Dental Materials Journal 2021; 40(3): 758–765

Effect of a chlorhexidine-encapsulated nanotube modified pit-and-fissure sealant on oral biofilm

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The purpose of this study was to characterize a chlorhexidine-encapsulated nanotube modified pit-and-fissure sealant for biofilm development prevention. HS (commercial control); HNT (HS+15wt%Halloysite®-clay-nanotube); CHX10% (HS+15wt% HNT-encapsulated with chlorhexidine 10%); and CHX20% (HS+15wt% HNT-encapsulated with CHX20%) were tested. Degree-of-conversion (DC%), Knoop hardness (KHN), and viscosity were analyzed. The ability of the sealant to wet the fissures was evaluated. Specimens were tested for zones of inhibition of microbial growth. *S. mutans* biofilm was tested by measuring recovered viability. Data were statistically analyzed ($p<0.05$). DC% was significantly higher for the HNT-CHX groups. For KHN, CHX10% presented a lower mean value than the other groups. Adding HNT resulted in higher viscosity values. The biofilm on CHX10% and CHX20% sealants presented remarkable CFU/mL reduction in comparison to the HS. The experimental material was able to reduce the biofilm development in *S. mutans* biofilm without compromising the sealant properties.

Keywords: Pit-and-fissure sealant, Viscosity, Biofilm, Chlorhexidine, Hardness

INTRODUCTION

Data from the National Center for Health Statistics (NCHS/2011–2012) showed that about 23% of children age 2–5 have experienced dental caries in primary teeth[1]. It was also reported that three in five adolescents age 12–19 have experienced dental caries in permanent teeth[1]. A recent study[2], assessing the effect of family income, parent's educational level and visit to the dentist, showed the importance of the development of more effective prevention strategies, especially for kids from lower economic classes. The use of sealants is beneficial not only for children, but also for adolescents and young adults. The Community Preventive Services Task Force[3] released an article supporting the use of dentin sealants as a result of the strong evidence of effectiveness in preventing dental caries among children. Data from 2011–2012 showed that one-half of children ages 9–11 and 43% of adolescents ages 10–12 had at least one dental sealant on a permanent tooth[1]. The U.S. Navy also encourages the placement of pit-and-fissure sealants on recruits with high caries risk[4,5]. Sealants can prevent caries initiation and arrest caries progression by creating a physical barrier that inhibits the collection of food particles and microorganisms in pits and fissures[6]. Preventive measures are less expensive and therefore are more likely to be implemented in communities with limited resources.

Although the sealant retention is an important factor in the prevention of dental caries, clinical trial published in 2019[7], using a sealant combined with antimicrobial, showed that this combination could be the best option for patients with high caries risk as a result of inappropriate oral hygiene. In addition, a second clinical and SEM[8] study, showed the presence of gaps between sealant and tooth and small fractures that suggest degradation of adhesion. In this case, to have a sealant that could protect the tooth through the release of antimicrobial until the next visit to the dentist would be beneficial to the patient.

Our recent studies[9-12], have shown that the incorporation of Halloysite® aluminosilicate clay nanotubes ($Al_2(OH)_4Si_2O_5 \cdot 2H_2O$, HNTs) into dentin adhesives can serve as a suitable reservoir for encapsulation and drug release of guest molecules[10,11]. Halloysite® clay nanotubes are a natural occurring polymorph of kaolinite with predominantly hollow nanotubular structures and a high length/diameter ratio[13-15]. HNTs contain a multi-layered tubular structure with the external surface mainly composed of siloxane groups and the internal surface of a gibbsite-like array of aluminol groups[13,15]. According to a previous publication[14], polymer nanocomposites reinforced with nanotubes have increased mechanical strength (*i.e.* tensile strength, flexural strength and modulus of elasticity), thermal stability, and biocompatibility[14]. In addition, HNT is capable of entrapping substances for controlled or sustained release[10,15,16]. Thus, many substances such as doxycycline, chlorhexidine and tetracycline have been loaded into HNTs and the drug

Color figures can be viewed in the online issue, which is available at J-STAGE.
Received Jun 19, 2020: Accepted Sep 7, 2020
doi:10.4012/dmj.2020-241 JOI JST.JSTAGE/dmj/2020-241

release has been reported to last longer than the drug alone or when loaded into other carriers[10,14,17].

