Antibacterial and Mechanical Properties of Pit and Fissure Sealants Containing Zinc Oxide and Calcium Fluoride Nanoparticles

Dara Lakshmi Swetha, C. Vinay, K. S. Uloopi, Kakarla Sri RojaRamya, Rayala Chandrasekhar

Department of Pedodontics and Preventive Dentistry, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India

Abstract

Context: Pit and fissure sealants (PFSs) are the most effective preventive materials in dentistry. Secondary caries around the sealed pits and fissures at the material–tooth interface and the wear of the material remains the common problems. To address these problems, efforts have been made by incorporating zinc oxide (ZnO) and calcium fluoride (CaF_2) nanoparticles (NPs) into the sealants to develop caries-inhibiting and stress-bearing sealants. **Aim:** Evaluation of antibacterial and mechanical properties of PFS containing ZnO and CaF_2 NPs. **Settings and Design:** This was an *in vitro* study. **Materials and Methods:** A total of 196 fissure sealant samples were divided into six test groups and a control group. The test group samples were prepared by incorporating two concentrations (0.5 wt% and 1 wt%) of ZnO and CaF_2 NPs into the sealants. The antibacterial activity was evaluated by direct contact test; compressive and flexural strengths were evaluated by a universal testing machine. **Statistical Analysis Used:** Statistical analysis was done by one-way ANOVA and *post hoc* Tukey test. **Results:** Sealants containing 1 wt% ZnO and CaF_2 NPs and their mixture exhibited significantly higher antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* when compared to control group ($P < 0.001$). Samples with ZnO NPs exhibited similar mechanical properties as conventional sealant (control group); however, the samples with CaF_2 NPs showed inferior mechanical properties ($P < 0.05$). **Conclusion:** The observations of the study infer that sealants containing 1 wt% ZnO and CaF_2 NPs and their mixture exhibited superior antibacterial activity. The mechanical properties of samples containing ZnO and mixture of ZnO and CaF_2 particles remained comparable to the conventional sealants.

Keywords: *Calcium fluoride, nanoparticles, pit and fissure sealants, zinc oxide*

Introduction

Occlusal pits and fissures are the most susceptible sites for dental caries because of their morphological complexity. Occlusal surfaces develop more than two-thirds of total caries in children in spite of constituting only 12.5% of all the teeth surfaces.[1] The susceptibility of occlusal surfaces of the permanent molars to dental caries has increased the applicability of pit and fissure sealants (PFS), since the application of topical fluoride is not effective on these surfaces.

Application of PFS is a cornerstone in preventive dentistry practice for the prevention of caries. Retention of sealants on the tooth surface is a critical factor for sustained protection. Consideration of mechanical properties of fissure sealants is a crucial requisite for their longevity. Secondary caries may form around the sealed pits and fissures at material–tooth interfaces, either due to partial loss of material or microleakage induced by polymerization shrinkage.[2]

Biofilm accumulation on resin restorations results in high prevalence of secondary caries.[3] Resin materials do not have inherent antibacterial property and it is learned that microorganisms can metabolise set resin matrix.[4] Antibacterial activity of the resin-based materials with fluoride and chlorhexidine was investigated and found that they initially possess strong antibacterial activity, but their release rates do not last long.[5,6]

Applications of nanotechnology led to the development of novel strategies in preventive dentistry, especially in the management of bacterial biofilms and improving the mechanical properties of various materials. Nanoparticles (NPs) provide wider range of interactions with

Address for correspondence:
Dr. Kakarla Sri RojaRamya,
Department of Pedodontics & Preventive Dentistry, Vishnu Dental College, Bhimavaram, West Godavari District, Andhra Pradesh, India.
E-mail: roja.ramya86@gmail.com

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the microorganisms due to their nanoscale dimensions, thereby increasing the antibacterial activity.[7] NPs provide higher surface-to-volume ratio and are highly reactive, which makes them unique in newer therapeutic strategies. NPs of zinc oxide (ZnO) are shown to have good antibacterial activity against *Streptococcus mutans* and *Lactobacillus*.[8] Similarly, calcium fluoride (CaF_2) NPs have better fluoride-releasing property that can impair bacterial acid production.[9]

Secondary caries, lack of abrasion resistance and fracture under compressive or flexural stresses are the most common clinical problems with sealants. It is ideal to have a sealant with better mechanical properties and antibacterial activity to create a favorable environment, which in turn increases the longevity of the restoration. Hence, a study was designed wherein ZnO and CaF_2 NPs are added to the conventional fissure sealant to evaluate the antibacterial activity and also to check whether addition of these NPs in minute amounts would influence the mechanical properties.

