

## Decrease in *Streptococcus mutans* after applying sealants to the occlusal surfaces of permanent teeth in adults

### *Decrecimiento de Streptococcus mutans después de la aplicación de sellantes en superficies oclusales de molares permanentes en adultos*

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#### ABSTRACT

**Introduction:** The main strategy for the prevention of caries disease is the use of pit and fissure sealants, which are indicated for posterior occlusal surfaces that are difficult to clean and cannot be protected very well by fluoride. This strategy is considered to be very important in caries prevention, especially in patients at high cariogenic risk. **Objective:** Evaluate whether the count of colony forming units of *S. mutans* per cm<sup>2</sup> on the occlusal surface of posterior permanent teeth changes after applying resin-based sealant. **Methods:** A study was conducted of 38 patients of both genders aged 18-30 years. The patients were at high cariogenic risk and had an indication of occlusal sealant application on at least one permanent posterior tooth. Two samples were taken of dental biofilm from the same teeth before (group T0) and after (group T1) applying sealant obtained by direct impression by tray technique with trypticase yeast extract, cysteine,

sucrose with bacitracin (TYCSB), and agar previously made and solidified. The samples were incubated at 37 °C for 48 hours. The colony forming units (CFU/cm<sup>2</sup>) were counted. The results were statistically analyzed with the Wilcoxon test at 95 % confidence. **Results:** Average expression in CFU/cm<sup>2</sup> before applying the sealant (T0) and standard deviation was 13.48 (± 14.2), whereas after applying the sealant (T1) it was 5.37 (± 8.90). There was a statistical difference between the two measures T0 and T1 (p< 0.05). **Conclusions:** Sealant application on occlusal surfaces of posterior permanent teeth significantly reduces the count of CFU/cm<sup>2</sup> of *S. mutans*.

**Keywords:** pit and fissure sealants; *Streptococcus mutans*; permanent dental restoration; composite resins.

## RESUMEN

**Introducción:** La estrategia principal para la prevención de la enfermedad de caries es el uso de selladores de fosas y fisuras, que están indicados para las superficies oclusales posteriores que son difíciles de limpiar y no se pueden proteger muy bien con fluoruro. Esta estrategia se considera muy importante en la prevención de caries, especialmente en pacientes con alto riesgo cariogénico. **Objetivo:** Evaluar si el recuento de unidades formadoras de colonias de *S. mutans* por cm<sup>2</sup> en la superficie oclusal de los dientes permanentes posteriores cambia después de aplicar un sellador a base de resina. **Métodos:** Se seleccionaron 38 pacientes con alto riesgo cariogénico, de ambos sexos entre 18 y 30 años de edad, con al menos un diente posterior permanente con una indicación de sellador oclusal. Se tomaron dos muestras de biofilm dental de los mismos dientes antes (grupo T0) y después (grupo T1) de aplicar sellador obtenido con una técnica de impresión directa por bandeja con extracto de levadura de tripticasa, cisteína, sacarosa con bacitracina (TYCSB), agar previamente preparado y solidificado, y se incubaron a 37 °C durante 48 horas. Se contaron las unidades formadoras de colonias (UFC/cm<sup>2</sup>). Los resultados se analizaron estadísticamente mediante la prueba de Wilcoxon con una confianza del 95 %. **Resultados:** El promedio de expresión en UFC/cm<sup>2</sup> antes de aplicar el sellador (T0) y la desviación estándar fue de 13,48 (± 14,2), y después de aplicar el sellador (T1) fue de 5,37 (± 8,90). Hubo una diferencia estadística entre las dos medidas T0 y T1 (p< 0,05). **Conclusiones:** La aplicación de sellador en las superficies oclusales de los dientes permanentes posteriores reduce significativamente el recuento de UFC/cm<sup>2</sup> de *S. mutans*.

**Palabras clave:** selladores de fosas y fisuras; *Streptococcus mutans*; restauración dental permanente; resinas compuestas.

