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# Comparative evaluation of Apical Microleakage of Bioceramic sealer versus Bioceramic sealer mixed with Chitosan nanoparticles – An In-Vitro Study.

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**ABSTRACT:**

The successful outcome of the treatment depends upon good apical seal which will prevent microleakage. The goal of this study was to assess the addition of chitosan nanoparticles to Bioceramic sealer's apical sealing capacity. A total of 20 single rooted single canal teeth were used. The root canals were prepared using rotary files up to #30/.06 and irrigated with 2.5% NaOCl and normal saline. Samples were divided into 2 groups. Group I was obturated with gutta-percha using Bioceramic sealer and Group II was obturated with gutta-percha using Bioceramic-Chitosan-nanoparticles sealer. All samples were immersed in methylene blue 2% solution. The specimens were sectioned, observed under stereomicroscope and the dye

leakage was measured. The data were analysed. The results of Unpaired t-test/Independent samples t-test showed that mean microleakage is significantly lower in group II as compared to group I. This study concludes that Chitosan nanoparticles incorporation improves the efficacy of Bioceramic sealer.

## INTRODUCTION:

The microbial invagination of the root canal system has a critical role in causation of Endodontic re-infection. In Endodontics, sealer is the interfacial material which adapts the obturating material with the canal wall [1] Currently, the usage of Bioceramic sealer in obturation has increased in Endodontics due to its enhanced sealing ability. Bioceramic is the material which is biocompatible and it's advantage is crystalline structure and properties similar to bone and tooth apatite, which forms Hydroxyapatite resulting in effective bonding between sealer and root dentin [2,3].

In addition, there are different Nanoparticles available like Chitosan-nanoparticles which is a natural biopolymer and biocompatible material derived by deacetylation of chitin, occurs in the exoskeleton of the crustaceans [4,5]. These are hydrophilic in nature making it Bio adhesive, adsorbing better on to the canal wall. Chitosan-nanoparticles are prospective substance in the field of endodontics; hence study is required to investigate its potential.

The current study will evaluate the effect of the addition of chitosan Nanoparticles on Bioceramic sealer to the apical sealing ability of Root canal Obturating material.

## MATERIALS AND METHODS:

A total 20 single rooted, single canal extracted teeth for orthodontic/periodontic reasons, with fully developed mature apices and non-carious teeth were included. The soft tissues, dental calculus and stains were removed from the teeth and stored in normal saline till further use. All specimens were decoronated at the level of cementoenamel-junction with a diamond disc under water coolant [Figure(1)and(2)].

Figure 1: Illustrating specimen decoronated at the level of cementoenamel junction with a diamond disc.

Figure 2: Showing prepared specimens

Working length was measured by placing size 15 K-file. Chemomechanical preparation was done using Crown down technique with ProTaper instruments till F2[Figure 3]. The root canal was lubricated using 17%EDTA gel, irrigated using 2 mL of 5.25% NaOCl solution and 2 mL normal saline. Prepared specimens were randomly divided into 2 groups (n=10) for single cone obturation. Samples from Group I were obturated using gutta-percha points with Bioceramic sealer, and Group II were obturated using gutta-percha points with Bioceramic-chitosan-nanoparticles sealer. Sealer was mixed according to manufacturer's instructions.

Figure 3: Showing ProTaper Instruments Sx,S1,S2,F1,F2(from Left to right)

Bioceramic-chitosan-nanoparticles sealer was obtained by incorporating chitosan nanoparticles[Figure 4] with the powder of Bioceramic sealer in the ratio of 15 mg:100 mg. This powder was mixed with liquid of Bioceramic sealer using Stainless steel spatula and applied into the canal using lentulo-spiral.

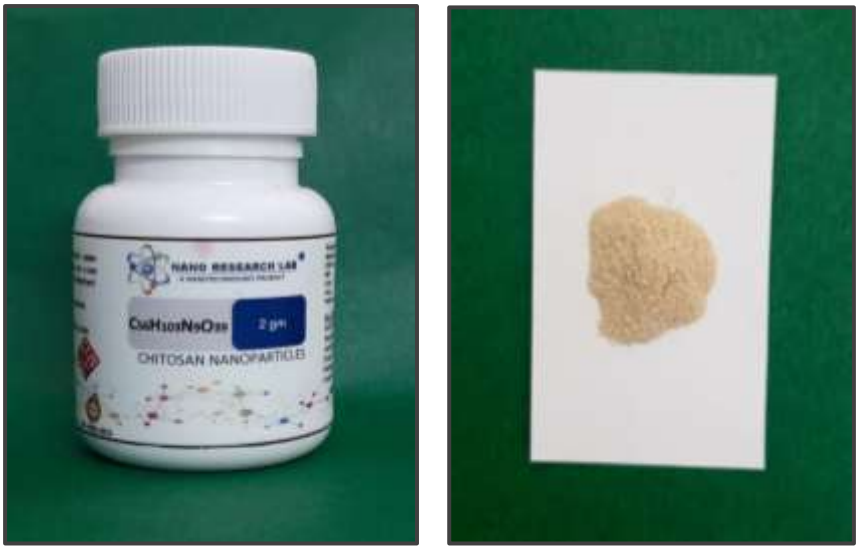


Figure 4: Chitosan Nanoparticles.