Materials and Methods

Preparation of samples

A total of 196 PFS samples were prepared and allocated to six test groups and a control group ($n = 28$). The samples of four test groups were prepared by incorporating two concentrations (0.5 wt% and 1 wt%) of ZnO and CaF_2 NPs and two groups with equal mixture. The NPs powder was added to the sealant and homogenously mixed in a dark room for 15 min with a glass spatula and stored in dark colored bottles until tests were performed.

Group allocation

The groups were designated as Group I - PFS with ZnO NPs (0.5% wt); Group II - PFS with CaF_2 NPs (0.5 wt%); Group III - PFS with ZnO and CaF_2 NPs (0.5 wt%); Group IV - PFS with ZnO NPs (1 wt%); Group V - PFS with CaF_2 NPs (1 wt%); Group VI - PFS with ZnO and CaF_2 NPs (1 wt%); and Group VII - plain fissure sealant samples (control). The samples in each group ($n = 28$) were equally ($n = 7$) allocated to test parameters, such as antibacterial activity against *S. mutans* and *Lactobacillus acidophilus*, compressive strength (CS) and flexural strength (FS).

Antibacterial activity

The antibacterial activity against *S. mutans* and *L. acidophilus* was determined by direct contact test. These bacteria were isolated from the saliva of an individual with active caries lesions and cultured on selective media Mitis Salivarius and Rogosa Agar, respectively. The obtained colonies were later subcultured to isolate bacterial strains which were confirmed by Gram staining. The bacteria were cultured aerobically overnight in 5 ml of brain–heart infusion (BHI) broth at 37°C.

Direct contact test was carried out by coating equal amount of sealant material on to the walls of eppendorf tubes. The materials were polymerized for 280 s with an overlapping regimen by light-emitting diode light-curing unit in seven 40 s cycles from top to bottom of the tubes from outside. Ten microliters of bacterial suspension was placed on the surface of each sample. The tubes were then incubated in vertical position for 1 h under a sterile condition. During the incubation period, the suspension liquid was evaporated to obtain a thin layer of bacteria, ensuring direct contact between the bacteria and the sample. Then, 300 µl of BHI broth was added to each tube. After 24 h, aliquots of 2 µl of the mixture was spread on Mitis Salivarius and Rogosa Agar plates and incubated at 37°C for 24 h and 48 h, respectively. The bacterial colonies were expressed as colony-forming units (CFU).

Compressive strength

Sealant samples measuring 4 mm in diameter and 6 mm in height were prepared using cylindrical plastic molds and stored in distilled water. The CS was then determined with the universal testing machine at a cross-head speed of 1 mm/min. All the specimens were placed with their flat ends between the plates of testing machine to apply the compressive load progressively along the long axis of the specimens. The maximum load applied to fracture the specimens was recorded and CS was calculated in MPa.

Flexural strength

Samples measuring 2 mm × 2 mm × 25 mm dimensions were prepared using rectangular plastic molds and stored in distilled water. Three-point bending test was performed using the universal testing machine at a cross-head speed of 0.5 mm/min. The FS was calculated in MPa.

Statistical analysis used

The data obtained showed normal distribution; hence, parametric statistical tests were used to analyze the data. Intergroup comparisons were done using one-way ANOVA test followed by *post hoc* Tukey test. Statistical significance was computed at $P \leq 0.05$ as significant and $P \leq 0.001$ as highly significant.

Results

Antibacterial activity

NPs incorporated groups exhibited lower mean CFU of *S. mutans* and *L. acidophilus* compared to control group. Intergroup comparison using one-way ANOVA test confirmed that there is a difference in mean CFU of both the bacteria among the groups [Table 1].

