Study Protocol

Comparison of Root End Sealing Ability of three Retrograde Filling Materials in Teeth with Root Apices Resected at 90^0 using dye penetration method under fluorescent microscope: An In-Vitro Study

Abstract:

Background: Inadequate seal at the apex is the substantial cause for surgical endodontic dissatisfaction. The retrograde filling material which is used should prevent the egress of potential contaminants into periradicular tissue.
Objectives: To evaluate the ability of MTA Angelus, Zirconomer and Bioactive bone cement to seal the root end as retrograde filling material; and to compare root end sealing ability of these three different retrograde filling materials with apices resected at 90^0 angles using dye penetration method under fluorescent microscope.
Methodology: Thirty six extracted upper anterior teeth are to be cut horizontally at the CEJ. After following the standard protocols of, "cleaning, shaping and obturation" with gutta percha and "AH Plus sealer", the samples will be resected 3mm at the apical end at 90 degree angle along the long axis of the tooth with the help of diamond disc. A root end cavity of depth 3mm will be made with Diamond coated ultrasonic surgical tip S12 90 ND. The teeth will be randomly categorized in 3 groups: Group 1: MTA angelus; Group 2: Zirconomer; Group 3: Bioactive Bone Cement. Following which the roots to be coated with nail varnish except the tip. Each material will be compressed in the root end cavity with the help of small pluggers. All the samples which are retrofilled will be kept in acrydine orange for a duration of 24 hours, following which cleaning & bucco-lingual sectioning(vertical) will be done. Fluorescent microscope will be used for observation of sectioned root samples.
Expected Results: Bioactive bone cement is expected to have better sealing ability of the retrograde cavity preparation with minimal or no microleakage followed by MTA Angelus and then Zirconomer.
Conclusion: If this study proves correct, this would be helpful for the clinicians to choose better and the most efficient retrograde filling material with best sealing ability and minimal microleakage in a retrograde preparation which will aid in success of the root canal treatment further resolving the infection.
Keywords: Retrograde Filling, Sealing Ability, Microleakage, Periapical Infection, In Vitro

Introduction:

Successful endodontic treatment is achieved by eliminating complete microbes from the root canal along with achieving a fluid tight seal by using materials of sufficient compatibility.[1,2,3] Although with the advent of latest techniques for endodontic treatment, with newer efficacious materials and instruments, the resolution of some cases[4] of

periapical diseases is not achieved which necessitates surgical intervention.[5] This process comprises of exposing the root apex which is involved, trailed by resecting of the root apically while preparing the retrograde cavity & placing of a compatible retrograde filling material. Most ramifications (98%) are present at the most apical part of the root with (93%) lateral canals. Therefore, to achieve the healing of apical bone, resection of the root in apical third (3 mm) is advised. [6,7] The leakage taking place at the apex could probably be because of two reasons; The chief cause being the apical microleakage which is the leakage taking place at the junction in the middle of the filling root end material & the walls of the canal. The 2nd path is because of movement of fluid & constituents lengthwise with the dentinal tubules which are open towards the root end which is resected. The total leakage from 2 pathways may be called as "Apical leakage" [8]

Historically, various root end filling materials have been recommended but each having few limitations to their usage.[9] Recently MTA has been suggested and proven to be good biocompatible retrograde filling material with minimum bacterial leakage and upgraded adaptation to the walls of the cavity. It has been proved to be a gold standard for retrograde filling material. *MTA-Angelus Reparative Cement* is an endodontic reparative cement, comprising of mineral trioxide aggregate (MTA). The release of Calcium ions from this material helps in promoting biological healing along with the formation of root cement in cases of root perforations.[10] There has been introduction of newer formulations of GIC, which are original materials, such as Zirconomer and zirconomer improved that contains ceramic & zirconia reinforced GIC which was able to combat the disadvantages of amalgam along with tooth colored restorative materials. The superior strength of amalgam is exhibited & it maintains the fluoride releasing capacity of GICs which can also be tested for "retrograde filling material". Bone cement is a viable newer repair and retrograde filling material which is being investigated in endodontics recently. Bioactive Bone cement comprises of characteristics that make it well suited as a repair material for variety of endodontic treatments [11] Still, no documentation has been there, of comparison of sealing ability of MTA along with 2 acknowledged root end filling materials that is zirconomer & Bioactive cement. Therefore, this current study committed to assess and compare the capability to seal the root end by using three various root end placing materials in teeth whose apices of the root were resected at an angle of 90 degree using "fluorescent dye penetration method".

Rationale:

Resection of the most apical three millimeters of the root is done as it helps in removing "98% of the apical ramifications and 93% of the lateral canals." The retrograde filling serves the purpose of providing an ideal hermetic seal of the root canal which essentially helps in inhibition of leakage of pathogens coronally along with their products into the surrounding periradicular tissues, thus promoting healing and cementum formation on the sectioned dentin surface.

Aim :

This study aims at evaluating and comparing capability of the three retrograde filling materials White MTA, Zirconomer and Bioactive bone cement to seal the apex in teeth with

resected root at 90 degree angulation using the method of penetration of the dye under "fluorescent microscope".

