Submitted: 23 August, 2024 Accepted: 31 October, 2024 Published: 03 July, 2025 DOI:10.22514/jocpd.2025.087

ORIGINAL RESEARCH

Journal of Clinical Pediatric Dentistry

In vitro effects of different sugar alcohol concentrations on the cariogenic biofilm formed on fissure sealants

Samyeli Sarihan[1,2,*], Merve Yildirim Ucuncu[3], Nursen Topcuoglu[3], Betul Kargul[2,4], Figen Eren[2]

[1]Department of Pediatric Dentistry, Institute of Health Sciences, Marmara University, 34854 Istanbul, Türkiye
[2]Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, 34854 Istanbul, Türkiye
[3]Department of Basic Medical Sciences, Faculty of Dentistry, Istanbul University, 34134 Istanbul, Türkiye
[4]Queen Mary University of London, E1 2AD London, UK

***Correspondence**
samyelisarihan@marun.edu.tr
(Samyeli Sarihan)

Abstract

Background: This study aimed to evaluate the effects of erythritol and xylitol, alone or in different combinations, on cariogenic biofilm formation on various fissure sealants. **Methods**: The triple biofilm formation of *Streptococcus mutans* ((American Type Culture Collection) ATCC-25175), *Streptococcus sobrinus* (ATCC-33478), and *Scardovia wiggsiae* ((German Collection of Microorganisms and Cell Cultures) DSM-22547), was evaluated on samples of hydroxyapatite (HA) disks and disks prepared from resin-based and glass ionomer fissure sealants. The biofilm formations on the samples were determined by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). **Results**: The lowest biomass and biofilm thickness, indicating a notable inhibitory effect on bacterial growth, corresponded to the combination of 2.5% xylitol + 7.5% erythritol. In addition, biofilm formation on glass ionomer surfaces was lower than on HA and resin-based surfaces. **Conclusions**: Based on these results, erythritol shows antimicrobial activity at high concentrations and, combined with xylitol, it seems to exhibit a synergistic bacteriostatic effect useful for anti-cariogenic agents. Nevertheless, validation using well-designed clinical trials is needed to assess the long-term safety and efficacy of these sugar alcohols in oral health applications.

Keywords

Biofilm; Xylitol; Erythritol; Fissure sealant

1. Introduction

The World Health Organization (WHO) Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with three out of four people affected living in middle-income countries. Globally, an estimated 2 billion people suffer from caries of permanent teeth, and 514 million children suffer from caries of primary teeth [1]. Dental caries is an infectious disease caused by the acids produced by the fermentation of carbohydrates by bacteria in dental plaque. The local demineralization of dental tissues is primarily due to the effects of lactic acid [2]. Mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus* in humans) and sucrose are considered the main regulators of cariogenic biofilm development [3]. Glycosyltransferases (Gtfs) from *S. mutans* can produce extracellular polysaccharides (EPS) from sucrose and starch, allowing bacterial adaptation to acidic environments and other stresses. Sucrose fermented by oral bacteria creates an acidic microenvironment leading to enamel demineralization [4].

In 1975, Mäkinen *et al*. [5] first reported that xylitol could substantially reduce dental caries by inhibiting the growth of *Streptococcus mutans*. Thus, replacing all or part of sucrose in the human diet with xylitol reduced the incidence of dental caries by more than 85%. According to Mäkinen *et al*. [5], most *S. mutans* strains transport xylitol into the cell via the phosphotransferase system, where xylitol is phosphorylated to xylitol-5-phosphate and subsequently expelled from the cell. This energy-consuming pathway is thought to be responsible for inhibiting the growth of *S. mutans*. Erythritol was also reported to prevent dental caries [6], but fewer studies have evaluated its efficacy.

Erythritol is the newest type of polyol (sugar alcohol) used as a sweetener. Erythritol is rapidly and almost completely absorbed in the small intestine, without being metabolized, and is excreted unchanged in the urine, unlike other sugar alcohols such as sorbitol and xylitol. Because of its sweet taste and high digestive tolerance, erythritol is used as a sugar substitute in foods, substantially reducing their calorie content. All dental and oral biological studies conducted to date have shown that erythritol does not contribute to dental caries formation [7].

Combinations of xylitol and erythritol have been proposed to reduce caries incidence more effectively than any single polyol used alone; however, few studies have examined the synergistic inhibitory effect of xylitol and erythritol on cariogenic bacteria [3, 8]. A previous study that evaluated erythritol and xylitol in combination found that the effects of these sugar alcohols were not synergistic, suggesting a potential additive effect. However, that investigation assessed these mixtures' impacts on individual bacterial species [8]. By contrast, the

current study examines these cariogenic bacteria collectively, aligning with the examination of the oral microbiota.

Studies have demonstrated that fluoride application and individual oral care alone are insufficient to halt pit and fissure caries. Because of their anatomical structure, pits and fissures are more prone to caries formation compared to the tooth's smooth surfaces. Therefore, researchers have focused on developing specialized treatments to address this issue. For instance, pit and fissure sealants are thought to prevent the accumulation of food debris in those structures [9]. However, no studies have examined the effects of sugar alcohols on biofilm formation on fissure sealants used in pediatric dentistry.

Therefore, the current study aims to investigate the effects of erythritol and xylitol, prepared alone and in combinations at various concentrations, on cariogenic biofilm formation on fissure sealants. Furthermore, this study includes *Scardovia wiggsiae*, a less-studied but important pathogen in early childhood caries. *S. wiggsiae* has been identified in infected pulp samples from pediatric patients, suggesting a potential role as a key member of the microbial consortium associated with the progression of deep dentinal caries [10]. A study reported increased biofilm formation by *S. wiggsiae* in the presence of polyols, suggesting that these sugar alcohols may not exert the same effect on all cariogenic bacteria [3]. Thus, we incorporated *S. wiggsiae* into our study to address this knowledge gap. This study is expected to contribute to the literature by examining the biofilm characteristics of the three main bacteria responsible for dental caries: *S. mutans* (ATCC-25175), *S. sobrinus* (ATCC-33478) *and S. wiggsiae* (DSM-22547), using confocal laser scanning microscopy and scanning electron microscopy.