Pairwise comparison using *post hoc* test confirmed that there is a statistically highly significant ($P < 0.001$) lower mean CFU of *S. mutans and L. acidophilus* in groups containing ZnO and CaF_2 compared to control group.

Sealants containing mixture of both ZnO and CaF_2 showed statistically significant ($P < 0.05$) antibacterial activity against *L. acidophilus* compared to other groups [Table 2].

Mechanical properties

Comparison of mechanical properties between the groups revealed that there is a highly significant difference in mean values of both CS and FS ($P < 0.001$) [Table 3].

On pairwise comparison, sealants containing both ZnO and CaF_2 NPs (Group III, VI) showed superior CS, whereas CaF_2 0.5 wt% (Group II) showed significantly lower CS compared to other groups. However, all the NPs containing test group samples except Group II (CaF_2 0.5 wt%) exhibited CS comparable to control group [Table 4].

Samples with a mixture of ZnO and CaF_2 NPs (0.5 wt%, Group III) exhibited higher mean FS compared to other groups ($P < 0.01$). Although other test groups showed similar FS compared to control group, Group V with 1 wt% CaF_2 NPs showed significantly lesser FS ($P < 0.05$) [Table 4].

Table 1: Intergroup comparison of mean colony-forming unit of *Streptococcus mutans* and *Lactobacillus acidophilus*

Groups	Sample size	Mean±SD	
		Streptococcus mutans	***Lactobacillus acidophilus***
Group I - PFS with ZnO NPs (0.5% wt)	*n*=7	8.71±5.894	7.64±1.909
Group II - PFS with CaF_2 NPs (0.5 wt%)		12.21±2.612	8.50±4.223
Group III - PFS with ZnO and CaF_2 NPs (0.5 wt%)		1.50±1.190	2.43±0.673
Group IV - PFS with ZnO NPs (1 wt%)		0.93±0.976	3.21±1.113
Group V - PFS with CaF_2 NPs (1 wt%)		5.07±2.244	2.93±0.886
Group VI - PFS with ZnO and CaF_2 NPs (1 wt%)		0.57±0.450	0.64±0.690
Group VII - Plain PFSs (control)		129.29±26.552	53.07±7.829
One-way ANOVA test (*P*)		0.000 (HS)	

HS: High significance; SD: Standard deviation; PFSs: Pit and fissure sealants; ZnO: Zinc oxide; NPs: Nanoparticles; CaF_2: Calcium fluoride

Table 2: Pairwise comparisons of mean colony-forming unit of *Streptococcus mutans* and *Lactobacillus acidophilus*

Group comparisons	*P*	
	Streptococcus mutans	***Lactobacillus acidophilus***
Group I (PFS with ZnO NPs [0.5% wt]) versus		
Group II	0.995	0.999
Group III	0.848	0.102
Group IV	0.797	0.238
Group V	0.994	0.178
Group VI	0.762	0.009 (S)
Group VII (control [PFS])	0.000 (HS)	0.000 (HS)
Group II (PFS with CaF_2 NPs [0.5 wt%]) versus		
Group III	0.472	0.034 (S)
Group IV	0.409	0.094
Group V	0.854	0.066
Group VI	0.372	0.002 (S)
Group VII	0.000 (HS)	0.000 (HS)
Group III (PFS with ZnO and CaF_2 NPs [0.5wt%]) versus		
Group IV	1.000	1.000
Group V	0.995	1.000
Group VI	1.000	0.961
Group VII	0.000 (HS)	0.000 (HS)
Group IV (PFS with ZnO NPs [1 wt%]) versus		
Group V	0.989	1.000
Group VI	1.000	0.812
Group VII	0.000 (HS)	0.000 (HS)
Group V (PFS with CaF_2 NPs [1 wt%]) versus		
Group VI	0.983	0.882
Group VII	0.000 (HS)	0.000 (HS)
Group VI (PFS with ZnO and CaF_2 NPs [1 wt%]) versus		
Group VII	0.000 (HS)	0.000 (HS)

Post hoc Tukey's test. S: Significance; HS: High significance; PFS: Pit and fissure sealant; ZnO: Zinc oxide; NPs: Nanoparticles; CaF_2: Calcium fluoride

