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Inhibitory Effect of Oral Thin Films (OTFs) Containing Xylitol Against *Streptococcus mutans*

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ABSTRACT

Dental Caries is a chronic disease affecting half of the global population, causing pain and discomfort due to progressive damage to the teeth. Whilst xylitol has been studied for its effecton dental caries prevention, current practices present few limitations for its successful oral delivery, including short residence time in the mouth and poor patient compliance. Recently, oral thin films (OTFs) emerged as an alternative to delivering xylitol in the oral cavity. This research aims to develop novel OTFs containing xylitol with extended-release properties (as determined by the disintegration time) and to investigate its effect on a cariogenic bacterial strain, *Streptococcus mutans*. The minimum inhibitory concentration (MIC) of xylitol was determined. Employing the microdilution broth method, the antibacterial activity of the oral thin films containing xylitol for oral *S. mutans* was performed with simulated salivary fluid, incubated at 1, 4, and 10 h. The MIC of xylitol was found at 10%. Meanwhile, there was no significant difference in the inhibition of *S. mutans*($p > 0.05$) between the control, OTFs (10 h), and xylitol-OTF (1 h), with the latter, demonstrated only 16.58% inhibition. Interestingly, when compared to xylitol-OTF (1 h), xylitol-OTF showed significant inhibition ($p < 0.05$) to *S. mutans* after four h (+53.24 %) and almost a completeinhibition after ten h (-92.58 %). These results suggest that the OTFs demonstrated a gradual release of xylitol and inhibited oral biofilm formation by decreasing the growth of *S. mutans* in a time-dependent manner. Most importantly, the study indicated the successful developmentof a novel xylitol-OTF with potential as an oral health biotherapeutic agent.

Keywords: *Xylitol, oral thin film, Streptococcus mutans, dental caries*

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INTRODUCTION

Dental caries is a common chronic infectious disease that affects 2.3 billion people worldwide, and it is multifactorial in origin [1,2]. Caries is derived from the *Latin* word for rot or rotten. It results from the interaction between the host factors, oral microorganisms present in dental plaque, carbohydrate diet, and societal and environmental factors [3,4]. Dental plaque is composed of a complex biofilm community where various bacterial populations are present as separated micro-colonies in physiologically diverse environments [5].

Dental Caries is characterized by demineralization of the tooth's inorganic component and destruction of the organic material of the tooth, is a bacterial infection of the calcified tissues of the teeth. It had been identified as a disease caused by bacteria fermenting food, producing acids and destroying tooth surface material. Mutans *streptococci* (MS) such as *S. mutans*, *Streptococcus sobrinus* are common causal bacteria responsible for enamel caries although there are also certain *Actinomyces* bacteria [6].

Streptococcus mutans as a primary etiology of the dental caries, poses a strong adhesive ability to attach to the tooth enamel surface, which is decisive in the initial step of colonization, biofilm formation and caries development. As *Streptococcus Mutans* multiply in the mouth, they can contribute to dental caries, plaques, delayed speech, chewing trouble, endocarditis, infections, behavioural issues socialization and concentration difficulties problems and have severe health effects and that influence our daily life [7].

Since dental caries is an increasingly global issue, there is significant interest in applying antimicrobial agents to prevent and treat dental caries [8]. Risk inhibitors may reduce the probability of an individual developing dental caries. For instance, reducing the amount and frequency of fermentable carbohydrate ingestion and executing the proper tooth brushing technique [9]. Also, natural mechanisms of the oral cavity include the salivary flow, which eliminates food debris in the oral cavity [6]. However, with the growing consumption of high carbohydrate and sugar diets at the present day, extra measures must be taken to decrease the rate of caries progression. For example, using fluoride toothpaste and antibacterial therapy such as chlorhexidine rinses [10], and sugar substitutes in food products like chewing gum and sweets [11].

A variety of sugar substitutes are implemented in today's food market, such as xylitol, sorbitol, mannitol, and erythritol. Xylitol ($C_5H_{12}O_5$) in particular has been widely explored. It is a sugar alcohol found in many fruits and vegetables and is extracted from xylan-rich plants such as birch wood and beechwood. They are fructose-free sugar containing 2.4 calories per gram and does not increase blood sugar or insulin quantity. Interestingly, it is non-cariogenic as oral bacteria do not ferment it. Like sorbitol, xylitol in chewing gums has similar beneficial effects in promoting

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enamel remineralization in short-term in-situ experiments. Despite that, studies have shown that xylitol has better anti-caries properties when compared to sorbitol. Long period (over 12 months) xylitol use on *S. mutans* showed mixed results. In comparison, a short period (6 months or less) daily xylitol dose over 6 g has been more superior than to lower oral MS levels compared to the latter [12].