2. Materials and methods

2.1 Determination of study groups and preparation of sugar alcohols

Five study groups were established to determine the effects of different concentrations of sugar alcohols on the cariogenic bacteria included in the study: 10% erythritol (CAS No. 149-32-6, Sigma, St. Louis, MO, USA), 10% xylitol, 5% xylitol + 5% erythritol mixture, 7.5% xylitol + 2.5% erythritol mixture, and 2.5% xylitol + 7.5% erythritol mixture. These sugar alcohol solutions were prepared according to the method of Kõljalg *et al*. [3]. In addition, a control containing 5% sucrose was included as a sixth group, based on the method of Staszczyk *et al*. [11]. Different stock solutions of sugar alcohols were prepared at the Faculty of Dentistry, Department of Basic Sciences, Marmara University. The desired concentrations of sugar alcohols were obtained by sterilizing them by filtration and then adding them to a brain-heart infusion (BHI) broth; this procedure was conducted at the Faculty of Dentistry, Microbiology Research Laboratory, Istanbul University.

2.2 Preparation of bacterial suspensions

Streptococcus mutans (ATCC-25175), *Streptococcus sobrinus* (ATCC-33478) and *Scardovia wiggsiae* (DSM-22547) were the strains used in this study. These strains were obtained from the bacterial collection of the Faculty of Dentistry, Microbiology Research Laboratory, Istanbul University. *S. wiggsiae* was cultured on Brucella agar (Merck KGaA, DE, Darmstadt, Germany) supplemented with 5% sheep blood, 5 mg/mL hemin, and 5 mg/mL vitamin K. *S. mutans* and *S. sobrinus* were cultured on brain-heart infusion (BHI) agar (Merck) in an anaerobic chamber with a gas mixture of 80% nitrogen (N_2), 10% carbon dioxide (CO_2) and 10% hydrogen (H_2) at 37 °C for 48 h. Bacterial suspensions were prepared by transferring colonies from the incubated plates to BHI broth supplemented with yeast extract, hemin, and vitamin K, and then diluting 100-fold to a final concentration of 10^6 CFU/mL.

2.3 Preparation of the disks

Three types of disks representing different surfaces (hydroxyapatite (HA), resin-based fissure sealants, and glass ionomer fissure sealants) were used in this study. Resin-based and glass ionomer fissure sealants were prepared according to the manufacturer's instructions.

Forty-eight sterile standard HA samples (HAD40, HiMed, USA) with a diameter of 5 mm and a thickness of 1.8 mm were purchased and used in the triple biofilm studies, without polishing.

Resin-based samples (Clinpro, 3M ESPE, St. Paul, MN, USA) with a diameter of 5 mm and a thickness of 1.8 mm were placed in silicone molds. Pressure was applied with glass slides and the samples were polymerized for 20 s, using a light-curing device (GC D-Light Duo LED Curing Light, Tokyo, Japan). The samples were then exposed to light from the opposite side for an additional 10 s.

Capsulated glass ionomer components (Fuji Triage, GC Dental, Tokyo, Japan) were mixed using a vibrator (Silamat S5, Vivadent, Schaan, Liechtenstein) for 10 s. Then, specimens with a diameter of 5 mm and a thickness of 1.8 mm were obtained by dispensing the mixture into silicone molds, using a capsule applier (capsule dispenser, GC Dental, Tokyo, Japan). Mylar strips were placed at the bottom and top of the molds, over glass slides. The samples were cured for 2 min and 30 s. Subsequently, a surface conditioner (Fuji Coat, GC Dental, Tokyo, Japan) was applied to both sides and polymerized for 20 s with the light-curing device (GC D-Light Duo LED Curing Light). The samples were not polished.

Each study group included eight HA disks, eight Clinpro fissure sealant disks, and eight Fuji Triage fissure sealant disks. Because there were six solution groups, a total of 48 HA disks, 48 Clinpro disks, and 48 Fuji Triage disks were obtained. The prepared materials were stored at 37 °C for 24 h under conditions that mimicked the moist oral environment with reduced oxygen availability to allow for complete polymerization of the resin and glass ionomer. The prepared materials were sterilized using the hydrogen peroxide gas plasma sterilization method.

2.4 Preparation of artificial saliva

Artificial saliva was prepared according to the formula of Pratten *et al*. [12]. The formula contained 1 g/L Lab-Lemco powder, 2 g/L yeast extract, 5 g/L proteose-peptone, 2.5 g/L porcine gastric mucin, 0.35 g/L sodium chloride, 0.2 g/L calcium chloride, and 0.2 g/L potassium chloride in distilled

water. After autoclaving, 1.25 mL of a 40% urea solution was added. The pH of the saliva substitute was adjusted to 7. The solution was then passed through a filter (0.22 μm) into a sterile container.

2.5 Biofilm formation experiment with fissure sealant materials

Sterile 48-well tissue culture plates (Greiner, Sigma-Aldrich, Kremsmünster, OÖ, Austria) were used for the *in vitro* triple biofilm experiment. Sterile fissure sealant and HA disks were placed in the wells. Then, 500 μL of the prepared artificial saliva was added to the disks, followed by incubation at 37 °C for 1 h on a shaker. Subsequently, the disks were rinsed with phosphate-buffered saline (PBS) and placed in new, sterile 48-well tissue culture plates containing 1.6 mL of six different sugar alcohol solutions and 200 μL of bacterial suspension. The plates were incubated anaerobically at 37 °C for 24 h. Next, the plates were washed with 2 mL of PBS and transferred to new wells. All experiments performed in at least duplicate.

2.6 Confocal laser scanning microscope (CLSM) evaluation

Seven samples from each of the 18 groups, for a total of 126 disks, were evaluated using a CLSM (Leica CLSM, Leica Microsystems, Heidelberg, BW, Germany). The disks were transferred to sterile 24-well tissue culture plates (Greiner, Sigma-Aldrich, Kremsmünster, OÖ, Austria) and 20 μL of the LIVE/DEAD BacLight Kit (lot number: 1910798, Invitrogen, Thermo Fisher Scientific, Eugene, OR, USA) staining solution was added. Plates containing samples were covered with aluminum foil and kept in the dark at room temperature for 15 min, then stored in a refrigerator at 4 °C until use. The samples were imaged using the CLSM with optical lenses at a 10× magnification and at wavelengths of 488 nm and 532 nm. Images of three different points on each sample were obtained, and a series of photographs, showing bacterial viability in red and green colors, were taken. The biomass and thickness of the triple biofilm on the sample surface were measured using the COMSTAT software (version 2.0, Technical University of Denmark, Kongens Lyngby, Denmark) ($\mu m^3/\mu m^2$).