The use of antimicrobial agents in dental biomaterials has been suggested to prevent the growth of microorganisms involved in dental caries[18,19]. Chlorhexidine is a well-known antimicrobial, commonly found in mouthwash solutions and toothpastes. The successful antimicrobial effect of chlorhexidine is a result of its capability to penetrate and disrupt the bacterial cytoplasmic membrane causing the leakage of cytoplasmic components[20]. However, chlorhexidine used as a mouthwash has several side effects such as increased pigmentation of the teeth or tongue and changes in the ability to taste, among others[21-23]. CHX may present promising results if combined with pit-and-fissure sealants designed for the prevention of oral biofilm formation in populations with high caries-risk. Previous studies showed that small volumes of CHX could be incorporated into a resin blend. A study[3] showed that by mixing chlorhexidine 1% into the resin matrix of a pit-and-fissure sealant (Clinpro, 3M ESPE, St. Paul, MN, USA) antibacterial activity is achieved without affecting the mechanical properties of the sealant. A previous study[24] showed that the incorporation of chlorhexidine (0.1 and 0.2 wt%) in resin infiltrants did not affect the materials' properties (*i.e.* degree of conversion and microhardness) while presenting satisfactory inhibition of planktonic *Streptococcus mutans*. Another study[25] reported that when chlorhexidine is added and released from copolymers based on BIS-GMA, changes in flexural strength and elastic modulus are detected and all the groups presented a 24 h burst of drug release. Although promising, it is clear that the volume of CHX added to the resin blend is critical to maintain the properties of the material (up to 1 v/v % can be added) and the CHX concentration should be low to avoid possible cytotoxic effects. Therefore, the slow release, while still effective, of site-specific chlorhexidine would be highly desirable as a co-adjuvant therapy for caries prevention in pit-and-fissure sealants. A previous research showed that when CHX10% and CHX20% is encapsulated into HNTs and incorporated into dental adhesives, no cytotoxicic effects were observed for dentin pulp stem cells[26].

Thus, the purpose of this study was to develop a chlorhexidine modified pit-and-fissure sealant material and to assess its physicochemical and biological properties. The effect of the modified material was evaluated by an established *S. mutans* biofilm model[27]. The hypothesis tested was that the pit-and-fissure sealant mixed with different concentrations of chlorhexidine encapsulated nanotubes would not present improved physicochemical and biological properties when compared to the commercially available sealants.

MATERIALS AND METHODS

Modified HNT-CHX pit-and-fissure sealant preparation

1. Chlorhexidine solution preparation

Chlorhexidine (chlorhexidine digluconate solution 20% in H_2O (Lot#BCBS7878V) Sigma-Aldrich, St. Louis, MO, USA) was encapsulated into the HNT (Halloysite® aluminosilicate clay nanotubes, Dragonite 1415JM, Applied Minerals., New York, NY, USA) according to the experimental groups described in Table 1. HNTs were used as carriers for the CHX. The HNT encapsulation was based on our previous reports[10,11,16]. Briefly, 1.25 g of HNT and 5 mL of 10% or 20% CHX solution (according to each group/Table 1) were mixed, sonicated and submitted to vacuum (25 mmHg). Lastly, the tube was centrifuged at 3,500 rpm at room temperature. The mixed material was dried using an incubator (7 days/37°C) and sieved at 45 μm in order to obtain the final product —a HNT-CHX powder.

2. Formulation of the HNT-CHX modified resin-based sealant

Dried 0%, 10%, and 20% HNT-CHX powder was mixed (15 wt%) with the commercial pit-and-fissure sealant (Lot#W31701, Helioseal® light-curing fissure sealant, Ivoclar-Vivadent, Amherst, NY, USA) using a motor-powered mixer (Roti-Speed, Roth, Karlsruhe, Germany). The experimental pit-and-fissure sealant and control groups were prepared under a filtered light system.

