Original Article

Antibacterial activity of new hydrophilic sealants: *In vitro* study

Federica Veneri, Elena Bardellini, Francesca Amadori, Emanuela Gobbi[1], Raffaella Belotti[2], Alessandra Majorana

Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Dental Clinic, School of Paediatric Dentistry and Unit of Oral Medicine, University of Brescia, Piazzale Spedali Civili, [1]Department of Molecular and Translational Medicine, Agrifood and Environmental Microbiology Platform (PiMiAA-AgroFood Lab), University of Brescia, Piazza del Mercato, [2]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Dental Clinic, University of Brescia, Piazzale Spedali Civili, Brescia, Italy

ABSTRACT

Background: Pits and fissures sealing and modulation of oral microbiota through probiotics are important preventive measures against dental decays. The aim of this study was to investigate the antibacterial activity of the Embrace™ WetBond™ Pit and Fissure Sealant (Pulpdent, USA) and UltraSeal XT® Hydro™ (Ultradent, USA) against selected oral bacteria and probiotics. **Methods:** The antibacterial effect of both sealants was tested both through planktonic growth inhibition test – 96-well microtiter plates and agar disk diffusion assay containing light-cured Embrace™ and UltraSeal XT® against *Streptococcus mutans* and two oral probiotics (*Streptococcus salivarius* and *Lactobacillus reuteri*). **Results:** Embrace™ showed a stronger and broad activity against all the bacterial strains tested ($P < 0.05$) in planktonic growth inhibition test even at its lowest dose (10 µl), with inhibition rates higher than 90% in all cases. UltraSeal XT® Hydro™ showed a mild antibacterial activity against *L. reuteri*, with growth inhibition rates being 19% and 23% for 20 µl and 50 µl, respectively. Regarding agar disk diffusion test, both sealants showed exclusively an antibacterial activity by contact. **Conclusions:** According to these findings, it is recommended to carefully plan the timing for the administration of different preventive interventions, such as oral probiotics assumption and sealant application, to maximize their specific effectiveness. We suggest prescribing oral probiotics first and putting off the Embrace™ sealant application to the end of probiotic treatment. On the contrary, it is possible to administer *L. reuteri* simultaneously with the application of UltraSeal XT® since it elicits a minimal antibacterial action against this oral probiotic.

KEYWORDS: Dental sealants, hydrophilic sealants, pits and fissures sealing, preventive dentistry

Address for correspondence:
Dr. Federica Veneri,
Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Dental Clinic, School of Paediatric Dentistry and Unit of Oral Medicine, University of Brescia, Piazzale Spedali Civili 1, 25123, Brescia, Italy.
E-mail: veneri.fed@gmail.com

Access this article online

Quick response code

Website: www.jisppd.com

DOI: 10.4103/JISPPD.JISPPD_442_20

Introduction

Dental decay is a multifactorial pathological process that affects approximately 90% of the world population, being the most common infectious disease.[1] Prevalence of caries in childhood ranges from 30% to 60% in developed countries and approximately 50% of preschool children show carious lesions.[2,3] This disease has a negative impact on health, on quality of life both of the children and the family and it is considered a

How to cite this article Veneri F, Bardellini E, Amadori F, Gobbi E, Belotti R, Majorana A. Antibacterial activity of new hydrophilic sealants: *In vitro* study. J Indian Soc Pedod Prev Dent 2020;38:387-92.

Submitted: 08-Oct-2020 **Revised:** 28-Dec-2020
Accepted: 28-Dec-2020 **Published:** 31-Dec-2020

public health issue.[4] Dental caries, as a multifactorial disease, results from a complex interaction of cariogenic oral flora with fermentable dietary carbohydrates on the tooth surface over time and host susceptibility, as described in Keyes–Jordan diagram.[5]

Dental decay consists of surface demineralization, followed by cavitation of the hard tissues of the teeth. If not treated, it can progress to pulp involvement, with pain, swelling, abscess, and systemic signs and symptoms. Mineral loss is caused by prolonged periods of low pH, usually induced by bacteria with acidogenic metabolism. In fact, many oral microorganisms cause fermentation of dietary simple carbohydrates, such as sucrose. Historically, *Streptococcus mutans* and *Lactobacillus* species are considered the main bacterial agents of dental caries.[6]

Treatment of active cavitated lesions require the removal of decayed and demineralized hard tissues, followed by restoration with suitable materials. Yet, dental caries management currently focuses on prevention, especially in childhood. Early therapeutic management includes behavioral changes, reparative strategies, and protective materials.