2.7 Scanning electron microscope (SEM) imaging and analysis

One disk from each of the 18 groups was placed in 48-well tissue culture dishes. Then, 1 mL of 4% formaldehyde was added, and the dish was covered with parafilm for preparation for SEM imaging. The samples were fixed in 4% formaldehyde for 40 min, washed with water, and dehydrated using a graded series of ethylene glycol solutions (50%, 75%, 90% and 2 × 100%). The dehydrated samples were coated with 15 nm gold, using the coating machine at the Department of Electrical-Electronic Engineering, Biomedical Imaging and Diagnostic Systems Laboratory, Marmara University. Then, the samples were examined by SEM (Zeiss EVO MA10, Carl Zeiss AG, Oberkochen, BW, Germany) at 20 kV and 3000× and 5000× magnifications.

2.8 Statistical analysis

The data were analyzed using IBM's SPSS Statistics 22 software (version 22, IBM Corporation, Armonk, NY, USA). The normality of the parameter distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Because the data were normally distributed, a two-way Analysis of Variance (ANOVA) test followed by a *post-hoc* Tukey test was used to evaluate the effects of the different solutions and materials on the biomass, average thickness, and maximum thickness values. Significance was determined at $p < 0.05$.

3. Results

3.1 Evaluation of biofilm formation using CLSM and COMSTAT

A CLSM analysis of images of live and dead cells in bacterial biofilms formed on three different surfaces (HA, Clinpro, and Fuji Triage) was performed. In the live/dead cell-staining procedure, the green dots indicate live cells, the red dots indicate dead cells, and the yellow dots indicate both live and dead cells. After evaluating all surfaces and solutions, the highest cell density was observed in the 5% sucrose solution. By contrast, the lowest cell densities were observed in the 2.5% xylitol + 7.5% erythritol and 5% xylitol + 5% erythritol solutions. In the case of the fissure sealants, the cell density of the triple biofilm on the Clinpro surface was higher than that on the Fuji Triage surface (Fig. 1).

Using CLSM and COMSTAT, we evaluated the biomass, average thickness, and maximum thickness of the triple biofilms formed by *Streptococcus mutans*, *Streptococcus sobrinus* and *Scardovia wiggsiae* on HA, Clinpro, and Fuji Triage surfaces (Fig. 2). Considerable differences in the biofilm characteristics were observed among the different solutions and materials, highlighting the efficacy of xylitol and erythritol in biofilm inhibition.

Significant differences were observed in the biomass values of the dead cells, live cells, and total cells among the solutions, materials, and their combinations ($p < 0.05$) (Table 1).

The 2.5% xylitol + 7.5% erythritol solution resulted in the lowest live-cell biomass of the triple biofilm formed on HA. The live-cell biomass in the 5% xylitol + 5% erythritol solution was also significantly lower than those in the 10% xylitol, 2.5% erythritol + 7.5% xylitol, and 5% sucrose solutions ($p < 0.05$).

The live- and total-cell biomass of the triple biofilm on the Fuji Triage material was significantly higher in the 10% xylitol solution than in the 5% xylitol + 5% erythritol and 2.5% xylitol + 7.5% erythritol solutions. The live- and total-cell biomasses of the triple biofilm in the 10% erythritol solution were significantly higher than those in the 2.5% xylitol + 7.5% erythritol solution ($p < 0.05$).

The live- and total-cell biomasses of the triple biofilms on Clinpro in the 10% erythritol, 5% xylitol + 5% erythritol, and 2.5% xylitol + 7.5% erythritol solutions were significantly higher than those on HA and Fuji Triage ($p < 0.05$) (Fig. 2, Table 2).

The lowest average thickness of the live-cell triple biofilm on HA was observed in the 2.5% xylitol + 7.5% erythritol solution ($p < 0.05$).

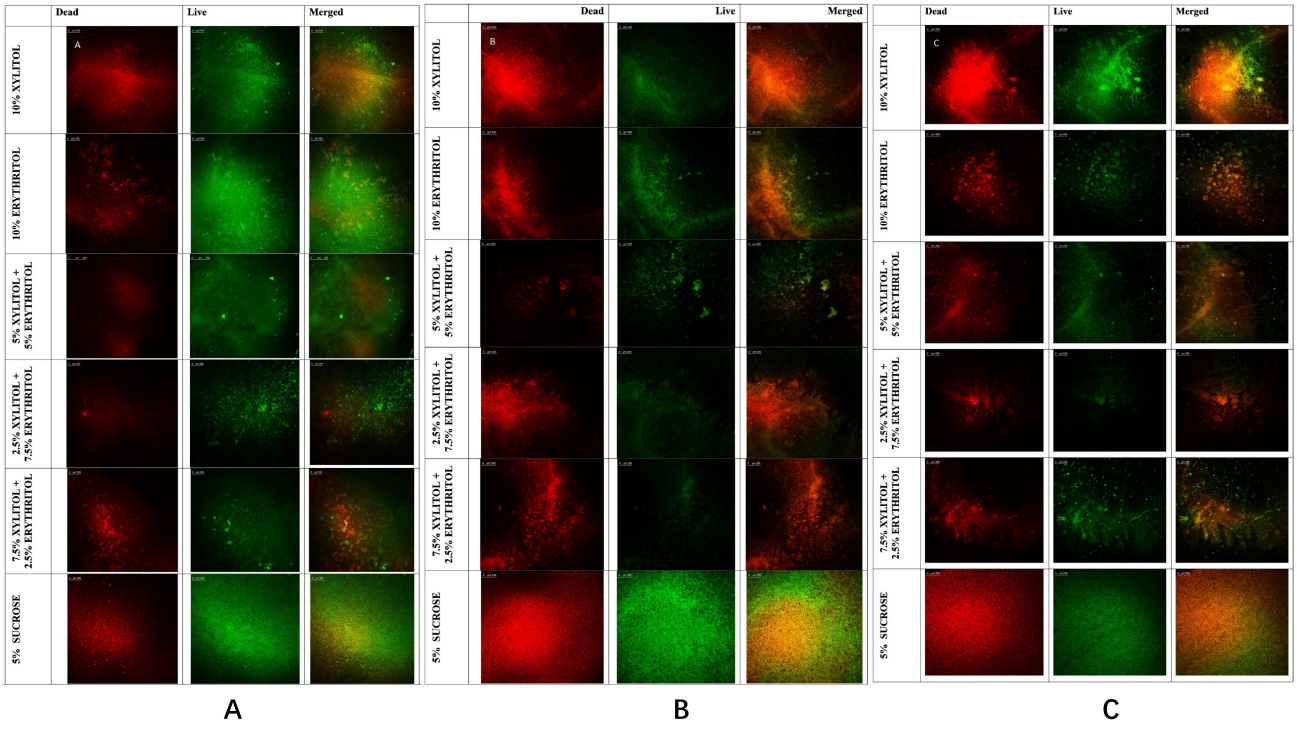

FIGURE 1. Confocal laser scanning microscopy (CLSM) images of the triple biofilms formed on the disk surfaces (the bar denotes 250 μm). (A) Hydroxyapatite surface. (B) Clinpro surface. (C) Fuji Triage surface.

