic surgery procedures usually involve the placement of material in root-end cavities to block the pathways between the root canal and the peri-radicular tissues and aim to repair root defects such as resorptions and perforations (1). As expected the material should be nonresorbable, radiopaque, biocompatible and dimensionally stable. Also the ability of the material to induce regenerative activity is highly preferred (2). To date various materials such as amalgam, Super EBA, zinc-oxide-eugenol cements, composite resin, and glass-ionomer cements (3) have been used for retrograde fillings and perforation repairs. However none of the

Posljednjih se godina čini da mineralno-trioksidni agregat (MTA), kao novi "endodontski reparatorni cement, ima sva svojstva idealnog materijala - visoko je biokompatibilan, može se vezati za dentin te potiče cijeljenje i osteogenezu (5). Također ima antimikrobno djelovanje (6) te se zbog hidrofилности može koristiti u kirurškim područjima kontaminiranim krvlju. Prvotno se zagovaralo korištenje MTA-a kod zatvaranja perforacija te kao materijal za punjenje korijenskih kanala (7). No, zbog povoljnih svojstava predložene su druge primjene, uključujući izravno prekrivanje pulpe (8), apeksifikaciju u jednom posjetu (9) ili liječenje vanjske resorpcije korijena (10). Prema prijašnjim istraživanjima propuštanja boje i bakterija, bolji su rezultati postignuti kod perforacija zatvorenih MTA-om u usporedbi s amalgamom, IRM-om, ZOE-om ili cementom Super EBA (11,12).

Komercijalno dostupan MTA mješavina je trikalcijeva silikata, trikalcijeva aluminata, dikalcijeva silikata, kalcijeva sulfata dihidrata i bizmutova oksida (ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, SAD). Navedeno je da slične kemijske sastave imaju MTA i Portlandski cement, osim ako je riječ o bizmutnom oksidu koji je odgovoran za radiokontrastnost MTA-a. Kad se prašak MTA-a pomiješa sa sterilnom vodom, stvara se koloidni gel koji se, u kliničkim uvjetima, stvrdne za oko tri sata. Premda sporo stvrdnjavanje može značiti bolju adaptaciju i manje skupljanje (13), integritetu materijala trebalo bi posvetiti posebnu pozornost, jer bi ga mogle isprati tekućine u usnoj šupljini prije nego što se stvrdne (3). Kalcijev klorid (CaCl_2), kalcijev format i kalijev klorid dio su kemikalija koje se koriste kako bi se ubrzalo stvrdnjavanje Portlandskog cementa (14). Sličnost između MTA i Portlandskog cementa potaknula je stručnjake da u MTA dodaju CaCl_2 kako bi ubrzali vrijeme njegova stvrdnjavanja (15). No, postoji nekoliko istraživanja o učincima dodataka na biološka svojstva MTA-a.

Visok pH i količina otpuštanja kalcijevih iona pripisuje se biološkim svojstvima MTA-a. (16,17). Zato je svrha ovog istraživanja bila procijeniti učinak dodavanja CaCl_2 u odnosu prema pH-u i otpuštanju kalcijevih iona.

Materijali i načini rada

Kontrolna skupina dobivena je miješanjem ProRootova MTA (ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, SAD) sa sterilnom vodom. U ispitivanim

above mentioned materials fulfill those requirements (2, 3). Recently, Mineral Trioxide Aggregate (MTA), a new 'endodontic repair cement', appears to have all of the characteristics of an ideal repair material. MTA is highly biocompatible (4), has the capability to adhere to dentin and stimulate healing and osteogenesis (5). It also exhibits antimicrobial activity (6) and its use on blood contaminated surgical fields is possible due to its hydrophilic characteristic. Originally MTA was advocated to be used for perforation repair and as a root filling material (7). Due to its favourable properties, some other applications of MTA have been suggested which include direct pulp capping (8), one-visit apexification (9) or external root resorption repair (10). According to previous studies, MTA repaired perforations leaked less when compared with amalgam, IRM, ZOE or Super EBA when evaluated with dye and bacteria leakage methods (11,12).