Table 3: Intergroup comparison of mean compressive strength and mean flexural strength

Groups	Sample size	Mean±SD	
		Compressive strength	Flexural strength
Group I - PFS with ZnO NPs (0.5% wt)	*n*=7	228.90±49.526	64.26±6.440
Group II - PFS with CaF$_2$ NPs (0.5 wt%)		131.09±47.992	60.85±20.232
Group III - PFS with ZnO and CaF$_2$ NPs (0.5 wt%)		296.58±44.589	91.63±7.467
Group IV - PFS with ZnO NPs (1 wt%)		313.84±12.465	56.91±14.955
Group V - PFS with CaF$_2$ NPs (1 wt%)		247.33±10.104	48.20±11.572
Group VI - PFS with ZnO and CaF$_2$ NPs (1 wt%)		300.27±42.010	60.35±5.009
Group VII - PFSs (control)		242.76±92.868	71.54±18.323
One-way ANOVA test (*P*)		0.000 (HS)	

HS: High significance; PFSs: Pit and fissure sealants; ZnO: Zinc oxide; NPs: Nanoparticles; CaF$_2$: Calcium fluoride; SD: Standard deviation

Table 4: Pairwise comparisons of mean compressive and flexural strength

Group comparisons	*P*	
	Compressive strength	Flexural strength
Group I (PFS with ZnO NPs [0.5% wt]) versus		
Group II	0.011 (S)	0.999
Group III	0.171	0.006 (S)
Group IV	0.040 (S)	0.942
Group V	0.992	0.281
Group VI	0.129	0.998
Group VII (control [PFS])	0.998	0.944
Group II (PFS with CaF$_2$ NPs [0.5 wt%]) versus		
Group III	0.000 (HS)	0.002 (S)
Group IV	0.000 (HS)	0.998
Group V	0.001 (HS)	0.563
Group VI	0.000 (HS)	1.000
Group VII	0.002 (S)	0.736
Group III (PFS with ZnO and CaF$_2$ NPs [0.5 wt%]) versus		
Group IV	0.995	0.000 (HS)
Group V	0.524	0.000 (HS)
Group VI	1.000	0.001 (HS)
Group VII	0.418	0.091
Group IV (PFS with ZnO NPs [1 wt%]) versus		
Group V	0.187	0.877
Group VI	0.999	0.999
Group VII	0.132	0.389
Group V (PFS with CaF$_2$ NPs [1 wt%]) versus		
Group VI	0.438	0.608
Group VII	1.000	0.030 (S)
Group VI (PFS with ZnO and CaF$_2$ NPs [1 wt%]) versus		
Group VII	0.339	0.694

Post hoc Tukey's test. S: Significance; HS: High significance; PFS: Pit and fissure sealant; ZnO: Zinc oxide; NPs: Nanoparticles; CaF$_2$: Calcium fluoride

Discussion

Polymerization shrinkage is more with fissure sealants as it consists predominantly resin matrix which may lead to weakening of the bond, resulting in microleakage that increases with time, leading to secondary caries formation.[2] Therefore, the fate of bacteria is of significance. Additional protection could be afforded against any subsequent deterioration of the bond at the resin–tooth interface if the sealant possesses some antibacterial property.[10] Incorporation of metal oxide particles such as silver oxide and ZnO into the restorative materials has been tried to impart the antibacterial property to the resin.[11,12] Zinc is a known inhibitor of acid production by mutans streptococci and also a potential enzyme inhibitor of *Streptococcus rattus* and *Streptococcus salivarius*.[13] CaF$_2$ materials are of significant interest in dentistry as a source of labile fluoride in caries prevention.[9]

Nanoscale-based approaches are being widely used and have been proven to be more effective in elimination of biofilm and inhibition of dental caries.[14-16] ZnO showed significant antibacterial activity over a wide spectrum of bacterial species when particle size is reduced to nanometer range.[17] Nanoscale dimensions of ZnO and CaF_2 materials allow considerable broader gamut of interactions with microorganisms increasing their antibacterial property.[18] Hence, we considered addition of ZnO and CaF_2 NPs to equip sealant with an additional antibacterial property and the other objective is to check whether addition of these NPs would influence the mechanical properties.

