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Original Research Paper

Endodontics

IMPACT OF BLOOD CONTAMINATION ON MİCROLEAKAGE OF MTA PLUS, BİODENTİNE AND BİO-C-REPAİR AS A ROOT-END FİLLİNG MATERİAL.

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ABSTRACT

Aim: To compare, in vitro, the microleakage of MTA Plus, Biodentine and Bio-C-Repair as root-end filling materials in the presence and absence of blood, using a dye penetration method under a stereomicroscope. **Materials And Methods:** Sixty single-rooted teeth with single canals underwent endodontic treatment and were incubated (37°C, 100% humidity, 48 hours). Root ends were resected, root end cavities were prepared with ultrasonic retrotips, and samples were randomly assigned to three material groups: MTA Plus, Biodentine and Bio-C-Repair filled with/without blood. Microleakage was assessed after 72 hours in 2% methylene blue via stereomicroscopic measurement of longitudinal sections. Statistical analysis used ANOVA and Scheffe's test **Results:** Biodentine showed less microleakage than MTA Plus in dry conditions ($p<0.05$). With blood, Biodentine and Bio-C-Repair had similar microleakage ($p>0.05$), but both leaked less than MTA Plus. **Conclusions:** Biodentine and Bio-C-Repair can be considered promising root-end filling materials, particularly in blood-contaminated conditions. Further clinical trials are required to confirm these in vitro results.

KEYWORDS : Biodentine, Bio-C-Repair, MTA Plus, Microleakage, Root end filling materials, Stereomicroscope

INTRODUCTION

The success of a root canal procedure is contingent upon the triad of accurate diagnosis, meticulous cleaning of pulpal space, and establishment of proper fluid-tight seal, thereby preventing any communication between canal space and periradicular tissues. [1] While non-surgical endodontic treatment typically yields a high success rate, failures may occur due to diverse factors, including variations in dental anatomy, persistent infection, and procedural errors. In such instances, surgical endodontic intervention becomes requisite Apicoectomy involves exposing the root apex, removing lesions, resecting the root end, preparing a cavity, and placing a biocompatible root-end filling. This retrograde filling aims to create an adequate apical seal, preventing bacteria and toxins from exiting the root canal system..[2]

A plethora of materials are used for root-end filling, like amalgam, zinc oxide eugenol, intermediate restorative material (IRM), composite resin, zinc phosphate cement and glass ionomer, but often lack biocompatibility and good sealing ability. To address these shortcomings, newer materials were introduced in 1969, emphasizing forming apatite in phosphate-containing physiological fluids. [3] In 1993, Torabinejad introduced MTA. To address clinical limitations, MTA Plus, with a finer particle size and larger surface area for faster setting, was launched in 2012. Biodentine, a bioactive calcium silicate material for root-end filling, appeared in 2010. More recently, Bio-C-Repair (2020), another calcium silicate bioceramic, offers a good biological seal and promotes biomineralization.[4] For the assessment of the quality of the apical seal accomplished by root-end filling materials, dye penetration with methylene blue has proven to be helpful in endodontic therapy, enabling better penetration.[5][6]

The rationale behind this study is to compare root-end filling microleakage with and without blood to simulate periapical surgery conditions. Achieving a dry surgical field is often challenging, leading to blood contamination that can affect material adaptation and increase leakage. Understanding material performance in different environments helps validate claims and informs clinicians on optimal material selection for better patient outcomes and fewer complications by ensuring a quality apical seal and minimising post-treatment complications.

Since no study has compared the microleakage of Mineral Trioxide Aggregate Plus (MTA Plus), Biodentine and Bio-C-Repair in the presence and absence of blood contamination, The present study compared the microleakage of Mineral Trioxide Aggregate Plus (MTA Plus), Biodentine, and Bio-C-Repair in the presence and absence of blood contamination using a dye penetration test. The null hypothesis states that there is no difference in microleakage between the MTA Plus, Biodentine and Bio-C-Repair, bioactive root-end filling materials in the absence and presence of blood-contaminated conditions.

MATERIALS AND METHODS

A sample size of 60 single-rooted teeth extracted for orthodontic/periodontal reasons was considered, which was calculated using G*Power (90% power, 0.05 significance). Teeth were cleaned with 5.25% sodium hypochlorite (Prime Dental, India) and ultrasonic scaling, then stowed in saline up to its use. Pre-operative X-rays and microscopic examination ensured no decay, cracks, fractures, curvature, or calcifications.

