Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/19791
DOI URL: http://dx.doi.org/10.21474/IJAR01/19791

RESEARCH ARTICLE

INFLUENCE OF ADDITION OF SILVER NANOPARTICLES TO ENDODONTIC SEALERS – AN IN VITRO STUDY

Dr. Manjusha D.[1] and Dr. Jai Kiran K.[2]

1. MDS, Department of Pedodontics and Preventive Dentistry GITAM Dental College and Hospital, Visakhapatnam.
2. MDS, Department of Pedodontics and Preventive dentistry GITAM Dental College and Hospital, Visakhapatnam.

Manuscript Info

Manuscript History
Received: 29 August 2024
Final Accepted: 30 September 2024
Published: October 2024

Key words:-
Root Canal Therapy, Antimicrobial Agents, Nanomaterials, Nanotechnology, Root Canal Sealants

Abstract

Background / Introduction: The main aim of root canal treatment is to significantly eradicate the microorganisms, and achieve hermetic seal. Silver nanoparticles are shown to have positive effect on inhibiting the growth of various oral pathogenic bacteria. Hence present study is designed to evaluate the antimicrobial efficacy of different endodontic sealers incorporated with silver nanoparticles.

Aim: To assess and compare the antimicrobial efficacy of four different root canal sealers against E. faecalis.

Methodology: Four different commercially available root canal sealers were used in the present study. Aliquots of the suspension containing E. faecalis were spread on Mueller-Hinton agar medium. Freshly mixed sealers and silver nanoparticle incorporated sealers were placed into wells of petri dishes. All plates were incubated for 72 h at 37°C under aerobic conditions, and zones of inhibition were measured at 24, 48, and 72 h.

Results: All the four commercial endodontic sealers when used alone and in combination with silver nanoparticles showed antimicrobial activity against E. faecalis. The antimicrobial activity of the sealers at the end of 72 hours is as follows: Zinc Oxide Eugenol >Endomethasone N > Nishika Canal Sealer BG > AH Plus. When the sealers were incorporated with silver nanoparticles, antimicrobial efficacy of AH Plus and Endomethasone N was enhanced.

Conclusion: Incorporation of silver nanoparticles had positive effect on antimicrobial efficacy of sealers, especially AH Plus and Endomethasone N, although the effectiveness of all the root canal sealers against E. faecalis decreased with time.

Introduction:-

Achieving optimal disinfection and a fluid-tight seal within the root canal system are indeed crucial aspects of successful endodontic treatment. Endodontic sealers play a key role in this process by filling the space between the obturating material and the root dentin, thereby creating a hermetic seal that prevents the ingress of microorganisms and fluids (Munitić M. et al, 2019) Despite thorough disinfection efforts during root canal treatment, reinfection

Corresponding Author:-Dr. Manjusha D.
Address:-MDS, Department of Pedodontics and Preventive Dentistry GITAM Dental College and Hospital, Visakhapatnam.

remains a significant concern and a leading cause of treatment failure. This is attributed to various factors, including the complex anatomy of root canals, which may harbor residual bacteria even after meticulous cleaning, and the presence of pathogenic bacteria that possess remarkable survival abilities and can form biofilms, rendering them more resistant to disinfection strategies (Tabassum S et al, 2016). Endodontic sealers with adequate antimicrobial activity can help create an environment within the root canal system that discourages microbial growth and reduces the risk of reinfection (Rathi CH et al, 2020).

To address this challenge, ongoing research is focused on developing endodontic sealers with enhanced and long-lasting antimicrobial properties. Incorporating antimicrobial agents, such as silver nanoparticles or antibiotics, into sealers is one approach being explored to improve their efficacy against microorganisms. Additionally, advancements in material science and nanotechnology may lead to the development of novel sealers with superior antimicrobial activity and prolonged effectiveness.

The incorporation of silver nanoparticles (Ag NPs) into endodontic sealers represents a promising application of nanotechnology to enhance antimicrobial efficacy in root canal treatments. This was because, silver shows low toxicity to mammalian cells and high toxicity towards bacterial microorganisms. They can also be used for prolonged drug delivery at the required site of action (D. Chandra Lekha et al 2021). Previously, Ag NPs have been used in dentistry to treat early carious lesions, regulate the plaque bacteria and also as root canal irrigants to eliminate the pathogenic residual bacteria. Among many properties of these nanoparticles, these are some important properties that make them more acceptable to use in endodontic therapy (Emmanuel R et al, 2015 and Bhandi.S et al, 2021).

Hence the present study is designed to assess and compare the antimicrobial activity of four different root canal sealers against E. faecalis in conventional form and after they are incorporated with Ag NP. The reason for using E. faecalis for assessment of antimicrobial activity is that although Enterococcus species comprise a small proportion of the initial flora in infected root canal, they are most commonly recovered from unsuccessful endodontic treatment and has also been associated with existing root canal infections (Farahat et al, 2022).