Behavioral approach should promote home care and effective oral hygiene maneuvers such as brushing and flossing daily. Moreover, diet should tend to avoid excessive sugar intake or snacking too frequently. Nonetheless, several studies demonstrated that behavioral changes are extremely difficult to achieve.

Preventive or early reparative strategies are based on remineralization and the best scientific evidence is currently supporting fluoride application.[7,8] Whereas fluoridated water has proven to reduce the overall decay rate in population, the most effective form of fluoride application is either with toothpaste, gel, or varnish.[9,10] Several studies also support the anticaries effect of xylitol, especially in combination with fluoride strategies. In fact, xylitol has been found to potentiate even small amounts of fluoride.[11] Current remineralization research focuses on various forms of calcium phosphate such as casein phosphopeptides, amorphous calcium phosphate complexes, and nanoparticle hydroxyapatite, that is a biomimetic material which promotes natural blocks building of enamel and reduces biofilm formation.[12,13]

Other preventive approaches include antimicrobial and potentially probiotic categories. Antimicrobial strategies are based on the use of chlorhexidine and less frequently, povidone-iodine rinses, but biofilms may be extremely resistant to changes and they usually need to be mechanically disaggregated.[14]

Probiotics are living microorganisms, primarily bacteria, safe for human consumption, that have favorable effects on oral health. To be effective as oral probiotics, these bacteria should adhere to tooth surfaces and colonize oral cavity. They are able to antagonize oral pathogens, including cariogenic bacteria, and to inhibit biofilm formation.[15] Although probiotics might offer a potential strategy for the future, their application in caries prevention still needs additional evidence.[16]

From a primary prevention perspective, pits and fissures sealing is considered to be one of the most effective procedures and it is strongly recommended. In fact, deep and narrow groves and fissures on occlusal surfaces of permanent molar are likely to retain food and to promote the presence of bacterial biofilm, increasing the risk of caries development.[17] Penetrating and sealing these surfaces with adequate materials can prevent carious lesions, as part of a comprehensive caries management approach.[18]

Current evidence shows that sealants are also effective in secondary prevention, as they inhibit progression of early noncavitated carious lesions.[19]

Dental sealants are either resin based or glass ionomer (GI) based. According to Anusavice *et al*., sealants can be classified as follows:[18]

- Resin-based sealants: urethane dimethacrylate or bisphenol A-glycidyl methacrylate monomers polymerized by either a chemical activator or light of a specific wavelength
- GI sealants: cements that were developed for their fluoride-release properties, stemming from the acid-base reaction between a fluoroaluminosilicate glass powder and an aqueous-based polyacrylic acid solution
- Polyacid-modified resin sealants (compomers): combine resin-based material found in traditional resin-based sealants with the fluoride-releasing and adhesive properties of GI sealants
- Resin-modified GI sealants: GI sealants with resin components. This type of sealant has similar fluoride-release properties as GI, but it has a longer working time and less water sensitivity than do traditional GI sealants.

The clinical procedure for sealants placement varies based on type and brand of the sealant. In general, manufacturers recommend thorough cleaning, isolation of the tooth surface, and a dry environment. Acid etching and the use of bonding agents are also encouraged for resin-based sealants.[17]

The most common reasons for sealants failure are salivary contamination, scarce retention, and adherence to the tooth surface, which depends on adequate adhesive procedures during sealant placement and curing including good isolation and dry environment.[20]

Modern research on sealants has tried to overcome these limitations, by developing hydrophilic systems, instead of hydrophobic traditional materials.

These particular hydrophilic sealants are fluoride releasing, light-curing resin materials, which are able to adhere to tooth surface also in the presence of humidity and liquids, without the use of dental dam. In fact, the polymer gets activated by water and it establishes a chemical bond with the tooth surface.

