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COMPARATIVE EVALUATION OF CALCIUM RELEASE OF THE APICAL PLUGS FORMED BY MINERAL TRIOXIDE AGGREGATE, BIODENTINE, AND ENDOSEQUENCE ROOT REPAIR MATERIAL WITH AND WITHOUT 2% TRIPLE ANTIBIOTIC POWDER: AN IN VITRO STUDY

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ABSTRACT:

Background: There are many excellent alternatives to the traditional method of apexification, including the use of MTA, Biodentine, and EndoSequence root repair material (RRM). MTA and Biodentine have been fortified with antibacterial substances to increase their antimicrobial potency. To combat the large variety of bacterial species seen in root canal infections, a combination of potent antimicrobials such as TAP is required. Clinical effectiveness is excellent due to the release of calcium from these cements.

Aim: Apical plugs made by MTA, Biodentine, and EndoSequence RRM were analyzed and compared in the study with and without the addition of 2% TAP.

Methods: A total of ninety single-established teeth were culled from a total of fifteen Two percent TAP was added to each group's MTA (Group A), MTA (Group B), Biodentine (Group C), EndoSequence RRM (Group D), and EndoSequence RRM (Group E). Ionized water was used to wet the teeth in 10 cc increments. On days 7, 15, and 30, a nuclear retention spectrophotometer was used to measure calcium discharge in the system. Information was analyzed using one-way evaluation of change (OEC), post hoc test (POST), and unpaired t-test (UNPAIRED).

Results: At days 7, 15, and 30, calcium release was highest in Group E in comparison to Group F (P 0.05), highest in Group C in comparison to Group D, and highest in Group A in comparison to Group B (P 0.05).

Conclusion: Adding 2 percent TAP to MTA, Biodentine, and EndoSequence RRM increased the amount of calcium particles formed.

Keywords: Apexification, apical plug, atomic absorption spectrophotometry, bioceramic cements, Biodentine, calcium release, EndoSequence root repair material, immature tooth, mineral trioxide aggregate, triple antibiotic powder

INTRODUCTION:

As with "iceberg" phenomena, tooth eruption in the oral cavity may be compared to There are still a few phases of tooth growth to go before the crown may be considered fully formed. In the years after a tooth's clinical eruption, roots continue to grow slowly and the apex continues to close. Any mechanical, physical, or microbiological assaults to the tooth at this crucial time might cause it to fail. any kind of trauma or significant caries, as well as pulpal pathosis during this vital phase, will stop the development of dentin and result in an open-apex tooth Since these teeth lack an apical barrier, reviving the necrotic pulp in them will be difficult. In these circumstances, calcium hydroxide apexification is the therapy of choice because of its high pH and antibacterial properties. Long treatment times, several sessions, and the use of calcium hydroxide over an extended period of time make the tooth more prone to root fractures when using the Calcium Hydroxide Apexification Technique [1]. It is thus necessary to use a one-step procedure to seal the open apex of an open tooth in order to prevent further decay.

As an alternative to calcium hydroxide apexification, mineral trioxide aggregate (MTA) is often utilized. It is a substance that is both biocompatible and sealant-friendly. Blood and dampness may also trigger the onset of MTA. Slow setting times, dentin discolouration, and complex handling features are some of the downsides of MTA. [3] To meet the growing need for dental materials, bioceramic cements have been created. As a result of its excellent physical and mechanical qualities, which resemble those of tooth dentin, biodentine is often referred to as "dentin in a capsule. As a result, it is easy to use and has a high biocompatibility and bioactivity. Also, metal impurities are avoided, therefore Biodentine does not stain the dentin like MTA, which is a big benefit. In immature teeth, Brasseler's Endo Sequence root repair material (RRM) is advised for the apical plug as a new generation bio ceramic material (four). This product is simple to use, as well as biocompatible and bioactive. It contains osteoinductive and osteoconductive capabilities, which make it able to promote tissue regeneration.

