Effect of Silver Nanoparticle-Added Pit and Fissure Sealant in the Prevention of Dental Caries in Children

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Objective: *The objective of this study was to evaluate the effects of pit and fissure sealant mixed with silver nanoparticles on dental caries, by means of monthly measurement of fluorescence with DIAGNOdent over six months.* ***Study Design:*** *This study was divided in two phases: experimental and clinical. In the experimental phase, the adhesion and microleakage of the pit and fissure sealant experiment were evaluated. Two groups of 10 teeth, without serious carious lesions, were included. Conventional (group A) and silver nanoparticles (group B) were added to the pit and fissure sealant. For the clinical phase, a split-mouth study was performed on 40 children aged 6-10 years old with healthy, erupted permanent first molars. A conventional pit and fissure sealant or a silver nanoparticle-mixed sealant was randomly placed. Repeated measures analysis was performed.* ***Results:*** *Conventional sealant presented an average microleakage of 30.6%, and the silver nanoparticle-mixed sealant showed 33.6% (P=NS). A three times greater reduction in fluorescence was found in the silver nanoparticles group compared to the conventional group (P<0.05). No sex- or age-based associations were found.* ***Conclusions:*** *The silver nanoparticle-mixed sealant reduced tooth demineralization significantly and likely increased remineralization, compared to the conventional sealant.*

Key words: *Caries, Sealant, Prevention, Silver Nanoparticles, DIAGNOdent*

INTRODUCTION

Dental caries, a carbohydrate-modified local infection that destroys the hard tissues of the teeth, has a multifactorial etiology, in which there is an interaction of various factors, including the host, agent, substrate, and time.[1] Caries is characterized by the destruction of hard dental tissue as it is demineralized by the acids generated by bacterial plaque, which forms during the degradation of the carbohydrates in the diet.[2] *Streptococcus mutans* has been implicated as the principal pathogen involved in the development of dental caries, which is why many epidemiological studies in dentistry have focused on isolating this microorganism to determine its pathogenicity;[3–5] as a result, preventive measures directed toward the elimination or reduction of this microorganism in the oral cavity have been developed.[6]

Ninety percent of carious lesions originate in the pits and fissures of the occlusal surfaces of permanent molars.[7,8] One preventive option is to seal "imperfections in the enamel", such as pits and fissures, by applying sealant in the form of mechanically retained resins on the etched surface of the enamel, with which the anatomical defects of the enamel are sealed.[9–12] It has been shown that the use of sealants on the first permanent molar reduces the possibility of receiving further restorations and prevents the emergence of dental caries.[13] However, longitudinal studies in teenagers and young adults have demonstrated evidence of the presence of caries even below the pit and fissure sealant.[14]

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Silver nanoparticles (NNPs) have recently been shown to possess bactericidal and bacteriostatic properties in various species of microorganisms and viruses.[15] In previous studies, we showed that *Streptococcus mutans*, the principal organism associated with tooth decay, is targeted by NNPs.[16] It has been determined that the minimum inhibitory concentration of NNP against *Streptococcus mutans* is 4.86 µg/ml, and the minimum bacteriostatic concentration is 6.25 µg/ml, employing NNPs of 40 and 60 nm in size, respectively.[16] These microbiological qualities remain when added to Gantrez, an adhesive used in toothpaste that does not stain the tooth structure.[17,18] Additionally, NNP toxicity has been avoided in periodontal tissue with a nanoparticle size of 50-100 nm, with no changes in cell viability.[19]

The objective of this study was to evaluate the protective effects against caries of pit and fissure sealant mixed with NNPs.

MATERIALS AND METHOD

Manufacturing of nanoparticles

The procedure involved reactions of colloidal solutions with oversaturation of salts and the creation of a precipitate by homogeneous or heterogeneous nucleation. After nucleation, the nanoparticles' growth occurred by diffusion. The concentration gradient, temperature changes, agitation, and surfactants allowed for the modification of the size increment. We produced monodispersed, non-agglomerated particles with a uniform size. First, we prepared a solution of silver, with sodium tetrahydroborate ($NaBH_4$) included as a reducing agent. The ratio between silver and the reducing agent was greater than one. After the reaction, we added a stabilizer to control size and uniformity. For the experiment, the mixture was prepared with 98 µg/mL of NNPs (10^{-3} mol) with Clinpro™ pit and fissure sealant (3M Espe, St. Paul, MN, USA) and was returned to the same sealant container. The size of the nanoparticles was between 40 nm and 80 nm.

