(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 5, Issue 2, Page No: 263-269
March-April 2022

IJMSCR

Comparative Evaluation of Antibacterial and Adhesive Properties Of Provisional Cements Incorporated With Two Different Types Of Nanoparticles

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Statement of problem: Frequently provisional restorations require long term permanence in the oral cavity; thus, an antibacterial effect is desirable. This effect may be achieved by incorporating nanoparticles into provisional cements.

Purpose: The purpose of this invitro study is to compare and evaluate the antibacterial and adhesive properties of provisional cements incorporated with silver zeolite and chitosan nanoparticles.

Objectives:
To evaluate the antibacterial efficacy of provisional cements incorporated with Silver Zeolite and Chitosan. To evaluate the bond strength of provisional cements incorporated with Silver Zeolite and Chitosan.

Methods: The nanoparticles (Silver Zeolite and Chitosan) incorporated at a conc of 2.5% w/w into provisional cements (ZOE, IRM, Non- Eugenol) was studied in vitro. The antibacterial effect against *Streptococcus. mutans* was tested using direct contact test. The bond strength was tested using Universal Testing Machine. The data was analyzed using the ANOVA test, with the Dunnett test for multiple pairwise comparisons.

Results: Addition of different nanoparticles into 3 commercially available provisional cements showed significant increase in antimicrobial efficacy and bond strength.

Conclusion: Silver Zeolite showed significant antibacterial property and improved bond strength when incorporated into provisional cements. Therefore, the addition of nanoparticles into provisional cements rendered all 3 provisional cements antibacterial against *Streptococcus mutans* without compromising bond strength with all 3 cements (ZOE, IRM, NE).

Keywords: nanoparticles, provisional cement, antibacterial property, bond strength.

Introduction

Provisional cementation of provisional restorations is widely practiced for a variety of clinical reasons, including the desire to make further occlusal adjustments, continuation of periodontal therapy, inspection of abutment teeth for support and these provisional restorations are of great importance in determining the prognosis of prosthetic treatment. They provide protection to the pulp and exposed dentin and serves for diagnostic and therapeutic purposes. Provisional cements are used for the cementation of provisional restorations to the prepared tooth. Most of the currently used dental restorative materials do not form a long-lasting perfect seal with the margins of the prepared tooth. Indeed, it has been shown that gap formation and microleakage are not completely prevented, even when using state- of-the art bonding systems. Gaps between restorative margins and cavity walls are colonized by oral microorganisms and may result in secondary caries and pulp diseases such as irreversible pulpitis. Secondary caries has been identified as the major factor responsible for the longevity of dental restorations. Provisional cements are usually temporary as they are used for crown fixation temporarily. Frequently, they require long term permanence in the oral cavity, thus an antibacterial effect is required along with the strength without compromising any other mechanical properties[1]. Hence, an attempt is being done by incorporating nanoparticles into provisional cements which are highly antimicrobial. The aim of our present study is to compare the antibacterial and adhesive properties of three commercially available provisional cements incorporated with Silver Zeolite and Chitosan nanoparticles.

Clinical implications: Incorporation of nanoparticles may prevent caries and inflammation, and thereby improve the results of the prosthetic treatment by enhancing physical properties. Further investigation is necessary on the effect on mechanical properties and clinical relevance.

Materials And Methods Provisional Cements

Commercially available '3' provisional cements Zinc oxide Eugenol (ZOE), Intermediate restorative material (IRM) and Non – Eugenol Zinc Oxide (NE)were used in the study. Approximately 0.25g of provisional cement is required for luting. Hence, 0.25g of provisional cements were used.2.5% of Silver Zeolite and Chitosan exhibited significant antimicrobial efficacy. Therefore, 2.5% concentration of Silver Zeolite (AgZ) and Chitosan (CHS) nanoparticles were taken. In order to obtain homogenicity, nanoparticles were added into powder and mixed thoroughly according to the manufacturer's instructions. Parameters studied were Antibacterial and Bond strength. The samples are divided into groups as follows.