Materials and Methodology:-

Four different commercially available root canal sealers were used in the present study.Sub grouping of samples was done in the following manner: (Table 1)

The microorganisms are grown in sterile solid media and standard strains of E. faecalis were obtained. Mueller Hinton agar medium is commonly used for testing the antimicrobial susceptibility of bacteria. This microbial suspension was spread evenly on Mueller Hinton agar and incubated at 37°C for microbial growth. After 24 hours, E. faecalis growth was observed. Each agar plate was divided equally and a 5mm diameter well was created at equidistant places using a sterile stainless steel cylinder by removal of agar. All the wells in each plate were filled with one of the eight subgroups of sealers. All the plates were incubated at 37°C under aerobic conditions for 72 hours, and zones of inhibition were measured at 24, 48, and 72 hours (Dalmia S et al, 2018).

Results:-

The obtained values were tabulated and subjected to statistical analysis. When observed individually, the antimicrobial efficacy was as follows: ZOE >Endomethasone N > Nishika BG > AH Plus. When the antimicrobial activity of the sealers incorporated with Ag NP was compared to that of the conventional sealers, there was increase in antimicrobial activity of AH Plus and Endomethasone N whereas addition of AgNP didn't show any improvement in antimicrobial activity in the other 2 sealers.

On intergroup comparison, AH Plus group showed highly significant value after incorporation of silver nanoparticles, Endomethasone and Nishika BG sealer showed a significant value. On intragroup comparison, both the groups showed highly significant values. (Table 2,3,4,5)

Discussion:-

Advancements in endodontic treatment have indeed been propelled by the development of new root canal filling materials. These materials are designed with enhanced capabilities to seal off bacterial in-growth and prevent fluid inflow, both of which are essential for successful treatment outcomes (Farahat et al, 2022).In recent years,

nanotechnology has been increasingly integrated into the field of dentistry, including endodontics. Nanoparticles, due to their minuscule size, provide a larger surface area relative to their volume. This characteristic enhances their effectiveness even when used in small doses. Moreover, nanoparticles exhibit antibacterial properties that are not limited to specific bacterial species (Bhandi.S et al, 2021).

Studying the antibacterial properties of nanoparticles, particularly silver nanoparticles, has been a significant focus in dental research. Silver has long been recognized as an effective antibacterial agent in dentistry, owing to its ability to release silver ions which have a detrimental effect on bacterial membranes, as they are electrostatically attracted to sulfur proteins, leading to membrane adhesion and deterioration (Bhandi.S et al, 2021). The objective of this study is to investigate whether the incorporation of silver nanoparticles into traditional root canal sealers can enhance their ability to combat pathogens.

The agar diffusion test is indeed a commonly utilized method for evaluating the antimicrobial efficacy of various endodontic sealers, and it was employed in the present investigation. The agar diffusion test offers a visual representation of the results, making it easier to interpret and compare the antimicrobial activity of different sealers. Zones of inhibition surrounding the sealers on the agar plates observed, allowing for a qualitative assessment of which sealer is most effective in eradicating microorganisms from the local microenvironment of the root canal system (Dalmia S et al, 2018).

AH Plus is indeed regarded as the gold standard among endodontic sealers, primarily due to its superior adhesion properties. It forms covalent bonds with dentin collagen, ensuring a strong and durable seal within the root canal system (Rathee G et al, 2020). Nishika canal sealer, on the other hand, incorporates bioactive glass in its composition as reported helps in reducing microbial colonization within the root canal system, thereby aiding in the success of the treatment (Washio A et al, 2019). Zinc Oxide Eugenol and Endomethasone both derive their antimicrobial activity from eugenol, which is the main constituent in their liquid composition. Additionally, in Endomethasone, the presence of hydrocortisone may provide an additional advantage due to its anti-inflammatory properties (JeanneauC et al, 2019).

The observed differences in antimicrobial activity among plain endodontic sealers can indeed be attributed to their respective compositions. In the case of Zinc Oxide Eugenol (ZOE) and Endomethasone N, both sealers exhibited the highest antimicrobial activity. This heightened activity is largely due to the presence of eugenol, a key component in their composition. Conversely, AH Plus showed the least antimicrobial activity in the study. This difference can be attributed to the composition of AH Plus, which includes epoxy resin and amine ingredients. During the polymerization process, these components release formaldehyde, which possesses antimicrobial properties. However, the antimicrobial activity of formaldehyde released from AH Plus may be comparatively lower than that of eugenol in ZOE and Endomethasone N, resulting in AH Plus exhibiting the least antimicrobial activity among the sealers tested and freshly mixed AH plus has a higher antimicrobial efficacy compared to set AH plus as observed by (Zhang et al, 2009)**.** The findings of (Gomes et al, 2004)study corroborate the observations regarding the antimicrobial activity of eugenol-based sealers compared to resin-based and MTA-based sealers.