The commercially available formulation of MTA is a mixture of tricalcium silicate, tricalcium aluminate, dicalcium silicate, calcium sulfate dihydrate, and bismuth oxide (ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, OK). It has been reported that the chemical compositions of MTA and Portland cement resemble each other except the ingredient bismuth oxide which is responsible for the radiopacity of MTA (13). When MTA powder is mixed with sterile water a colloidal gel is obtained which hardens in approximately 3 hours in clinical conditions. Although slow setting may mean better adaptation and less shrinkage (13), special care should be taken to protect the integrity of the material as it may be washed out with oral fluids before setting is accomplished (3). Calcium chloride (CaCl_2), calcium formate, and potassium chloride are among those chemicals used to accelerate the setting of Portland cement (14). The similarity between MTA and Portland cement have encouraged researchers to add CaCl_2 to MTA to speed up the setting time of the material (15). However there are few studies regarding the effects of additives on the biological properties of MTA.

The biological properties of MTA have been attributed to its high pH and calcium release (16, 17). Therefore, the aim of this study is to evaluate the effect of adding CaCl_2 in terms of pH and calcium ion release.

Materials and Methods:

The control group was obtained by mixing ProRoot MTA (ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, OK) with sterile water. In the test groups,

skupinama MTA je sadržavao 5-postotni ili 10-postotni udjel kalcijeva klorida (CaCl_2).

Za ovo istraživanje koristilo se 7,5 gr ProRootova MTA, uključujući pet uzoraka s 500mg ProRootova MTA po skupini.

Materijali su pomiješani kako slijedi:

Skupina 1: 500 mg ProRootova MTA s 0,2 ml sterilne vode

Skupina 2: 500 mg ProRootova MTA s 0,15 ml sterilne vode i 25 mg CaCl_2

Skupina 3: 500 mg ProRootova MTA s 0,15 ml sterilne vode i 50 mg CaCl_2

Materijali su bili 30 sekundi mehanički miješani i zatim stavljeni u polietilenske epruvete te uronjeni u 10 ml deionizirane vode. Svih 15 uzoraka bilo je pet minuta centrifugirano u stroju na 3000 okretaja (Universal 16 A, Hettich, Njemačka).

Vrijednosti pH zabilježene su pH-metrom (Sentron 2001, Integrated Sensor Technology, Roden, Nizozemska) i to odmah nakon miješanja te nakon jednog, tri i 24 sata.

Prije toga je pH-metar bio kalibriran s otopinama poznatoga pH (7,0 i 4,0).

Između svakog mjerenja elektroda je bila oprana i osušena. Otpuštanje kalcijevih iona bilo je izmjereno atomskim apsorpcijskim spektrometrom (Perkin-Elmer 1100 B, Weiterstadt, Njemačka).

Deionizirana voda korištena je za kalibraciju stroja za nultu apsorbenciju.

Uzorci su bili razrijeđeni u deioniziranoj vodi i vrijednosti za otpuštanje kalcijevih iona zapisane su odmah nakon miješanja te nakon jednog, tri i 24 sata.

Rezultati

Rezultati istraživanja razrađeni su prema F-testu, jednostranom ANOVA-om i Tukeyevim testom.

Tablica 1. pokazuje pH vrijednosti skupina odmah nakon miješanja te nakon jednog, tri i 24 sata. Miješanje MTA s 10-postotnim kalcijevim kloridom uzrokovalo je povišenje pH-a odmah nakon miješanja u usporedbi s MTA-om bez dodataka ($p > 0,01$).

Povišeni pH u skupini s 10-postotnim udjelom CaCl_2 bio je statistički znatan u odnosu prema kontrolnoj skupini i skupini s 5-postotnim udjelom CaCl_2 ($p < 0,01$).

Dodatak CaCl_2 poticao je otpuštanje više kalcijevih iona u usporedbi s kontrolnom skupinom.

Razlika otpuštanja kalcijevih iona između skupine s 5-postotnim udjelom CaCl_2 i skupine s 10-postotnim udjelom CaCl_2 bila je također velika ($p < 0,01$), Tablica 2.

MTA contained either 5% or 10% CaCl_2 .

For this study were used 7.5 gr ProRoot MTA, including 5 samples with 500 mg ProRoot MTA for each group.

The materials were mixed as shown below:

Group 1: 500 mg ProRoot MTA with 0.2 ml sterile water

Group 2: 500 mg ProRot MTA with 0.15 ml sterile water and 25 mg CaCl_2

Group 3: 500 mg ProRoot MTA with 0.15 ml sterile water and 50 mg CaCl_2

The materials were mixed mechanically for 30 seconds and placed in polyethylene tubes, which were immersed in 10ml in deionized water. All 15 samples were centrifuged in 3000 rot/5 min. in apparatus (Universal 16 A, Hettich, Germany).

pH values were recorded at the baseline, 1, 3, and 24 hours with a pH meter (Sentron 2001, Integrated Sensor Technology, Roden, Netherlands).

pH meter was previously calibrated with pH=7.0 and 4.0 solutions.