3. Degree of conversion (DC)

The DC was calculated based on our previous report[10,11]. The modified and commerical sealants were evaluated using a Fourier Transform infrared spectrometer (FTIR, model 4100, JASCO International, Tokyo, Japan) equipped with an attenuated total reflection device in the absorbance mode (8 cm^{-1} resolution and 2.8 mm/s mirror speed)[10,11]. For that, specimens (3/group) were prepared and measured, before and after being light-activated for 20 s (as recommended by the manufacturer)[11] (3 measurements/specimen) using a single emission peak LED light-curing unit (LCU, Demi Ultra, Kerr, Orange, CA, USA). To calculate the DC (%), the absorbance bands at 1,637 cm^{-1} (methacrylate group) and 1,607 cm^{-1}

Table 1 Control and experimental groups

Group	Description
HS	Helioseal Clear
HNT	Helioseal Clear+15 wt% Halloysite® clay nanotubes
CHX10%	Helioseal Clear+15 wt% Halloysite® clay nanotubes encapsulated with chlorhexidine 10%
CHX20%	Helioseal Clear+15 wt% Halloysite® clay nanotubes encapsulated with chlorhexidine 20%

(aromatic ring in Bis-GMA) were used according to the following equation[28]:

$$DC\ (\%)=1-\frac{\text{cured (area under 1637/area under 1607)}}{\text{uncured (area under 1637/area under 1607)}}\times 100$$

4. Knoop hardness (KHN)
Disk-shaped specimens were fabricated for each sealant group (n=5; 5 readings per specimen) using a Teflon mold (6 mm diameter×1 mm thickness) and light-cured for 20 s (top and bottom). Next, the specimens were stored at 37°C for 24 h. The specimens were subjected to hardness testing (LECO, M-400, St. Joseph, MI, USA) using a Knoop diamond indenter[11,12]. The diagonal lengths were measured after each indentation and the values converted to KHN numbers (kg/mm^2).

5. Viscosity
The effect of the addition of HNT and CHX on the resin based material was determined using a viscometer (Brookfield, DV-II, Middleboro, MA, USA) (n=3 readings/group). The material was held in place and subjected to shear with a spindle (CPE-52). After preliminary testing, a shear rate of 20 L/s was selected and used to determine the values of viscosity for each experimental group.

6. Sealant ability to wet the fissures
Human permanent molars were obtained after approval by the IU Institutional Review Board (IRB#1802193325) according to the inclusion criteria of absence of cracks, caries or restorations. Teeth (n=5/group) were autoclaved in water and cleaned before use and ramdomly divided into 4 groups according to the type of sealant. For the bonding protocol, the enamel surfaces were cleaned with a non-fluoride pumice paste (Lot#050817AP, nada pumice paste, Preventech Technologies, Indian Trail, NC, USA). Etching gel was applied for 30 s, rinsed thoroughly with distilled water and dried until the enamel presented a matte-white appearance. The sealant was applied with a disposable brush and after 15 s, the sealant was light-cured for 20 s. The marginal adaptation between sealant and enamel interface was checked for the presence of gaps and excess of material before storage. The specimens were stored for 24 h at 37°C in distilled water. Next, specimens were sectioned longitudinally in a bucco-lingual direction using a diamond blade mounted on a saw machine (ISOMET 1000, Buehler, Lake Bluff, IL, USA). After the cut, penetration of the sealant was analyzed according to the different type of fissure anatomy (V-fissure shape; U-fissure shape; and mixed shape —fissures type I and inverted Y). For that, one calibrated examiner evaluated occlusal fissure penetration by examining under optical microscope at 8× magnification (Leica MZ 125, Leica Microsystems, Wetzlar, Germany) and with a digital camera (Canon EOS Rebel T3, Canon, Melville, NY, USA). The presence of bubbles, gaps and other failures were qualitatively determined for each group.

Antimicrobial activity
For the agar diffusion and biofilm development assays, 12 disk-shaped sealant specimens (6.2 mm diameter×1 mm thick) were prepared using a metallic mold and light-cured (20 s per each side). The specimens were kept at 37°C for 24 h and disinfected using ultraviolet light exposure (30 min per each side). The disinfection method was previously confirmed (data not shown).

1. Agar diffusion
For the agar diffusion method[29], the inoculum procedures of *Streptococcus mutans* were appropriate to provide a semi confluent growth of the microorganisms tested (1–2×10^8 colony-forming units (CFUs)/mL) onto a brain heart infusion agar plate. Six specimens were placed on the inoculated agar plates and incubated at 37°C for 48 h in a 5% CO_2 incubator. The zones of inhibition of microbial growth around the specimens (HS, HNT, CHX10%, CHX20% and CHX 0.12% solution as a positive control) were measured. The inhibitory zone was considered the distance (mm) from the outside margin of the initial point of microbial growth[29].