Objectives:

1. To evaluate ability of MTA Angelus to seal the root end as "retrograde filling material".
2. To evaluate ability of Zirconomer to seal the root end as "retrograde filling material".
3. To evaluate ability of Bioactive bone cement to seal the root end as "retrograde filling material".
4. To compare the ability to seal the apex of the root of the above three retrograde filling materials with resection of apex at 90^0 angulation using penetration of the dye method under fluorescent microscope.

Source of the Data:
After taking Informed consent from the patients whose teeth will be removed for orthodontic reason, to use their extracted teeth for the study. Human permanent mandibular premolars extracted teeth for orthodontic purpose will be collected from the Department of Oral Surgery, Sharad Pawar Dental College, Sawangi (M), Wardha. Among these, thirty six sound healthy teeth with absence of caries, restoration, cracks and white spots will be selected.

Methodology:
Thirty six extracted upper anterior teeth are to be cut horizontally at the CEJ. After following the standard protocols of, "cleaning, shaping and obturation" with gutta percha and "AH Plus sealer", the samples will be resected 3mm at the apical end at 90 degree angle along the long axis of the tooth with the help of diamond disc. A root end cavity of depth 3mm will be made with Diamond coated ultrasonic surgical tip S12 90 ND. The teeth will be randomly categorized in 3 groups: Group 1: MTA angelus; Group 2: Zirconomer; Group 3: Bioactive Bone Cement. Following which the roots to be coated with nail varnish except the tip. Each material will be compressed in the root end cavity with the help of small pluggers. All the samples which are retrofilled will be kept in acrydine orange for a duration of 24 hours, following which cleaning & bucco-lingual sectioning(vertical) will be done. Fluorescent microscope will be used for observation of sectioned root samples.

Inclusion Criteria:

- Freshly extracted single rooted maxillary teeth with sound root.

Exclusion criteria

- Teeth having calcified canals
- Teeth with fracture of the root
- Teeth having several canals

Sample Size Calculation:
Based on mean value of dye penetration in table no. 1 of the reference article the minimum sample size calculated is 11 in each of the 3 groups with α-value = 5%, β-value = 20% and

80% power of the study. 12 in each group can be taken to further minimise errors or loss in data.

Table 1. Group ratio

Group 1	12
Group 2	12
Group 3	12
Total	36

Sample Size	
Group 1	11
Group 2	11
Total	**22**

Study Parameters	
Mean, group 1	1.4
Mean, group 2	2.4
Alpha	0.05
Beta	0.2
Power	0.8

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(0.843^2 + 0.843^2/1)(1.96 + 0.84)^2}{1^2}$$

$$n_1 = 11$$

$$n_2 = K * n_1 = 11$$

$\Delta = |\mu_2 - \mu_1|$ = absolute difference between two means
σ_1, σ_2 = variance of mean #1 and #2
n_1 = sample size for group #1
n_2 = sample size for group #2
α = probability of type I error (usually 0.05)
β = probability of type II error (usually 0.2)
z = critical Z value for a given α or β
k = ratio of sample size for group #2 to group #1

Distribution of samples:

Group I: Retrograde filling with MTA angelus

n=12

Group II- Retrograde filling with Zirconomer

n=12

Group III – Retrograde filling with Bioactive bone cement

n=12

Materials:

- White MTA-angelus (Angelus , Londrina,PR, Brazil)
- Zirconomer (Conventional GIC, Shofu,Japan)
- Bioactive bone cement (Surgical Simplex P, Stryker)
- Angelus Silano silane coupling agent
- Diamond coated ultrasonic surgical tip S12 90 ND (Satelec/Aceton, Mergnac, France)
- Acrydine orange dye

Procedure:

This study will be conducted in Sharad Pawar Dental College, in Department of conservative Dentistry and endodontics.

i. For sample Preparation, 36 maxillary anterior extracted teeth will be selected for this study.
ii. An intra oral radiograph (both mesio distal and bucco lingual) will be taken before the procedure to analyse the presence of a solitary canal, and check for absence of resorption (internal/external) or calcification, canals which are filled & already instrumented & completely formed apex.
iii. The extracted teeth will be stored in "0.2% thymol solution" & will be submerged in "5% sodium hypochlorite" for 30 minutes to eliminate organic tissue debris.
iv. The coronal part of the teeth will be sectioned & teeth length to be standardized to 16 mm (from apical - coronal point).
v. Following preliminary radiographs, standardized access cavities will be prepared & enlargement of cervical portion of the root with "ISO size 70 to ISO size 90 GG drills" will be done.
vi. Canals to be prepared till ISO size 70 K-File keeping it shorter of the apical foramen by 1mm.
vii. While instrumentation, the canals to be irrigated by 2 ml of 1% NaOCl after every alteration of file.
viii. Canals to be parched and packed with 17% EDTA (pH 7.2) for time span 3 minutes for smear layer removal.
ix. The canal which is cleaned & shaped is to be dried out with paper points & obturation will be done with gutta percha by means of lateral condensation technique.

x. Access cavity to be made impenetrable with temporary filling material.

xi. Roots to be then kept at 100% humidity for one week at room temperature.
xii. The root end resection will be done by eradicating 3 mm in all 3 groups with the help of diamond disc with continuous irrigation with NaCl solution at 90 degree angle to long axis of the tooth.
xiii. A root end cavity with the depth of 3mm will be prepared using with Diamond coated ultrasonic surgical tip S12 90 ND.
xiv. Periodontal probe would be used to check depth of the root end cavity.
xv. Nail varnish would be applied all over the root surface excluding the tip, the place where retrograde filling material is placed.
xvi. Small pluggers will be used to condense the material in the cavity which will be prepared.
xvii. Fluorescent dye in the form of an aqueous solution will be used for exposing the root, for 24 hours, which will be further cut in a longitudinal plane & then measurement of dye penetration will be done with fluorescent microscope.