As a reaction product, MTA, Biodentine, and EndoSequence RRM create calcium hydroxide, which further dissociates into calcium and hydroxide ions, respectively. Hard and soft tissues may be disinfected since the high alkaline pH has an adverse effect on bacteria development. For these three drugs' mechanisms to work, calcium release is a necessary cofactor. [5]

Tooth decay is most often caused by a root canal infection including many strains of bacteria. MTA and Biodentine's antimicrobial activities have been improved by the addition of antibacterial chemicals such as 2 percent chlorhexidine, 10 percent doxycycline, and 2 percent silver zeolite powder. Given the disease's polymicrobial nature, it is unlikely that a single antimicrobial drug integrated into an apical plug-forming dental material could adequately sterilize the root canal. [8] With so many microorganisms, a TAP (triple antibiotic powder) is more likely to be needed. Because 2 percent TAP is added to the MTA, Biodentine, and EndoSequence RRM utilized to produce apical plugs in critical conditions of young teeth, it is a novel technique. [11]

Sterilization of wounds and stimulation of tissue regeneration are two of TAP's most well-known applications. The standard TAP regimen includes minocycline/doxycycline, ciprofloxacin, and metronidazole. the antibiotic doxycycline, which is a tetracycline, tends to form chelates with calcium ions liberated from apical plugs. Doxycycline may damage apical plugs created by MTA, Biodentine, and Endo Sequence RRM. [12] In the modified TAP version of the trial, cefaclor was used in place of doxycycline as the active ingredient. [13]

To find out whether incorporating TAP into MTA, Biodentine, and EndoSequence RRM altered their cardinal calcium release feature, this study compared the calcium release potentials of each of these materials.

METHOD:

Using convention number 0255/2017-2018, this was an in vitro study that was carried out with the approval of the moral panel. Biodentine (Septodont, St.-Maur-des-Fosses) and Endo Sequence RRM (Brasseler, USA) were used to shape apical plugs in teeth arranged to mimic youthful teeth with open apices in this study. Selection criteria for these innovative endodontic concretes included their clinical effectiveness as well as their biocompatibility and bio smart behaviour.

We used 90 human single-pull teeth that had been freshly removed for the design but were free of decay and other defects. Teeth with root break, prior deformities, or breaks on the root surface, as well as teeth with physical anomalies, were not included in the review.

The teeth were collected and stored in a 0.5 percent chloramine T arrangement. Using a low-speed handpiece, a cleaning brush and prophylaxis glue were used to clean the teeth before they were put into service.

By embedding a 25 mm size 10 Kerr record (K document), the root trench's integrity was confirmed, and its length was not fixed. The biomechanical planning was done with the goal of having the apical portion of the root trench ready to measure 40 K and the coronal erupting finished by the size 80 K document. Before and after each instrumentation procedure, the root trenches were flooded with two ml of 5.25 percent sodium hypochlorite (NaOCl). A jewel plate was used to create an isolation between the teeth's cementoenamel intersection and the tooth foundations while the examples were being created. The recreation of an open peak was completed by removing the apical 2 mm of root with the help of a jewel plate and a rapidly rotating hand piece. It was decided that the root lengths of all the examples should be 12 millimeters. Ethylene diaminetetraacetic corrosion and 5.25 percent NaOCl were used to flush root channels and flush out the NaOCl, followed by a flush of refined water to remove the remaining NaOCl from the trenches completely. After that, the pipes were dried with the aid of legitimate paper focuses. Pre-arranged teeth were autoclaved and stored in clean, impermeable containers at 37 degrees Fahrenheit in a hatchery for future use.

To prepare the new TAP, we replaced the doxycycline in regular TAP (ciprofloxacin + metronidazole + doxycycline) with ciprofloxacin and metronidazole, and then replaced it with cefaclor. Traditional medicines were used in their powdered form to maintain the blend's grain size and consistency. CIP+M+Cefaclor was stored in an impermeable container after it had been rearranged.

The following (n = 15) is how apical fittings (4 mm in diameter) were framed in each of the pre-arranged root example test groups:

- Group A: MTA + 2% TAP (4 mm of apical plug formed using MTA with the incorporation of 2% TAP)

- Group B: MTA (4 mm of apical plug formed using MTA)
- Group C: Biodentine + 2% TAP (4 mm of apical plug formed using Biodentine with the incorporation of 2% TAP)
- Group D: Biodentine (4 mm of apical plug formed using Biodentine)
- Group E: EndoSequence RRM + 2% TAP (4 mm of apical plug formed using EndoSequence RRM with the incorporation of 2% TAP)
- Group F: EndoSequence RRM (4 mm of apical plug formed using EndoSequence RRM).

MTA, Biodentine, and EndoSequence RRM were all used in accordance with the manufacturer's instructions for apical plug placement in instances from Groups B, D, and E, and 4 mm of the apical fitting was framed with endodontic pluggers.

TAP was included into MTA, Biodentine, and EndoSequence RRM to form a 4 mm apical fitting in instances of Group A, Group C, and Group F, which were all governed by the manufacturer's instructions.

