Evaluation of Effect of silver nano particles on antimicrobial efficacy of novelretrograde filling materials against *E. Faecalis In-vitro* study

Dr. Srinjal Suman[1], Dr. Raju Chauhan[2], Dr. Pradyumna Misra[3] and Dr. Preeti Shukla

[1]Postgraduate student,Department of Conservative Dentistry & Endodontics,Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India. E-mail:-shrinjalsuman@gmail.com [2]Professor,Department of Conservative Dentistry & Endodontics,Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India. E-mail:-drrschauhan@saraswaticolleges.com [3] Professor and Head of Department, Department of Conservative Dentistry & Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India. E-mail:-drpmisra4lko@gmail.com , [4]Associate Professor,Department of Conservative Dentistry & Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India. E-mail:-pretishukla@gmail.com

Corresponding Author

Dr.Preeti Shukla

Associate Professor,Department of Conservative Dentistry & Endodontics,Saraswati Dental College & Hospital, 233, Tiwari Ganj, Faizabad Road, Lucknow–227105 Uttar Pradesh, india E-mail:- **pretishukla@gmail.com** Mobile:- **+91-9176217514**

Running Title

Antimicrobial efficacy of retrograde filling matrials

ABSTRACT

Background :

When endodontic treatments fail, a surgical intervention and root end filling procedure are required. Thus, search for the best restorative materials continues. Very few studies have compared effect of silver nano particles with bioceramic restorative materials for antibacterial activities, which is the goal of the study against *E. Faecalis.*

Aim :

To check the effects of silver nanoparticles on antibacterial activity of 3 novel bioceramic root end filling materials.

Materials and Methods :

An *In-vitro* study was conducted using three bioceramic based cements; Pro root MTA, Biodentine, and Bio C Repair with and without silver nano particles against *E. Faecalis.* Agar Disc diffusion was used as test technique. *E. Faecalis* was grown on Muller Hilton Agar (MHA) media. At 34 °C, the samples were incubated and examined for zones of inhibition at 24 hrs.

Statistical Analysis :

Intra and intergroup comparison was performed using Anova test, which was substantiated with Tukey's Post Hoc analysis to perform pair-wise comparison of data.

Results :

Anova test revealed that at 24hrs, compared to positive control there is highly significant difference in the efficacy of the three materials and Biodentin has best results. Pro root MTA and Bio C Repair have insignificant variation. Significant difference was found between results of plain and silver nano added materials.

Conclusion :

Based on the results of this study, adding silver nanoparticles to the bioceramic filling materials improved their antibacterial efficacy.

Key words :

Antibacterial efficacy, Biodentin, Bio C Repair, Pro root MTA, Silver nano particles.

INTRODUCTION

Major etiological factor for the pulp and periradicular infection are the micro-organisms, which are also responsible for the failure of an endodontic treatment. So, to eradicate the cause root canal treatment is performed. (1)

The outcome of successful endodontic treatment depends on a thorough diagnosis, proper biomechanical preparation and obtaining a hermetic seal of the root canal. Most periradicular infections are best approached non surgically. (2)

It's a well known fact that no cleaning protocol allows the eradication of 100 % bacteria in an infected root canal.[3] So, in case of endodontic failures , re- rct is done non surgically. If infection still persists and re-intervention is not possible then apical surgery is often the last resort to maintain tooth with periradicular lesion.[4] Surgical resection of apical third of root that is a microbial hub [5] followed by retrograde restoration ensures no further infection spread and periapical healing.

Numerous authors have identified *E. Faecalis* as the predominant microorganism found in root treated canals displaying persistant periapical disease. (6) The difficulty in eliminating *E. Faecalis* from root canal is due to its ability to adapt to environmental changes while retaining its pathogenicity. Previous studies have reported a prevalence of *E. Faecalis* ranging from 24-77% in teeth with failed endodontic treatment. (7)

To be clinically successful, the root end filling material should have antimicrobial activity as well as good apical seal and biocompatibility to inhibit microbial growth and ensure regeneration of periradicular tissues as well. The material used therefore should afford all of these criteria along with dimensional stability, proper setting time, and biomimmetic properties under static and functional conditions. (2)

Among inorganic restorative materials that have gained attention in regenerative dentistry, calcium silicate based cements have been used the most but antibacterial efficacy is still not completely evident with these materials. (1) So, a constant search for the best root end restorative material goes on which has it all.

