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EFFECT OF CAVITY PREPARATION AND BLOOD CONTAMINATION ON MARGINAL ADAPTATION OF ENDOSEQUENCE ROOT REPAIR MATERIAL USED AS ROOT-END FILLING: RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Aim of the study was to evaluate effect of root end cavity preparation either using conventional bur or ultrasonic diamond retro-tip in presence or absence of blood contamination on marginal adaptation of EndoSequence Root Repair material.

Materials and Methods: Forty four fully formed human maxillary central incisors were used in this experiment; after proper access cavity preparation, root canal instrumentation using ProTaper Universal rotary system, obturation using gutta-percha with AH plus, and apical 3mm of the roots were resected, the teeth were randomly divided into two main **groups I &II** of 22 samples each; root end cavities in group I were prepared using inverted cone burs and in **group II** using ultrasonic diamond retro- tip. Each main group was then randomly divided into two subgroups according two blood contaminations of the root end cavities where **subgroups IA** and **IIA** the retrograde cavities were coated with fresh human blood, while **subgroups IB** and **IIB** cavities left uncontaminated. For all samples root end cavities were filled using putty ERRM and incubated at 37°C and 100% humidity after immersing in molds filled with heparinized blood for one week. Samples were split longitudinally and examined under SEM and size of gaps between the root-end filling materials and the dentin were recorded.

Results: The highest mean gap value of ERRM was observed in **subgroups IA**& **IB** in which root end cavities were prepared using conventional bur either contaminated or not contaminated with blood than that prepared using ultrasonic diamond retro-tip in **subgroups IIA**&**IIB**, with no statistical difference between contaminated or non-contaminated cavities.

Conclusions: ERRM as root end filling is better to be applied in root end cavities prepared using ultrasonic tip, and not negatively affected by blood contamination.

KEYWORDS: Marginal gap**,** marginal adaptation, ERRM, ultrasonic retro-tip, blood contamination.

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INTRODUCTION

Endodontic surgery is the treatment modality indicated for clinical cases where conventional endodontic treatment or retreatment is unsuccessful. Improvement in surgical techniques and invention of newer equipment and retrograde filling materials makes surgical endodontics a predictable treatment for such resistant cases.[1]

Apical surgical procedures include root apex exposure, root end resection, retrograde cavity preparation and filling using retrograde filling material.[2] Root end filling materials in addition to be an inert non-toxic, biocompatible, bioactive, bioinductive, and manipulated easily, it should perfectly adhere to prepared root end cavity walls to form a tight seal preventing passage of microorganisms and irritants from the root canal system to periapical tissue, thus long-term clinical success of apical surgery is affected by adaptation between retrograde material and root dentin.[3,4]

EndoSequence Root Repair Material (ERRM) is specifically formulated as a condensable premixed putty mass which utilizes new bioceramic nanotechnology, ERRM putty consistency suitable to difficult fields as retro-fills. No mixing required since setting reaction of ERRM started by the moisture naturally found in the dentinal tubules, this favorable handling quality shortened the setting time, increased strength and makes it highly resistant to washout. ERRM was reported to has antibacterial effect due to its pH (12+) and is extremely biocompatible, osteogenic, posses excellent mechanical and biological properties, radioopaque , insoluble, metal-free that do not cause stain and hydrophilic forms hydroxyapatite after setting and chemically bonds to dentin.[5,6]

Root-end cavity preparation is a critical step for successful periapical surgery. Ideal retrograde cavity should be prepared with 3mm depth, parallel to root long axis and centered in the root, this is important to preserve proper wall thickness, retain retrograde filling material and achieve hermetic apical seal.[7,8] Class I cavity was traditionally applied after root end resection using conventional bur on a contra angle handpiece, it was reported that this traditional technique using burs were generally of accepted quality, however, clinically achieving these criteria is hard due to inaccessible root apex, unavailable apical bone root access, and tenacity of hand piece.[9, 10]