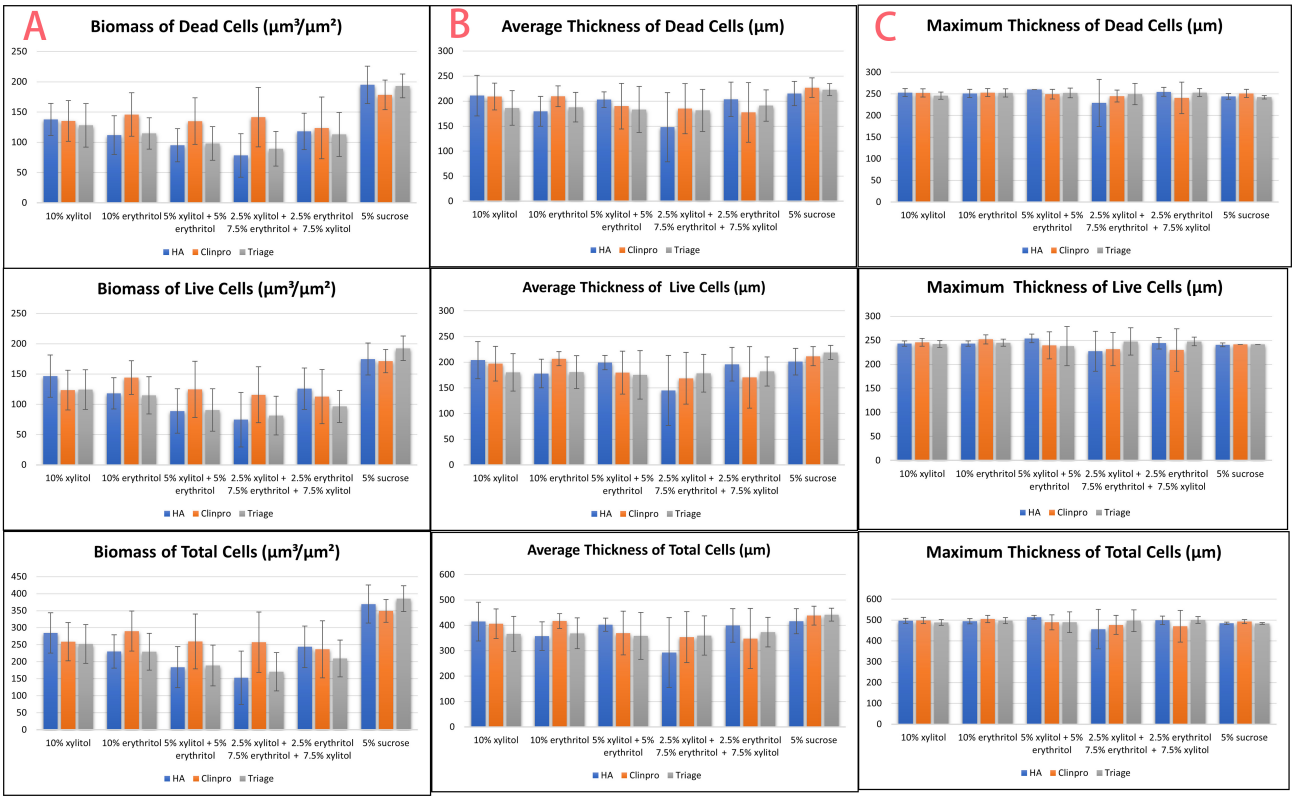

FIGURE 2. Quantitative analysis of the biomass and thickness of triple biofilms formed on three different surfaces, using confocal laser scanning microscopy. (A–C) Biomass ($\mu m^3/\mu m^2$) and thickness (μm) of dead, live, and total bacterial cells in the biofilms formed on three different surfaces: HA, Clinpro, and Fuji Triage. The data are presented as mean values with standard deviations. The measurements comprise the biomass and thickness of dead cells (top row), live cells (middle row), and total cells (bottom row) across the different conditions. HA: hydroxyapatite.

TABLE 1. Results of the Tukey test that was conducted to evaluate the effects of the different solutions and materials on the biomass ($\mu m^3/\mu m^2$) values.

Biomass ($\mu m^3/\mu m^2$)	Source	Type III sum of squares	*df*	Mean square	*F*	*p*
Dead cells						
	Solution	301,378.20	5	60,275.65	53.093	0.001*
	Material	35,772.05	2	17,886.02	15.755	0.001*
	Solution × material	53,909.43	10	5390.94	4.749	0.001*
Live cells						
	Solution	308,110.60	5	61,622.12	52.685	0.001*
	Material	15,436.94	2	7718.47	6.599	0.002*
	Solution × material	54,600.50	10	5460.05	4.668	0.001*
Total cells						
	Solution	1,211,083.00	5	242,216.70	63.081	0.001*
	Material	96,034.20	2	48,017.10	12.505	0.001*
	Solution × material	201,175.30	10	20,117.53	5.239	0.001*

*Two-way ANOVA test. *p < 0.05.*

TABLE 2. Effects of different solutions and materials on the biomass ($\mu m^3/\mu m^2$) values of the biofilms.