The aim of this *in vitro* study was to assess the antibacterial activity of two modern hydrophilic sealants. In particular, Embrace™ WetBond™ Pit and Fissure Sealant (Pulpdent, USA) and UltraSeal XT® hydro™ (Ultradent, USA) were evaluated for their activity against *S. mutans*. In addition, antibacterial activity against main oral probiotics, such as *Streptococcus salivarius* and *Lactobacillus reuteri*, was also investigated. In fact, since these strategies for dental caries prevention can be implemented simultaneously, it is desirable to obtain synergic therapeutic effects, rather than conflicting effects. The antibacterial effect of sealants was tested both in a planktonic growth inhibition assay and an agar diffusion assay.

Methods

Bacterial strains

ATCC25175 *S. mutans* strain (Microbiologics, Minnesota, USA); M18 DSM 14865 *S. salivarius* strain, also known as BLIS M18 (BLIS Technologies, Dunedin, New Zealand) distributed in Italy as Carioblis® (Omeopiacenza-Pontenure, Italy) and a mixture of DSM17938 and ATCC PTA5289 strains of *L. reuteri* (Prodentis; BioGaia, Lund, Sweden) were used as test microorganisms.

Brain hearth infusion broth (BHIB), being suitable for selected microorganisms, was used as nonselective culture medium.

Embrace™ WetBond™ Pit and Fissure Sealant (Pulpdent, USA) and UltraSeal XT® hydro™ (Ultradent, USA) were used as sealants.

Quantitative assessment of planktonic growth inhibition

Experimental setup included four 96-well microtiter plates containing light-cured Embrace™ (10 µl and 20 µl) and UltraSeal XT® (20 µl and 50 µl), respectively.

The plates were prepared by applying the sealant material on the side walls of 96-well microtiter plates. The material was light cured for 60 s to allow adhesion and solidification. The plates were then rinsed with 200 µl of sterile distilled water which was left in place for 30 min, incubating at 37°C. Water was then removed with a sterile pipette and substituted with chosen culture medium.

BHIB (200 µl) was added to each well and inoculated with 3×10^4 bacterial cells. The plates were incubated at 37°C for 24 h. After this time, growth inhibition was assessed with spectrophotometric technique. In particular, the plates were set up as shown in Table 1.

Assessment of antibacterial activity in disk diffusion test

BHIB was stored at 8°C–15°C then sterilized at 120°C for 15 min. Tested bacteria underwent overnight growth at 37°C. Bacterial sample were then placed in Petri dishes with 30 ml of sterilized BHIB allowed to solidify and subsequently added with soft agar.

Two samples for each tested bacterium were prepared, each of which containing two sealant disks. A control sample (sealant disks without bacteria) for each sealant was also set up.

Sealants disks (UltraSeal XT®-50 µl; Embrace™-20 µl) were light cured for 20 s and placed directly into Petri plates with sterile tweezers. Petri plates were incubated at 37°C for 48 h. Inhibition of bacterial growth was observed by the formation of halos with no visible bacteria, which were measured in millimeters.

Data analysis

The experimental protocol was repeated twice. Test–retest reliability was evaluated by assessing the intraclass correlation coefficient (ICC) based on the two repetitions. Mean values between the two repetitions were calculated and considered for the analysis. All data were recorded in Microsoft Excel datasheets, and statistical analysis was performed using IBM SPSS Statistics (v25, Inc., Chicago, IL, USA). Parametric methods were used as data followed a normal distribution verified using the Kolmogorov–Smirnov test. To compare two variables, the t-Student test was applied. For nonparametric data, the comparison of two variables was performed with the Mann–Whitney U-test. The significance level was set at $P < 0.05$.