Materials used in this study, namely Pro root MTA, Biodentin, and Bio C Repair show less microleakage and better antibacterial efficacy than other cements as seen in previous researches.

Pro root MTA which is a calcium silicate based material, is currently the choice of material for long term follow up and used in many fields of endodontics. But due to its few shortcomings Biodentin has been promoted which can be used as an endodontic repair material. (8)

Bio C Repair (Angelus, Londrina, Pr, Brazil) (BCR) a recently launched bioceramic restorative material also fulfills the same criteria and is available in a premixed syringe form and is bioactive, antibacterial, non-staining and promotes healing. (9)

Now a days we are in an era of nanotechnology. Silver nano particles play a crucial role in providing apical seal and inhibiting bacterial growth in aqueous and solid media because of their high reactivity due to large surface to volume ratio.[10] So, addition of silver nano particles with all its goodness to these restorative materials will probably end the quest and bring a new era of restorative dentistry.

MATERIALS & METHODS

Experimental materials taken in study were Pro root MTA (Dentsply Sirona, USA), Biodentin (Septodont, France), Bio C Repair (Angelus, Brazil) and Silver Nano Particles (nano carbon technologies,Nanotech lab, 30-50nm). After separate evaluation of the plain bioceramic filling materials, materials were evaluated by addition of silver nano particles to them for increased antimicrobial efficacy.

Groups were randomly divided according to the material to be tested :-

Group 1 - Plain Pro root MTA

Group 2 - Plain Biodentin

Group 3 - Plain Bio C Repair

Group 4 - Pro root MTA with 5% Ag Nano particles

Group 5 - Biodentin with 5% Ag Nano particles

Group 6 - Bio C Repair with 5% Ag Nano particles

Group 7 - Positive Control

Group 8 - Negative Control

To measure proper amount an advanced digital weighting machine was used and silver nano particles were mixed with the materials in 5% by weight.

Antimicrobial assessments were performed on *E. Faecalis* bacteria. For preparation of microbial inocula, *E. Faecalis* (ATCC 29212) strains were freshly grown on McConkey Agar plates overnight at a temperature of 37^0C. Two or three colony forms were chosen from the freshly grown agar plates and mixed with brain heart infusion broth, approximately 2-3 microlitres. 0.5 McFarland standards (1.5×10^8 CFU) were used as a reference to adjust the turbidity of the microbial suspension.

Antimicrobial efficacy test was conducted in Cytogene Research and Development Lab (Lucknow, India) by Kirby- Bauer's Disc Diffusion method against *E. Faecalis.* Test name was Agar Disc Diffusion Assay.

For the isolation of bacteria, initially Muller Hinton Agar (MHA) was prepared as per the standard composition given by HI-media that is 38gm of the media was suspended in 1L distilled water and the media was autoclaved at 121^0C and 15psi for 15minutes in autoclave (Gentek India Pvt. Ltd.).

After the sterilization media was poured in sterile glass petri dishes inside the Laminar airflow (Toshiba, India) using the aseptic techniques, each plate was poured with 20ml of the culture media.

The plates were then allowed to solidify properly, then the media was inoculated with the respective bacterial isolate, which was *E. faecalis* by spread plate technique, for which 100µl of the culture broth of isolate was added over the media and uniformly spread using sterile glass rod.