2. Biofilm culture
Streptococcus mutans UA159 biofilms were prepared as described elsewhere[30]. The specimens were used as substrates for the 3 day biofilm formation and development in tryptone soy broth with yeast extract (TSB+YE) media with 1% sucrose. Fresh media was replaced every 24 h.

3. Counting of viable colony forming units (CFU/mL)
After 3 days of biofilm development[30], the specimens containing the biofilms were inserted in glass tubes with 2 mL of 0.89% NaCl solution and biofilm disruption was performed in an ultrasound bath for 10 min in cold water. After disruption, the discs were scratched with a spatula to remove the remaining biofilm. Discs were discarded. The biofilm suspensions were transferred to 15 mL conical tubes and 3 mL 0.89% NaCl was added[31]. The biofilm suspension was then sonicated for 30 s and submitted to serial dilution. Serially diluted aliquots were inoculated in blood agar (TSA II 5% SB) and incubated at 37ºC, 5% CO_2 for 48 h. The colonies were counted to determine the number of CFU/mL[31].

Statistical analysis
Data from degree of conversion (DC%), hardness (KHN), viscosity (mPa.s), zone of inhibition (in mm) and biofilm ($\log_{10}$ CFU/mL) were analyzed using one-way ANOVA followed by Tukey test (p<0.05) (GraphPad Prism version 7.03). The sealant ability to wet the fissures was submitted to a qualitative analysis.

RESULTS

The results of degree of conversion, Knoop hardness and viscosity for each pit-and-fissure sealant are presented in Table 2. The DC (%) was significantly higher for CHX10% and CHX20%. Hardness was not affected by

Table 2 Mean±SD of DC%, KHN and viscosity [mPa.s]

	Degree of Conversion (%)	Knoop hardness (KHN)	Viscosity [mPa.s]
HS	55.52±1.42[b]	54.19±7.86[ab]	557.12±12.78[a]
HNT	54.89±3.26[b]	55.93±4.68[a]	764.43±24.42[b]
CHX10%	64.97±4.22[a]	43.5±5.88[b]	726.04±66.42[b]
CHX20%	71.77±2.69[a]	55.94±4.68a	768. 42±53.15[b]

Superscript letters identify statistically similar groups in each column ($p<0.05$).