## INTRODUCTION

Caries is one of the most common diseases affecting the oral cavity, and it has been described as a complex and chronic disease that slowly progresses in most patients.<sup>(1)</sup> It is not self-limiting; therefore, it can progress as far as complete destruction of the tooth. There are several factors involved in the process of dental caries, including the

bacteria that are immersed in the biofilm. Since of their virulence, they attach to the tooth surface, producing acids that lead to the loss of minerals from the dental surface. These bacteria include the *Mutans Streptococci* group, in which *Streptococcus mutans* (*S. mutans*) has been considered the main etiological agent of caries disease.<sup>(2)</sup>

Currently, the main strategy for the prevention of caries disease is the use of pit and fissure sealants, which are indicated for posterior occlusal surfaces that are difficult to clean and cannot be protected very well by fluoride. This strategy is considered to be very important in caries prevention, especially in patients with high cariogenic risk. There are two principal types of sealant materials: resin-based and glass ionomer-based. Existing evidence indicates that resin-based sealants are more effective in reducing caries on permanent teeth in children and adolescents; for this reason, resin-based sealants are the first choice for the sealing of pits and fissures.<sup>(3)</sup>

As sealant is a dental material that performs in the same way as any other resin restorative material, its surface properties affect bacterial adhesion and colonization. Evidence from *in vitro* and *in vivo* studies show that resin-based material tends to accumulate more bacteria and biofilm than the tooth enamel itself, moreover it has been reported that the activity of *S. mutans* could accelerate the degradation process of the resin composite and also the sealants.<sup>(4)</sup> Furthermore in the case of pit and fissure sealants, the research of Baca et al. showed a decrease of *Mutans* streptococci in saliva after the application of sealants in children without caries. This study did not specify the type of sealant material studied. Thus, it is important to clinically determine whether resin-based sealant truly has an effect in decreasing *S. mutans* on occlusal surfaces in adult patients with high cariogenic risk.<sup>(5)</sup>

A recent study show that there is adherence of *S. mutans* colonies to different restorative biomaterials. In both, the methodology of the tray technique was used, a simple method to be able to quantify colonies of *S. mutans*.<sup>(6)</sup>

The hypothesis proposed was that the number of *S. mutans* colony forming units (CFUs) per cm<sup>2</sup> on the occlusal surfaces of posterior permanent teeth decreases after the application of resin-based sealant. This clinical experimental study has principal objective to evaluate if the count of colony forming units of *S. mutans* by cm<sup>2</sup> on the occlusal surface of posterior permanent teeth change after applying resin-based sealant by the tray technique.

## METHODS

An observational clinic study was made since march to December of 2014. The size of the sample was calculated using the statistical program G\*Power©, Version 3.1.3

(Enrich-Heine, Universität Düsseldorf, Düsseldorf, Germany), based on the comparison of two dependent groups with a confidence level of 95% ( $\alpha = 0.05$ ) and statistical power of 80%. The size effect was moderate ( $\rho$  of 0.5). The analysis indicated that at least 35 patients were required, and the final N was 38 to accommodate possible patient drop-out. A total of 228 patients were examined at the clinic of the Universidad de Chile Dental School, and 143 of these were selected according to the inclusion and exclusion criteria of the study. Participants were documented in a Microsoft Office Excel spreadsheet, and through its randomized function, 38 patients were selected and documented in another Microsoft Office Excel spreadsheet (Version 2010).

**Inclusion criteria:** Patients between 18 and 30 years old who had at least one permanent posterior tooth with an indication for occlusal sealant; that is, if the permanent adult tooth had deep pits and fissures or if they were stained. Patients with high cariogenic risk according to the Cariogram Program 2.01 software (Mälmo, University, Mälmo, Sweden).

**Exclusion criteria:** Patients doing any of the following: taking drugs likely to reduce salivary flow; undergoing treatment with a mouthwash, antimicrobial gel, or fluoride toothpaste with 2500 ppm of fluorine ion or higher during the last three months; being under antibiotic treatment; having been classified as ASA III according to the American Society of Anesthesiologists; using fixed or removable prosthetics; using fixed or removable orthodontics, an acrylic device; chewing gum at least four or more days per week; or having a physical disability that precludes them from being responsible for their own hygiene.

