Comparative Evaluation Of The Antibacterial And Physicochemical Properties Of Bioceramic Apexit Plus Sealer Mixed With Cationic Nanoparticles. - In Vitro Study.

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Abstract

Aim: *To evaluate and compare the antibacterial efficacy and physicochemical properties of Bioceramic sealer(BC Endosequence) and calcium hydroxide based sealer(Apexit Plus) mixed with chitosan nanoparticles* ***Objectives:*** *To evaluate and compare the antibacterial efficacy of Bioceramic sealer(BC Endosequence) and Calcium hydroxide sealer(Apexit Plus with and without addition of chitosan nanoparticles.(against E.faecalis) 3. To evaluate and compare the physicochemical properties ie.Dissolution, Setting time, Rheologic property of both the sealers.*

Materials and Methods: *Horizontal sectioning of the teeth was done at the cemento enamel junction. Root portion of the teeth was used for vertical sectioning of the teeth. Pilot holes of 3mm in diameter and 2mm in depth was prepared in dentin blocks. Dentin blocks was treated with EDTA and sodium hypochlorite to remove smear layer and organic debris. Sealers were mixed and CS-NP was added into the sealers. After complete setting of sealers, samples were placed in water bath for 24hrs. They were checked on 1st, 3rd,and 7th day. Test samples were weighed using precision balance machine before and after placement in water bath and the difference in weight was calculated and subjected to statistical analysis.*

Results: *Bioceramic and Apexit plus sealer completely inhibited the growth of E. faecalis.*

Conclusion: *This study showed that Bioceramic sealer (BC Endosequence) and calcium hydroxide based sealer(Apexit Plus) when mixed with chitosan nanoparticles completely inhibited the growth of E. faecalis.*

Keywords - *Bioceramic sealers, Apexit plus sealer, cationic nanoparticles, antibacterial property , physiochemical property.*

Introduction

The success of root canal treatment is directly related to the elimination of micro-organisms through mechanical cleaning and shaping, supplemented by antibacterial irri-gants, adequate filling of the empty space, and use of antimicrobial dressings (with calcium hydroxide) between appointments, if neces-sary.[1,2] However, these procedures do not result in complete sterility of the root canal space.[3] The major cause of endodontic failure is the survival of microorganisms in the apical portion of root filled teeth. E.faecalis can adhere to the root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms.[4]

Endodontic sealers are used in the obturation of root canal systems to achieve a fluid-tight or hermetic seal throughout the canal including the apical foramen and canal irregularities and minor discrepancies between the dentinal wall of the root canal and the core filling material.[5] Therefore, sealers help prevent leakage, reduce the possibility of residual bacteria from the canal to invade the periapical tissues, and resolve the periapical lesion. The properties of root canal sealers have an impact on the quality of the final root filling. Various kinds of endodontic sealers according to these standards have been extensively studied, including the working time, setting time, flow, film thickness, solubility, dimensional change, and radiopacity. [6]

Most important requirements of sealers are biocompatibility, excellent seal, adequate adhesion, and antimicrobial property. A constant search to increase the antibacterial properties of sealers is on.Various antibacterial agents have been added to sealers to enhance this property.

Chitosan (CS) is a nontoxic biopolymer derived by the deacetylation of chitin. Chitin is a natural polymer occurring in the exoskeleton of the crustaceans. It is a bioadhesive that readily binds to negatively charged surfaces and has excellent antimicrobial and antifungal activities.

Antibacterial nanoparticulates are found to have higher antibacterial activity than antibacterial powders. This is because of the higher surface area and charge density of nanoparticulates, which

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enable them to achieve a greater degree of interaction with the negatively charged surface of bacterial cells.

Till today we don't have a sealer which fulfills all the ideal requirements. So this study is being done to investigate the antibacterial effect of addition of chitosan nanoparticles to newer bioceramic sealer and apexit and its effect on the solubility ,flow and setting time of these sealers.

Materials And Methods

Preparation of chitosan nanoparticle

Chitosan was dissolved in 1v/v% acetic acid solution at concentration of 0.5w/v% and the Ph was raised to 4.6 to 5 with 10N NaOH.

Chitosan nanoparticle (CS-NP) were formed upon adding 5ml 0.25% sodium tripolyphosphate in water to 15ml chitosan soln under stirring at a speed of 1000rpm.

The nanoparticle were separated by centrifugation at 20,000 rpm for 30min

The supernatant was discarded and chitosan nanoparticle was rinsed with water to remove NaOH and then froze dried before further use.

Preparation of sealers with chitosan nanoparticle-.

Two sealers ie.Apexit Plus and Bioceramic sealers were used.In group I and II the sealers were mixed according to manufactureres recommendations.In group III and IV chitosan np was added in the ratio of 15:100 of the sealer.