Direct contact test was preferred over agar diffusion test for the evaluation of antibacterial activity because studies have shown that in agar diffusion test, sufficient amount of ZnO could not leach to the surrounding environment due to its insolubility.[3,7] We found that ZnO and CaF_2 NPs could endow the resin with good antibacterial activity. ZnO, CaF_2 NPs and their mixture in both the concentrations (0.5 wt% and 1 wt%) exhibited stronger antibacterial activity against *S. mutans* and *L. acidophilus*. Similar results were demonstrated when ZnO NPs were mixed with flowable and regular composite resins.[7,19]

The possible explanations that could be responsible for antimicrobial behavior of ZnO NPs are (1) production of active oxygen species such as H_2O_2 which inhibit the growth of bacteria by internalization into the bacterial cell membrane causing destruction of cellular components such as lipids, DNA, and proteins;[20] (2) zinc ions interfere with the bacterial enzyme systems by displacing magnesium ions which are essential for enzymatic activity of the dental plaque;[21] and (3) interaction between the NPs and bacteria caused by electrostatic forces which are produced by light exposure.[22]

Fluoride-releasing property of CaF_2 NPs could be the main reason for antibacterial activity. These fluoride ions act directly or in the form of metal complexes, to inhibit many enzymes.[23] Fluoride ions combine with hydrogen ions forming the hydrogen fluoride molecule, which can eventually inhibit the glycolytic enzymes such as enolases in *S. mutans*.[15,24] In addition, fluoride ion hinders the proton extrusion by the formation of F-ATPases, thereby lending a proton back into the cell.[23,25] The release of fluoride ions from CaF_2 might have suppressed the glucan production ability and acid production by the bacteria.

Strong mechanical properties of the resin materials are essential for their longevity. Whenever an effort is made to alter the composition of a given material to improve any of the properties, necessarily, it should not cause changes in the other beneficial properties. It is learned that addition of NPs to resins in higher concentrations would affect the mechanical properties.[7] Hence, tests were performed to evaluate the CS and FS of sealants added with ZnO and CaF_2 NPs and compared with conventional sealant.

Samples added with CaF_2 NPs (0.5 wt%) resulted in decreased CS. Leaching of fluoride might have resulted in voids in the matrix, leading to decrease in the strength of the material. The CS of the samples added with ZnO and the mixture of ZnO and CaF_2 NPs in both concentrations (0.5 wt% and 1 wt%) remained comparable to conventional sealant. Similar finding of significant increase in the CS was reported with 1 wt% of ZnO NPs incorporated flowable resin.[7]

Samples with CaF_2 NPs showed decreased FS probably due to the defects produced during the dispersion of NPs, causing weak zones, resulting in reduced strength. The samples containing ZnO and the mixture of ZnO and CaF_2 NPs (0.5 wt% and 1 wt%) showed FS comparable to conventional sealant.

Sealants added with mixture of 0.5 wt% ZnO and CaF_2 NPs exhibited higher FS compared to the samples with 1 wt% ZnO and CaF_2 NPs, and this could be due to decrease in the depth of cure. Similarly, Tavassoli Hojati *et al*. had reported a decrease in depth of cure in flowable composite when ZnO NPs were incorporated in higher concentrations.[7]

To summarize, the addition of ZnO and CaF_2 NPs to sealants could significantly inhibit the growth of *S. mutans* and *Lactobacillus*. The mechanical properties of sealants containing NPs were comparable to the conventional sealants except CaF_2 NPs added samples. Addition of these NPs may alter the flow and color of the material; hence, the present study gives further impetus to investigate these physical properties and also the other mechanical properties such as marginal integrity and wear resistance.

Conclusion

The addition of ZnO and CaF_2 NPs alone or in mixture to fissure sealants could significantly inhibit the growth of *S. mutans* and *L. acidophilus* without affecting the mechanical properties. Although CaF_2 NPs added samples provided favorable antibacterial property, the mechanical properties were compromised.

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Conflicts of interest

There are no conflicts of interest.

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