**Collecting samples:** In the first session, the dental surfaces were cleaned with a brush and oil-free pumice to remove any food and biofilm particles after thorough visual examination with a number 5 mirror (Hu-Friedy Mfg Co. LLC. USA). The indication for sealant application was the presence of demineralized white or yellow-light brown areas around pits or fissures, which represent earlier enamel caries that are not decay. The use of a sharp probe was not necessary to detect early caries lesions. A recent bite-wing x-ray was used (at least 6 months) for all patients to confirm the diagnosis. This stage was performed by operator 1 (EF). In the second session, 2-3 hours after patients brushed their teeth, a sample of the biofilm was obtained with the tray technique from permanent premolar or molar teeth (used for their minimum area to determine the count) that were indicated for sealants (Group T0). Then, the same operator applied the occlusal sealant to the same tooth. A resin-based sealant without fluoride (Concise®, 3M Dental Products, St Paul, MN, USA) was applied to the enamel surface, which had previously been cleaned with a brush and oil-free pumice, etched with 37% phosphoric

acid for 15 seconds, then rinsed thoroughly with water for 30 seconds and dried, using rubber dam isolation (two operators NA-GM). After one month, a biofilm sample was obtained from the sealed tooth using the tray technique (Group T1), before to verify that the sealant fulfilled the conditions according to Ryge modified criteria (table) (marginal adaptation, anatomic form, surface roughness, luster)), the sealant was evaluated by the fourth calibrated operator (PV) (Cohen´s Kappa interoperators 0.75). After this sample was obtained, all other occlusal surface premolars and molars with sealant indication were sealed.

**Table - Ryge Criteria**

<b>Clinical characteristic</b>	<b>Alpha</b>	<b>Bravo</b>	<b>Charlie</b>
Marginal Adaptation	Explorer does not catch or has one way catch when drawn across the restoration/tooth interface	Explorer falls into crevice when drawn across the restoration/tooth interface	Dentin or base is exposed along the margin
Anatomic Form	The general contour of the restorations follows the contour of the tooth	The general contour of the restoration does not follow the contour of the tooth	The restoration has an overhang
Surface Roughness	The surface of the restoration does not have any surface defects	The surface of the restoration has minimal surface defects	The surface of the restoration has severe surface defects
Luster of Restoration	The restoration surface is shiny and has an enamel-like, translucent surface	The restoration surface is dull and somewhat opaque	The restoration surface is distinctly dull and opaque and is esthetically displeasing

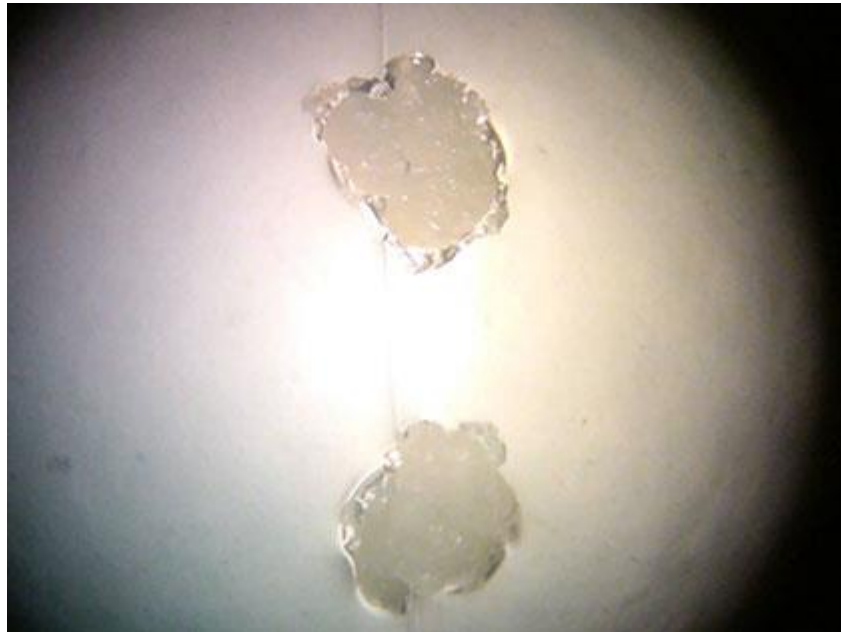
All sealed teeth were photographed. All photos were analyzed with the ImageJ® computational program (<http://rsb.info.nih.gov/ij/index.html>) to measure the occlusal areas of the premolars and molars. To determine the average of these values, the percentage of the occlusal surface area occupied by sealant was calculated. This stage was performed by operator number 5 (JRS).