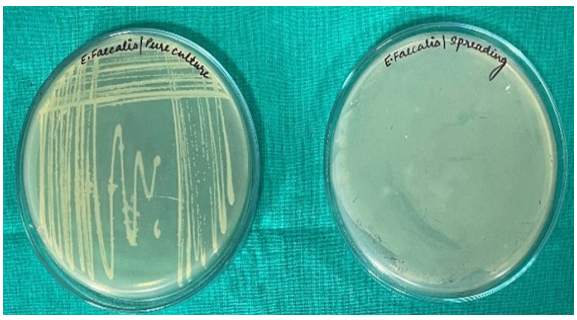

Figure 1- *E. Faecalis* grown on culture plate and plain sterile culture plate before inoculation.

Ten minutes after spreading, disc was placed onto the media plates using sterile forceps and then each disc was loaded with respective sample on separate plates after manipulation according to manufacturer's instruction and being allowed to set.

The samples were allowed to diffuse through the disc into the media and then the plates were sealed with paraffin and incubated at 34^0C for 24hrs.

The plates had two additional discs in which one of the discs was positive control having 20 µl ciprofloxacin and another was negative control which was loaded with distilled water.

Next day after incubation the plates were observed for the clear zone around the disc, known as the zone of inhibition, and the diameter of these zones was measured in mm and recorded at 24hrs. 10 repetitions done of each sample for gathering authentic data and accurate statistical analysis.

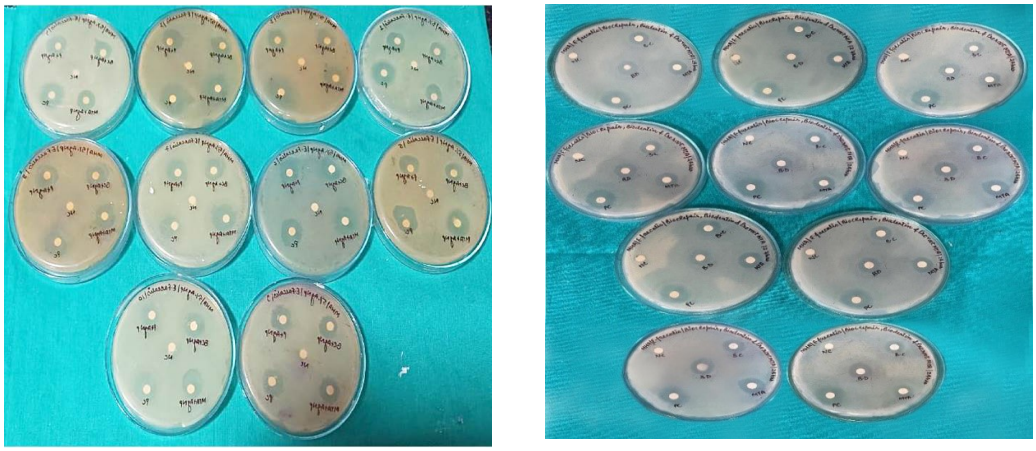

Figure 2- Inhibition zones observed in plain and Ag nano particle added materials and 10 repetitions done.

RESULTS

The data was gathered and entered in MS Excel spreadsheet. Data was analyzed using SPSS version 26. Data analysis was carried out using Descriptive statistics and other relevant tests of significance. The p value was set at 0.05 to be significant and value less than 0.01 was considered as highly significant. Confidence level was set at 95% and power of the study was fixed at 80%. Data was analysed to assess the zone of inhibition of the different restorative materials at 24 hours. Intra and intergroup comparison was performed using Analysis of variance or ANOVA, which if found to be significant was substantiated with Tukey's Post Hoc analysis to perform pair-wise comparison of data. Finally, a subset table was made to understand the order of groups.

Data was analysed to assess the zone of inhibition of the different restorative materials at 24 hours with and without 5% addition of Ag Nano Particles.

After Tukey's post hoc analysis and subset table interpretation the order for efficacy here is :

For plain materials :

Positive control > Biodentin > Bio C Repair = Pro root MTA

For materials added with 5% silver nano particles :

Positive Control = Biodentin > Bio C Repair > Pro root MTA

It can be interpreted that there is a highly significant difference in the antibacterial efficacy of plain bioceramic based filling material compared to positive control. The efficacy is increased upon addition of silver nano particles to the same materials compared to plain materials.