Between each measurement the electrode was washed and dried. Ca^+ ion release was measured with the atomic absorption spectrophotometer (Perkin-Elmer 1100 B, Weiterstadt, Germany).

Deionized water was used for calibration of the apparatus for zero absorbency.

The samples were diluted in deionized water and the values of Ca ion release were recorded at the baseline, after 1 hour, 3 hours and 24 hours.

Results

The results of this study were elaborated according to F-test, one way Anova and Tukey's test.

Table 1 - illustrates the pH values of the groups at baseline, 1, 3 and 24 hours. Mixing MTA with 10% CaCl_2 caused an increase in pH at the baseline when compared with MTA with no additives ($p < 0.01$).

The increase of pH in 10% CaCl_2 group was statistically more significant than the control and 5% CaCl_2 groups ($p < 0.01$).

Addition of CaCl_2 favored more calcium release when compared with the control group.

The difference between 5% CaCl_2 and 10% CaCl_2 groups in terms of Ca release was also significant ($p < 0.01$), Table 2.

Tablica 1. Prosječna i standardna devijacija vrijednosti pH za ProRootov MTA s CaCl₂ ili bez njegaTable 1 Average and standard deviation of pH values for ProRoot MTA with or without CaCl₂

pH	Baseline Mean ± SD	1 hour Mean ± SD	3 hours Mean ± SD	24 hours Mean ± SD
ProRootov MTA	12,0 ± 0,22	11,4 ± 0,25	11,66 ± 0,28	11,72 ± 0,13
ProRootov MTA+ CaCl ₂ 5%	11,8 ± 0,12	11,76 ± 0,11	11,56 ± 0,13	11,76 ± 0,09
ProRoot MTA+ CaCl ₂ 10%	12,44 ± 0,11	12,24 ± 0,11	12,3 ± 0,10	12,42 ± 0,16
F-test	21,79	29,31	22,83	44,66
P	0,0001	<,0001	<,0001	<,0001

Tablica 2. Prosječna i standardna devijacija vrijednosti otpuštanja kalcijevih iona za ProRoot s CaCl₂ ili bez njegaTable 2 Average and standard deviation of Ca ions release values for ProRoot MTA with or without CaCl₂

Ca ione release (mg/dl)	Baseline Mean ± SD	1 hour Mean ± SD	3 hours Mean ± SD	24 hours Mean ± SD
ProRootov MTA	0,09 ± 0,02	0,11 ± 0,01	0,13 ± 0,03	0,15 ± 0,02
ProRootov MTA+ CaCl ₂ 5%	0,13 ± 0,01	0,16 ± 0,02	0,16 ± 0,01	0,30 ± 0,02
ProRootov MTA+ CaCl ₂ 10%	0,27 ± 0,01	0,31 ± 0,02	0,34 ± 0,01	1,09 ± 0,02
F-test	155	271	207,7	3198,75
P	<,0001	<,0001	<,0001	<,0001

Rasprava

Mineralno-trioksidni agregat (MTA) proizveden je devedesetih godina kao materijal za retrogradno punjenje. Od tada se preporučuje za mnogobrojne kliničke primjene. Mnoga *in vitro* i *in vivo* ispitivanja pokazala su da su rubno zatvaranje i biokompatibilnost MTA-a superiorniji u usporedbi s amalgamom, IRM-om i Super EBA-om (18-19). Dostupne su dvije komercijalne formulacije - sivi i bijeli MTA. Premda postoje neke razlike u sastavu, obje su biokompatibilne (20).

Dokazano je da se dodavanjem kalcijeva klorida u MTA, smanjuje vrijeme stvrdnjavanja materijala (15,21). No, MTA s kalcijevim kloridom pokazao je smanjene vrijednosti tlačne jačine, što se smatra važnim čimbenikom (15). Dodaje se da bi vrijeme stvrdnjavanja bilo važnije od vrijednosti tlačne jačine ako bi se MTA koristio za retrogradno, naknadno punjenje. A kraće vrijeme stvrdnjavanja, čini se, bilo bi korisnije kada je kirurško područje vlažno i materijal može isprati okolna tekućina.

PH ima važnu ulogu u biološkim svojstvima MTA-a.

U ovom istraživanju kombinacija ProRootova MTA s 5-postotnim kalcijevim kloridom nije pokazala povišenje vrijednosti pH-a, no kod ProRootova MTA s 10-postotnim udjelom CaCl₂ odmah nakon miješanja uočena je povišena vrijednost pH-a, a slično je bilo i u drugim vremenskim intervalima.