**Microbiological procedure:** According the method previously validated,<sup>(7)</sup> the trays were prepared before the impression was taken. For this application, disposable fluoride gel application trays (Deepak Products Inc., Miami, FL, USA) were used, each of which was sterilized in a biosafety hood (Esco Technologies, Inc., Harboro, PA, USA) under ultraviolet light for 30 minutes. Each tray was then charged with 7.5 ml of TYCSB agar (L-cysteine 0.2 g/L, Bacto Casein 15.0 g/L (Difco Laboratories Inc., MI, Detroit USA)), yeast extract 5.0 g/L, sodium sulfite 0.1 g/L, sodium chloride 1.0 g/L, sodium phosphate 12 hydrate 2.0 g/L, sodium bicarbonate 2.0 g/L, sodium acetate trihydrate 20.0 g/L, sucrose 50.0 g/L, agar 15.0 g/L, distilled water (qsp) and 0.2 U/mL bacitracin selective medium for *S. mutans*. Subsequently, to individualize the sample, the trays were cut to fit no more than 3 teeth. Immediately after this process, the trays were placed in sterile petri plates and placed in sealed plastic bags in the refrigerator for storage. Before use, the trays were placed in an incubator/stove (LabTech Co.ZDP-A2080, Korea) for 24 hours at 37.°C as a quality control measure. Each sampling was performed by gently pressing the tray for 20 seconds over the tooth's occlusal surface. Then, the sample trays were stored in sterile Petri plates, transported at 4 °C and incubated at 37 °C in a microaerophilic container (jar candle CO<sub>2</sub> 10%) for 48 hours. Each tray was assigned a number by the sixth operator, who it entered the number into the database. (NA).

**Isolation, identification and counting of *S. mutans*:** This procedure was performed by the seventh operator (PP), who was blinded to the study parameters. The operator was calibrated for *S. mutans* counting before this analysis (Cohen's Kappa 0.8). After 48 hours of incubation, macroscopic counts of colonies compatible with *S. mutans* were performed according to the macromorphology and agar adherence, and the colonies were observed under an optical microscope (Stemi 2000 Carl Zeiss Microscopy, Thornwood, NY, USA) with a light source (Schott KL 1500, Carl Zeiss Microscopy, Thornwood, NY, USA). Later, Gram staining was performed to determine the micromorphology of the colonies.

The *S. mutans* count was expressed in colony forming units (CFUs) of *S. mutans* from the plates and trays with the TYCSB agar (Figure 1). Later, selected colonies compatible with *S. mutans* adhesion and morphological characteristics were suspended in Todd-Hewitt broth (Difco Laboratories Inc., MI, Detroit, USA) and incubated at 37 °C for 48 hours according the preciously validated methodology.<sup>(7)</sup> The colonies were then subjected to biochemical tests to identify the species of Mutans streptococci and to distinguish *S. mutans* from *Streptococcus sobrinus* (*S. sobrinus*), which has similar morphological characteristics but whose results differ in these tests. The biochemical tests included the raffinose fermentation, melibiose fermentation and esculin hydrolysis