Biomass ($\mu m^3/\mu m^2$)	Hydroxyapatite (Mean ± Standard Deviation (SD))	Clinpro (Mean ± SD)	Fuji Triage (Mean ± SD)	*p*
Dead cells				
10% xylitol	137.75 ± 26.54	135.29 ± 33.67	128.01 ± 35.91	0.599
10% erythritol	111.85 ± 31.86	145.86 ± 35.99	114.65 ± 25.89	0.001*
5% xylitol + 5% erythritol	95.08 ± 27.41	134.99 ± 38.30	97.97 ± 27.76	0.001*
2.5% xylitol + 7.5% erythritol	78.22 ± 35.98	141.58 ± 49.20	89.09 ± 28.63	0.001*
2.5% erythritol + 7.5% xylitol	118.03 ± 30.09	123.66 ± 51.01	112.92 ± 36.44	0.688
5% sucrose	194.87 ± 31.01	178.28 ± 24.65	193.11 ± 19.56	0.077
p	0.001*	0.001*	0.001*	
Live cells				
10% xylitol	146.57 ± 34.95	123.50 ± 32.89	124.30 ± 32.88	0.048*
10% erythritol	118.02 ± 25.76	144.06 ± 27.68	114.79 ± 30.67	0.002*
5% xylitol + 5% erythritol	88.92 ± 36.84	124.73 ± 46.46	90.58 ± 35.02	0.007*
2.5% xylitol + 7.5% erythritol	74.53 ± 45.14	115.73 ± 46.27	81.50 ± 32.00	0.005*
2.5% erythritol + 7.5% xylitol	125.83 ± 34.24	112.75 ± 44.69	96.55 ± 26.41	0.036*
5% sucrose	174.64 ± 26.39	171.25 ± 19.28	192.46 ± 20.32	0.006*
p	0.001*	0.001*	0.001*	
Total cells				
10% xylitol	284.32 ± 59.38	258.79 ± 56.47	252.31 ± 57.10	0.173
10% erythritol	229.87 ± 48.97	289.93 ± 58.74	229.44 ± 53.98	0.001*
5% xylitol + 5% erythritol	184.00 ± 60.32	259.72 ± 80.49	188.55 ± 60.02	0.001*
2.5% xylitol + 7.5% erythritol	152.75 ± 78.24	257.32 ± 88.94	170.59 ± 56.45	0.001*
2.5% erythritol + 7.5% xylitol	243.86 ± 61.15	236.40 ± 83.77	209.46 ± 54.31	0.230
5% sucrose	369.50 ± 56.10	349.54 ± 33.42	385.57 ± 37.96	0.033*
p	0.001*	0.001*	0.001*	

*Two-way ANOVA test. *p < 0.05.*

The average thickness of the live-cell triple biofilm on Clinpro was significantly higher in the 10% erythritol solution than in the 2.5% xylitol + 7.5% erythritol ($p = 0.028$), 10% xylitol ($p = 0.028$), and 2.5% xylitol + 7.5% xylitol ($p = 0.043$) solutions ($p < 0.05$).

In addition, when 10% erythritol was used, the average thickness of the total-cell triple biofilm on Clinpro was significantly higher than that on HA ($p = 0.001$) and Fuji Triage ($p = 0.009$) (Fig. 2, Table 3).

The 2.5% xylitol + 7.5% erythritol solution resulted in the lowest maximum thickness of the live- and total-cell triple biofilm on HA ($p < 0.05$).

Nevertheless, there were no significant differences in the maximum biofilm thicknesses observed on Clinpro and Fuji Triage exposed to the various solutions ($p > 0.05$) (Fig. 2, Table 4).

3.2 SEM analysis

SEM images of the prepared disks were taken at 3000× and 5000× magnifications to examine the inhibition of bacterial growth. Triple biofilm formation was prominent in the 5% sucrose control group, whereas bacterial growth was inhibited in the solutions prepared with sugar alcohols. In particular, the 2.5% xylitol + 7.5% erythritol group exhibited substantially reduced bacterial growth compared to the other groups (Fig. 3).

4. Discussion

The effects of the various concentrations of xylitol and erythritol, both alone and in combination, on the growth of three cariogenic bacterial species (*S. mutans* (ATCC-25175), *S. sobrinus* (ATCC-33478) and S. wiggsiae (DSM-22547)) were investigated across three different surfaces. Previous studies have examined the effects of these sugar alcohols on the growth of various bacterial strains; however, few studies have evaluated biofilm formation by different types of bacteria together. Moreover, no study has evaluated the effects of sugar alcohols on cariogenic biofilm formation on the surfaces of fissure sealants. We found that the 5% sucrose group (*i.e.*, the control) exhibited the highest biomass and biofilm thickness on all evaluated surfaces. This result is consistent with the premise that sucrose promotes the production of extracellular polysaccharides that facilitate bacterial adhesion and biofilm formation [13].

A previous study [14] reported 36%–77% growth inhibition

TABLE 3. Effects of the different solutions and materials on the average biofilm thickness (μm).

Average thickness (μm)	Hydroxyapatite (Mean ± Standard Deviation (SD))	Clinpro (Mean ± SD)	Fuji Triage (Mean ± SD)	*p*
Dead cells				
10% xylitol	210.90 ± 40.70	209.16 ± 26.93	186.23 ± 34.63	0.042*
10% erythritol	179.60 ± 29.75	209.80 ± 20.85	187.82 ± 29.75	0.002*
5% xylitol + 5% erythritol	202.96 ± 15.51	189.88 ± 45.52	183.31 ± 46.03	0.249
2.5% xylitol + 7.5% erythritol	147.87 ± 69.17	184.88 ± 50.15	181.52 ± 42.05	0.062
2.5% erythritol + 7.5% xylitol	203.45 ± 34.24	177.35 ± 59.43	190.94 ± 31.33	0.160
5% sucrose	215.22 ± 24.40	226.73 ± 19.48	222.84 ± 12.14	0.155
p	0.001*	0.001*	0.001*	
Live cells				
10% xylitol	146.57 ± 34.95	123.50 ± 32.89	124.30 ± 32.88	0.048*
10% erythritol	118.02 ± 25.76	144.06 ± 27.68	114.79 ± 30.67	0.002*
5% xylitol + 5% erythritol	88.92 ± 36.84	124.73 ± 46.46	90.58 ± 35.02	0.007*
2.5% xylitol + 7.5% erythritol	74.53 ± 45.14	115.73 ± 46.27	81.50 ± 32.00	0.005*
2.5% erythritol + 7.5% xylitol	125.83 ± 34.24	112.75 ± 44.69	96.55 ± 26.41	0.036*
5% sucrose	174.64 ± 26.39	171.25 ± 19.28	192.46 ± 20.32	0.006*
p	0.001*	0.001*	0.001*	
Total cells				
10% xylitol	284.32 ± 59.38	258.79 ± 56.47	252.31 ± 57.10	0.173
10% erythritol	229.87 ± 48.97	289.93 ± 58.74	229.44 ± 53.98	0.001*
5% xylitol + 5% erythritol	184.00 ± 60.32	259.72 ± 80.49	188.55 ± 60.02	0.001*
2.5% xylitol + 7.5% erythritol	152.75 ± 78.24	257.32 ± 88.94	170.59 ± 56.45	0.001*
2.5% erythritol + 7.5% xylitol	243.86 ± 61.15	236.40 ± 83.77	209.46 ± 54.31	0.230
5% sucrose	369.50 ± 56.10	349.54 ± 33.42	385.57 ± 37.96	0.033*
p	0.001*	0.001*	0.001*	

*Two-way ANOVA test. *p < 0.05.*

TABLE 4. Effects of the different solutions and materials on the maximum thickness (μm) of the biofilm.