Group I – ZOE Control (ZOE);

Group II – Non- Eugenol Control (NE); Group III - IRM Control (IRM);

Group Ia – ZOE + 2.5% AgZ Group Ib – ZOE + 2.5% CHS Group IIa – NE + 2.5% AgZ Group IIb – NE + 2.5% CHS Group IIIa – IRM + 2.5% AgZ Group IIIb – IRM + 2.5% CHS

Bacteria

The lawn cultures of 'Streptococcus mutans' a facultative anaerobic strain that is resistant to bacitracin was obtained by inoculating bacterial suspension (109 CFU mL-1) onto the surface of Brain Heart Infusion agar and was incubated at 37°C.

Antibacterial Property

Antibacterial efficacy was assessed by using Agar disc diffusion test. Culture plates containing Mueller Hinton Agar were prepared. BHI broth containing bacterial suspension (Streptococcus. mutans) was also prepared. Both the preparations were incubated prior for 24 hours at 37°C. After incubation, Culture media was streaked using cotton swab containing bacterial suspension. Wells of uniform dimensions were prepared using a borer in a culture media. The homogenous mix of the control and samples were introduced into punch holes using a dropper and incubated at 37°C for 24 hours and were allowed for the development of a uniform 'lawn' of colonies. The inhibition of bacterial growth was easily detected as a clear circular area around the provisional cement sample in which the bacterial growth was inhibited. The size of each inhibition zone was determined by measuring the largest diameter from the midpoint of the sample to border of Zone of Inhibition. The result obtained was expressed in mm and used as a parameter to compare the inhibition potentials of the cement samples. Large inhibition zone indicated high antibacterial activity of the sample, whilst 0 inhibition zone showed no antibacterial activity.

Bond Strength

A prepared tooth die was used. Putty impression was made and was poured with die stone. Then the models were placed in resin block of uniform dimensions (10*12*12mm) as required for testing bond strength. Crowns along with the hook were fabricated for the prepared die model using provisional restorative resin (DPI). Luting cements which were incorporated with nanoparticles were used for fixation of crown to the model. Measured amount of cements (0.25g) and nanoparticles (2.5%) were taken and mixed thoroughly to obtain a homogenous mix in luting consistency. For Control groups, no addition of nanoparticles was done and were mixed. After mixing each cement, cement was spread uniformly on the crown. The crown was then fixed to the tooth and excess cement is removed (Fig.1.). Soak them in artificial saliva for 24 hours.

After 24 hours, the samples were subjected to test bond strength which was evaluated using Universal Testing Machine (Fig.2.)

Fig.1. Cementation of crown

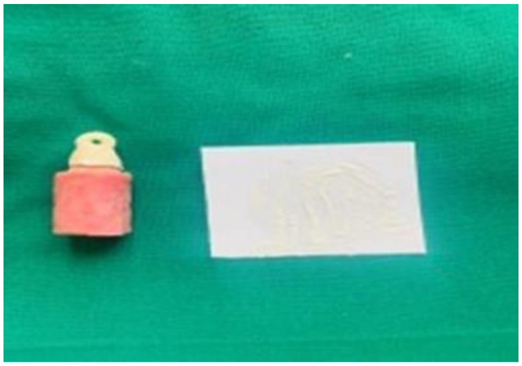
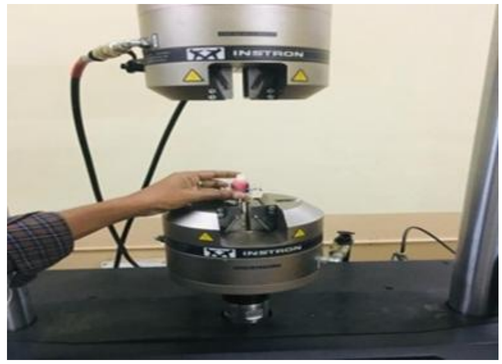


Fig.2. Evaluation of bond strength



Statistical Analysis

The results of the agar diffusion test were analysed using three-way ANOVA followed by post hoc turkey test. The results of the tensile strength test were analysed using One-way ANOVA and multiple pairwise comparisons using Dunnett test. The level of Significance was set at $P < 0.05$.

Results

The results obtained showed the antibacterial efficacy of provisional cements against the selected microorganism *Streptococcus.mutans.* Silver Zeolite and Chitosan incorporated into provisional cements exhibited dose-dependent antibacterial efficacy. The mean diameter values of zone of inhibition measured against tested microrganisms for control and test samples are presented in Table.1. The control specimens of ZOE and IRM showed significant increase in antibacterial efficacy. The control specimens of non-Eugenol did exhibit increase in antibacterial efficacy but it is very minimal.