Ultrasonic retro-tips were introduced with multiple shapes and angulations to be an alternative to classic burs, it can be selected according to the root anatomy and location with improvement in root end cavity preparation. Ultrasonic retrotips were reported to produce retrograde cavities that are cleaner, smaller, centrally located, more retentive, and aligned with the root canal direction with little damage of the surrounding tissue .[11, 12]

Exposure to blood is common to occur during clinical surgical situation including periapical surgery, this blood contamination might affect the setting reaction with subsequent effect on the physical and biological properties of the retrograde filling material and also it's sealing ability to root canal dentin.[13, 14]

The null hypothesis of this study that there is no difference between conventional or ultrasonic retro-tip root end cavity preparation either contaminated or not contaminated with blood on marginal adaptation of Endosequance Root Repair Material.

Aim of the study

This study was designed to evaluate the effect of root end cavity preparation either using conventional bur or ultrasonic diamond retro-tip in presence or absence of blood contamination on marginal adaptation of EndoSequence Root Repair Material.

MATERIALS AND METHODS

Study design

The present study was designed as randomized controlled trial, conducted in Endodontic Department, Faculty of Dental Medicine for Girls, Al-Azhar University. The experiment was designed, analyzed and interpreted according to the Consolidated Standards of Reporting Trials (CONSORT). Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from Research Ethic CommitteeApproval Code (REC-PD-22-03), Faculty of Dental Medicine for Girls, AL Azhar University.

Samples size calculation

Sample size was calculated according to the following (Wittes 2002)[15] formula:

Sample size (N) = (Zα/2+1–β)2 *2*SD2/ Δ2,

Where SD= Standard deviation of the size of the gap observed in ERRM putty for longitudinal replica using ultrasonic tip preparation estimated from previous study[16] was 1.16 μ.m, and Δ 2 = effect size estimated between the samples, α = Level of significance, and 1–β = Power. Thus N= (1.96+0.84)2*2*(1.16)2/ (1)2=21.1

Accordingly, at least we will work with 22 fully formed human maxillary central incisors in each group with total sample 44. Each group was then randomly divided into two equal subgroups according to blood contaminations of the root end cavities.

Teeth selection

Forty four extracted permanent, fully formed human maxillary central incisors with straight roots were examined visually and radiographically to be free from root caries, root cracks, root canal filling, and of Vertucci's type I root canal configuration and selected for this study. The external surfaces of all teeth were cleaned using ultrasonic scaler to remove attaching remnants and debris, then stored in distilled water until use

Specimens' preparation

After proper access cavity preparation and checking the root canal patency, the working length was determined by introducing K-file ISO #10 (MANI Inc., Japan) and confirmed by using radiograph. The root canal preparation was completed using ProTaper Universal rotary system (Dentsply, Maillefer, Switzerland) which were used according to manufacturers' recommendations up to F4 file. The root canals were irrigated after each file using 2.6% NaOCl solution and final irrigation was performed by using 17% EDTA 5ml for 1min. After dryness of the root canal with paper points, it was obturated with master gutta-percha size F4 and AH plus (Dentsply, Konstanz,Germany) sealer using cold lateral condensation technique then teeth were incubated at 37˚C and 100% humidity for 48 hours. The apical 3 mm of each root was resected at 90˚ to tooth long axis using fissure bur mounted in high-speed hand piece under continuous air/ water coolant.

Specimens' grouping

The forty four Specimens were divided randomly using closed envelops to two groups (**Group I** and **II**) (n=22) according to root end cavity preparation and then each group divided to two subgroups A and B according to contamination of root end cavity with blood.

Group I: (Conventional bur group)(n=22 samples) in which #34 inverted cone burs in slow-speed contra-angle hand piece with continuous water cooling in up and down soft motion was used to prepare the retrograde cavities with 3mm depth down the long axis of the canal which was measured using the periodontal probe.

Group II: (Ultrasonic group) (n=22 samples) in which root end cavities of 3mm depth were

prepared using ultrasonic diamond coated retrograde tip (Satelec, Cedex, France) at medium power of ultrasonic unit and under water spray.