Maximum thickness (μm)	Hydroxyapatite (Mean ± Standard Deviation (SD))	Clinpro (Mean ± SD)	Fuji Triage (Mean ± SD)	*p*
Dead cells				
10% xylitol	252.93 ± 9.24	252.05 ± 9.42	245.86 ± 8.11	0.025*
10% erythritol	251.16 ± 9.50	252.93 ± 9.24	252.05 ± 9.42	0.830
5% xylitol + 5% erythritol	260.01 ± 0.00	249.39 ± 11.10	252.05 ± 11.10	0.001*
2.5% xylitol + 7.5% erythritol	229.05 ± 54.57	244.97 ± 13.92	249.39 ± 24.62	0.157
2.5% erythritol + 7.5% xylitol	254.70 ± 10.41	240.55 ± 36.43	252.93 ± 9.24	0.094
5% sucrose	244.09 ± 6.66	251.16 ± 9.50	242.32 ± 4.05	0.001*
p	0.001*	0.186	0.052	
Live cells				
10% xylitol	243.20 ± 5.59	245.86 ± 8.11	242.32 ± 7.14	0.244
10% erythritol	243.20 ± 5.59	252.05 ± 9.42	244.97 ± 7.47	0.001*
5% xylitol + 5% erythritol	253.82 ± 8.97	239.67 ± 28.11	237.90± 40.95	0.161
2.5% xylitol + 7.5% erythritol	227.28 ± 41.49	231.71 ± 34.56	247.62 ± 28.37	0.153
2.5% erythritol + 7.5% xylitol	244.09 ± 12.16	229.94 ± 44.14	247.62 ± 8.97	0.088
5% sucrose	240.55 ± 4.05	241.43 ± 0.00	241.43 ± 0.00	0.374
p	0.001*	0.062	0.632	
Total cells				
10% xylitol	496.13 ± 11.95	497.90 ± 15.11	488.17 ± 13.32	0.053
10% erythritol	494.37 ± 12.43	504.98 ± 17.24	497.02 ± 14.27	0.061
5% xylitol + 5% erythritol	513.82 ± 8.97	489.06 ± 35.88	489.94 ± 49.65	0.046*
2.5% xylitol + 7.5% erythritol	456.34 ± 94.62	476.68 ± 45.24	497.02 ± 52.17	0.159
2.5% erythritol + 7.5% xylitol	498.79 ± 20.59	470.49 ± 76.42	500.56 ± 16.06	0.072
5% sucrose	484.64 ± 5.59	492.60 ± 9.50	483.75 ± 4.05	0.001*
p	0.001*	0.062	0.488	

*Two-way ANOVA test. *$p < 0.05$.*

in the late log phase for *S. mutans* and *S. sobrinus* strains when 4% xylitol and 4% erythritol were used. That study also reported that xylitol and erythritol not only inhibited the growth of polysaccharide-producing oral streptococci but also reduced their adhesion to glass surfaces [14]. In another study, erythritol reduced *S. mutans* biofilm formation by 31.32%, whereas xylitol reduced it by 3.55% [15]. In the current study, 10% erythritol alone resulted in lower bacterial biomass on fissure sealant surfaces compared to 10% xylitol. This outcome indicates that erythritol is more effective than xylitol as a biofilm inhibitor.

Erythritol has also been found to be highly effective against periodontal pathogens. A previous study [16] reported that erythritol inhibited the adhesion of *Porphyromonas gingivalis* to an *Streptococcus gordonii* substrate and, in turn, markedly reduced biofilm formation. That study demonstrated that erythritol is effective not only against cariogenic bacteria but also against periodontal pathogens. In addition to its ability to suppress bacterial growth as a result of DNA and RNA depletion, erythritol exerts dual-species inhibition effects on biofilm development through various pathways. These include attenuating the extracellular matrix production and modifying the amino acid metabolism through dipeptide uptake [16]. However, erythritol's effectiveness changes according to the bacterial strains because of variations in bacterial membrane permeability and enzyme systems, which can influence how erythritol impacts metabolic pathways. By contrast, xylitol's primary inhibitory mechanism is phosphorylation to xylitol-5-phosphate in the bacterial cell, disrupting glycolysis because this phosphate compound cannot be further metabolized. This futile cycle depletes the bacterial cell's energy resources, inhibiting bacterial growth [17]. However, variations in the phosphoenolpyruvate phosphotransferase system (PTS) across different bacterial species may affect how efficiently xylitol is taken up and metabolized, potentially leading to strain-specific differences in effectiveness.

The different effects of xylitol and erythritol on bacteria raised the question of whether they could be more effective when used in combination. A recent study [8] evaluated the synergistic effects of high concentrations of sugar alcohols on *S. mutans* and *S. sobrinus*, and although the results did not show synergistic inhibition, an additive effect was observed. That report emphasized that the different sensitivities of various cariogenic bacterial strains to polyols made combination treatment more appealing [8]. Another work [3] reported that 10% polyol combinations were effective against mutans