All the samples were sealed with Intermediate restoration and were coated with nail polish including the access restoration but leaving 2 mm at the apical area [Figure 5(a)].

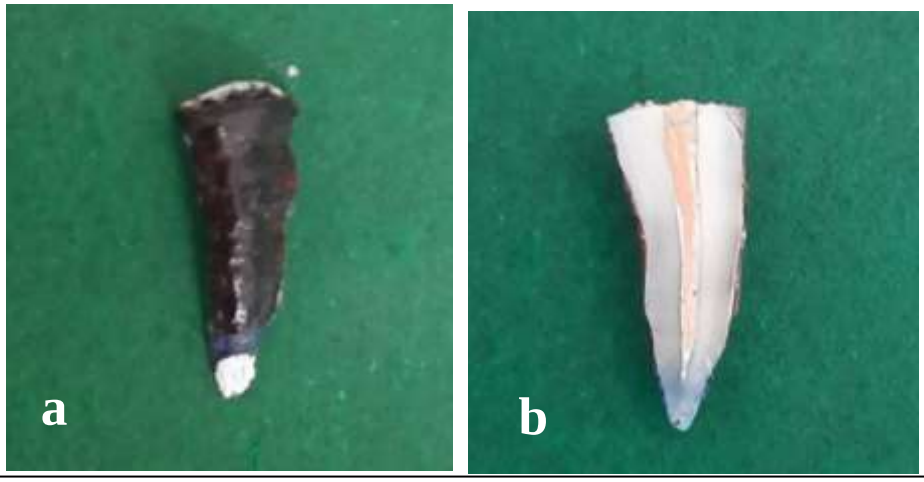


Figure 5: showing (a)the sample coated with nail polish leaving 2 mm at the apical area. (b)Specimen immersed in 2% Methylene Blue dye and longitudinally sectioned.

Samples were subjected to thermocycling, immersed in 2% Methylene Blue dye for 72 hours and then thoroughly rinsed with water. Specimens were cut in halves longitudinally using Diamond disc, observed under stereomicroscope for microleakage [Figure 5(a)(b)]. Dye penetration was measured in millimetres from apical point to the most coronal part of dye with the help of inbuilt scale in Stereomicroscope[Figure 6].

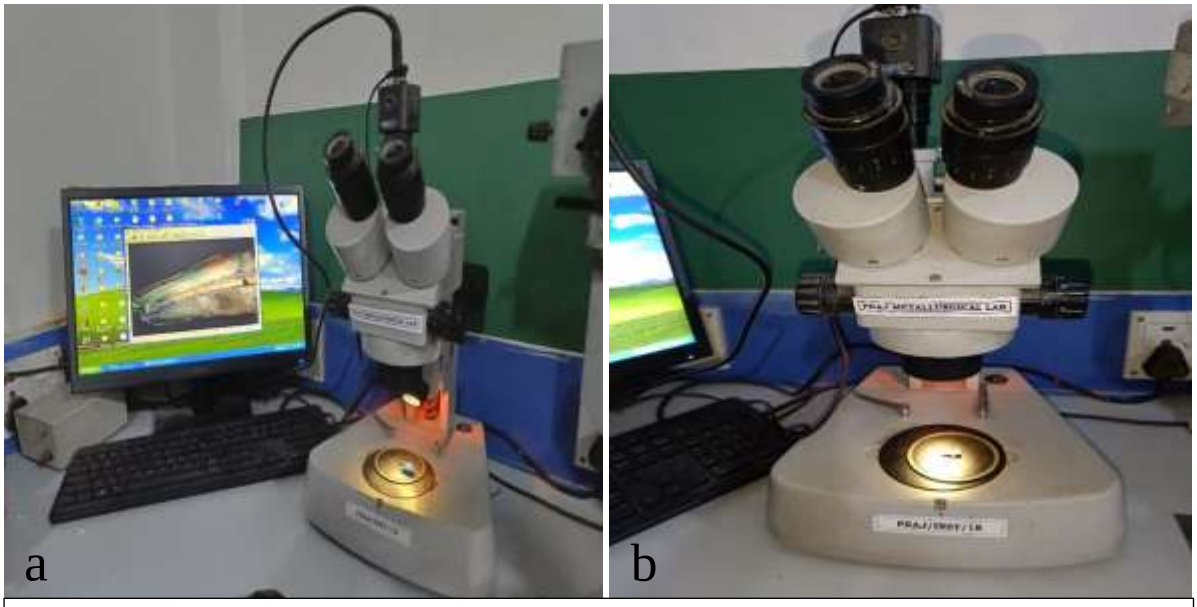


Figure 6:(a,b) showing Stereomicroscope.