Nail stain was given to the outer root surface of all root samples after the apical plugs had been arranged, with the exception of the 2 mm surrounding the apical foramen, in order to equalize the evaluation of calcium particles provided only via the apical aperture of the apical fittings.

After the final adjustment of the apical fittings, each specimen was immersed in a deionized water test tube. 370°C was the temperature at which the tubes were fastened and hatched. Days 7, 15, and 30 were used to complete the calcium discharge assessment using a nuclear retention spectrophotometer (15 of the samples in each group were also divided into three testing periods, i.e. n = 5). One-way ANOVA, Post hoc tests, and unpaired t-tests were used to analyze the results. Statistical Package for the Social Sciences (SPSS) was used to perform all of the factual analyses (Version 21.0. Armonk, NY: IBM Corp).

RESULTS:

Apical plugs made from MTA, Biodentine, and EndoSequence RRM, as well as those made

with 2% TAP incorporation in MTA, Biodentine, and EndoSequence RRM, released calcium during the course of the study's 30-day duration. At day 7, all the apical plugs emitted the most calcium ions, a trend that continued through days 15 to 30 of the experiment.

It was shown that Group F (EndoSequence RRM) had the highest concentration of calcium ions produced on days 7, 15, and 30 compared to Group D (Biodentine) and Group B (MTA).[Table 1].

Groups	**Time duration (day)**	Mean	SD	P value
Group B (MTA)	7. day	5.34	0.1	<0.05
	15. day	3.45	0.07	
	30. day	1.23	0.05	
Group D	7. day	7.01	0.02	
(biodentine)	15. day	4.43	0.14	
	30. day	1.54	0.05	
Group F	7. day	10.34	0.16	
(Endo Sequence	15. day	9.47	0.06	
RRM)	30. day	5.45	0.04	

One-way ANOVA and *post hoc* test, accepting $P < 0.05$, showed that the mean value of calcium ions released was maximum for Group E (EndoSequence RRM + 2% TAP) on days 7, 15, and 30 as compared to Group C (Biodentine + 2% TAP) and Group A (MTA + 2% TAP) ($P < 0.05$) [Table 2].

Groups	Time duration (days)	Mean	SD	P value
Group A (MTA	7. day	5.34	0.01	<0.05
+ 2% TAP)	15. day	3.56	0.03	
	30. day	1.45	0.05	
Group C	7. day	8.56	0.34	
(Biodentine +	1.5. day	4.35	0.22	
2% TAP)	30. day	1.67	0.34	
Group E	7. day	10.46	0.11	
(EndoSequence	1.5. day	9.46	0.03	

RRM + 2%	30· day	5.23	0.02	

The unpaired *t*-test, accepting $P < 0.05$, showed that the calcium ions released by Group A (MTA + 2% TAP), Group C (Biodentine + 2% TAP), and Group E (EndoSequence RRM + 2%TAP) were greater than calcium ions released by Group B (MTA), Group D (Biodentine), and Group *F* (EndoSequence RRM), respectively, on days 7, 15, and 30 ($P < 0.05$). A substantial increase in the calcium ion release potential was seen when TAP at a concentration of 2% was added to MTA, Biodentine, and EndoSequence RRM.

DISCUSSION:

During a tooth's basic development, any damage, caries, or other pulpal pathosis that impairs root development must be given special concern and treatment. Thin dentinal barriers make biomechanical instrumentation ineffective. Because of the peak's unique shape, it is impossible to guarantee a complete debridement and the lack of apical choke, which allows endodontic irrigants, medicaments, root channel fills and their consequences to exit beyond the zenith without risk of infection. An apical boundary that may act as a stop for the endodontic materials has to be strengthened on the board of a nonvital tooth with an open peak. Because of their superior physical and mechanical qualities, these three materials have become excellent alternatives to long-term apexification systems. In this review, we have selected these ingenious bioactive materials.

The multimicrobial character of the root trench disease is well-known. [6],[7] Even after trench sanitization, safe bacteria like Enterococcus faecalis may be discovered thriving in root channels. To get by in draw trenches, Enterococcus faecalis has to deal with basic conditions including glucose shortage. These "uncommon survivors" were referred to in the root trenches as such because of this. [14] As a result, the inclusion of Enterococcus faecalis (ATCC 29212) as a trial microorganism in this evaluation seemed appropriate.

Antibacterial additives such as 2 percent chlorhexidine, 10 percent doxycycline, and 2 percent silver zeolite powder have been applied to the apical fittings to improve their antimicrobial properties. [8] A combination of EndoSequence RRM and MTA was also employed to generate the apical plugs in juvenile teeth.