Table 1: Intergroup comparison of antimicrobial efficacy of plain bioceramic based filling materials after 24 hours.

Groups	Number	Mean	Std. Deviation	95% Confidence Interval for Mean		Min.	Max.	P value
				Lower Bound	Upper Bound			
Gp 1 : Pro root MTA	10	9.18	0.12293	9.0921	9.2679	9	9.3	0.000**
Gp 2 : Biodentin	10	10	0.11547	9.9174	10.0826	9.8	10.1	
Gp 3 : Bioc repair	10	9.2	0.20548	9.053	9.347	9	9.5	
Gp 7 : Positive Control	10	14	0.41647	13.7321	14.3279	13.2	14.5	

'P' stands for Probability value denoting data occurred under null hypothesis

***Table.2 Intergroup comparison of antimicrobial efficacy of bioceramic* based filling materials with 5% addition of Ag nano particles after 24 hours.**

Groups	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Min.	Max.	P value
				Lower Bound	Upper Bound			
Gp 4a : Pro root MTA	10	10.35	0.10801	10.2727	10.4273	10.2	10.5	0.000**
Gp 5a : Biodentin	10	13.75	0.71995	13.235	14.265	13	15	
Gp 6a : Bioc repair	10	12.91	0.29231	12.7009	13.1191	12.5	13.2	
Gp7: Positive Control	10	14.03	0.41647	13.7321	14.3279	13.2	14.5	

'N' stands for number of samples.
'P' stands for Probability value denoting data occurred under null hypothesis.

DISSCUSSION

Successful outcome of root canal therapy depends upon thorough elimination and disinfection of root canal system, failure in doing so can lead to reinfection sooner or later. To overcome this, a complete coronal, apical and lateral seal with appropriate usage of root canal filling is important.[11]

In endodontics, *E. Faecalis* is the most frequently isolated robust micro-organism in root filled teeth with root canal failures than in primary cases. Its commonly associated with persistent apical periodontitis and can also bear prolonged starvation, [12] and thus justifying its use as a test organism in this study.

Different methods have been used to investigate the antimicrobial effects of dental materials. Agar disc diffusion assay was used in this study because its the most standard, simple and easy

way to find antimicrobial activity, used worldwide for last 60 years, also has been used in previous studies to facilitate comparison of data.[13]

Among the Most highlighted root end filling materials so far, in the 1990s MTA was developed with purpose to serve as root end filling material and to seal lateral root perforations and has favorable properties like providing good seal, biocompatibility, low toxicity and high ph. (Torabinezad et al, 1995)[14] However, it has some drawbacks such as long setting time, tooth discoloration, difficult handling, limited antimicrobial activity and high cost. (Parirokh M et al)[15]

In such case, researches go on for material with best antibacterial efficacy as no retrograde filling material provides perfect seal and Biodentin, a calcium silicate based bioceramic cement introduced in 2010 claims improvement of some drawbacks of Pro root MTA like long setting time and handling.

Bio C Repair cement (Angelus) was recently made available on the market. According to the manufacturer, its qualities surpass previous bioceramic products, especially because it comes in premixed syringe form, ultimately saving time. Besides the physical seal provided by the expansion of the cement, it causes a biological seal by the formation of an intermediate layer of mineralization. Setting of Bio C Repair will depend on the presence of moisture at the site as it sets in presence of moisture or blood. Chemical reactions involve the hydration of calcium Silicate compounds to produce a hydrated Calcium Silicate (C-S-H) gel.[8]

Silver nano particles used in this study are one of the most widely used nano particles, most notably serving for medical applications. Stowe *et al.*[16] also found in their study that silver nano particles have antimicrobial property because of silver ions interaction with thiol groups and possess deadly effect on bacterial enzymes, their growth and subsequent cell division resulting in damage of cell wall, leading to cell wall lysis.