All cavities were cleaned of gutta-percha remnants, rinsed with water, dried with paper points, and examined under sterio-microscope (Carl Zeiss, Germany) and exclude and replace all samples that has any cracks.

The main **Groups I**& **II** were then divided into two subgroups **(A** and **B**) according to blood contamination of the retrograde cavities. Human blood was obtained by phlebotomy from the first author. In subgroups IA and IIA (n=11 samples) the root end cavities were contaminated with blood by filling it with fresh blood and then slowly aspirated, thus the cavities were coated with blood and filled with ERRM putty form (Brasseler USA, Savannah, GA, USA) which was applied in small increments and packed into root end cavities using prefitted Schilder pluggers (Dentsply Caulk, Milford, DE). The samples then were immediately immersed in molds filled with heparinized blood and incubated at 37°C and 100% humidity for one week.

While in **subgroups IB** and **GIIB** (n= 11 samples) the root end cavities were left without blood application. ERRM putty was incrementally applied as in **IA** and **IIA** subgroups and also incubated at 37°C and 100% humidity for one week.

After setting of ERRM, each sample was sectioned longitudinally using isomet 4000 micro saw (Buehler, USA) and samples were viewed under a stereomicroscope to confirm the integrity of root apices and exclude the samples with cracks extending to the ERRM-dentin interface, and then mounted on an aluminum stub and marginal gap between retrograde material and dentin interference was examined and recorded IN micrometer using SEM (National Research Centre (QUANTA FEG250) under magnification 2000X and the gap between retrograde material and dentine wall was recorded at three selected points and the average was recorded for each sample.

Statistical analysis:

All data were collected, tabulated and analyzed. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, followed by t-test which was used to compare between the subgroups in non-related samples and three-way ANOVA tests were used to test interactions between different variables. The significance level was set at $P \leq 0.05$.

RESULTS

Mean marginal gap obtained at the interface of dentine root-end filling materials was recorded and summarized for the four subgroups in ***table (1)***.

The scanning electron microscope examination for longitudinal sections of root end cavities filled with ERRM showed mean marginal gap obtained at dentin –root filling interface which were analyzed

TABLE (1): The mean values and standard deviations of marginal gap for ERRM applied in conventional and ultrasonic prepared cavities in presence or absence of blood contamination.

Variables	ERRM				P-Value
	With blood		Without blood		
	Mean	SD	Mean	SD	
Rotary bur (GI)	3.90	1.22	3.08	0.93	**0.110 ns**
Ultrasonic tip (GII)	2.50	0.75	2.63	0.76	**0.716 ns**
P-Value	**0.060 ns**		**0.249 ns**		

and summarized for four subgroups in ***table (1)***. Comparison of two **groups (I&II)** showed high mean value (3.90&3.08) and standard deviation (1.22&0.93) in **group I** cavities prepared using bur in both contaminated and non-contaminated **subgroups (A&B)**; than the mean value (2.50 &2.63) and standard deviation (0.75 &0.76) in **group II** cavities prepared using ultrasonic retro-tip in two **subgroups (A&B)**, there is no statistically significant difference between two main groups in either contaminated (P=0.060) condition or non-contaminated (P=0.249) ($p<0.05$).

With regard to the effect of blood contamination in each group; in **group I** cavities, **subgroup IA** showed high mean value(3.90) and standard deviation (1.22) of marginal gap compared to mean value (3.08) and standard deviation (0.93) **subgroup IB** that not smeared with blood and there is no statistically significant difference between the two subgroups (P= 0.110) ($p<0.05$). In **group II** the **subgroup IIB** in which the ultrasonic prepared cavities not contaminated with blood showed high mean value (2.63) and standard deviation (0.76) than **subgroup IIA** mean value (2.50)and SD (0.76) which smeared with blood, there was no statistically significant difference between the two subgroups (P= 0.716) ($p<0.05$).