The cardinal calcium particle discharge mechanism of MTA, Biodentine, and EndoSequence RRM is assumed to be inherent in their cytocompatibility, biocompatibility, and regenerative/reparative potential. [15] Consequently, the purpose of this study was to evaluate the capacity of MTA, Biodentine, and EndoSequence RRM to discharge calcium, as well as to determine whether the inclusion of 2 percent TAP in these materials affects their ability to discharge calcium.

Using nuclear retention spectrophotometry, Jacinto et al. found that drenching in 10 ml of neutral pH deionized water on days 7, 15, and 30 yielded accurate calcium particle concentration estimates without contamination from the submersion solution. [5]

Material was placed in polyethylene tubes and bathed in 10 ml deionized water in previous tests to determine the amount of calcium particles released. Root tests with contoured apical fittings were used in our evaluation. As a result of this new approach, calcium discharge evaluation has become more accurate and relevant. Calcium particles provided via dental materials may be studied using the technique of nuclear retention spectrophotometry. [16],[17]

Calcium particles provided by EndoSequence RRM and EndoSequence + 2 percent TAP apical fittings were more conspicuous than those delivered by other review groups, according to the current in vitro study. According to the most recent research, the addition of 2% TAP to MTA, Biodentine, and EndoSequence RRM improved the particles' ability to release calcium.

It has been shown that bioceramic endodontic materials can provide calcium particles for a longer period of time, which can be inferred from their fundamental signature that calcium silicate included in them is hydrolyzed to generate a gel and hydroxide of calcium. Hydroxyl particles are also delivered into the medium by the calcium hydroxide, which separates and delivers calcium particles. Hydroxyapatite and water crystallize faster when these particles combine with the phosphate particles in the tissue fluids. Because of the water's continued response with calcium silicates, more gel-like calcium silicate hydrate forms and more

hydroxyapatite pearls are precipitated. [5] When all of the survey items sought to distribute calcium particles consistently throughout a 30-day period, a similar example was identified.

Days 7, 15, and 30 were the worst for EndoSequence RRM after EndoSequence RRM, Biodentine, and MTA. MTA + 2 percent TAP produced the least calcium particles, followed by EndoSequence RRM + 2 percent TAP and Biodentine + 2 percent TAP. Alkalinity was shown to be coordinated with EndoSequence RRM's most prominent calcium release by Abu Zeid and Mokeem Saleh, Candeiro et al., and others to be similar. [18],[19]

Biodentine provided more calcium particles than MTA-Angelus, according to Aprillia et alconclusion's in this evaluation. [20] Particle discharge peculiarity was supported by Gandolfi and colleagues, who argued that design and mineral constituents of the materials are key factors. Last but not least, it is up to them whether or not the substance retains water or is soluble. In biodentine formations, calcium phosphate particles that are less than one micrometer in diameter are present, resulting in a thinner surface layer. MTA Angelus, on the other hand, has a larger molecule (1-5 in width). When compared to MTA, Biodentine's calcium particle discharge was shown to be much more than that of MTA's. [21]

The addition of antibacterial agents to dental materials may alter the material's physical qualities, which may affect its clinical display. The fusion of such antimicrobial dental professionals is thus possible legitimate when it works on the antimicrobial sufficiency without sacrificing the fascinating physical and morphological qualities of that dental material. a. MTA, Biodentine, and EndoSequence RRM have their calcium discharge capability increased by fusing 2% TAP to these materials.

An evaluation of calcium discharge properties of bioceramic materials, which are valued at $1,000,000 in their system, indicated a truly enormous increase in the calcium particles provided by materials with a 2% TAP solution than the calcium particles delivered by materials alone. Thus, in the case of apical fittings, 2 percent TAP in MTA, Biodentine, and EndoSequence RRM may be used to work on their antibacterial adequacy without affecting and rather to further enhance their cardinal calcium discharge system.

This combination, formed by combining 2 percent modified TAP including cefaclor in MTA, Biodentine, and EndoSequence RRM to frame one stage apical module of an open peak necrotic juvenile tooth, requires more in-vivo investigations to show its effect and survivability.

CONCLUSION:

Endo Sequence RRM showed a lower calcium particle discharge limit than Bio dentine or MTA for all exploration spans. Bio dentine + 2 percent TAP and MTA + 2 percent TAP were shown to transport less calcium into the apical fitting than Endo Sequence RRM + 2 percent TAP. In order to generate apical fittings for an endodontic treatment of a young necrotic tooth, 2% of the weight of changed TAP including cefaclor with MTA, Biodentine, and Endo Sequence RRM may be presented. This may help.

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