In the present *in-vitro* study comparison of effect of antimicrobial efficacy was done between plain bioceramic materials Pro root MTA, Biodentin and Bio C Repair and after addition of 5% silver nano particles in each of the bioceramic materials.

In the present study 5% concentration of silver nano particles was used as many previous studies have shown insignificant results on lower concentration. (M Eskandarinezhad et al) [17]

The data was gathered and p value was set at 0.05 to be significant, less than 0.01 was considered as highly significant. Data was analysed to assess the zone of inhibition of the different restorative materials at 24 hours.

Statistical analysis was carried out. The Intra and intergroup comparison was performed using ANOVA test, which if found to be significant was substantiated with Tukey's Post Hoc analysis.

Comparison of zone of inhibition of different plain sealing materials after 24 hours:

Positive control>Biodentin>Bio C Repair=Pro root MTA

Compared to positive control there is highly significant difference in the efficacy of the three materials. Pro root MTA and Bio C Repair have insignificant variation.

Here best value is of Biodentin apart from positive control and least is of Pro root MTA which can be justified according to study conducted by H Bakhtiyar et al **[18]** that Biodentin is less soluble and faster setting compared to Pro root MTA while in Pro root MTA it leads to partial material loss and alteration in interface.

Han et al **[19]** reported that Ca/P ratio significantly increases in Biodentin and Pro root MTA groups at 1,7,30 and 90 days compared with control. Biodentin presents higher Ca levels than Pro root MTA at all times and biomineralization of Biodentin is much more prominent than Pro root MTA. Also, results of many studies conducted on antimicrobial property of MTA remains controversial. (M Samiei et al, 2013) [20]

Comparison of zone of inhibition of materials added with 5% silver nano particles after 24 hours:

Positive control=Biodentin with Ag nano>Bio C Repair with Ag nano>Pro root MTA with Ag nano particles.Here compared to the positive control, there is no significant difference in the zone of inhibition of Biodentin.Among Bio C Repair with Ag and group 6 there is a significant difference in the zone of inhibition.A previous study conducted by Yousara N *et al.* [21] denoted this change occurring due to change in setting time of MTA when added nano particles to it due to acceleration of hydration process.Samiei M *et al.*[20] showed that Ag nano particles effectively enhanced antimicrobial efficacy of MTA against microbial species. Ag NPs affected structural integrity of the biofilm and prevented DNA replication.

CONCLUSION

Within the limits of the study it was observed that tricalcium silicate based bioceramic materials have antibacterial efficacy against *E. Faecalis* and Biodentin was found to be most effective in killing the bacteria compared to other two materials which had insignificant difference.

The addition of silver nano particles to the material drastically increased the efficacy of the three compared to their plain counterparts. Most significant effect was observed on Biodentin. Bio C Repair has also shown noticeable results especially the increased time and additional benefits of clinical application but very few studies have been conducted on the same and further studies with different techniques and time intervals are needed.

This study supports the hypothesis that the test materials are bioactive and their bioactivity increases over time; however, this is an in vitro study and the long term impact of their bioactivity on the tooth should be evaluated and further in vivo investigations should be done.