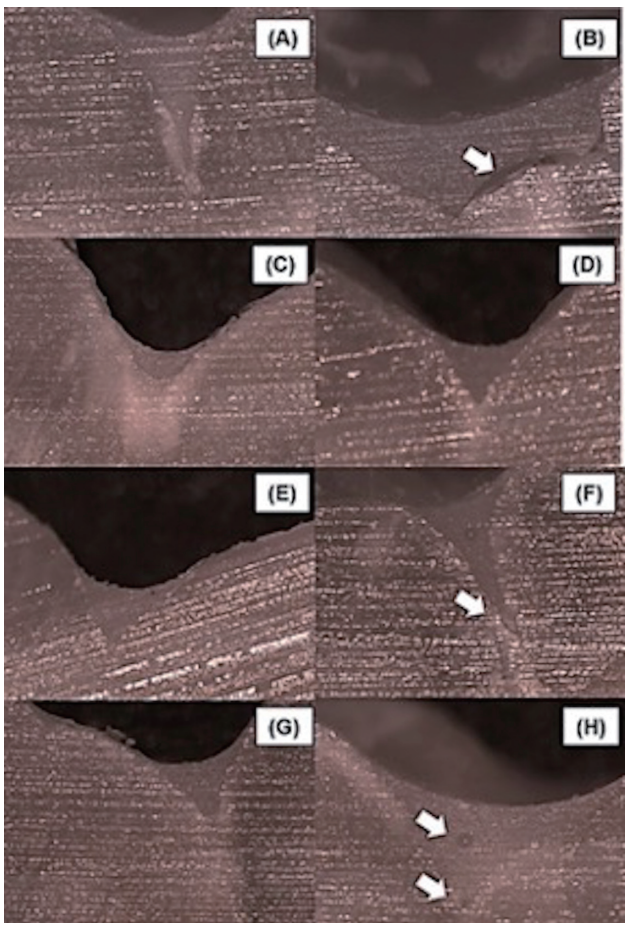

Fig. 1 Illustrative micrographs of the penetration of the pit and fissure sealant in different enamel substrates using optical microscope at 8× magnification: (A) HS completely wet the V-shaped depression on the occlusal surface; (B) The arrow indicates the presence of a void between the enamel and HS; (C and D) HNT completely wet the U-shaped and V-shaped depression on the occlusal surface; (E) CHX10% completely wet the V-shaped depression on the occlusal surface; (D) In group CHX10%, a void was observed (indicated by the arrow) in the I-shaped depression on the occlusal surface; (G) CHX20% completely wet the V-shaped depression on the occlusal surface; (H) In group CHX20%, a bubble (round and well defined) was observed within the bulk of the sealant and a void in the I-shaped depression on the occlusal surface.

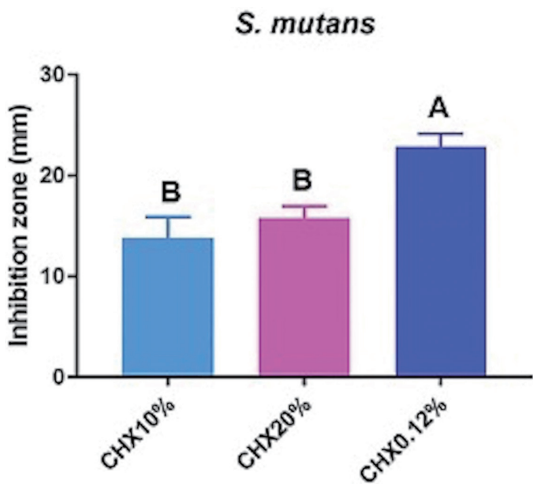

Fig. 2 Grown inhibition zone of *S. mutans* UA159 in mm. Antimicrobial activity of the experimental groups containing chlorhexidine encapsulated nanotubes (CHX10% and CHX20%) and CHX0.12%* (*positive control) against *S. mutans*.
Upper case letters indicate statistically similar groups ($p<0.05$).

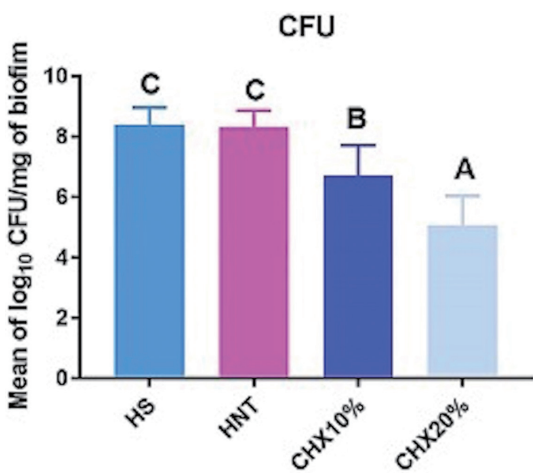

Fig. 3 Chlorhexidine encapsulated nanotube (CHX10% and CHX20%) and control groups (HS and HNT) effects on *S. mutans* biofilm reported by the recovery of $\log_{10}$ of CFU/mL after 3 days of biofilm development.
Upper case letters indicate statistically similar groups ($p<0.05$).

the incorporation of HNT with and without CHX (HNT, CHX10% and CHX20%) when compared to the control group, without HNT (HS) ($p<0.05$). Pit-and-fissure sealants containing HNT (with and without CHX) presented higher viscosity values than the control group (without nanotubes). At a shear rate of 20, group HS showed viscosity values that were statistically lower than HNT, CHX10% and CHX20%. Regarding the qualitative analysis of the ability of the experimental pit-and-fissure sealant to wet the fissures (Fig. 1), for the HS group, one tooth presented a void between the sealant and enamel (Fig. 1B) and two teeth presented voids in the deepest portion of a V shaped enamel sulcus. For the HNT group, the experimental sealant completely wet the sulcus/grooves independent of the shape for all the specimens; For the CHX10%, two teeth presented a small gap between the material and the enamel and two teeth presented voids in the deepest portion of a Y shaped enamel sulcus. For the CHX20%, two teeth presented a small void between the material and the enamel and one tooth presented a small void in the deepest portion of a Y shaped enamel sulcus.