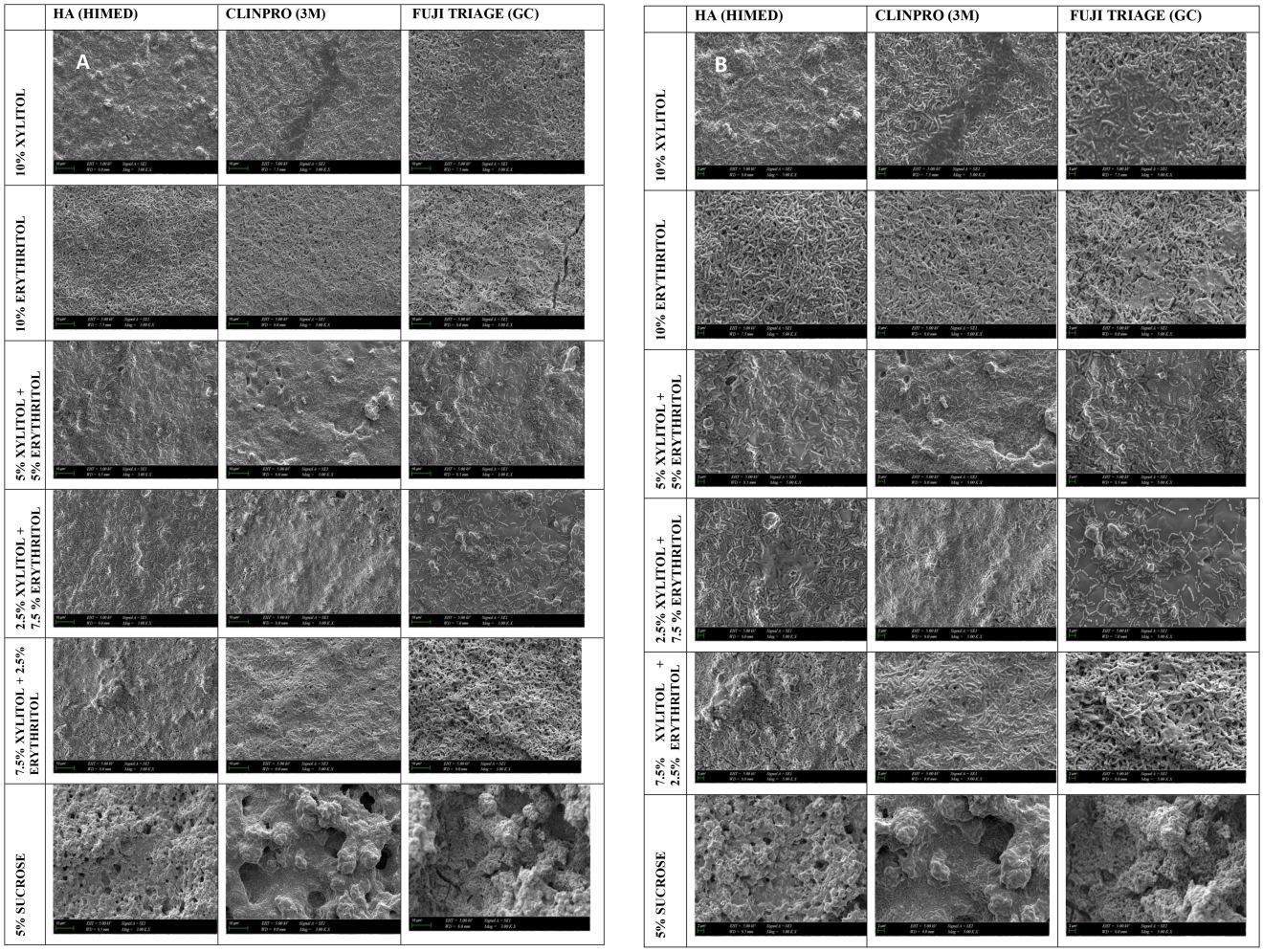

FIGURE 3. Scanning electron microscope (SEM) images of the triple biofilms formed on the disk surfaces (HA, Clinpro, and Fuji Triage): (A) 3000× magnification; (B) 5000× magnification. HA: hydroxyapatite.

streptococci, although an increase in *S. wiggsiae* biofilm formation was observed: 10% polyol combinations resulted in an increase of 2%–15% in the latter, whereas 10% and 15% single polyol solutions resulted in increases of 1%–24% and 8%–34%, respectively. These observed increases suggest that *S. wiggsiae* may have different response mechanisms to polyols [3]. Therefore, in our study, bacteria with different sensitivities to polyols were used together. As a result, the combination of 2.5% xylitol + 7.5% erythritol resulted in the lowest live-cell biomass and biofilm thickness. The 5% xylitol + 5% erythritol solution also reduced live-cell biomass levels compared to the 10% erythritol and 10% xylitol solutions alone. This result suggests that the combined use of sugar alcohols at different ratios may be more effective against the different bacterial strains in the mouth. Although our study focused on *S. mutans*, *S. sobrinus* and *Scardovia wiggsiae*, other cariogenic bacteria and oral pathogens may also be affected by xylitol and erythritol. Thus, future studies should investigate the effects of these sugar alcohols on a wider range of oral microorganisms to provide a more comprehensive understanding of the polyols' potential benefits.

Sealants prevent caries on tooth surfaces and are increasingly considered active agents for controlling and managing caries on the occlusal and proximal surfaces of teeth [18]. The effects of the evaluated fissure sealants on biomass and biofilm thickness provide insights into the role of surface materials in biofilm formation. Glass ionomer sealants contain fluoride and are thought to prevent caries through fluoride release; however, their main disadvantage is inadequate retention. Nevertheless, glass ionomer sealants may prevent caries development even after the visible loss of the material through fluoride release [18]. Hence, glass ionomer-based materials are effective in inhibiting biofilm formation. To our knowledge, no studies have evaluated the biofilm formed by bacteria on fissure sealants in the presence of sugar alcohols; thus, it may not be appropriate to compare the results of this study with other works. However, a similar study [11] using restorative filling materials was recently published. The mentioned study demonstrated that the combination of erythritol and glass ionomer could prevent secondary caries by inhibiting biofilm formation. The ability of clinical *Streptococcus mutans* strains isolated from 40 pediatric patients to form biofilms on both composite and glass ionomer materials in the presence of different sugar alcohol solutions was evaluated. As a result, glass ionomer materials were more effective in inhibiting biofilm formation than composite materials [11]. Similarly, we observed lower biomass levels on the glass ionomer surface of Fuji Triage than on the HA and Clinpro surfaces. Surface roughness is a crit-

ical factor influencing the adhesion and biofilm formation of primary colonizers, with higher roughness promoting biofilm growth. Here, although glass ionomer exhibited higher surface roughness than the other tested materials, biofilm reduction was more pronounced, potentially because of the release of fluoride ions [18].

While our study provides valuable insights, there are limitations that should be considered. The *in vitro* nature of the experiments may not fully replicate the complex environment of the oral cavity. Factors such as saliva flow, the presence of other microorganisms, and individual variations in oral microbiota can influence the effectiveness of sugar alcohols in real-world settings. Therefore, future research should include *in vivo* studies to confirm the efficacy of these sugar alcohols in a clinical setting.

Furthermore, although our study focused on *S. mutans*, *S. sobrinus* and *S. wiggsiae*, other cariogenic bacteria and oral pathogens might also be affected by xylitol and erythritol. Thus, future studies should investigate the effects of these sugar alcohols on a broader range of oral microorganisms to provide a more comprehensive understanding of their potential benefits.