**METHOD OF STATISTICAL ANALYSIS:**

Statistical analysis was performed using Statistical package for social sciences (SPSS) software (IBM Corp) (v.21.0). Descriptive statistics was performed. Independent samples t-test/Unpaired t-test was performed to assess significant differences between the 2 groups. A p-value of less than 0.05 was considered as statistically significant at 95% confidence intervals.

**RESULTS:**

Intergroup comparison of Microleakage(in mm) between 2 groups was performed using Independent samples t-test/Unpaired t-test.

Table 1:

| | **Group I** | | **Group II** |
|---|---|---|---|
| Sr no | Gutta Percha using Bioceramic sealer | Sr no | Gutta Percha using Bioceramic-Chitosan nanoparticles sealer |
| 1 | 0.65 | 1 | 1.2 |
| 2 | 1.64 | 2 | 1.93 |
| 3 | 0.6 | 3 | 0.00 |
| 4 | 1.16 | 4 | 2.17 |
| 5 | 2.51 | 5 | 0.77 |
| 6 | 1.72 | 6 | 1.11 |
| 7 | 1.87 | 7 | 1.32 |
| 8 | 1.61 | 8 | 1.54 |
| 9 | 1.75 | 9 | 1.34 |
| 10 | 1.89 | 10 | 1.43 |

Table 1: represents Descriptive statistics of individual values of Microleakage(mm) in different groups.

Table 2:

| Groups | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| **Group I : Gutta Percha using Bioceramic sealer** | 10 | .65 | 2.51 | 1.5480 | .75867 |
| **Group II : Gutta Percha using Bioceramic-Chitosan nanoparticles sealer** | 10 | .00 | 2.17 | 1.2830 | .47947 |

Table 2:Represents Descriptive statistics (Mean and Standard deviation) of Microleakage(mm) in different groups.

Table 3:

| Parameter | Comparison Groups | N | Mean | Mean difference | t value | df | p value |
|---|---|---|---|---|---|---|---|
| **Microleakage (in mm)** | **Gutta Percha using Bioceramic sealer** | 10 | 1.5480 | .26500 | .934 | 18 | 0.036* |
| | **Gutta Percha using Bioceramic-Chitosan nanoparticles sealer** | 10 | 1.2830 | | | | |

Table 3:Represents Intergroup comparison of Microleakage(mm) between 2 groups

Table 1 represents Descriptive statistics of individual values of Microleakage in different groups. Table 2 showed mean and standard deviation of microleakage in 2 groups. This comparison showed statistically significant differences between the 2 groups(p value <0.05). Table 3 showed Intergroup Comparison between 2 groups with Statistical significant difference. Thus, the mean microleakage is significantly lower in group II as compared to group I, inferring that group II shows better results[Figure 7].