The tooth was marked to receive a 15 mm standardised length

and decoronated with the diamond disc (Kerr Dental Products, California, USA) at the cementoenamel. As the preparation for access was done, the #10 K file (Mani, Inc., Tochigi, Japan) was used, the glide path was established, and the working length was measured. The Coronal Flaring was done using ProTaper SX and chemomechanically prepared up to Protaper Gold F3 (Maillefer, Dentsply, Ballaigues, Switzerland) using E connect Endomotor, and irrigation was carried out with 5.25% NaOCl and 17% EDTA (Prevest Denpro, India) followed by saline rinse, and drying with paper point. Obturation was done using gutta-percha (Maillefer, Dentsply, Ballaigues, Switzerland) and AH plus sealant (Maillefer, Dentsply, Ballaigues, Switzerland). The access cavity was sealed with GIC restoration (GC FUJI IX GP) (GC Corporation, Tokyo, Japan). Samples were stored in an incubator with 100% humidity at 37°C for 48 hours to simulate an oral situation.

Later on, the apical 3 mm was cut perpendicular to the long axis of the tooth with high-speed impact air handpiece (3,50,000 rpm) using #557 carbide fissure bur (SS white, Japan) and a Gutman Class-I root end preparation with a depth of 3 mm cavity was prepared by making light brushing movements towards the long axis of the teeth using the RG4 ultrasonic retro tips (Cricdental, India) under the dental operating microscope (DOM).

The prepared samples were randomized into three groups (n=20) by root end filling materials (Group I- MTA Plus (Prevest Denpro, India), Group II-Biodentine (Septodont, Saint-Maur-des-Fosses, France), and Group III- Bio-C-Repair (Angelus, Londrina, Brazil), with 20 teeth in each group. Each material group was divided into two subgroups (n=10): A (without blood contamination) and B (blood-contaminated). Root end filling materials were placed per manufacturer instructions. Subgroup B received 1 ml of human blood obtained by the researcher through phlebotomy using a 23-gauge needle by authorised personnel prior to filling.

After 24 h, all samples except the excised apical portion were covered with two layers of nail varnish to seal off all other accessible portals and permitted to dry. In the incubator, samples were dangled by dental floss in a test tube and allowed to remain immersed in the 2% methylene blue dye for 72 hours. Then, they were removed, and the excess dye was rinsed off with running water for 15 minutes. After that, with the help of a diamond disc, one longitudinal groove on the buccal surface and the other on the lingual surface was made parallel to the long axis of the tooth. Linear dye penetration (mm) was then measured using Digimizer software and examined under a stereomicroscope at 1.8x magnification.).

Statistical Analysis

Statistical analysis was performed on the collected data, including calculating 95% confidence intervals. One-way ANOVA and post-hoc Scheffe's tests were used to determine statistically significant differences ($p \leq 0.05$) between groups using SPSS version 20.00.

RESULTS

The mean values for linear dye penetration in each of the subgroups are shown in Figure 1.

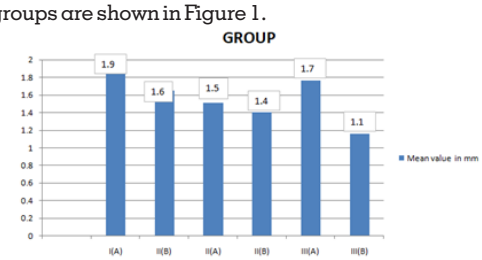

Figure 1: Mean values for linear dye penetration in each of the subgroups

The inter-group and intra-group analysis for three materials in the absence and presence of a blood-contaminated environment was carried out to determine whether the mean results obtained were statistically significant or not, for which post hoc Tukey's test was conducted. p-value ≤ 0.05 was considered to be statistically significant.

In a dry environment, that is, without blood contamination, when Group I(A) was compared with Group II(A) (p = 0.012), the result was statistically significant. Meanwhile, when Group I(A) was compared to Group III(A) and when Group II(A) was compared to Group III(A), the result was non-significant, as represented in Table 1.

Table 1: Post Hoc Tukey's Test For Intergroup Analysis Of Three Materials In Absence Of Blood

Group (I)	Group(J)	Mean difference(I-J)	Std. Error	Sig.(p)
I(A)-MTA Plus	II(A)-Biodentine	0.39075	0.09970	0.012
I(A)-MTA Plus	III(A)-Bio-C-Repair	0.14050	0.09970	0.850
II(A)-Biodentine	III(A)-Bio-C-Repair	0.25025	0.09970	0.286

In a blood-contaminated environment, when Group I(B) was compared with Group III(B) (p = 0.0), the result was statistically significant. Meanwhile, when Group I(B) was compared to Group II(B) and when Group II(B) was compared to Group III(B), the result was non-significant, as represented in Table 2.