The mean diameters of ZOE, IRM and Non- Eugenol are 8.5,10.8 and 2. The antibacterial efficacy of provisional cements against *S.mutans* significantly increased with the incorporation of Silver zeolite and Chitosan nanoparticles ($P< 0.5$). 2.5wt% of Silver Zeolite in ZOE, IRM and Non- Eugenol showed the mean diameters of 20.7,22.3 and 6.5. 2.5 wt% of Chitosan in ZOE, IRM and Non- Eugenol showed the mean diameters of 11.9, 14.7 and 4.

The mean bond strength values for both control and test samples are presented in Table.2. The control specimens of ZOE, IRM and Non-Eugenol showed mean bond strength of 12.4,15.3 and 20.4. The provisional cements incorporated with nanoparticles showed increase in bond strength but it is not significant.

Therefore, between the 3 control groups tested for antimicrobial efficacies, IRM showed statistically significant increase in antibacterial efficacy followed by ZOE and NE. Among the cements incorporated with Silver Zeolite, IRM+ AgZ showed highest antibacterial efficacy followed by ZOE+AgZ and NE+AgZ. Among the cements incorporated with Chitosan, IRM+CHS showed highest antibacterial efficacy followed by ZOE+CHS and NE+CHS. When all groups are considered and compared, IRM+AgZ showed highest antibacterial efficacy followed by ZOE+AgZ, IRM+CHS, ZOE+CHS, IRM, ZOE, NE+AgZ, NE+CHS and NE. (as shown in Graph.1.)

When control groups tested for bond strength were compared, non-Eugenol showed statistically significant increase in bond strength. Among the cements incorporated with Silver Zeolite, NE+AgZ showed increase in bond strength followed by IRM+AgZ and ZOE+AgZ but it is not statistically significant. Among the cements incorporated with Chitosan, NE+CHS showed highest bond strength followed by IRM+CHS and ZOE+CHS. Therefore, when all the groups were compared, NE+ AgZ showed highest bond strength followed by NE+CHS, NE, IRM+CHS, IRM +AgZ, IRM, ZOE+CHS, ZOE+AgZ and ZOE.(as shown in Graph.2.)

Volume 5, Issue 2; March-April 2022; Page No 263-269

Mean diameter of zone of inhibition (mm)

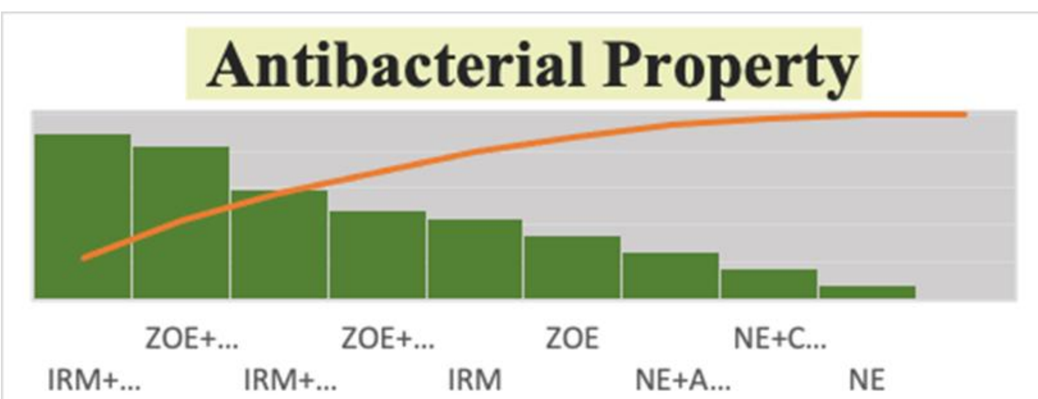
ZOE	Non Eugenol (NE)	IRM	ZOE + silver zeolite	ZOE + chitosan	NE + silver zeolite	NE + chitosan	IRM + silver zeolite	IRM + chitosan
8.5	2	10.8	20.7	11.9	6.5	4.0	22.3	14.7

Table. 1.Mean diameter of Zone of Inhibition for all the groups of provisional cements incorporated with nanoparticles.