Adhesion and microleakage of the pit and fissure sealant trial

Two groups of 10 molars and/or premolars, without carious lesions and extracted for orthodontic reasons or because of periodontal disease, were used. Group A received conventional Clinpro™ pit and fissure sealant (3M Espe, St. Paul, MN, USA), and group B received NNPs mixed with Clinpro™ pit and fissure sealant. Both groups were subjected to occlusal load thermocycling process of 250 cycles at temperatures of 5°C-37°C -55°C, each with duration of 30 seconds in order to take a better clinical simulation. The presence of macroscopic detachment was verified, and the teeth in both groups were covered with pink wax on all of their surfaces, except for the surface on which the sealant was placed, and then the teeth were immersed in a 2% methylene blue stain for four hours. They were then washed under tap water for 5 minutes. The teeth were sliced mesiodistally, using a #7016 fine diamond rotary disc at low speed under constant irrigation. The sliced molars (40 halves) were observed under a stereoscopic microscope (Leica EZ40, Wetzlar, Germany), by which the presence of leakage of methylene blue in the sealant/dental tissue interface was assessed by two independent observers. The amount of microleakage was calculated by dividing the stained surface by the total area occupied by the pit and fissure sealant, using a digital vernier.

Clinical study

This study was conducted in accordance with the Declaration of Helsinki, and the study design was approved by the local ethics committee. A split-mouth, double-blind clinical trial was performed on the first mandibular molars without caries of 40 children aged 6-10 years old, with consent for participation from their parents. Half of the mouth received NNP-added pit and fissure sealant in one molar, while the contralateral molar received conventional pit and fissure sealant. The presence of carious lesions was assessed using a pen-type laser fluorescence device, the DIAGNOdent pen (Kavo, Biberach, Germany), with which greater fluorescence equaled greater tooth decalcification, which is an initial step in the formation of caries.[20] Five measurements were obtained from each molar — mesial, central, distal, buccal and cuspid — and the average was calculated.

First, the enamel of the occlusal surface of the molars was etched with 35% phosphoric acid for 15 seconds. The enamel was then rinsed for 15 seconds and dried. The NNP-added or conventional sealant was then applied. The sealant was polymerized for 20 seconds and then was applied on the contralateral molar following the same indications. Monthly revisions were performed over the following 6 months to assess fluorescence and detachment of the sealant.

Statistical analysis

The fluorescence scores were estimated with both treatments, and the scores were compared using multivaried repeated measures analysis (R, version 2.12.2).

RESULTS

Regarding adhesion and microleakage in the pit and fissure sealant trial, conventional sealant presented an average microleakage of 30.6%, and the silver nanoparticle-added sealant showed microleakage of 33.6% (P=NS). Figure 1 shows views of representative samples. There were no differences in adhesion between the two groups.

For the clinical trial, a total of 80 first mandibular molars from 40 children were included; the average age of the patients was 8.2 years old, ranging from 6 to 10 years of age. Sixty percent of the patients were female.

Figure 2 shows the overall average during the entire follow-up of measured fluorescence in both groups: conventional (control) and NNP-added sealants. The average difference was more than 25% between the groups. Figure 3 shows initial reductions in fluorescence in both groups, although the NNP group had a tendency to diminish its fluorescence faster, which could suggest that the teeth tended to remineralize when the microorganism causing its demineralization was eliminated. It was also observed that there were no interactions between the groups.

FIGURE 1. Microleakage of the pit and fissure sealant trial (left: conventional sealant; right: NNPs added to sealant).