Cultivation and Innoculation of Bacteria-

The direct contact antibacterial assays was adopted in this study.E.Faecalis was incubated overnight at 37degrees under agitation in BHI medium.

An aliquol 2ml of the culture was added to the broth and incubated for 6 to 8 hrs until the exponential growth is reached.The bacterial conc in the medium was then adjusted to an optical density of 0.1(at 600nm)

For Testing Antibacterial Property-

Sample size- 32 8 wells/group

Two sealers,apexit plus and bioceramic sealer were used. The sealers were prepared according to manufactureres instructions, weighed and mixed with CS-NP approx 15ul of sealer.

For placing bacteria in direct contact with test material, direct contact test was used. Each group were placed in eight wells in a microplate.

Plate preparation involves applying a thin layer of equal amounts of fresh sealer on the walls of well. Each sealer was mixed with or without CS-NP making four groups.

Then the sample were innoculated with 10ul E.faecalis bacterial suspension and was allowed to dry in direct contact with sealers, and innoculation of the plates was done at 37degree.Then the growth of micro organisms was observed on 1st,3rd and 7th day.

After checking the growth ,reading were taken and checked on microplate reader and subjected to statistical analysis.

To Check The Solubility Of Sealers

Preparation of sample for testing dissolution property-

Sample size- 32 8 teeth/4 groups

Extracted teeth were autoclaved. Horizontal sectioning of the teeth was done at the cemento enamel junction.

Root portion of the teeth was used for vertical sectioning of the teeth. Sections of 4-5mm in thickness was prepared.

Pilot holes of 3mm in diameter and 2mm in depth was prepared in dentin blocks.Dentin blocks was treated with EDTA and sodium hypochlorite to remove smear layer and organic debris. Sealers were mixed and CS-NP was added into the sealers.

After complete setting of sealers, samples were placed in water bath for 24hrs. They were checked on 1st, 3rd,and 7th day.

Test samples were weighed using precision balance machine before and after placement in water bath and the difference in weight was calculated and subjected to statistical analysis.

For Testing Rheologic Property-

Sample size - 32 3 times

All cements were manually prepared by mixing the powder with liquid component. Cements were delivered via a syringe onto a glass plate.

Three minutes after the commencement of mixing a second glass plate was placed on top of the sealer, followed by a weight to make a total mass of 120gm on the sealer.

Ten minutes after the commencement of mixing, the weight was removed and the diameter of the sealer disc was measured. The experiment was repeated three times

For Testing Setting Properties-

Sample size- 32 8 discs/group

Eight plasters of cast rings with an internal diameter of 10mm and a thickness of 2mm were prepared for each group. The external borders of the mold was fixed with wax on glass plate.

The molds were filled with the material and transferred to a chamber with 95%relative humidity and temp of 37deg.After 150 sec from onset of mixing a Gilmore type needle with a mass of 100gm and a flat end of 2.0mm in diameter was carefully lowered vertically onto the horizontal surface of testing sample.

The needle tip was cleaned and the probing was repeated until indentations ceased to be visible.The time used from start of mixing to this point was recorded.If the results differ by more than +5%,the test was repeated.

Results

Table No.2: Distribution of mean and SD values of Antibacterial Efficacy before and after 1st day, 3rd day and 7th day in Control and Experimental groups: (Student's Paired 't' test)

	Group A (Bioceramic sealer)(Control) (n=8)	Group B (Bioceramic + Chitosan nanoparticle) (n=8)	Group C (Apexit Plus sealer) (Control) (n=8)	Group D (Apexit Plus + Chitosan nanoparticle) (n=8)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1st day	-0.0072±0.015	0.0018±0.186	-0.0218±0.0305	-0.0158±0.0224
3rd day	-0.0105±0.015	0.0141±0.013	-0.0476±0.0347	-0.0006±0.0036
7th day	-0.004±0.0067	0.0051±0.0098	-0.008±0.0057	-0.0029±0.0047
Student's Paired	0.24	3.02	0.22	3.64
't' test value				
'p' value and	p>0.05,	p<0.05,	p>0.05,	p<0.05,
significance	Not significant	significant	Not significant	significant

By applying Student's Paired 't' test there is no significant decrease in mean values of antibacterial efficacy from 1st day to 7th day in Groups A and C (Control groups) (i.e. $p>0.05$) and significant decrease seen in Groups B and D (Experimental groups) (i.e. $p<0.05$)

Table No.10: Distribution of percentage change values of Solubility Testing before and after 1st day, 3rd day and 7th day in Control and Experimental groups:

Percentage change (%)(Decrease)	Group A (Bioceramic sealer) (Control)(n=8)	GruopB(Bioceramic + Chitosan nanoparticle) (n=8)	Group C(Apexit Plus sealer)(Control)(n=8)	Group D(Apexit Plus + Chitosan nanoparticle)(n=8)
From before to 7th day	2.45%	3.91%	18.91%	21.63%
From 1st day to 7th day	2.00%	2.92%	12.07%	14.82%
From 3rd day to 7th day	0.95%	1.39%	6.89%	6.45%

Discussion

The purpose of inserting a root canal sealer into the canal is to provide an apical seal that is able to inhibit the leakage of irritant substances or pathogen microorganisms from the root canal system into the periradicular tissues[7]. Poggio C et al stated that sealability helps to hamper the creation of a locus minoris resistentiae prone to the colonization by microorganisms of oral microflora or by new emerging pathogens thus preventing inflammation and infection.

