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ORIGINAL ARTICLE

Sucrose and Xylitol-Induced *Streptococcus mutans* Biofilm Adherence

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Abstract

Objective: To study the adherence of *Streptococcus mutans* biofilm after induction with sucrose and xylitol. **Material and Methods:** Laboratory experimental study incorporating posttest-only control group design. *S. mutans* biofilm was generated for 24 hours at a temperature of 37°C using BHIB with 5% sucrose and BHIB with 1% xylitol. An adherence assay was conducted in accordance with the method applied previously. The quantity of adhered bacteria was measured by means of a spectrophotometer at 570 nm. The data were presented as mean and standard deviation. **Results:** A biofilm induced with sucrose has a higher adherence level (0.9294 ± 0.0431) compared with one induced with xylitol (0.5095 ± 0.0392). Sucrose induces adherence levels by increasing glucan binding protein and glucosyltransferase of the bacteria, whereas xylitol will inhibit the glycolysis process of the bacteria. **Conclusion:** The adherence of sucrose-induced *S. mutans* biofilm is higher than that of xylitol-induced *S. mutans* biofilm.

Keywords: Dental Plaque; *Streptococcus mutans*; Sucrose; Xylitol.

Introduction

According to Indonesia's National Basic Health Research Department (RISKESDAS), in 2018, the national prevalence of oral disease was 57.6%, with a DMF-T index of 8.43. Such an elevated rate requires unprecedented promotive and preventive action on the part of health workers and dentists. Caries are the result of the demineralization of the teeth caused by metabolic activity within the bacterial biofilm covering its surface. Several bacteria are involved in caries development, but the predominant strain is *Streptococcus mutans*, which demonstrates the ability to metabolize carbohydrate as a food source. The end product of the metabolic activity is lactic acid, which can result in the demineralization of teeth [1].

Sucrose is a carbohydrate formed by two types of monosaccharides, namely glucose and fructose [2]. Sucrose, constituting the most cariogenic of all carbohydrates [3], is highly soluble in water and can diffuse readily into a biofilm. *S. mutans* produces soluble glucan, while insoluble glucan is derived from sucrose metabolism. This correlates with bacterial attachment, matrix formation and sucrose fermentation that forms lactic acid in the biofilm. Bacterial attachment can occur by means of fimbriae or Extracellular Polysaccharides (EPS). Biofilm adherence is one of the main problems currently subject to research, which is being conducted as a means of promoting an understanding of how bacterial colonization occurs both within and outside the oral cavity [4].

Caries prevention is being promoted on a global scale, one approach being the use of sugar substitute, also known as artificial sugar, believed to be capable of reducing the incidence of caries [5]. Sugar alcohols, such as manitol, sorbitol and xylitol, are commonly employed as sugar substitutes. Xylitol can reduce the incidence rate of caries by increasing salivary flow and oral pH, suppressing cariogenic bacteria and reducing oral plaque [6]. Bacterial attachment can occur by means of fimbriae or EPS. Biofilm adherence is one of the main problems currently being researched in order to promote an understanding of how bacterial colonization occurs both within and outside the oral cavity [4].

The effect of xylitol on *S. mutans* requires further investigation because that conducted to date has produced contradictory results with regard to how it affects this particular bacterium [7]. This research was undertaken in order to analyze *S. mutans* biofilm adherence after induction with sucrose and xylitol.

Material and Methods

Study Design

This research constituted a laboratory experimental study incorporating a posttest-only control group design. The study was conducted at the Microbiology Laboratory, School of Medicine, Brawijaya University.

Data Collection

S. mutans was cultured in Brain Heart Infusion Broth (BHIB) (Research Center, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia) and subsequently incubated for 24 hours at a temperature of 37°C inside an incubator [7]. An adherence assay was conducted in accordance with the method applied previously, although with slight modifications [8].

The bacteria were cultivated for 24 hours in polystyrene tubes at 37°C and an angle of 30° before being divided into three groups: a control group, treatment group I, and treatment group II. The Control Group contained cultured bacteria and BHIB, Group I contained cultured bacteria, BHIB and 5% sucrose, while Group II contained cultured bacteria, BHIB and 1% xylitol. The bacteria culture was then carefully poured from the tubes. Adhered cells were removed from the tube surfaces with NaOH 0.5M before being

centrifuged, washed, suspended in saline and quantified using a SmartSpec Plus Spectrophotometer (Bio-Rad, Life Science California, USA) at 570 nm [9].

$$\text{Percentage adherence} = \frac{\text{O. D of adhered cells x 100\%}}{\text{O. D. of total cells}}$$

Data Analysis

The data were presented as mean and standard deviation. The statistical analysis was performed using SPSS 21 Software.

Ethical Aspects

Ethical clearance from the Bioethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya (260/HRECL FODM/IX/2018).

Results

The optical density (OD) of adherence in BHIB with *S. mutans* as the control group was lower than that with 5% sucrose or BHIB with 1% xylitol. Sucrose-induced *S. mutans* biofilm demonstrated a higher adherence level than xylitol-induced biofilm (Table 1).