Results

The overall ICC (test-retest reliability) based on the two experimental protocol repetitions was

Table 1: Experimental setup included four 96-well microtiter plates containing light-cured Embrace™ WetBond™ (10 µl and 20 µl) and UltraSeal XT® Hydro™ (20 µl and 50 µl) respectively

Tested effect	Composition
Test (antibacterial activity)	BHIB–*S. mutans*–sealant
Test (antibacterial activity)	BHIB–*S. salivarius*–sealant
Test (antibacterial activity)	BHIB–*L. reuteri*–sealant
Control (bacterial growth)	BHIB–*S. mutans*
Control (bacterial growth)	BHIB–*S. salivarius*
Control (bacterial growth)	BHIB–*L. reuteri*
Control (culture medium)	BHIB
Control (sealant)	BHIB–sealant

BHIB=Brain hearth infusion broth; *S. mutans=Streptococcus mutans; S. salivarius=Streptococcus salivarius; L. reuteri=Lactobacillus reuteri*

0.91, thus supporting the consistency of the two measurements.

Planktonic growth inhibition test

Optical density (OD) resulting from spectrophotometric analysis was used as bacterial quantification parameter. OD mean values with standard deviation (SD) both for bacterial growth control samples and for antibacterial activity samples are reported in Table 2 for Embrace™ and in Table 3 for UltraSeal XT®; *P* values are also presented.

The difference between OD mean values of control samples (BHIB with and without sealant) is only 0.02. Therefore, sealant dispersion results to be extremely low.

Embrace™ sealant shows a strong antibacterial activity against all of the three tested bacterial strains ($P < 0.0001$). In fact, it inhibits microorganism growth even at its lowest dose (10 µl), resulting in growth inhibition percentages higher than 90% in all cases.

UltraSeal XT® does not show growth inhibition activity against *Streptococcus spp*., neither at 20 µl and 50 µl doses. On the other hand, it has a mild antibacterial activity against *L. reuteri*, with growth inhibition rates being 19% and 23% for 20 µl and 50 µl, respectively ($P < 0.001$).

Antibacterial activity in disk diffusion test

Regarding UltraSeal XT® (50 µl) antibacterial activity against *L. reuteri, S. mutans,* and *S. salivarius*, growth inhibition halos were not noticeable around sealant disks. Once sealant disks were removed, inhibition by contact was evident, resulting in halos directly underlying the disks [Figure 1].

Given the planktonic growth inhibition test results, Embrace™ disk-diffusion test was performed with the lowest dose technically achievable (20 µl). The results showed exclusively an antibacterial activity by contact, since an inhibition halo was present only under the disks, after their removal [Figure 2].

Discussion

The aim of this study was to investigate the antibacterial activity of two hydrophilic sealants (Embrace™ and UltraSeal XT®) both in solid and in liquid assays, to perform a qualitative and quantitative analysis, respectively. To the best of our knowledge, there are no such studies reported in literature.

Control samples containing BHIB and bacteria showed high OD values, testifying a good growth of microorganisms. The difference of OD mean values between control BHIB sample and BHIB samples

Table 2: Embrace™ WetBond™ pit and fissure sealant mean and standard deviation of optical density resulting from planktonic growth inhibition tests

Embrace™ WetBond™ pit and fissure sealant-3×10⁴ cells						
Sample	**10 µl**	**Inhibition rate (%)**	***P****	**20 µl**	**Inhibition rate (%)**	***P****
S. mutans+sealant	0.097±0.011	95	<0.0001	0.102±0.018	97	<0.0001
S. mutans	0.798±0.043			0.660±0.09		
S. salivarius+sealant	0.099±0.010	95	<0.0001	0.103±0.014	98	<0.0001
S. salivarius	0.839±0.023			0.773±0.083		
L. reuteri+sealant	0.108±0.008	94	<0.0001	0.121±0.034	95	<0.0001
L. reuteri	0.899±0.038			0.798±0.055		
BHIB+sealant	0.087±0.006					
BHIB	0.067±0.005					

*Mann–Whitney U-test, $P < 0.05$. BHIB=Brain hearth infusion broth; *S. mutans=Streptococcus mutans; S. salivarius=Streptococcus salivarius; L. reuteri=Lactobacillus reuteri*

Table 3: UltraSeal XT® Hydro™ mean and standard deviation of optical density resulting from planktonic growth inhibition tests