The observed enhancement in antimicrobial activity in AH Plus and Endomethasone N when incorporated with silver nanoparticles (Ag NP) aligns with previous discussions regarding the antimicrobial properties of these sealers (Zhang et al, 2009). AH Plus, despite its initial lower antimicrobial activity compared to eugenol-based sealers, may benefit from the addition of Ag NP, which possess strong antimicrobial properties on their own. This addition likely enhances the overall antimicrobial efficacy of AH Plus, particularly considering that set AH Plus might not retain significant antimicrobial activity on its own (Faris et al, 2018). Similarly, Endomethasone N, which already demonstrated optimal antimicrobial properties in its plain form due to the presence of eugenol, may further amplify its antimicrobial activity with the incorporation of Ag NP. This combination of eugenol and Ag NP could synergistically enhance the antimicrobial action of Endomethasone N.

The absence of significant enhancement in antimicrobial activity in ZOE and Nishika sealers after incorporation with Ag NP could be attributed to several factors. One possibility is that the antimicrobial activity of ZOE and Nishika sealers in their plain form is already robust, possibly due to the presence of eugenol in ZOE or bioactive glass in Nishika. This inherent antimicrobial activity might overshadow any additional effect conferred by the Ag NP, resulting in no significant improvement. Additionally, the interactions between the components of the sealers and Ag NP may vary, influencing the overall antimicrobial efficacy. Further research may be needed to elucidate the

specific mechanisms underlying the observed outcomes and to explore potential strategies for optimizing the antimicrobial activity of all types of endodontic sealers, including those incorporating Ag NP.

Conclusion:-

The conclusion drawn from the study suggests that the incorporation of silver nanoparticles (Ag NP) into endodontic sealers enhances their antimicrobial activity. This finding implies that the use of sealers containing Ag NP could offer a promising approach to minimize endodontic failures, particularly those related to microbial re-infection or persistence. However, it's essential to acknowledge the limitations of the study and interpret the findings within that context. Further research may be warranted to validate these results, explore potential long-term effects, assess biocompatibility, and evaluate the clinical applicability of sealers containing Ag NP.

Limitations of the present study

The size of the inhibition zones depends on the ability of the material to diffuse through the particular medium. In turn, the diffusibility is mainly affected by three factors which include: (1) hydrophobicity or hydrophilicity of the material, (2) the rate of release from the matrix in which the material is placed, and (3) size. These factors are difficult to control in in vitro studies and the agar medium doesn't exactly replicates the tooth structure and its surrounding conditions/medium. Also the size of inhibition zone doesn't exactly measure the antimicrobial activity of a particular material.

Table list:

Subgroup 1	1a AH Plus
	1b AH Plus + AgNP
Subgroup 2	2a Endomethasone
	2b Endomethasone + AgNP
Subgroup 3	3a Nishika BG
	3b Nishika BG + AgNP
Subgroup 4	4a Zinc Oxide Eugenol
	4b Zinc Oxide Eugenol + AgNP

Table 1 - Subgrouping of endodontic sealers

Sealers	24 hours Mean (mm)	48 hours Mean (mm)	72 hours Mean (mm)
Sub group 1a (AH Plus)	5.57	5.26	5.26
Sub group 1b (AH plus + Ag NP)	8.52	7.86	7.51
Sub group 2a (Endomethasone)	10.13	9.26	8.82
Sub group 2b (Endomethasone + Ag NP)	15.13	14.42	12.96
Sub group 3a (Nishika BG)	9.22	9.08	8.8
Sub group 3b (Nishika BG + Ag NP)	8.7	8.33	8.06
Sub group 4a (Zinc Oxide Eugenol)	20.42	19.8	18.58
Sub group 4b (Zinc Oxide Eugenol + Ag NP)	19.62	19	18.01

Table 2 - Zones of inhibition observed (in mm) at 24, 48 and 72 hours.

Pair of comparision	Mean inhibition zone	SD	SE	t	P value
Sub group 1a – 1b	5.36 7.96	0.25 1.16	0.436	-1.21	0.0 (highly significant)
Subgroup 2a – 2b	9.40 14.17	0.65 5.47	1.81	-2.28	0.04 (significant)
Sub group 3a – 3b	9.03 8.36	0.73 0.31	0.26	2.74	0.04 (significant)
Sub group 4a – 4b	19.60 18.87	1.27 0.71	0.46	1.24	0.20 (not significant)

Table 3 - Intergroup comparision of antimicrobial activity of endodontic sealers.