SEM images of marginal gap in longitudinal section at magnification 2000X for each subgroup are shown in ***figure (1).***

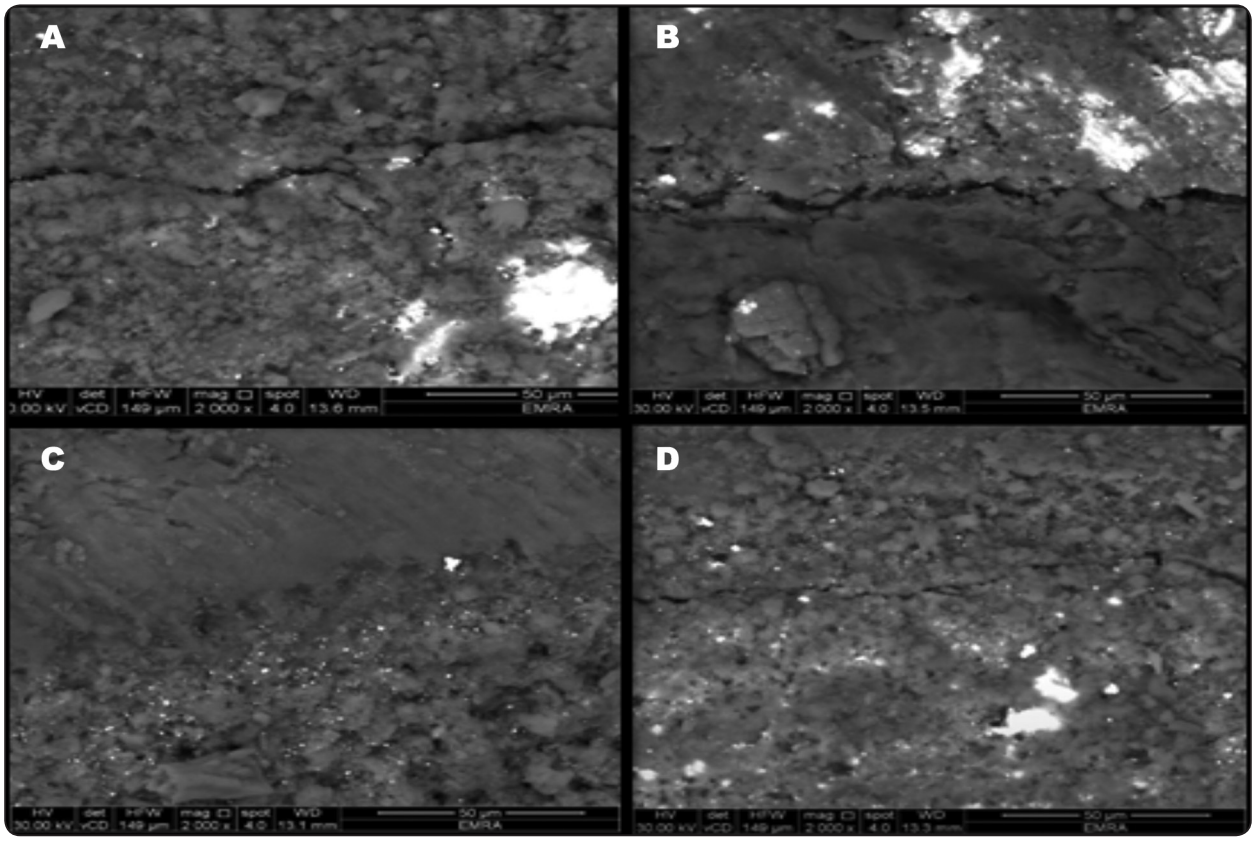

Fig. (1): Scanning electron microscopic (SEM) images showing marginal gap of ERRM-dentin interface at magnification 2000X : (A) conventional bur prepared cavity contaminated with blood, (B) conventional bur prepared cavity not contaminated with blood, (C) ultrasonic retro-tip prepared cavity contaminated with blood, and (D) ultrasonic retro-tip prepared cavity not contaminated with blood.