Discussion

Mineral trioxide aggregate (MTA) was developed in the 1990s as a root-end filling material. Since then it has been proposed for many clinical applications. Several *in vitro* and *in vivo* studies have demonstrated that the sealing ability and biocompatibility of MTA are superior to that of amalgam, IRM and Super EBA (18-19). Two commercial formulations are available: gray MTA and white MTA. Although both materials have some differences in terms of composition, both formulations have been shown to be biocompatible (20).

It has been reported that adding CaCl₂ to MTA decreased the setting time of the material (15, 21). However MTA with CaCl₂ displayed lower compressive strength which was considered as an important factor (15). It was also added that when MTA was used as a root-end filling material, setting time would be more of concern than the compressive strength values. Therefore faster setting time appears to be advantageous when the surgical environment is wet and there is a possibility of the material to be washed away with surrounding fluids.

pH plays a major role in biological properties of MTA.

In our study, combination of ProRoot MTA and 5% CaCl₂ did not show any increase of pH values, but the combination of ProRoot MTA and 10% CaCl₂ immediately increased the values of pH, which were similar during other intervals.

Rezultati dobiveni u ovom istraživanju slažu se s onima Bortoluzzija i njegovih suradnika (21) koji su pokazali da je 10-postotni kalcijev klorid u MTA-u trenutno znatno povišio pH.

U slučaju upale tkiva, pH bi se mogao znatno smanjiti do 5,5 ili 5,6. Navedeno je da bi kisela okolina kod pH 5 bila štetna za fizička svojstva i hidratno ponašanje MTA-a (22).

Torabinejad i Chivian (23) naveli su da MTA može ostati nestvrdnut u slučaju jake upale. U istraživanju pretražnim elektroničkim mikroskopom (SEM-om) i difrakcijom rendgenskih zraka (XRD), uzorci MTA s pH 5 nisu mogli postići konačnu tvrdoću, jer su bile kompromitirane kristalne strukture MTA (24). Također je navedeno da se trikalcijski silikat, kao osnovna komponenta MTA-a, rastopio u 20-postotnoj maleičnoj ili salicilnoj kiselini (25). Osim toga, Lee i suradnici predložili su da se prije negoli se MTA unese u upalno područje obavi predtretman s alkalnim preparatom kao što je $\text{Ca}(\text{OH})_2$, kako bi se neutralizirao pH okoline. Prema tome, u slučaju akutne upale, kada je pH okoline snižen, povišenje pH materijala moglo bi biti povoljno za stvrdnjavanje i otpornost MTA-a.

MTA nije inertan materijal, nego bioaktivan kad je u doticaju s tkivnim tekućinama (16). Fizičko-kemijska osnova biološkog svojstva MTA donedavno se pripisivala stvaranju hidroksiapatita, nakon što bi otpušteni kalcijevi ioni iz MTA došli u doticaj s tkivnim tekućinama (16). Drugi popratni proizvod hidratiziranog MTA-a je kalcijev hidroksid (20). Zato je otpuštanje kalcijevih iona iz materijala odgovorno za biološka svojstva MTA-a. Navedeno je da je dodatak CaCl_2 poboljšao svojstvo ProRoota za otpuštanje kalcija (21).

Naš pokus pokazuje da ProRootov MTA s 5-postotnim udjelom CaCl_2 , otpušta više kalcijevih iona nego čisti MTA u 24 sata. No, dodatak 10-postotnog kalcijeva klorida u ProRootov MTA omogućuje veće otpuštanje kalcijevih iona nego 5-postotni kalcijev klorid u razdoblju od 24 sata.

Ti su rezultati u skladu s onima Bortoluzzija i suradnika (21). Ti su znanstvenici otkrili da bi se lakše radilo s MTA-om kada bi bio pomiješan s kalcijevim kloridom, jer bi bilo više otpuštanja kalcijevih iona te bi se smanjilo vrijeme stvrdnjavanja.

Abdulah i suradnici (26) naveli su da bi Portlandski cement s 5-postotnim ili 10-postotnim udjelom kalcijeva klorida, možda mogao poboljšati regeneraciju kosti i smanjiti vrijeme stvrdnjavanja, a pokazao je i biokompatibilnost te netoksična svojstva.

Rezultate dobivene u ovom istraživanju možemo objasniti dodatnim kalcijem iz kalcijeva klorida.