USFDA has defined Oral Thin Films (OTFs) as, "a thin, flexible, non-friable polymeric film strip containing one or more dispersed active pharmaceutical ingredients (APIs) which is intended to be placed on the tongue for rapid disintegration or dissolution in the saliva prior to swallowing for delivery into the gastrointestinal tract". This has gained popularity as statistics have shown that four out of five patients prefer OTFs over conventional solid oral dosages forms. Advantages include convenience and patient compliance as well as fast disintegration [13]. However, limited studies have been conducted regarding the use of OTFs to deliver sugar substitutes such as Xylitol, particularly for the prevention and treatment of oral health problems. Moreover, collagen as a unique ingredient for OTFs is acknowledged for the application in pharmaceutical and biomedical field because of its outstanding biodegradability, biocompatibility, and cell attachment proficiencies and poor antigenicity. Usage of collagen is because of its capability to self-assemble *in vitro* into numerous strong formations [14].

In light of the above, this study aimed to formulate an oral thin film containing xylitol made up of fish collagen as one of the unique strip-forming ingredients. The minimum inhibitory concentration (MIC) of xylitol was first assessed, and the antibacterial effect of the developed oral thin film containing xylitol on oral *S. mutans* were subsequently investigated.

EXPERIMENTAL

Materials

The following Table 1 shows the materials for the preparation of Oral Thin Films (OTFs).

Table 1: Materials for the preparation of Oral Thin Films (OTFs)

Material	Brand	Origin
Hydroxypropyl methyl cellulose	ACROS Organics	Geel, Belgium
Hydrolyzed Fish Collagen	Xian Lukee BioTech	Weiyang, China
Glycerol	Sigma	Steinhelm, Germany

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Lactic Acid	Sigma	Steinhelm, Germany
Xylitol	XyloSweet	North America, USA
Tween 80	Sigma	Steinhelm, Germany

*All the listed ingredients are considered GRAS (Generally Recognized as Safe) for FDA(Food and Drug Administration) and regconized for food products preparation.

Bacterial Strain and Cultivation

S. mutans strain (ATCC 25175), obtained from Faculty of Dentistry, University Sains Islam Malaysia, were cultured with Mueller-Hinton (MH) Broth at 37 °C in anaerobic condition for 36 h. Using a micropipette, 30 µL of *S. mutans* solution was then placed into 3 bottles of 3 ml Bijou bottle with Mueller-Hinton (MH) Broth. The procedures were repeated for 5 times. 100 µL of *S. mutans* solution was then placed into one bottle of 10 ml Bijou bottlerespectively with Mueller-Hinton (MH) broth using a micropipette. *S. mutans* was then isolated onto each Mueller-Hinton (MH) agar using the streaking technique. This procedure was repeated for 5 times. All the Bijou bottles were sealed with parafilm and they were mixed using Vortex mixer. *S. mutans* were cultivated in each specific agar at 37 °C in anaerobic condition for 36 h.

Minimum inhibitory Concentration (MIC) of Xylitol

MIC for xylitol was determined by the microdilution broth method. A series of 96-wellplate (12 × 8 wells) containing 150 µL of culture medium was prepared for each bacterium tested. Mueller Hinton broth medium was used for bacteria, *S. mutans.* 150 µL ofeach xylitol concentration was added to their corresponding wells using a micropipette. 10 µLof *S. mutans* bacteria was then added to each well using a micropipette. The solution was homogenized and all the well plates containing xylitol and bacteria were incubated at 37 ºC for12 h & 24 h in an anaerobic condition. Growth was monitored by measuring the absorbance ata wavelength of 660 nm.

Preparation of Oral Thin Films (OTFs)

For Oral Thin Films production, 100 mL of distilled water was prepared in six 250 mL Schott bottles. Then the solutions were heated at 80 °C with constant magnetic stirring (WiseStirÂ® MSH-20D, Echel, Germany) at 500 rpm. Ten gram of HPMC powder was added. Then 5 mL of glycerol, 2 mL of lactic acid and 1 % of Tween liquid were added using micropipettes to each Schott bottle. Ten gram of Xylitol was then added into each Schott bottle, except for the control. Then, Fish Collagen were each added to the respective Schott bottle except for the control and OTFs containing xylitol **(**XTOTF), according to Table 2.