Mean of bond strength

ZOE	NE	IRM	ZOE + silver zeolite	ZOE + chitosan	NE + silver zeolite	NE + chitosan	IRM + silver zeolite	IRM + chitosan
12.4	20.4	15.3	13.8	14.0	22.5	21.4	16.1	16.6

Table. 2. Comparison of Mean bond strength among all the groups of provisional cements incorporated with nanoparticles



Graph. 1. Comparison of Mean diameter of Zone of Inhibition of provisional cements incorporated with nanoparticles

Graph. 2. Comparison of Mean bond strength among provisional cements incorporated with nanoparticles

Discussion

Provisional cements are the most commonly used restorative materials in dentistry in general and for cementation of crowns in prosthodontics in particular. They are usually temporary. Furthermore, situations such as full mouth rehabilitation, restorative phase of implant reconstructive procedures, temporomandibular joint dysfunction therapy and unforeseen events such as laboratory delay or patient's unavailability and to protect teeth against thermal insult, chemical irritation, to provide aesthetics and may also lead to the need for long term provisional restoration. In such situations, provisional cements are susceptible to cement washout, marginal leakage, bacterial infiltration and caries. Hence, the present study was designed to test the antibacterial capacities and tensile strengths of three commercially available provisional cements and to test the hypothesis that adding to them Silver Zeolite and Chitosan will render them more antibacterial than the same unmodified cements, without adversely affecting their tensile strength. 3 provisional cements i.e., Zinc Oxide Eugenol, IRM and Non- Eugenol Zinc Oxide were used in the study not only because they are most commonly used for clinical purpose but also for a minute Antimicrobial efficacy which can be seen with cement itself. Silver Zeolite and Chitosan nanoparticles were used as they were shown to exhibit highest antimicrobial efficacy in various studies.

Silver Zeolite shows more Zone of Inhibition than Chitosan because of presence of nano silver in its formation. In silver zeolite, alkaline or alkaline earth metal ion complexed with crystal aluminosilicate is partially replaced with silver ion by the ion-exchange method. Two mechanisms are proposed for the bactericidal action of silver zeolite. One is the action of silver ion itself released from zeolite and the other is that of reactive oxygen species generated from silver in the matrix. Positively charged silver ions binds with negative ions present on the bacterial cell membrane and forms pits in the bacterial cell wall causing cell death due to metal depletion.

Chitosan is a natural polysaccharide derived from Chitin mainly from the shells of shrimps and other crustaceans. It strongly binds with the amino groups of bacteria and causes cell death by disrupting the DNA.

IRM is more antibacterial than ZOE, due to the presence of thymol in its composition which has proven antibacterial efficacy. It shows high dimensional stability, low solubility, abrasion resistance, and good sealing properties due to the reinforcement with polymer.

Eugenol is a phenol derivative, in combination with Zinc oxide as pulp capping agents, temporary cements and root canal filling cement25. Eugenol exhibited its own antibacterial while freegenol did not have antibacterial property of its own3. Eugenol is less retentive than non-Eugenol. Eugenol shows poor retention after temporary cementation not only due to the presence of cement remnants, but also eugenol reacts with free radicals thereby inhibiting the polymerization of methacrylate monomers decreasing the bond strength. Higher the polymerization, higher is the bond strength15.

As the polymerization is decreased, bond strength also decreases in Eugenol-containing cements. Thus, making Eugenol- containing cements less retentive than non-Eugenol containing cements.

The results obtained for the present study are IRM among control and IRM + AgZ among samples showed highest Zone of Inhibition. Non-Eugenol among controls and NE + CHS among samples exhibited least Zone of Inhibition. When both controls and Samples were compared, IRM + AgZ exhibited highest Zone of Inhibition than controls and other sample groups.

The results of bond strength are non- eugenol did not alter bond strength than IRM and Zinc Oxide Eugenol among the control group. Non- Eugenol + AgZ showed no much change in bond strength when compared to other sample groups. When both controls & samples were compared, Non-Eugenol + AgZ showed highest significance in bond strength when compared to controls & other sample groups.

Conclusion

Within the limitations of the study, it can be concluded that IRM shows more antibacterial property than ZOE and Non-Eugenol. Silver Zeolite shows more antibacterial property than Chitosan. IRM with silver zeolite shows more antibacterial property. Non eugenol is more retentive compared to Eugenol and IRM.

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