tests. A positive result for all three tests indicated the presence of *S. mutans*. After 48 hours, each incubated broth sample was centrifuged (BD Sero-Fuge 2001, Clay-Adams Becton, Dickinson and Co., Sparks, MD, USA) for 5 minutes at approximately 1500 r.p.m. to obtain a pellet. The pellet was resuspended in 450  $\mu$ L of phosphate buffer (pH 7.2) to approximate a McFarland 5 standard. Then, 100  $\mu$ L of this suspension was inoculated in each biochemical test, e.g., esculin (Brain Heart Infusion, 1% esculin, Difco Laboratories Inc., MI, Detroit, USA), raffinose (1% of raffinose. Difco Laboratories Inc., MI, Detroit, USA) and melibiose (1% of melibiose, Difco, Laboratories Inc., MI, Detroit, USA), and incubated for 24 hours at 37 °C. Subsequently, two drops of ferric ammonium citrate were added to the melibiose and raffinose broths.



**Fig. 1** - *S. mutans* colonies on TYCSB agar exhibit their appearance (Observed under 4x magnification).

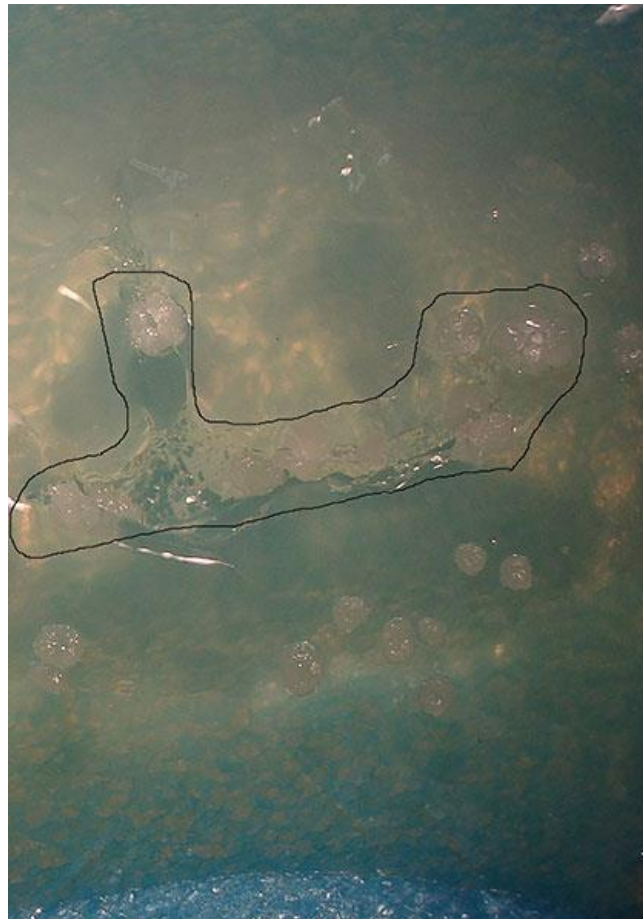
Statistical analysis was performed by a blinded operator who did not know which groups of *S. mutans* corresponded to T0 (before applying sealant) and T1 (After applying sealant). For the statistical analysis of the variables, the Statistical Package for Social Sciences (SPSS) version 15 for Windows (IBM. Inc. Chicago. IL, USA) was used, and to verify the data distribution, the *Shapiro-Wilk test* ( $p \leq 0.05$ ) was used. The *Wilcoxon test* was used with a significance level of 95%.

This research was approved by the ethics committee of the Dental School at the Universidad de Chile, number PRI-ODO 11-02. Each patient was informed in detail about the research and provided written informed consent. Since these patients had a high

cariogenic risk, they received preventive measures and a treatment plan according to their risk.