DISCUSSION

Marginal adaptation known as degree of approximation of filling material to dentine surface. The gap size between dentin and retrograde material represent the quantitative aspect of the material sealing capability thus seal is an outcome of marginal adaptation which is significant for success of root end filling. Therefore, the evaluation of marginal adaptation of retrograde materials by SEM can give expectations about its sealing property and a key factor in success of endodontic surgery.[18, 19]

EndoSequence root repair material (ERRM) has the same advantages of the golden root end filling material MTA and, moreover has improved handling properties, a shorter setting time and does not cause discoloration which are annoying disadvantages of MTA .19 ERRM can be applied directly to the root end cavity, doesn't contain minerals so does not cause discoloration, and the setting reaction by-product forms hydroxyapatite which provide good bond to dentine wall. It was reported that ERRM showed sealing ability comparable to that of white MTA root-end filling material. Another study showed less marginal gap with statistically significant difference to that of MTA when they were compared as retrograde materials.[20, 21]

The root-end cavity is critical step for success of surgical endodontics depending on the technique used. Traditionally small size burs were used, however ultrasonic retro-tip introduced to provide root end cavity that was reported to be better-shape, more smooth, deeper and centrally located. Also, during clinical procedures the root end filling material exposed to blood that might affect its setting reaction and subsequently the physical properties.[22, 23] Thus the aim of this study was to evaluate the effect of cavity preparation and blood contamination on marginal adaptation of ERRM as root end filling material.

In the current study the extracted teeth selected were maxillary central incisors with type I root canal system which confirmed by using radiograph, to allow more standardization for samples by decreasing chance of anatomical variation that may affect the study results. The investigation of marginal gap between retrograde filling material and inner cavity dentine wall was recorded using scanning electron microscope that was proved to be efficient method by providing superior magnifying power and good resolution which facilitate measuring the gap interface and analyzing the degree of material adaptation.[21, 24]

To mimic the clinical endodontic surgical procedure, apical 3mm of the root was resected perpendicular to the tooth axis using the bur, since it was reported large number of lateral canals and apical ramifications are found at least 3mm from the root apex, thus apical resection shorter than 3mm can cause reinfecti*on* and treatment failure. [25, 26] Also a hemorrhagic situation was simulated by using human blood, root end cavities smeared and finally immersed in container of heparinized blood.[27]

The results of the current study showed low mean value and standard deviation of marginal gap for root end cavities prepared using the diamond-coated ultrasonic retro-tip compared to that prepared conventionally using bur in either contaminated or non-contaminated cavities with blood which revealed better adaptation of ERRM to dentin wall cavities prepared by using ultrasonic tip. This result can be attributed to advantages of ultrasonic tips over conventional bur prepared cavities of being more centered, cleaner and deeper, however the conventional bur cavities have more dentine debris and thicker smear layer that may interfere with retrograde filling material adaptation.[8] Also the ultrasonic tip used in this study was diamond-coated ultrasonic type which was reported to be very efficient in removing the apical part of gutta-percha and dentine debris; in addition, its coated side tends to abrade dentin rather than chipping which

minimizes cracks formation the main disadvantages of ultrasonically prepared root end cavities.[28] This result was supported by study reported that root end cavity prepared using ultrasonic retro-tip showed better sealing ability than cavities prepared with conventional bur . However another study evaluated apical microleakage which is an indication for marginal adaptation, and also used MTA as root end filling material that has similar properties of ERRM.[29]

The results regarding effect of blood contamination on marginal adaptation of ERRM showed that there is no statistically significant difference of marginal gap in both ultrasonic or bur designed cavities. This result was in contrast to results reported that exposure of MTA during setting adversely affect its marginal adaptation, where MTA has the same chemical composition of ERRM8. However this result was in accordance with another finding reported that marginal gap measurement of ERRM root end filling not negatively affected by blood contamination, and no statistically significant difference between presence or absence of blood during its setting reaction.[31] The finding of this study confirmed the claims of ERRM developers who reported that moisture is important to initiate the setting reaction and aqueous environment is essential for its hardening.[32, 33]

CONCLUSIONS

Within the limitations of the present study; it can be concluded that EndoSequance root repair material better to be applied in ultrasonically diamond retro-tip prepared root end cavity and ERRM marginal adaptation not negatively affected by blood contamination during setting and it can be a promising root end filling material.

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