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The solutions were stirred for 20 min in order for all the formulations to dissolve well. The solutions were then cooled to at least 50 °C. 10 mL of the solutions was then poured into sterile petri dishes (90 mm x15 mm) with a 50 mL falcon tube to ensure uniform thickness after dehydrating. Dehydrating each different formulation was done in a laminar flow cabinet (ESCO, laminar flow cabinet, Singapore) at room temperature at 25 °C and were stored in the desiccator until analysis.

Table 2: Composition of Oral Thin Films (OTFs)

Components	Functions	Control	OTFs containing xylitol (XTOTF)	OTFs containing xylitol and fish collagen (XTFC5)
Xylitol	Active pharmaceutical ingredients	-	10 g	10 g
HPMC	Film forming polymers	10 g	10 g	10 g
Glycerol	Plasticizers	5 mL	5 mL	5 mL
Fish Collagen	Strip forming Polymer	-	-	5 g
Lactic acid	Saliva Stimulating agent	2 mL	2 mL	2 mL
Tween 80	Surfactants	1 %	1 %	1 %

Physical characterization of the Oral Thin films

All prepared films were checked for their appearances either they are transparent or opaque or presence of air bubble. The thickness of the patch was measured using digital Vernier Calliper with a least count of 0.01 mm. The thickness was measured at different strategic points of the film and average was taken. Weight variation is studied by individually weighing randomly selected films and calculating the average weight. Folding endurance was determined by repeated folding of the film at the same place till the strip breaks. The number of times the film is folded without breaking was computed as the folding endurance value. The film size required for dose delivery (4 cm^2) was placed on a glass Petri dish containing 10 ml of pH 6.8 phosphate buffer. The time required for the film to break was noted as in vitro disintegration time.

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Antibacterial Effect of OTFs containing Xylitol

The antibacterial effect of OTFs was measured by the microdilution broth method using Mueller Hinton Broth (MHB). OTFs (20 % *w/v*) was weighed and dissolved in 5 mL simulated salivary fluid for 1 h, 4 h and 10 h with agitation at 50 RPM. Simulated salivary fluid were prepared by mixing disodium hydrogen phosphate (2.4 g/L), potassium dihydrogen phosphate (0.19 g/L), and sodium chloride (8 g/L) in distilled water adjusted to pH 6.75 with phosphoric acid. The tubes were centrifuged at 2500 × *g* for 10 min to remove undissolved debris. The supernatants were collected and aliquot into a 12-well plate containing MH broth. The bacterial cell (1% *v/v*) were added to the 12-well plate (2 mL) and incubated for 24 h. The absorbance was read using a TECAN microplate reader at 660 nm.

Statistical Analysis

The minimal susceptibility of S. mutans to the Mueller-Hinton (MH) broth medium of the xylitol concentration at 5 %, 10 % and 20 % after 12 h and 24 h were compared using SPSS Statistics software (SPSS, IBM New York USA; version 21) The statistical significance difference on the mean absorbance (660 nm) for the minimum inhibitory concentration of xylitol between 5 %, 10 % and 20 % after 12 h and 24 h were determined using ANOVA and Bonferroni post hoc statistical analysis. The results were presented by P-values and considered as significant when $p < 0.05$.

RESULTS AND DISCUSSION

The qualitative optical, mechanical and physical observations were conducted to assess the translucency, fragility, flexibility, moldability and the thickness of the OTFs. Overall, OTFs containing xylitol and fish collagen (XTOTF-FC) were easier to manage than the two other type of films. According to Table 3, XTOTF-FC is less fragile and soluble with high flexibility and translucency than the other two OTFs. These properties indicated that this type of OTFs was more resistant to breakage and distortion, thus making it easier to handle and transport.

Its low solubility meant that the OTFs would have better extended-release properties that could retain the contents of the film for a longer duration of time. Figure 1 illustrates the various OTFs of different formulations. The high translucency of the XTOTF-FC film, when compared to OTF and XTOTF, showed that it was free of bubbles, which also contributes to its aesthetic appeal [15]. Therefore, for the desirable properties of XTOTF-FC, this type of film was chosen to be used in the remainder of the study.