No zone of inhibition was found for any specimen from the control groups (*i.e.*, HS and HNT; value=0.00), for this reason these groups were removed from the statistical comparison. The results of zone of inhibition are presented in Fig. 2. CHX20% and CHX10% presented similar inhibitory activity against *S. mutans* growth ($p<0.05$). The results of $\log_{10}$ CFU/mL of *S. mutans* are shown in Fig. 3. Significant $\log_{10}$ CFU/mL reduction in all CHX-containing groups was observed when compared to the control groups, HS and HNT. CHX20% presented statistically significant lower $\log_{10}$ CFU/mL than CHX10%, and both groups presented lower CFU/mL when compared to HS and HNT ($p<0.05$). There was no significant difference between the control groups HS and HNT ($p>0.05$).

DISCUSSION

In the present study, the degree of conversion, viscosity, sealant ability to wet enamel fissures and the antimicrobial effects of a modified chlorhexidine-encapsulated nanotube pit-and-fissure sealant developed for biofilm development prevention was evaluated. After analyzing the data, the hypothesis, that commercial sealant mixed with different concentrations of chlorhexidine encapsulated nanotubes would not present improved properties when compared to the commercial control group, was rejected.

Three significant components of resin based composite materials are the polymeric matrix, reinforcing fillers and chemicals active in the polymerization reaction[32]. The amount and dispersion of the filler that is added into the polymeric matrix plays an important role in the performance of the material. Furthermore, during the agar diffusion pilot test to define the optimum CHX concentration (unpublished data) it was possible to observe compromised material performance. Agglomeration of CHX-encapsulated nanotubes in certain areas of the pit-and-fissure sealant discs resulted in an irregular profile of inhibition zones. This information highlights the importance of the method of homogenization of the halloysite nanotube into the resin matrix, which was optimized for the further studies. Another issue with the agglomeration of particles would be the possibility that the degree of conversion would be affected due to light passing through the specimen being scattered by the filler particles (in this case agglomerated); the Knoop hardness and other mechanical properties of the material could also be compromised. Therefore, the methodology was optimized (section 2.1.2) and the inhibition zone helped not only to prove that the CHX was being released but supported an improvement in the effect of the homogenization of the filler into the resin matrix.

Regarding the degree of conversion, data showed that the incorporation of chlorhexidine encapsulated nanotubes led to a more effective monomer conversion when compared to the control group, HS. For the groups CHX10% and CHX20%, a degree of conversion higher than 60% was achieved while for HS and HNT the average conversion was around 55%. Previous work by our research group has shown that when up to 15 wt% HNT is added to resin matrix, it does not affect the DC[10-12]. Ultimately, the CHX-HNT containing pit-and-fissure sealant provided enough light transmission through the resin disks to result in polymerization higher than or similar to the commercial control group, HS.

The role of the Halloysite® clay nanotubes as a drug-carrier and as a filler for resin-based materials has been explored by research in different areas. HNTs can be loaded with various antibacterial disinfectants, drugs and substances for delivering at specific areas[33]. Studies[24,25] have reported that when water soluble agents such as 1% CHX are added into the resin-blend (without nanotubes), droplets can be formed resulting in intrinsic microporosities, release of residual monomers and decreased mechanical properties such as degree of conversion, hardness and water sorption. Our results showed that the use of CHX (10 and 20wt%) encapsulated into the nanotubes (as a filler) increased the DC while keeping similar results for KHN when compared to the control group (except for the group CHX10%), indicating a benefical relationship between the resin-matrix and experimental filler. Even though an increased degree of conversion was achieved for groups CHX10% and CHX20% it is important to highlight that as reported by a different study and in agreement with the present research, the DC is not necessarily responsible for improved mechanical properties of resins[12].

The failure of pit-and-fissure sealants is believed to be due to the presence bubbles and failures in the adaptation/retention between sealant and enamel[34,35]. One of the most critical steps when developing a sealant, especially with a modified filler/resin matrix ratio, is to create a material that will safely infiltrate the occlusal groove-fossa system to seal and protect the tooth. Occlusal pits-and-fissures are susceptible locations for the development of caries because microorganisms are