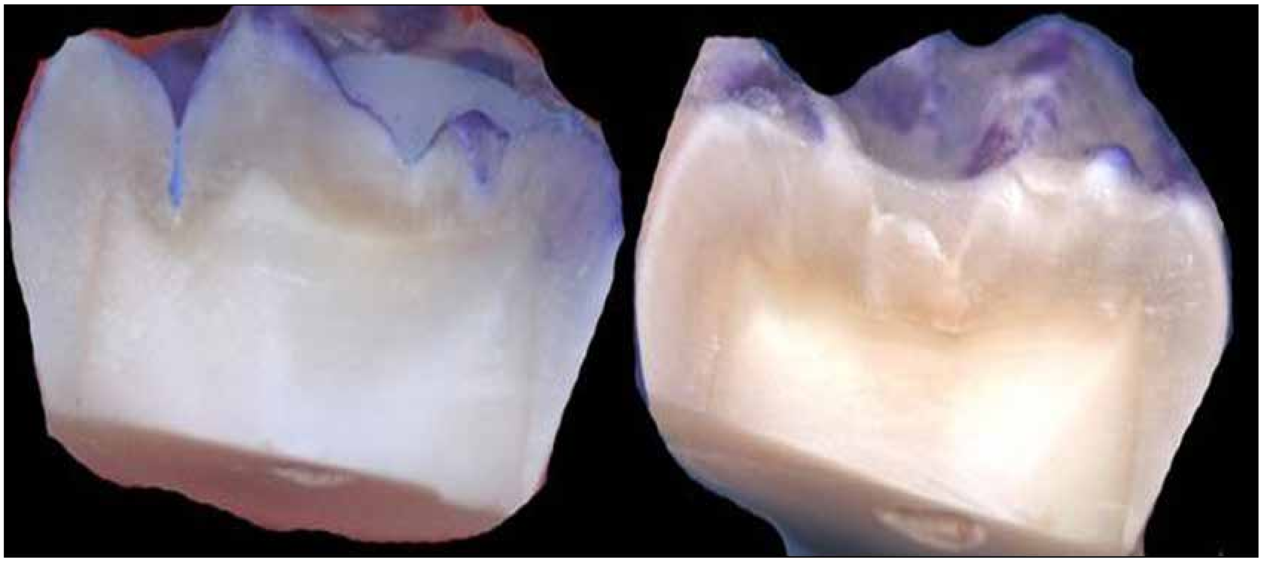
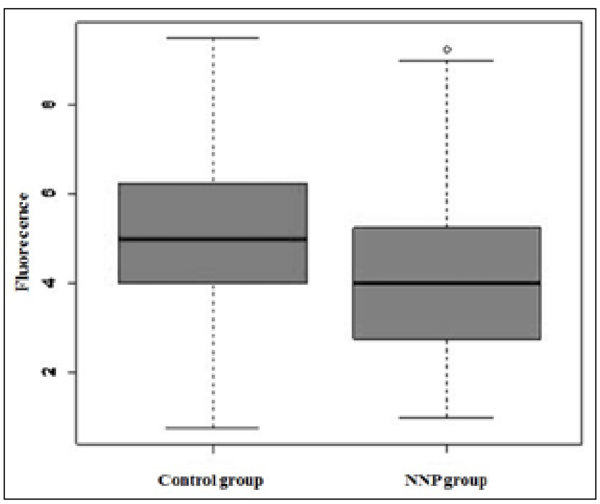


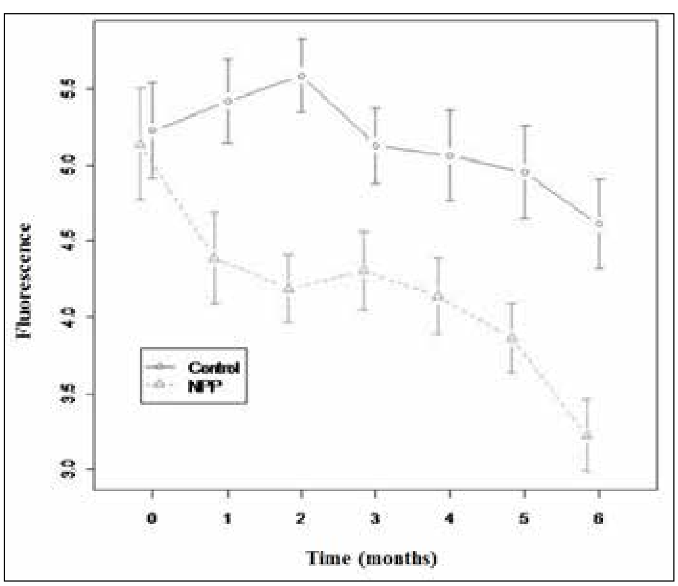
FIGURE 2. General fluorescence of molars with conventional sealant and mixed with NNPs.