UltraSeal XT® Hydro™-3×10⁴ cells						
Sample	**20 µl**	**Inhibition rate (%)**	***P****	**50 µl**	**Inhibition rate (%)**	***P****
S. mutans+sealant	0.847±0.015	−6	<0.0002	0.798±0.021	−6	<0.0001
S. mutans	0,766±0.026			0.724±0.047		
S. salivarius+sealant	0.883±0.044	−7	<0.001	0.962±0.053	−9	<0.0001
S. salivarius	0.793±0.023			0.854±0.027		
L. reuteri+sealant	0.73±0.028	19	<0.0001	0.7±0.04	23	<0.0001
L. reuteri	0.847±0.024			0.84±0.05		
BHIB+sealant	0.098±0.02					
BHIB	0.062±0.002					

*Mann–Whitney U-test, $P < 0.05$. BHIB=Brain hearth infusion broth; *S. mutans=Streptococcus mutans; S. salivarius=Streptococcus salivarius; L. reuteri=Lactobacillus reuteri*

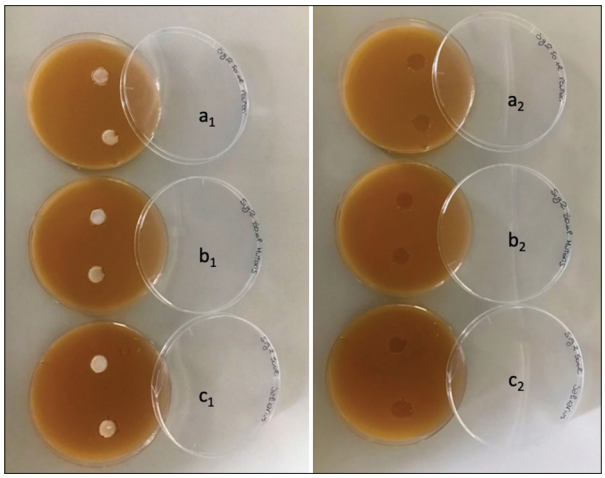

Figure 1: UltraSeal XT® disk diffusion test. Petri plates containing Brain hearth infusion broth added with *Lactobacillus reuteri* (a_1), *Streptococcus mutans* (b_1) and *Streptococcus salivarius* (c_1) and disks of UltraSeal XT® (50 µl) are shown on the left. On the right (a_2, b_2, c_2), Ultraseal sealant disks were removed from plates, showing underlying halos of growth inhibition by contact

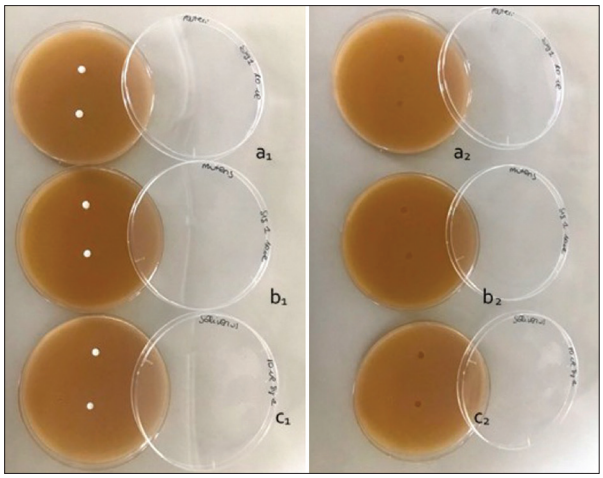

Figure 2: Embrace™ disk diffusion test. Petri plates containing Brain hearth infusion broth added with *Lactobacillus reuteri* (a_1), *Streptococcus mutans* (b_1) and *Streptococcus salivarius* (c_1) and disks of UltraSeal XT® (20 µl) are shown on the left. On the right (a_2, b_2, c_2), Ultraseal sealant disks were removed from plates, showing underlying halos of growth inhibition by contact

added with sealant is only 0.02, meaning that sealant dispersion is extremely low. Nevertheless, since this value increases the final OD measured, growth inhibition activity of tested sealants would probably be higher than the percentages experimentally obtained.

At planktonic inhibition test, Embrace™ showed a strong antibacterial activity against all of the three tested strains. Our previous pilot results showed that Embrace™ produces complete bacterial growth inhibition at higher doses (50 µl). Therefore, we decided to test it at lower doses (20 µl and 10 µl) to identify a possible inhibition threshold.