Torabinejad M stated that sealers accomplish the objective of providing a fluid tight seal: they must always be used in conjunction with the filling material, regardless of the technique or material used . Root canal sealers, even if used only as adjunctive materials in the obturation of root canal system, have been shown to influence the result of root canal treatment .

In 1988 Grossman outlined the criteria for an ideal sealer, even though none of the endodontic sealers currently available possess all these properties: the sealers and their components should cause neither tissue destruction nor cell death, so they could not be toxic; they should remain dimensionally stable on setting; they should provide adequate working time for manipulation and placement into the root canal; they should have adhesion, performing bonding between dentin and obturation material; sealers also should be visible on radiographs; they should not cause staining of the crown of the teeth;they should not encourage bacterial growth; they must create and maintain a seal apically, laterally and coronally; they should be soluble in a solvent (when retreatment is necessary) but they should contemporaneously not disintegrate when in contact with tissue fluids.[8]

Several types of root canal sealers are used in endodontic practice with each one having own merits and demerits. Sealers are basically selected based on their sealing ability, adhesive properties, biocompatibility & antimicrobial efficacy.

The newer sealers include MTAbased sealers, EndoSequence bioceramic sealer, Methacrylatebased resin sealer.

The sealers used in this study are Apexit plus sealer and BC sealer.

Apexit Plus is a two-component material, which sets by complex formation. For this complex formation the three components calcium hydroxide, salicylate and water are needed and the following reaction is postulated: Traces of water cause small quantities of Ca(OH)2 to dissolve releasing hydroxide ions that subsequently react with acidic phenol groups of the salicylate.

Apexit Plus differs from Apexit in that it is supplied in a more convenient delivery form and has a more hydrophylic formulation. Consequently, the material is more reliable if used in thicker layers,Studies on apexit plus have showen excellent tissue compatibility and good permanent seal.

EndoSequence BC Sealer has been designed as a non-toxic hydraulic calcium silicate cement that is easy to use as an endodontic sealer. Among the attributes of BC Sealer are improved convenience and delivery, and the advantage of utilizing the water inherent in the dentinal tubules to drive the hydration reaction (of the material) thereby shortening the setting time.

Chitosan Nanoparticles

Chitin is a polysaccharide of animal origin found abundantly in nature and characterized by a fibrous structure. It forms the basis of the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs and lobster. The chemical structure of chitin is similar to cellulose, having one hydroxyl group on each monomer substituted with an acetylamine group .[9] The unique character of nanoparticles for their small size and quantum size effect could make chitosan nanoparticles exhibit superior activities. Chitosan nanoparticles exhibit higher antibacterial activity than chitosan on account of the special character of the nanoparticles. The negatively charged surface of the bacterial cell is the target site of the polycation.Therefore, the polycationic chitosan nanoparticles with higher surface charge density interact with the bacteria to a greater degree than chitosan itself. Chitosan nanoparticles provide higher affinity with bacteria cells for a quantum-size effect. Because of the larger surface area of the chitosan nanoparticles, nanoparticles could be tightly adsorbed onto the surface of the bacteria cells so as to disrupt the membrane, which would lead to the leakage of intracellular components, thus killing the bacteria cells.[10]

For group A, there was significant increase in the antibacterial efficacy from 1st day to 3rd day.However from 3rd day to 7th day there was gradual decrease in the efficacy. The present study showed that it possessed potent antibacterial effect. The sealer is a complex form of calcium silicate cement, calcium phosphate, and calcium oxide. Moisture from dentin is supposed to facilitate the hydration reactions of calcium silicates to produce calcium silicate hydrogel and calcium hydroxide. Calcium hydroxide partially reacts with the phosphate to form hydroxyapatite and water . [11]

For group B, there was significant increase in the antibacterial efficacy from 1st day to 3rd day.However from 3rd day to 7th day there was gradual decrease in the efficacy.This difference in antibacterial activity may be attributed to the decrease in the release of CS-NP from the sealer and the difference in bactericidal mechanisms between them.The antibacterial action of chitosan has been attributed to its polycationic nature, which interacted with the negatively charged surface of bacteria, altering cell permeability and resulting in the leakage of intracellular components and cell death.