To determine the significance of the difference in fluorescence between the study groups by observation month, as well as the effects of age and sex on this difference, multivariate repeated measures analysis was performed. A multiple r^2 was estimated for the model, finding that the inclusion of these three variables explained approximately 74% of the observed difference and that it specifically presented an Eta of 0.43 for the effect of the addition of NNPs to sealant, which indicated a contribution to this difference of 43%.

Of the 40 sealed molars, 4 had the sealant detach, 2 from each group, and 2 patients did not attend any of their follow-up appointments. These losses to follow-up were included in the analysis.

FIGURE 3. Fluorescence by month (average, CI 95%) measured with DIAGNOdent in children's mandibular molars with conventional pit and fissure sealant and NNPs added to sealant.



DISCUSSION

In this study, there were differences observed between the teeth sealed with sealant mixed with NNPs and the teeth sealed without NNPs. The results found in the present study indicated significantly greater decalcification in the group without NNPs, indicating a greater risk of the development of dental caries, while in the NNP group, there likely was greater remineralization. Additionally, we did not find higher frequencies of detachment and microleakage compared to the conventional sealant. There were no color changes. This study used a split-mouth clinical trial design, which was suitable and it enrolled both control and experimental teeth from the same patients, thus controlling for factors that might have affected sealant retention and caries prevention.

In many countries, the prevalence of dental caries in children has diminished in recent years; however, caries continues to be an important public health concern. Approximately 25% of children and teenagers between the ages of 5 and 17 years old account for 80% of the dental caries in permanent teeth.[1]

Currently, research has focused on designing products that prevent this disease, most of them aiming to eliminate *S. mutans*, whether using preventive or therapeutic resources once the carious lesion has settled; these resources have included mouth rinses, fluoride varnish, fluoride foods, toothpaste, gels, laser application and use of pit and fissure sealants.[6, 21–25]

The use of fissure sealant is a common preventive measure used in many countries. Fissure sealants have made a significant contribution to decreased rates of dental caries. Pit and fissure sealant is a mechanically retained resin placed on the surface of the enamel that seals the tooth's anatomical "defects"; in molars, its preventive action has yielded good results. However, failure rates have been reported at between 5% and 10% each year.[26] Heidmann *et al* showed a 32% reduction in caries in sealed permanent first molars in 12-year-old patients after one year of follow-up and a 25% reduction during the second year.[27] Nazar *et al* evaluated the effectiveness of sealant retention and caries prevention with and without primer and bond below the fissure sealant in children between 6 to 9 years old from public schools. In this study, there were no differences observed in teeth sealed with primer and bond, compared to teeth sealed without primer and bond, regarding the enhancement of sealant retention and prevention of caries.[28] Dennison et al. analyzed an insurance claim database to evaluate the use and effectiveness of placing sealants on first and second permanent molars of children in private dental practices. Children who received sealant were compared with those who did not to determine post-sealant restorative outcomes after five years. Both the incidence of occlusal caries and the use of sealants were lower than expected. Additionally, molar occlusal surfaces were only 50% more likely to have been restored in sealed teeth than in non-sealed teeth after five years.[29]

In our population, in which dental health education is not adequate, and the majority of the population does not receive periodic revisions, the development of a pit and fissure sealant with characteristics such as ours is imperative, to minimize the therapeutic costs for the population. Silver has important antimicrobial effects, and these effects are dependent on superficial contact, by which silver can inhibit the enzymatic systems of the respiratory chain and alter DNA synthesis.[30] Both silver diamine fluoride and silver nitrate have been shown to have bactericidal effects against *S. mutans*. However, their use has been limited because of the dental pigmentation caused by high concentrations of silver precipitation, which can also cause pulp irritation due to high silver ion penetration into the dentin.[31,32] In the current work, we decided to evaluate the addition of NNPs to pit and fissure sealant, to increase the protective effects by eliminating the main causative microorganism of dental caries, *S. mutans*. By diminishing *S. mutans*, the decalcification caused by it was eliminated; therefore, the sealant indirectly had an additional protective effect against caries.

Currently, the individuals included in the study continue to attend follow-up visits every six months, for the evaluation of the development of caries and the aforementioned remineralization process. Future studies are necessary to evaluate the bactericidal effects of NNPs added to other resins, glass-ionomer cements and varnishes.

CONCLUSIONS

The silver nanoparticle-added sealant reduced tooth demineralization significantly and likely increased remineralization, compared to the conventional sealant.

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