likely to remain undisturbed in these areas[36]. While the different morphology of the grooves did not influence caries initiation and progression, it is well known that the microorganisms in the upper parts of the fissure are more metabolicly active making the progression rate faster in this area than in the deeper parts of the system[36,37]. Therefore, to prepare a material that can properly wet the enamel and protect the tooth from the upper to the narrow sites of the fissure would be ideal. Our previous research[10-12] established that 10–15 wt% HNT can be safely added into the resin matrix (dentin-adhesives), however, the viscosity of the final material was never evaluated. In the present study, the modified material (HNT, HNT+CHX10% and HNT+CHX20%) presented statistically higher viscosity when compared to the control group, without nanotubes (HS) ($p<0.05$). Afterwards a qualitative analysis was performed to evaluate the experimental and control sealants ability to wet the enamel grooves and fissures. The micrographs showed that although the sealant is more viscous it still behaves similarly to the control group in terms of adaptation and penetration (Illustrated in Fig. 1). Therefore, it was possible to determine that although the viscosity was higher, the *in-vitro* performance of the modified material was similar to the control group.

Regarding the effect of the modified pit-and-fissure sealant on antimicrobial activity, agar diffusion and *Streptococcus mutans* biofilm assays were used. A review of the new generation of antimicrobial and bioactive restorative materials stated that these new materials have the potential to prevent dental caries[38]. In the present study, the modified material was able to remarkably reduce the *S. mutans* biofilm formation and development independent of the CHX concentration (*i.e.* 10 or 20%) when compared to the control groups, without encapsulated CHX. Studies[24,25] have shown that without nanotubes, chlorhexidine release is drug-ratio dependent and proportional to the water sorption into the resin blends. In this case, the resin-blend modified by the addition of chlorhexidine presents a release burst during the first 24 h followed by a continuously low release for up to 5 weeks[25]. The early release of CHX from the resin matrix can prevent microbial biofilm formation, but based on reports[39,40] after this period the resin matrix could be compromised by increased wear, caused by the diffusion of CHX from the matrix, which would result in high biofilm formation over time.

Chlorhexidine has been recognized as the 'gold standard' product as a result of its superior antiplaque effect[21,41]. Research to investigate bacterial resistance to chlorhexidine incorporated into resin-based materials is still in the early stages[38]. A recent study[42] evaluated if the use of biocides such as chlorhexidine could contribute to the development of bacterial drug resistance. No increased resistance to cetylpyridinium chloride (CPC), and 12-methacryloyloxydodecylpyridinium bromide (MDPB) (antibacterial used to prevent caries and periodontal disease and used in dentin adhesives) was detected for *Streptococcus mutans* and *Enterocccus faecalis*[42]. However, the same study showed that repeated exposure of *E. faecalis* to CHX led to bacterial resistance[42].

Diet eating behaviors (*i.e.* by excessive sugar and fermentable carbohydrate intake) as well as demographics and environmental factors can contribute to the high prevalence of caries in early ages[43]. Current dental caries prevention includes the application of fluorides, plaque control and use of pit-and-fissure sealants[44]. Pit-and-fissure sealants have been recommended for children with high caries risk[45]. Considering the routes of administration and distribution to achieve therapeutic efficacy (oral local drug delivery or delivery on-site of action), when using CHX the local and slow delivery of this bisguanide, as offered by the modified material proposed in this study, could be recommended for children and young adults with high caries risk, particularly in areas with limited access to a dentist. The novelty is to show the ability to utilize nanotubes as a drug nanocarrier able to slow release guest molecules, preventing biofilm formation in long term. In the present study, the well-known antimicrobial and 'gold-standard' anti-plaque agent chlorhexidine was added as the test drug. Even though CHX was incorporated into the nanotubes, one of the limitations of the present study was to calculate the amount of chlorhexidine released both from the encapsulated nanotube as well as from the chlorhexidine-encapsulated nanotube modified pit-and-fissure sealant. Current research in our laboratory is focused on the understanding of drug release kinetics when using encapsulated Halloysite nanotubes

CONCLUSION

Approaches to promote oral health and prevent dental caries are less expensive than surgical interventions; they can affect the overall population regardless of economic factors and should be integrated into oral public health efforts. The modified chlorhexidine-encapsulated nanotube pit-and-fissure sealant presented similar or improved physicochemical and mechanical properties when compared to the commercial pit-and fissure sealant. Through the local, slow and effective release of chlorhexidine, the modified sealant was able to reduce biofilm development in an established matrix-rich biofilm model. *In situ and in vivo* studies are necessary to validate the use of the proposed modified materials.

ACKNOWLEDGMENTS

The authors would like to thank Alain Moulari for the laboratory support during the processing of the nanotubes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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