GroupA (Unmodified group)	Sum of Squares	df	Mean Square	F	P – value
Between Groups	39	17	2.294	32.118	0.00*
Within Groups	1	14	0.71		
Total	40	31			

Group B (Ag NP modified group)	Sum of Squares	df	Mean Square	F	P - value
Between Groups	34.3	16	2.144	5.641	0.01*
Within Groups	5.7	15	0.380		
Total	40	31			

Table 4 - Intragroup comparision of antimicrobial activity of endodontic sealers

Acknowledgements:-

- There is no source of funding to the study. The study was self funded.
- We thank Vanga V Narasimha Rao sir, Professor, Department of Pedodontics and Preventive Dentistry, GITAM Dental College for helping us with the conceptualisation of the study.
- We thank Department of Microbiology, GIMSR, Visakhapatnam for providing us the lab for performing our study.
- There are no conflicts of intrest.
- Author contributions -

1. Dr. Manjusha Dumpala - Substantially contributed to conception and design of the study. Contributed to the acquisition, analysis and interpretation of the data. Drafted the manuscript and gave the final approval. Agrees to beaccountable for all aspects of the work in ensuring that questions relating to the accuracyor integrity of any part of the work are appropriately investigated and resolved.

2. Dr. Jai kiran Killada - Substantially contributed to conception and design. Contributed to the analysis and interpretation of the data. Critically revised the manuscript for important intellectual content and gave final approval. Agrees to beaccountable for all aspects of the work in ensuring that questions relating to the accuracyor integrity of any part of the work are appropriately investigated and resolved.

References:-

1. Bhandi, S.; Mehta, D.; Mashyakhy, M.; Chohan, H.; Testarelli, L.; Thomas, J.; Dhillon, H.; Raj, A.T.; Madapusi Balaji, T.; Varadarajan, S.; et al. Antimicrobial Efficacy of Silver Nanoparticles as Root Canal Irrigant's: A Systematic Review. J. Clin. Med. 2021, 10, 1152-1163.
2. Charlotte Jeanneau, Thomas Giraud, Jean-Louis Milan, Imad About. Investigating endodontic sealers eugenol and hydrocortisone roles in modulating the initial steps of inflammation. Clinical OralInvestigations.2019:24(2).639-647.
3. D. Chandra Lekha, R. Shanmugam, K. Madhuri, L. Priyanka Dwarampudi, Mahendran Bhaskaran, Deepak Kongara, Jule Leta Tesfaye, N. Nagaprasad, V. L. Nirmal Bhargavi, Ramaswamy Krishnaraj,et.al. Review on Silver Nanoparticle Synthesis Method, Antibacterial Activity, Drug Delivery Vehicles, and Toxicity Pathways: Recent Advances and Future Aspects. Journal of Nanomaterials.2021.1-11.
4. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial efficacy of different endodontic sealers against Enterococcus faecalis: An In vitro study. J Int Soc Prevent Communit Dent 2018;8:104-9.
5. Emmanuel R, Palanisamy S, Chen SM, Chelladurai K, Padmavathy S, Saravanan M, Prakash P, Ajmal Ali M, Al-Hemaid FM. Antimicrobial efficacy of green synthesized drug blended silver nanoparticles against dental caries and periodontal disease causing microorganisms. Mater Sci Eng C Mater Biol Appl. 2015 Nov 1;(56):374-9.
6. Farahat, Mohamed &Elfaramawy, Medhat & Yehia, Tariq. The effect of addition of silver nanoparticles on the antibacterial effect of three different root canal sealers (an in vitro study). Egyptian Dental Journal.2022. 68. 1775-1779.
7. Faris, Awad & Khalifa, Ahmed & Emera, Radwa. The Antimicrobial Efficacy OfNanosilver Modified Root Canal Sealer. European Journal of Research in Medical Sciences. 2018. 6. 1-6.
8. Gomes BP, Pedroso JA, Jacinto RC, Vianna ME, Ferraz CC, Zaia AA, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of five root canal sealers. Braz Dent J. 2004;15(1):30-5.
9. Rathee G, Cheema J, Thapak G, Chauhan P, Kumar AV. Recent advancements in root canal sealers. Int J Res Health Allied Sci 2020; 6(4):46-49.
10. Rathi CH, Chandak M, NikhadeP.Functions of root canal sealers- a review. J.Evolution Med. Dent. Sci. 2020;9(17):1454-1458.
11. Sˇimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. 2019. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS ONE. 14(10):1-20.
12. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. Eur J Dent. 2016. Jan-Mar;10(1):144-147.
13. Washio A, Morotomi T, Yoshii S, Kitamura C. Bioactive Glass-Based Endodontic Sealer as a Promising Root Canal Filling Material without Semisolid Core Materials. Materials (Basel). 2019 Nov 29;12(23):3967:1-17.
14. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009 Jul;35(7):1051-5.