Even at low doses (10 µl), Embrace™ inhibits *L. reuteri*, *S. mutans,* and *S. salivarius* growth with extremely high rates (>90%) and with high significance level ($P < 0.0001$).

In the present study, the good performance of Embrace is in agreement with other studied which support its chemical, mechanical, and clinical effectiveness.[21-23]

Regarding UltraSeal XT® sealant, bacterial growth inhibition was not noticeable at low doses (10 µl). Therefore, we decided to test it with 20 µl and 50 µl volume.

UltraSeal XT® did not show any antibacterial activity against *S. mutans* nor *S. salivarius*, both at 20 µl and 50 µl volume. On the other hand, it showed a mild inhibition growth against *L. reuteri* both at 20 µl and 50 µl dose, with growth inhibition rates lower than 25% ($P < 0.01$).

This is in agreement with Güçlü *et al*. who reported that UltraSeal XT® discs failed to release any detectible free fluoride ions into deionized water during a 14-day observation period, indicating that any fluoride ion release was at a concentration below 0.001 ppm, being 0.0264 wt% the maximum fluoride ion concentration.[24]

At disk-diffusion solid assay, after 24 h 37°C incubation, all bacteria showed homogeneous growth around sealant disks, thus demonstrating a lack of antibacterial activity at distance.

Once the disks were removed, a halo underlying sealant disk was present in all cases. These findings suggest that, from a qualitative point of view, both Embrace™ (20 µl) and UltraSeal XT® (50 µl) elicit an antibacterial activity by contact against *L. reuteri, S. mutans,* and *S. salivarius*.

Therefore, pits and fissures sealing guarantees not only a mechanical seal, but also provides protection by contact to underlying and surrounding dental structures, thanks to continuous fluoride release.[25]

Embrace™ showed a strong antibacterial activity both in solid and liquid assays, either against examined cariogenic bacteria (*S. mutans*) and probiotic strains (*S. salivarius* and *L. reuteri*). Inhibitory action is due to fluoride release that acts against all tested bacteria indiscriminately. We suggest prescribing the probiotic first, to maximize its antagonism against cariogenic strains and putting off the Embrace™ sealant application to the end of probiotic treatment.

UltraSeal XT® has also shown antibacterial activity by contact against all three tested strains in disk-diffusion tests. In planktonic growth test, on the other hand, it elicited growth inhibition only against *L. reuteri*, with extremely low rates. UltraSeal XT® antibacterial

activity has not proven satisfactory, probably because of low fluoride content. Therefore, UltraSeal XT® can be applied both in the short term and during probiotic treatment, since its preventive effectiveness is exclusively due to mechanical seal.