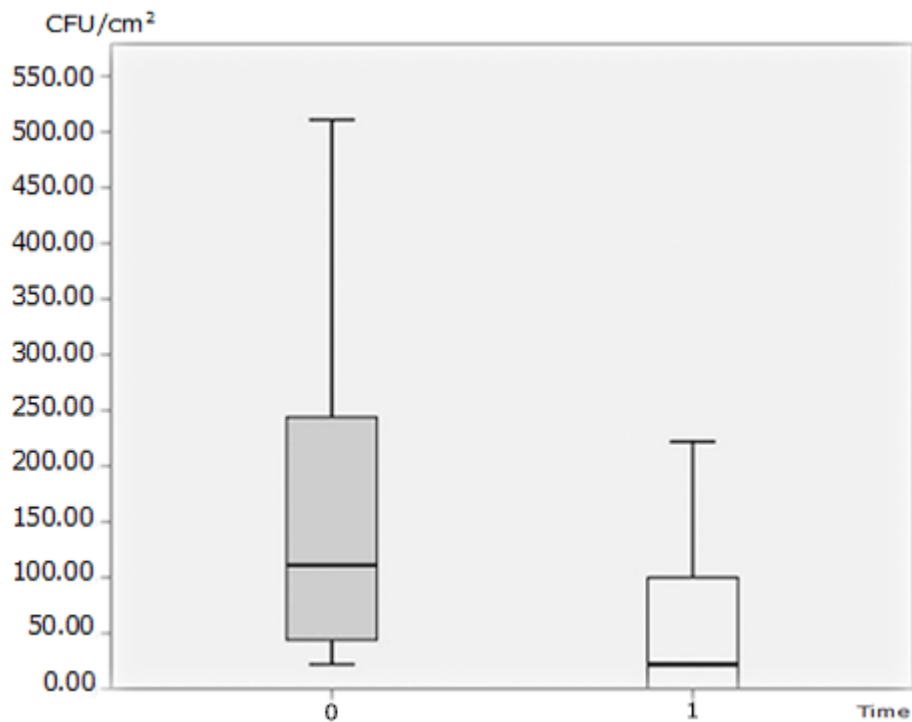
### RESULTS

Samples were obtained from 38 patients with mean age of 23.8 years. The total number of samples included 38 teeth ( $N= 38$ ), no patients dropped out, and no sealants were lost. Of the teeth, 57.9% (22) were permanent molars, and 42.1% (16) were premolars. The test gave positive results for *S. mutans* fermented raffinose, meliobiose (yellow coloration) and esculin (black coloration). The *S. mutans* isolated colonies were obtained with macroscopic properties (Fig. 1 and 2). The median CFU/cm<sup>2</sup> and standard deviation (DS) before applying sealant (T0) were 111.00 ( $\pm 199.42$ ), and these values after applying sealant (T1) were 22.00.54 ( $\pm 102.54$ ) CFU/cm<sup>2</sup> (Fig. 3). The Wilcoxon analysis showed a significant difference between the T0 and T1 measures ( $p < 0.05$ )



**Fig. 2** - *S. mutans* isolation from surface teeth using TR. The black line represents the outline of pit surface occlusal.





**Fig. 3** - CFUs of *S. mutans* distribution of groups T0 and T1.

## DISCUSSION

In the present clinical study, the counts of *S. mutans* CFU/cm<sup>2</sup> in the pit and fissure areas on the occlusal surfaces of posterior teeth were compared before and after applying resin-based sealant. A significant difference was found in the samples after applying sealant (T1), with a decrease in the CFU/cm<sup>2</sup> of *S. mutans*, and the proposed hypothesis was accepted.

The results of this study coincide with others reported by our research group, under similar conditions and with validated methodology. Vildósola et al. reported that on the surface of the composite resins adhere more *S* colonies of Mutans than on the surface of Amalgam restorations.<sup>(6)</sup> And in the present work, the sealant decreased the number of *S* Mutans colonies compared to before and after the application of a sealant on surfaces of premolars and molars. This could mean that the roughness of the surface would have a relevant effect on the adherence of *S. mutans* colonies. The amalgam is smoother and more polished than the composite resin, and the sealed surface is smoother and more polished than the unsealed tooth surface. This coincides with observational studies in the literature correlating the polish and smoothness of the surfaces.<sup>(8)</sup>