REFERENCES

1. Fouad AF, Zerella J, Barry J, Spangberg LS: Molecular detection of Enterococcus species in root canals of therapy-resistant endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005; 99(1):112-118.
2. Zarrabi MH, Javidi M, Naderinasab M, Gharechahi M. Comparative evaluation of antimicrobial activity of three cements: new endodontic cement (NEC), mineral trioxide aggregate (MTA) and Portland. J Oral Sci. 2009; 51(3):437-442
3. Kharouf N, Arntz Y, Eid A, Zghal J, Sauro S, Haikel et al. Physicochemical, antibacterial properties of novel,premixed calcium silicate based sealer compared to powder-liquid bioceramic sealer: An in vitro study. J Clin Med. 2020; 9(3096):1-17
4. Maria NL, Simona BPA, Mihai HR , Mona K , Elena CL. Comparison of three calcium silicate cements used as retrograde filling material.: An in vitro study. J Res and Clin Med.2020; 4(1):32-36
5. Antunes HS, Gominho LF, Andrade-Junior CV, Dessaune-Neto N, Alves FR, Rocas IN et al. Sealing ability of two root end filling materials in a bacterial leakage model: An in vitro study. Int Endo J. 2016; 49(10): 960-965
6. Zischka M, Kunne CT, Blom J. Comprehensive molecular, genomic and phenotypic analysis of a major clone of E. Fecalis MLST ST40. BMC Genomics. 2015; 16:175
7. Kandaswamy D, Venkatesh B, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, povidone iodine, and calcium hydroxide. Int Endod J. 2010; 43(5):419-423
8. Aggarwal V, Singla M, Miglani S, Kohli S. Comparative evaluation of push out bond strength of Pro Root MTA, Biodentin, and MTA Plus in furcation perforation. J conserv Dent.2013; 16(5):462-465

9. Toubes KS, Tonelli SQ, Girelli CFM, Azevedo CG, Thompson AC, Nunes E et al. Bio-C Repair - A New Bioceramic Material for Root Perforation Management: Two Case Reports. Braz Dent J.2021; 32(1):104-110
10. Degrazia FW, Leitune VCB, Garcia IM, Arthur RA, Samuel SMW, Collares FM. Effect of silver nano particles on the physicochemical and antimicrobial properties on orthodontic adhesives: an in vitro study. J App Oral Sci. 2016; 24(4): 404-410
11. Aminsobhani M, Ghorbanza A. Coronal microleakage in root canals obturated with lateral compaction, warm vertical compaction and guttaflow system. Iran Endod J. 2010; 5(2): 83-87
12. Figdor D, Davies JK, Sundqvist G. Starvation survival growth and recovery of E. Fecalis in human serum. Oral Microbiol Immunol. 2003; 18(4):234-239
13. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate based endodontic materials into root canal dentin. Int Endod J. 2011; 44(12): 1081-1087
14. Torabinejad M, Hong CU, Lee SJ, Monsef M, Ford TRP. Investigation of MTA for root end filling in dogs. J Endod.1995; 21(12):603-608
15. Parirokh M, Torabinezad M. Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. J Endod. 2010; 36 (3):400-413
16. Stowe TJ, Sedgley CM, Stowe B, Fenno JC. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial property of Pro Root MTA. J Endod. 2004; 30(6): 429-431
17. Eskandarinezhad M, Shahveghar-Asl N, Sharghi R, Shirazi S, Shakouie S, Milani AS et al. Sealing efficacy of MTA with and without nanosilver for root end filling : An in vitro bacterial leakage study. J Clin Exp Dent. 2017; 9(1):27-33
18. Bakhtiar H, Nekoofar MH, Aminishakib P, Abedi F, Moosavi FN, Esnaashari E et al. Human Pulp Responses to Partial Pulpotomy Treatment with TheraCal as Compared

with Biodentine and ProRoot MTA: A Clinical Trial. J Endod. 2017 Nov; 43(11):1786-1791

19. Han L, Kodama S, Okiji T. Evaluation of calcium releasing and apatite forming abilities of fast setting calcium silicate based endodontic materials. Int Endod J. 2015; 48(2):124-130
20. Samiei M, Aghazadeh M, Lotfi M, Shakoei S, Aghazadeh Z, Mahdi S et al. Antimicrobial efficacy of MTA with and without silver nano particles for root end filling: An in vitro study. Iran Endod J. 2013; 8(4):166–170
21. Nashaat YM, Labib AH, Omar N, Shaker M. Comparative study of the antibacterial effect of MTA, nano MTA, Portland cement and nano Portland cement. EDJ. 2019; 65(1):701-706