Timing: Nov 2020- Nov 2022

Scoring for dye penetration for apical microleakage:
0: No dye penetration.
1: Dye penetration into apical one third of retrograde filling material.
2: Dye penetration into apical middle third of retrograde filling material.
3: Dye penetration into full length of retrograde filling material.
4: Dye penetration beyond retrograde filling material.

Statistical Analysis:

Analysis: Descriptive and analytical statistics will be done. The normality of data will be tested by Shapiro-Wilk test. If the data followed normal distribution parametric tests (ANOVA test with Tukey's post hoc test) will be used and if the data does not follow normal distribution non-parametric test (Kruskal Wallis Test with Dunn post hoc test) will be used. The p value <0.05 will be considered statistically significant.
Software: SPSS (Statistical Package for Social Sciences) Version 20.1 (IBM Corporation, Chicago, USA)

Expected results:
Bioactive bone cement is expected to have better sealing ability of the retrograde cavity preparation with minimal or no microleakage followed by MTA Angelus and then Zirconomer.

Discussion:
Anurag Jain et al in 2016 compared the sealing capacity of 4 root-end filling materials in extracted teeth having the apex of their roots resected at various angles in teeth with root apices resected at different angles by selecting 100 upper front teeth (freshly extracted)

which were cut in a horizontal plane at CEJ. Standard procedure of "cleaning, shaping and obturation" with gutta percha was done followed by random distribution of tooth samples in two groups: Group I: resection of apex of root at 0º ;Group II: resection of apex of root at 45º. The root apices resection was done by cutting 2mm and 1mm length of the root in the above groups. This was followed by preparation of the retrograde cavity at the root apex at the depth of 3 mm and application of nail varnish all over the root surface excluding the root tip. Further, random distribution of samples from group 1 and 2 was done into 4 subgroups each: Pro root MTA, Portland Cement, Intermediate Restorative Material, Light cure nano GIC Ketac ; n=100 . After retro filling the teeth, acrydine orange dye solution was used to store these samples for 24 hours followed by vertically sectioning them bucco-lingual plane. The cut samples of the root were assessed under "fluorescent microscope". It was concluded that the root sealing capability of (MTA) was found to be better than portland cement, Intermediate restorative material and Light cure Glass ionomer.

Dr. Ajmal Mir et al(2018) studied thirty human maxillary central incisor teeth which were prepared and obturated using GP and AH plus sealer. After 24 hours, apical 3mm was removed from each tooth and root end cavities made with ultrasonic tips. Samples were distributed at random into three groups of 10 samples each based on root end filling material : Group i: Mineral Trioxide Aggregate, Group ii: Bioactive bone cement, Group iii: Glass ionomer cement. After one week of storage in PBS solution, specimens were subjected to dye extraction analysis and amount of dye absorbed was calculated in terms of absorption units using UV visible light spectrophotometer. Results showed that samples back filled with bioactive bone cement had absorbed lowest dye (0.69±0.078 au) followed by samples filled with MTA (0.72±0.108 au). Highest dye absorption values were shown by samples filled with GIC(1.53±0.200 au). Statistical analysis showed no significant difference between bioactive bone cement group and MTA group ($p = 0.872$) while as significant difference existed between these two and glass ionomer group ($p<0.001$).

Fatinder Jeet Singh et al (2020) studied 90 freshly extracted sound, upper incisor teeth which were subjected to RCT using standard protocols. Root resection was done at the apex followed by retrograde cavity preparation which was done using ultrasonic retrotip. The teeth samples were divided into 6 groups depending upon various root-end filling materials :Amalgam, Resin modified Glass ionomer cement, Cermet cement, Mineral trioxide Aggregate, Biodentine, Control group. In the above samples, leakage through the apex was observed and assessed under "confocal laser scanning microscope". It was established that biodentine showed excellent sealing capability followed by mineral trioxide aggregate, cermet cement and Resin modified Glass ionomer, whereas amalgam showed least sealing ability.

Implications:

If this study proves correct, this would be helpful for the clinicians to choose better and the most efficient retrograde filling material with best sealing ability and minimal microleakage in a retrograde preparation which will aid in success of the root canal treatment.

Scope:

Newer materials will have a better marginal adaptability and sealing ability in retrograde preparations preventing microleakage. Further in vivo studies should be carried out to provide more accurate results.

LIMITATIONS:

Since this is an invitro study, the results can differ when co related with the in vivo group.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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