For group C, there was significant increase in the antibacterial efficacy from 1st day to 3rd day.However from 3rd day to 7th day there was gradual decrease in the efficacy.

Apexit Plus is a calcium hydroxide– based material. Root canal sealers with integrated calcium hydroxide have enhanced antibacterial activity . The antimicrobial effect of this sealer stems from the release of hydroxide ions, which raise the pH to above 12.5. As the calcium hydroxide sealer sets, the pH declines to about 9.14, resulting in loss of the sealer's effectiveness . Apexit Plus exhibited a higher antibacterial activity and was effective against the tested microorganism.[12]

For group D, the antibacterial efficacy was found to be the highest on 1st day. However from 1rd day to 7th day there was gradual decrease in the efficacy of the sealer.

$Ca(OH)_2$ has limited ability to eradicate bacterial cells inside dentinal tubules. $Ca(OH)_2$ has low solubility, diffusibility, and dentin-buffering ability, which reduced its antibacterial effect. When $Ca(OH)_2$ is used alone without any combination with other antimicrobial agents, there is limited antimicrobial activity against *E. faecalis*. This could explain that there occurs limited antimicrobial effect of $Ca(OH)_2$ alone when compared with $Ca(OH)_2$ combined with chitosan against C. albicans and E. faecalis.

The distribution for % change values for 1st , 3rd and 7th day for Group A were 0.95%, 2% and 2.45% respectively.The tested material showed solubility within the limit allowed in the ISO 6876/2001 recommendations (3% mass fraction).Because the inorganic and radiopacifier components of the sealer are premixed with water-free liquid-thickening carriers, water is required for the sealer to reach its final set.

The distribution for % change values for 1st , 3rd and 7th day for Group Bwere 6.89%, 12.07% and 18.91% respectively. The tested material showed solubility which crossed the limit allowed in the ISO 6876/2001 recommendations (3% mass fraction). The differences between Endosequence BC sealer and Apexit plus probably reflect the fact that the former is a pure bioceramic sealer, whereas the latter is a Ca(OH)2 baesd sealer.[13] The solubility values for apexit were greater than those for bioceramic sealer.This suggests that there may be substantial breakdown.While this may be a problem in terms of sealing, there is likely to be a release of calcium and hydroxyl ions.This effect is similar to be noted by Tagger et al (1988). The weight loss after final dessication of the material shows the amount of material lost during test period.

The distribution for % change values for 1st , 3rd and 7th day for Group C were 1.39%, 2.92% and 3.91% respectively. The tested

material showed solubility within the limit allowed in the ISO 6876/2001 recommendations (3% mass fraction).However addition of CSNP increased the solubility values compared to when bioceramic sealer alone was used.

The distribution for % change values for 1st , 3rd and 7th day were 6.45%, 14.8% and 21.6% respectively. The tested material showed solubility which was not within the limit allowed in the ISO 6876/ 2001 recommendations (3% mass fraction).However addition of CSNP increased the solubility values compared to when apexit sealer alone was used.

The setting time is primarily a control test on the stable behavior of a product and is dependent on the sealer components, particle size, room temperature, and relative humidity.

The ANSI/ADA specification requires that the setting time of a sealer shall be within 10% of that stated by the manufacturers. In this study, BC sealer and Apexit Plus® were in agreement with ANSI/ADA standards. However addition of Csnp increased the setting time of sealers but within the limits of ADA specification standards.

Flow is an important physical property that allows the cement to fill spaces of difficult access such as isthmus and accessory canals.During the flow test no repetitions were required besides those standardized at the beginning of the experiment. According to the flow test, Endosequence BC Sealer cement (27.33mm) and Apexit sealer(23.5) demonstrated flow greater than 20 mm which is in agreement with ISO 6786/2001 recommendations Greater value for Bioceramic sealer may be attributed to its physical properties.

Its major inorganic components include tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, and calcium hydroxide. It uses zirconium oxide as the radiopacifier and contains water-free thickening vehicles to enable the sealer to be delivered in the form of a premixed paste . Flow of the sealers gradually decreased after addition of Csnp.

Conclusion

Within the limitations of the study, following conclusions can be drawn that this study showed that Bioceramic and Apexit plus sealer when freshly mixed with chitosan nanoparticles completely inhibited the growth of E. faecalis. Also, when allowed to set 1 day, 3 days and 7 days, the sealers continued to inhibit the growth of E. faecalis with efficacy gradually decreasing on 7th day. Sealers with Csnp were statistically better at eliminating E. faecalis than sealers without Csnp when fresh and set ($p < 0.01$). Addition of Csnp to both sealers improved the antibacterial property but at the same time it did not alter the physicochemical properties. However the flow property was reduced which was not within the ADA specification standards.

Further research is essential to offer new guidelines for the treatment protocol of endodontic infections. It should be kept in mind that the major factor contributing to the healing or maintenance of an infection involves the host immunological response.

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