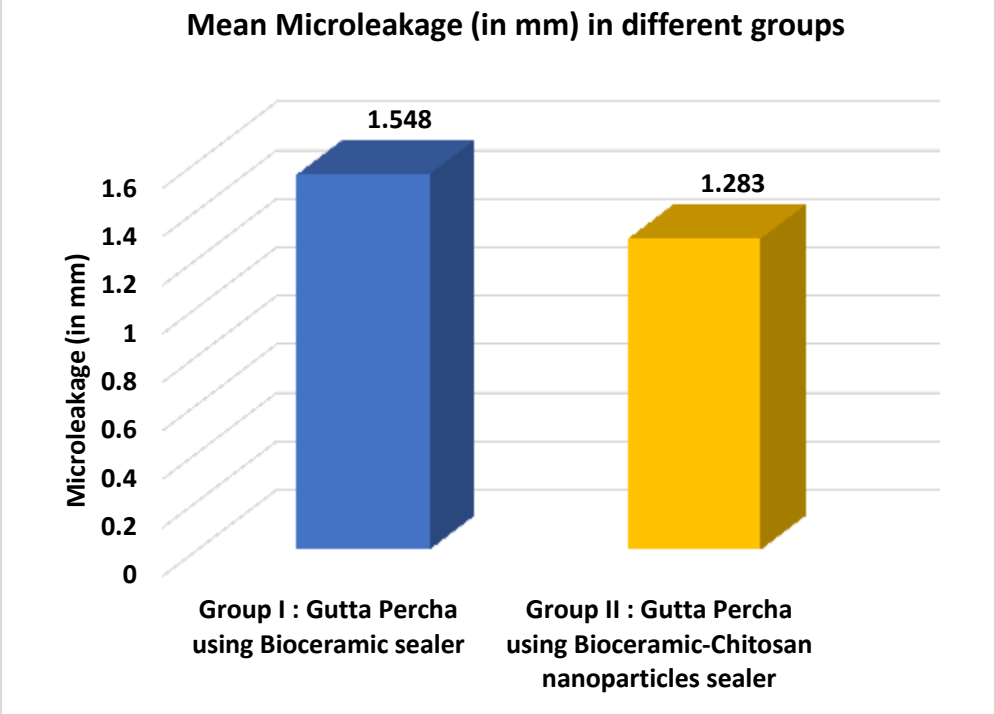


Figure 7: Showing Bar diagram comparing mean microleakage(mm) in 2 groups

[Figure 7]illustrates the bar diagram in which Group II showed less microleakage than Group I.

**DISCUSSION:**

The practitioners are looking for sealer which can result in a strong apical seal [7]. Grossman (1988), stated that sealers should not induce tissue degradation, maintain seal apically, must not foster bacterial development [8]. Sealers are used to fill voids, lateral and accessory canals [9,10,11]. If it is ineffective, microleakage might result in treatment failure [9,12,13].

The alleged mode of action for Bioceramic sealer is the particles diffuse tubularly into the dentinal tubules, forming mechanical interlocking linkages. After denaturing the collagen fibres, the mineral content of the sealer is absorbed into the intertubular dentin, resulting in a mineral infiltration zone and a potent alkaline sealer. Calcium silicates combines with moisture from the dentin to generate hydroxyapatite, which results in a partial reaction of phosphate with calcium silicate hydrogel and calcium hydroxide along the mineral infiltration zone [14]. Also, Donnermeyer D et al [15,16]stated that Hydroxyapatite crystals grow between the dentin and the sealer, making it difficult to separate these crystals from the dentin walls and dentinal tubules.

The term "nano material" refers to natural, incidental, or manufactured material that contains particles in an unbound state or as an aggregate or agglomerate, where 50% or more of the particles are in the size range of 1-100 nm in terms of number, size, distribution, or at least one external dimension [17,18]. According to Bernkop [19], Chitosan nanoparticles penetrate anatomical complexity by diffusing from the sealer. Chitosan is more easily absorbed into the canal wall because of its hydrophilic qualities, which allow it to be in close contact with root dentine.[1]. Darrag AM [20]claimed that chitosan has a great number of hydroxyl and amino groups, which cause chitosan to become cationic, facilitating ionic interactions with dentine

calcium ions. Chitosan's amino group can be protonated, allowing it to remove additional molecules for adsorption into the root dentine and infiltrate deeper into the dentinal tubules.

In this study, stereomicroscope with magnification upto x45[Figure 6] and 2% methylene blue dye was used as it has a smaller molecular weight than bacterial toxins and has leakage characteristics same as butyric acid, a microbial metabolic product with more penetration than Indian ink [21,22,23].Ahlberg KM et al. [24]stated that particle molecular size, pH, and chemical reactivity all have an impact on the extent of dye penetration. Also, method of longitudinal sectioning detects apical leakage more precisely where lot of aberrations are present [25,26].

Considering the Sealing ability of obturation material with the root dentin, the results of our study showed Bioceramic sealer incorporated with chitosan nanoparticles had lower microleakage. [table 1]shows Descriptive statistics of individual values of Microleakage(mm) in the groups. According to the results[Table 1], It was found out that the Minimum microleakage measured was 0.6mm whereas with group II, one of the sample showed no microleakage, suggesting Group II had better sealing ability. Based on statistical analysis, Table 3 showed Intergroup Comparison between 2 groups in which the mean microleakage of group I is 1.548mm and group II is 1.283mm with statistical significant difference of 0.036 ($p<0.05$). The Results of the Dye penetration revealed that there is apical microleakage observed in the obturation of both the groups. However, least microleakage was seen with the group in which Bioceramic sealer was incorporated with chitosan nanoparticles. This could be explained by greater diffusion of chitosan nanoparticles into the dentinal tubules facilitating the sealer to adapt and seal the root canal dentine. This result was consistent with other studies where chitosan increased the adaptability of Bioceramic sealer significantly [4].

In his work, Ratih et al tested the flowability of the sealer which was incorporated with chitosan nanoparticles, he stated that integration of chitosan nanoparticles with nanoparticle size was shown to alter the flowability of sealer [27,28]. Therefore, it can be considered that flowability is an important component and has impact in the outcome of this study, resulting in improved sealing ability.

**CONCLUSION:**

- This study concludes that Chitosan-nanoparticles incorporation improves the efficacy of Bioceramic sealer, showing better diffusion and adhesion of sealer into dentinal tubules, resulting in good sealing ability which lessens the microleakage. However, further studies are required to evaluate the results using different techniques of obturation.

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