Table 2: Post hoc Tukey's Test for Intergroup Analysis of three materials in the presence of blood

Group (I)	Group(J)	Mean difference(I-J)	Std. Error	Sig.(p)
I(B)-MTA Plus	II(B)-Biodentine	0.24600	0.09970	0.3035
I(B)-MTA Plus	III(B)-Bio-C-Repair	-0.49400*	0.09970	0.00
II(B)-Biodentine	III(B)-Bio-C-Repair	0.24800	0.09970	0.296

When Group III(A) was compared with Group III(B) (p = 0.0), the result was statistically significant. Meanwhile, when Group I(A) was compared to Group I(B) and when Group II(A) was compared to Group II(B), the result was non-significant, as represented in Table 3.

Table 3: Post hoc Tukey's Test for Intragroup Analysis of three materials in the absence and presence of blood

Group(I) (without blood contamination)	Group(J) (blood contaminated)	Mean difference (I-J)	Std. Error	Sig.(p)
I(A)-MTA Plus	I(B)-MTA Plus	0.25250	0.09970	0.276
II(A)-Biodentine	II(B)-Biodentine	0..10775	0.09970	0.947
III(A)-Bio-C-Repair	III(B)-Bio-C-Repair	0.60600	0.09970	0.00

Based on the above-mentioned statistics, Biodentine showed less microleakage than other compared materials in the absence of blood contamination, whereas there was no significant difference between Biodentine and Bio-C-Repair in the presence of blood.

DISCUSSION

Surgical endodontic therapy is indicated for salvaging teeth in failed nonsurgical endodontic cases, particularly when there is expulsion of irritants leading to persisting or emerging disease.[7] Achieving a good apical seal, facilitated by a well-adapted root-end filling material, is essential for preventing

leakage and ensuring the success of root-end surgery.[8]

While various methods exist for evaluating microleakage, dye penetration, particularly with 2% methylene blue, remains a popular and simple choice. Matloff et al. showed methylene blue penetrates obturated canals better than some radioisotopes. Its distinct colour contrast allows easy microscopic assessment of penetration into dentinal tubules. Additionally, it's easy to store, cost-effective, and has a good safety profile, making it suitable for leakage studies. [9]

Stereomicroscopy was chosen for its 3D surface imaging, avoiding the complex pre-treatment needed for Scanning Electron Microscopy. Furthermore, the incorporation of image analysis software served to minimise subjective interpretation of parameters. Critically, stereomicroscopy enabled unambiguous visualisation and precise quantification of dye penetration extent.[10][11]

Traditional root-end filling materials (amalgam, etc.) were used until the 1990s when bioactive materials spurred renewed interest. Bioactive materials, like MTA, induce reactions in living tissues, notably forming hydroxyapatite. While MTA showed better outcomes than older materials, its long setting time, washout, discolouration, and handling issues led to newer calcium-silicate materials (MTA Plus, Biodentine, Bio-C-Repair) designed to overcome these limitations.[12][13][14][15]

This study found less microleakage with Biodentine than MTA Plus in dry conditions, aligning with Gupta et al. (2021). This could be due to Biodentine's pre-dosed formulation having lower water: powder ratio, leading to smaller pores and better adaptation to the cavity, thus improving the seal and reducing leakage compared to MTA Plus.[16]

Additional factors contributing to Biodentine's performance, as elucidated by Saleh et al. and Huffman et al., include Biodentine's micromechanical retention and apatite deposition at the dentin interface, improving resistance to displacement. Its quick setting time (12 min), accelerated by moisture (like blood during filling), allows for rapid interfacial sealing, minimizing interface disruption and reducing bacterial entry and microleakage.[17][18]

Bio-C-Repair's small particle size (<2 m) promotes rapid release of Ca2+ and OH- ions for lesion repair. In contact with moisture/tissue fluids (including blood), it releases bioactive ions that interact with dentin matrices, forming a Mineral Infiltration Zone (MIZ). This MIZ creates a strong biological seal, similar to Biodentine, reducing bacterial entry. The material's low solubility further improves its ability to seal dental defects.[4]

Akcay et al. (2019) found blood contamination reduced Biodentine's push-out bond strength, likely due to hindered hydration and setting, causing surface irregularities. This might explain Biodentine's higher microleakage than Bio-C-Repair with blood in this study. However, the microleakage difference between Bio-C-Repair and Biodentine with blood was not statistically significant.[19]

CONCLUSION

Considering the constraints inherent in this in vitro study and the clinical challenge of maintaining a consistently dry apical field during endodontic surgery, the novel bioactive material Bio-C-Repair emerges as a potentially efficacious root-end filling due to its bioactive nature, similarities to Biodentine, and good handling. However, clinical trials are needed to confirm its long-term effectiveness.

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