Currently, although there is sufficient evidence to show those sealants are effective in reducing caries in children and adolescents, there are no clinical studies that show a similar association in adults and whether there is a real decrease in *S. mutans* on the occlusal surfaces of teeth, especially in high-risk patients.<sup>(3)</sup> The only study similar to was carried out by Baca, which had similar results to this study. The study reported by Baca et al. included saliva samples that were obtained from two groups of children, with and without caries, showing fewer *Mutans streptococci* only in the group " without caries " after sealant application. Despite this result, the study by Baca only saliva samples were considered, the individual caries risk of each patient was not identified, the study was conducted only in children, and the sealant material used was not specified (with or without fluoride).<sup>(9)</sup> The present study was conducted with defined methodology and data; for example, all patients were classified according their caries risk, the patients were adults with an average age of 24 years, all received the same type of resin-based sealant without fluoride, all were previously assessed by sampling *S. mutans* (T1) with alpha Ryge criteria rather than on potential retention factors that could affect the recount. These results show that any difference found between the microbiological counts of *S. mutans* was mainly due to the influence of the material itself used in patients with similar characteristics.<sup>(6)</sup>

It has been recognized that caries is a multifactorial disease in which a number of factors interact. The *S. mutans* count is only one of several factors that promote the development of dental caries, and this factor alone is not a predictor of future disease. Moreover, it is known that some authors question the true role of *S. mutans* as the principal bacterium that produces caries, but it remains the microorganism present in early disease, and its metabolism produces acids that promote the demineralization of tooth enamel. From this perspective, any mechanical or chemical barriers that may be brought to intervene in the action of *S. mutans* will favor its reduction and logically, will decrease the local risk despite this acid production.

The results of this study confirm the necessity of sealing pits and fissures because sealants act as a potential mechanical barrier against microorganisms and most likely promote the reduction of bacterial adhesion and colonization, especially of cariogenic bacteria. Thus, retentive areas, such as deep fissures, are eliminated, eliminating the ecological harbors of bacteria.

The main limitation of this study was that the count of *S. mutans* was evaluated in the short term (1 month), and some studies have mentioned that the chemical composition of resin-based materials may increase bacterial colonization on their surfaces by several mechanisms that were described previously. Therefore, it would be interesting to know

how oral conditions change the resin-based sealant surface in the long term due to reports that the action of enzymes in human saliva, such as esterases, lead to the degradation of the organic resin matrix on a molecular level, which may influence the generation of microscopic retention or rough areas and lead to an increase in bacterial aggregation on resin-based materials. Moreover, it would be interesting to investigate a possible solution to the problem described above, regarding the remains of the sealant in the long term, as well as the real effect of the development of new sealants containing antibacterial molecules or particles, such as selenium organ or chitosan, which have been shown to exhibit sustained release over time against cariogenic bacteria, especially *S. mutans*.<sup>(10)</sup>

The findings of this study provide clinical information on the real importance of sealant application in young adult patients with high caries risk because, although there is adequate evidence that sealants are effective in children at high risk, the research is limited for adults, so it is necessary to increase and to improve the clinical studies on the subject.

The conclusion is in adult patients, the application of sealant on the occlusal surfaces of posterior permanent teeth contributes to a reduction in the CFU/cm<sup>2</sup> counts of *S. mutans* after one month, as evidenced by the difference in the counts before and after sealant application.

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### Conflict of interests

The authors declare no conflict of interests.

### Authorship declaration

Patricio Vildósola Grez, Eduardo Fernandez y Jose Roberto Cury Saad, they had a part in the conception of the idea and methodology of the investigation.

Jose Roberto Cury Saad, Patricia Palma Fluxá, Natalia Acuña Zepeda, Gustavo Moncada Cortés, they were in charge of applying the research methods and executing them.

Eduardo Fernandez y Alain Manuel Chaple Gil, they were in charge of the methodological adequacy of the research, the writing, the search of the references and the discussion of the article.

All authors made a review and approved the final version of this paper.

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