Table 1. Biofilm adherence of *Streptococcus mutans* after induction with 5% sucrose and 1% xylitol.

Biofilm Adherence Inductor	N	Mean	Std. Deviation
BHIB + *Streptococcus mutans* (Control)	6	0.2708	0.1238
BHIB + *Streptococcus mutans* + 5% Sucrose	6	0.9294	0.0431
BHIB + *Streptococcus mutans* + 1% Xylitol	6	0.5095	0.0392

Discussion

Induction with 5% sucrose and 1% xylitol to *S. mutans* was proven to affect biofilm formation and its adherence. The result obtained from this research showed biofilm adherence after induction with 5% sucrose to be higher than that of 1% xylitol-induced biofilm adherence.

Adherence is one of the factors affecting biofilm formation. Bacterial adherence may occur because bacteria possess receptors that receive the adhesin produced [10]. Bacterial adherence is affected by multiple factors, such as nutrition and oral hygiene. The administering of sucrose to *S. mutans* increases its biofilm adherence. Excessive bacterial adherence can lead to the accumulation of bacteria on the surface of teeth. Such a build-up of bacteria, especially *S. mutans* in the oral cavity, can prove harmful to the host [11].

Biofilm formation depends on multiple factors, one of them being nutrition, which also plays an important role in bacterial viability. *S. mutans* is no exception since it demonstrates virulence factors, one of which is adhesion. For adhesion to work, bacteria require nutrition to produce EPS in order that the bacteria can form a bond between themselves and the tooth surface [12].

S. mutans adherence can occur through one of two mechanisms, namely, a sucrose-dependent mechanism or a sucrose-independent mechanism [13]. In the former, Glucosyltransferase (Gtf) will turn sucrose into glucan in the *S. mutans* cells, strengthen the bond between bacteria and increase bacterial adherence. In addition to Gtf, Glucan binding protein (Gbp) also plays a role in sucrose-dependent mechanisms by acting as a receptor for glucan on the cell surface. Glucan produced by *S. mutans* consists of two types,

namely; water-soluble glucan and water-insoluble glucan. Water-insoluble glucan increases the mechanic stability of biofilm by binding bacteria to the tooth surface [14].

The results of this study are consistent with those of the research conducted previously, which indicated an increase in the biofilm adherence of S. mutans bacteria induced by 3% sucrose and a decrease in biofilm adherence induced by 3% xylitol [15]. Previous research showed no significant difference between the treatment group *S. mutans* induced by 5% sucrose and the treatment group *S. mutans* induced by 1% xylitol. The addition of inductors to *S. mutans* affects its biofilm adherence [7].

Increased *S. mutans* adherence with a sucrose-independent mechanism is induced by Antigen I/II (Ag I/II). Ag I/II bonds with salivary agglutinin, which is gp-340. Gp-34 functions as a binder between the bacteria and viruses present in the body with the result that these are eliminated. However, if the gp-340 attaches to the tooth surface, its role will change as a receptor for Ag I/II and it will increase the adherence of *S. mutans* to the tooth surface [13]. Daily use of xylitol can reduce the incidence of caries by stimulating increased salivary flow, suppressing biofilm growth and decreasing the amount of *S. mutans* that plays an important role in the formation of biofilm and caries [16]. The reduced adherence of S *S. mutans* biofilms, induced by the application of 1% xylitol, can occur because when xylitol enters cells, it will be processed through a phosphoenolpyruvate-phosphotransferase (PEP-PTS) cycle and become xylitol-5-phosphate [17]. Xylitol-5-phosphate is toxic to S. mutans cells and, consequently, must be eliminated in its original form through a dephosphorylation mechanism, which will consume considerable amounts of energy [7].

Xylitol decreases the levels of *S. mutans* in plaque and saliva by disrupting their energy production processes, leading to a futile energy cycle and cell death. It reduces the adhesion of these microorganisms to tooth surfaces and also their acid production potential. *S. mutans* transports the sugar to the cell in an energy-consuming cycle that is responsible for growth inhibition. Xylitol is then converted to xylitol-5-phosphate via phosphoenolpyruvate: a fructose phosphotransferase system involving *S. mutans*, which results in the development of intracellular vacuoles and cell membrane degradation [5,7]. Moreover, xylitol inhibits the glycolysis process by competing with the phosphofructokinase enzyme leading to insoluble polysaccharides reduction that plays an important role in bacterial adherence [17]. Actions that can be taken to decrease bacterial adherence include brushing the teeth and maintaining oral hygiene through the use of xylitol. In previous studies, 3% of xylitol was proved capable of decreasing S. mutans biofilm adherence [15].

Conclusion

The adherence of sucrose-induced *S. mutans* biofilm is higher than that of xylitol-induced *S. mutans* biofilm.

Authors' Contributions

PN	0000-0003-4779-2714	Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft Preparation and Writing - Review and Editing.
SP	0000-0001-5266-8967	Investigation, Formal Analysis and Writing - Review and Editing.
ASP	0000-0003-3352-6167	Formal Analysis, Writing - Original Draft Preparation and Writing - Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

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