The results obtained in this study corroborate those found by Bortoluzzi et al. (21), who found that the presence of 10% CaCl_2 increased significantly the pH in the immediate period.

In case of an infective tissue the pH may display a dramatic decrease to 5,5-5,6 and an acidic environment such as pH 5 has been reported to be detrimental to the physical properties and hydration behavior of MTA (22).

Torabinejad and Chivian (23) reported that MTA may remain unset in highly infected cases. In another SEM and XRD study the pH 5 MTA specimens could not reach the ultimate hardness because crystalline structure of MTA was compromised (24). It was also reported that tricalcium silicate which is the basic component of MTA dissolved in 20% maleic or salicylic acid (25). In addition before applying MTA over an inflamed area, Lee et al (24) suggested pretreatment with an alkaline medication such as $\text{Ca}(\text{OH})_2$ to neutralize the environmental pH. Therefore in acute infective cases where pH of the environment is decreased, an increase in the pH of the material may favor the setting and strength of MTA.

MTA is not an inert material but it is bioactive when tissue fluids are encountered (16). The physicochemical basis for the biological properties of MTA have recently been attributed to the production of hydroxyapatite when the calcium ions released by the MTA come into contact with tissues fluid (16). Another byproduct of hydrated MTA is calcium hydroxide (20). Thus calcium ion release of the material is responsible for the biological properties of MTA. It was reported that addition of CaCl_2 improved the calcium release properties of ProRoot MTA (21).

Our experiment shows that addition of 5% CaCl_2 in ProRoot MTA favored more Ca ions release than pure MTA in 24 h period. Although, the addition of 10% CaCl_2 in ProRoot MTA allows more Ca ions release than 5% CaCl_2 in 24 h period.

These results are in accordance with those found by Bortoluzzi et al. (21), who revealed that MTA material became easier to handle when mixed with CaCl_2 , increase Ca ions release and decrease setting time.

Abdulah et al. (26) reported that the Portland cement with 5% or 10% calcium chloride was potentially able to promote bone repair, reduced hardening time and presented biocompatibility and nontoxic qualities.

The explanation for the results obtained in our study remains in the additional calcium from cal-

Ispitivane skupine kojima je dodana ta supstancija imale su veće otpuštanje kalcijevih iona. Iako dodatak CaCl_2 skraćuje vrijeme stvrdnjavanja MTA-a te povećava pH i količinu otpuštanja kalcijevih iona, potrebna su dodatna istraživanja kako bi se procijenilo njegovo djelovanje.

Zaključak

Dodatkom kalcijeva klorida ProRootovu MTA-u smanjilo se vrijeme stvrdnjavanja, što se smatra važim čimbenikom u sprječavanju ispiranja MTA tijekom korištenja u vlažnim kirurškim područjima.

Kombinacijom MTA-a s 10-postotnim CaCl_2 postiže se trenutačno povećanje vrijednosti pH-a, a dodamo li 5-postotni i 10-postotni CaCl_2 otpušta se više kalcijevih iona tijekom 24 sata, što dodatno poviše pH.

cium chloride. The materials that received this substance presented higher Ca ions release. Although accelerating the setting time of MTA increased the pH and calcium ion release, further studies are warranted to evaluate its effects.

Conclusion

The addition of CaCl_2 to Pro Root MTA decreased setting time which is considered as an important factor to prevent the washing out of MTA during use in wet surgical areas.

Combination of MTA with CaCl_2 10% achieves immediate increase of pH values and the addition of 5% and 10% CaCl_2 released more calcium ions within 24 hours which enhances the increase of pH.

Abstract

MTA has been proposed for various treatment alternatives due to its high biocompatibility. However delayed setting times may limit its use. **Objectives:** The aim of this study was to evaluate the effect of adding a setting accelerator 5% or 10% CaCl_2 to MTA on the pH and calcium ion release of MTA (ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, OK). **Materials and Methods:** MTA powder was mixed with sterile water with or without CaCl_2 followed by measurement of pH and calcium ion release at baseline, 1, 3 and 24 hours with a pH meter (Sentron 2001, Integrated Sensor Technology, Roden, Netherlands) and an atomic absorption spectrophotometer (Perkin- Elmer 1100 B, Weiterstadt, Germany). **Results:** 10% CaCl_2 added specimens revealed immediate elevation in pH which continued during all time intervals ($p < 0.01$). The highest calcium release was also observed in groups with CaCl_2 . **Conclusion:** The results of this study suggest that CaCl_2 as a setting accelerator enhances the biological properties of mineral trioxide aggregate by increasing pH and calcium ion release.

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