Regarding clinical relevance, our findings suggest that these sugar alcohols could be applied in dental products like toothpaste or mouthwash to help prevent caries and periodontal diseases. Nevertheless, clinical trials are necessary to determine optimal formulations for real-world applications.

5. Conclusions

Xylitol and erythritol are important sugar alcohols with the potential to improve oral and dental health. The findings of our study indicate that xylitol and erythritol can be used as effective agents against cariogenic bacteria. Erythritol's superior ability to inhibit biofilm formation and suppress bacterial growth supports its potential use in dental health products. However, the differing effects of sugar alcohols on various bacterial strains necessitate careful consideration in clinical applications. Therefore, long-term clinical studies on the safety and efficacy of these sugar alcohols are essential to developing dental health products, highlighting the need for further research.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

SS, FE, BK and NT—designed the research study; wrote the manuscript. SS and NT—performed the research. SS, FE, NT and MYU—analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

This study was based on the work performed by Samyeli Sarihan for the fulfillment of the degree of Doctor of Philosophy, University of Marmara, Istanbul, Turkey. The study was supported by a research grant from The Marmara University Scientific Research Committee (BAPKO), with project number TDK-2023-11029. The authors, therefore acknowledge with thanks BAPKO for technical and financial support.

FUNDING

This research was funded by a research grant from The Marmara University Scientific Research Committee (BAPKO), with project number TDK-2023-11029. This article is derived from the doctoral thesis titled "*In vitro* evaluation of the effects of different sugar alcohol concentrations on the biofilm formed on fissure sealants" conducted at the Institute of Health Sciences, Marmara University.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] World Health Organization. Global oral health status report: towards universal health coverage for oral health by 2030. 2022. Available at: https://www.who.int/publications/i/item/9789240061484 (Accessed: 02 September 2024).

[2] Luo BW, Liang NL, Townsend JA, Lo ECM, Chu CH, Duangthip D. Sugar substitutes on caries prevention in permanent teeth among children and adolescents: a systematic review and meta-analysis. Journal of Dentistry. 2024; 146: 105069.

[3] Kõljalg S, Smidt I, Chakrabarti A, Bosscher D, Mändar R. Exploration of singular and synergistic effect of xylitol and erythritol on causative agents of dental caries. Scientific Reports. 2020; 10: 6297.

[4] Ganter J, Hellwig E, Doerken S, Al-Ahmad A. *In vitro* evaluation of the cariogenic potential of rebaudioside a compared to sucrose and xylitol. Clinical Oral Investigations. 2020; 24: 113–122.

[5] Knuuttila MLE, Mäkinen KK. Effect of xylitol on the growth and metabolism of streptococcus mutans. Caries Research. 1975; 9: 177–189.

[6] Zen I, Martins TP, Sampaio C, Delbem ACB, Monteiro DR, Pessan JP. Use of polyols in dentistry: effects on tooth mineral dynamics and oral biofilms. In Chauhan DN, Singh PR, Chauhan NS, Shah K (eds.) Pharmacological studies in natural oral care (pp. 689–701). 1st edn. Wiley-Scrivener: Hoboken. 2023.

[7] Boesten DM, den Hartog GJM, de Cock P, Bosscher D, Bonnema A, Bast A. Health effects of erythritol. Nutrafoods. 2015; 14: 3–9.

[8] Cannon ML, Merchant M, Kabat W, Catherine L, White K, Unruh B, *et al*. *In vitro* studies of xylitol and erythritol inhibition of *streptococcus mutans* and *streptococcus sobrinus* growth and biofilm production. Journal of Clinical Pediatric Dentistry. 2020; 44: 307–314.

[9] Hashemi kamangar SS, Zareian H, Bahador A, Pourhajibagher M, Bashareh Z, Valizadeh S. Evaluation of antibacterial effects of fissure sealants containing chitosan nanoparticles. International Journal of Dentistry. 2021; 2021: 8975948.

[10] Tanner ACR. Anaerobic culture to detect periodontal and caries pathogens. Journal of Oral Biosciences. 2015; 57: 18–26.

[11] Staszczyk M, Jurczak A, Magacz M, Kościelniak D, Gregorczyk-Maga I, Jamka-Kasprzyk M, *et al*. Effect of polyols and selected dental materials on the ability to create a cariogenic biofilm-on children caries-associated streptococcus mutans isolates. International Journal of Environmental Research and Public Health. 2020; 17: 3720.

[12] Pratten J, Smith AW, Wilson M. Response of single species biofilms and microcosm dental plaques to pulsing with chlorhexidine. The Journal of Antimicrobial Chemotherapy. 1998; 42: 453–459.

[13] Wint WY, Miyanohara M, Terada-Ito C, Yamada H, Ryo K, Murata T. Effects of sucrose and farnesol on biofilm formation by streptococcus mutans and candida albicans. Microorganisms. 2024; 12: 1737.

[14] Söderling EM, Hietala-Lenkkeri AM. Xylitol and erythritol decrease adherence of polysaccharide-producing oral streptococci. Current Microbiology. 2010; 60: 25–29.

[15] Ghezelbash GR, Nahvi I, Rabbani M. omparative inhibitory effect of xylitol and erythritol on the growth and biofilm formation of oral Streptococci. African Journal of Microbiology Research. 2012; 6: 4404–4408.

[16] Hashino E, Kuboniwa M, Alghamdi SA, Yamaguchi M, Yamamoto R, Cho H, *et al*. Erythritol alters microstructure and metabolomic profiles of biofilm composed of Streptococcus gordonii and Porphyromonas gingivalis. Molecular Oral Microbiology. 2013; 28: 435–451.

[17] Loimaranta V, Mazurel D, Deng D, Söderling E. Xylitol and erythritol inhibit real-time biofilm formation of Streptococcus mutans. BMC Microbiology. 2020; 20: 184.

[18] Ng TC, Chu CH, Yu OY. A concise review of dental sealants in caries management. Frontiers in Oral Health. 2023; 4: 1180405.

How to cite this article: Samyeli Sarihan, Merve Yildirim Ucuncu, Nursen Topcuoglu, Betul Kargul, Figen Eren. *In vitro* effects of different sugar alcohol concentrations on the cariogenic biofilm formed on fissure sealants. Journal of Clinical Pediatric Dentistry. 2025; 49(4): 162-171. doi: 10.22514/jocpd.2025.087.