Conclusions

It is recommended to carefully plan the timing for the administration of different preventive interventions, such as oral probiotics assumption and sealant application, to maximize their specific effectiveness.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis-A comprehensive review. J Clin Periodontol 2017;44 Suppl 18:S94-105.
2. Mathur VP, Dhillon JK. Dental caries: A disease which needs attention. Indian J Pediatr 2018;85:202-6.
3. Corrêa-Faria P, Paixão-Gonçalves S, Paiva SM, *et al*. Incidence of dental caries in primary dentition and risk factors: A longitudinal study. Braz Oral Res 2016;30:???. [doi: 10.1590/1807-3107BOR-2016.vol30.0059].
4. Abanto J, Tsakos G, Paiva SM, Carvalho TS, Raggio DP, Bönecker M. Impact of dental caries and trauma on quality of life among 5- to 6-year-old children: perceptions of parents and children. Community Dent Oral Epidemiol 2014;42:385-94.
5. Keyes P, Jordan H. Factors influencing initiation, transmission and inhibition of dental caries. In: Mechanisms of Hard Tissue Destruction. New York: Academic Press; 1963. p. 261-83.
6. Kutsch VK. Dental caries: An updated medical model of risk assessment. J Prosthet Dent 2014;111:280-5.
7. Suwansingha O, Rirattanapong P. Effect of fluoride varnish on caries prevention of partially erupted of permanent molar in high caries risk. Southeast Asian J Trop Med Public Health 2012;43:808-13.
8. Divaris K, Preisser JS, Slade GD. Surface-specific efficacy of fluoride varnish in caries prevention in the primary dentition: Results of a community randomized clinical trial. Caries Res 2013;47:78-87.
9. Slade GD, Sanders AE, Do L, Roberts-Thomson K, Spencer AJ. Effects of fluoridated drinking water on dental caries in Australian adults. J Dent Res 2013;92:376-82.
10. Greig V, Conway DI. Fluoride varnish was effective at reducing caries on high caries risk school children in rural Brazil: Question: What is the efficacy of 5% fluoride varnish in preventing decayed and filled surfaces increments among high-caries-risk children? Evid Based Dent 2012;13:78-9.
11. Maehara H, Iwami Y, Mayanagi H, Takahashi N. Synergistic inhibition by combination of fluoride and xylitol on glycolysis by mutans streptococci and its biochemical mechanism. Caries Res 2005;39:521-8.
12. Hannig M, Hannig C. Nanotechnology and its role in caries therapy. Adv Dent Res 2012;24:53-7.
13. Tawakoli PN, Al-Ahmad A, Hoth-Hannig W, Hannig M, Hannig C. Comparison of different live/dead stainings for detection and quantification of adherent microorganisms in the initial oral biofilm. Clin Oral Investig 2013;17:841-50.
14. Featherstone JD, White JM, Hoover CI, Rapozo-Hilo M, Weintraub JA, Wilson RS, *et al*. A randomized clinical trial of anticaries therapies targeted according to risk assessment (caries management by risk assessment). Caries Res 2012;46:118-29.
15. Meurman JH, Stamatova I. Probiotics: Contributions to oral health. Oral Dis 2007;13:443-51.
16. Cagetti M, Mastroberardino S, Milia E, *et al*. The Use of Probiotic Strains in Caries Prevention: A systematic review. Nutrients 2013;5:2530-50.
17. Beauchamp J, Caufield PW, Crall JJ, Donly K, Feigal R, Gooch B, *et al*. Evidence-based clinical recommendations for the use of pit-and-fissure sealants: A report of the American Dental Association Council on Scientific Affairs. J Am Dent Assoc 2008;139:257-68.
18. Anusavice KJ, Shen C, Rawls HR. Phillip's Science of Dental Materials. 12th ed. St. Louis, Missouri: Elsevier; 2013.
19. Holmgren C, Gaucher C, Decerle N, Doméjean S. Minimal intervention dentistry II: Part 3. Management of non-cavitated (initial) occlusal caries lesions--non-invasive approaches through remineralisation and therapeutic sealants. Br Dent J 2014;216:237-43.
20. Locker D, Jokovic A, Kay EJ. Prevention. Part 8: The use of pit and fissure sealants in preventing caries in the permanent dentition of children. Br Dent J 2003;195:375-8.
21. Gawali PN, Chaugule VB, Panse AM. Comparison of Microleakage and Penetration Depth between Hydrophilic and Hydrophobic Sealants in Primary Second Molar. Int J Clin Pediatr Dent 2016;9:291-5.
22. Askarizadeh N, Heshmat H, Zangeneh N. One-year clinical success of embrace hydrophilic and helioseal-F hydrophobic sealants in permanent first molars: A Clinical Trial. J Dent (Tehran) 2017;14:92-9.
23. Panigrahi A, Srilatha KT, Panigrahi RG, Mohanty S, Bhuyan SK, Bardhan D. Microtensile Bond Strength of Embrace Wetbond Hydrophilic Sealant in Different Moisture Contamination: An In-Vitro Study. J Clin Diagn Res. 2015;9:ZC23-5. DOI: 10.7860/JCDR/2015/11662.6178.
24. Güçlü ZA, Dönmez N, Hurt AP, Coleman NJ. Characterisation and microleakage of a new hydrophilic fissure sealant - UltraSeal XT® hydro™. J Appl Oral Sci. 2016;24:344-51.
25. Hamilton I, Bowden G. Fluoride effects on oral bacteria. In: Fluoride in Dentistry. Copenhagen: Munksgaard; 1996. p. 230-51.