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Table 3: Physical evaluation of the Oral Thin Films (OTFs) of different formulations

Properties	OTF	XTOTF	XTOTF-FC
Translucency	Transparent	Semi-translucent	Opaque
Thickness (mm)	0.34 ± 0.012	0.41 ± 0.011	0.64 ± 0.019
Folding Endurance	134 ± 2.13	201 ± 4.88	412±5.33
Disintegration Time (s)	133 ± 4.45	109 ± 7.81	204 ± 10.14

*OTF: Control, oral thin film without xylitol and fish collage; XTOTF: OTF 6 xylitol; XTOTF-FC: OTF with xylitol and fish collagen.

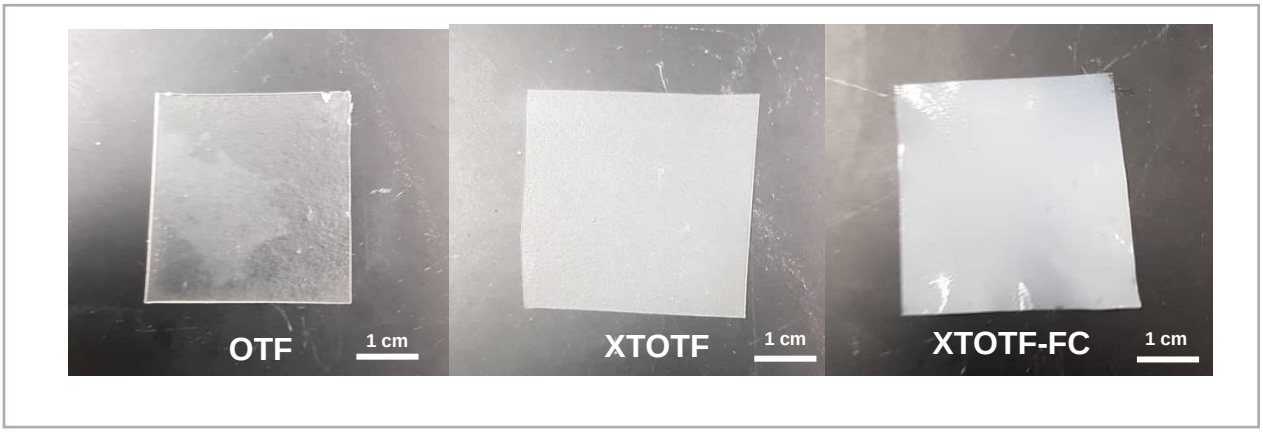

Figure 1: Different formulation of oral thin films (OTFs)

The minimum inhibitory concentration (MIC) of xylitol against *S. mutans* was first conducted to assess the effectiveness of xylitol in preventing dental caries. The purpose of finding the MIC was to determine which concentration of xylitol would be most effective in inhibiting the growth of *S. mutans*. Once this was identified, the chosen xylitol concentration would be incorporated into XTOTF-FC for further analysis. Table 4 summarizes the MIC of different concentrations of xylitol against *S. mutans* at 12 h and 24 h. Microdilution broth method was performed using various concentrations of xylitol in Mueller Hinton (MH) broth. There was a higher inhibitory percentage of xylitol against *S. mutans* after 24 h compared to 12 h.

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Table 4: Minimum inhibitory concentration of xylitol on the inhibition of *S. mutans*

Xylitol Concentration (*w/v*)	Mean Absorbance (660 nm)	Standard Deviation	Inhibitory Percentage (%)
After 12 h			
0%	0.0516	0.0119	-
5%	0.0487	0.0100	5.67
10%	0.0390	0.0060	24.45
20%	0.0240	0.0073	53.42
After 24 h			
0%	0.4385	0.1618	-
5%	0.1512	0.0904	65.53
10%	0.0449	0.0150	89.76
20%	0.0644	0.0240	85.32

It is illustrated in Figure 2 that there is no significant difference (p>0.05) between the different concentrations of xylitol at 12 h. However, after 24 h, there was a significant difference between the control group and 5 % xylitol concentration, indicating that the results of the control xylitol concentration and 5 % xylitol concentration were significant (p<0.05). Furthermore, there was a significant difference between the control group and xylitol at 10% concentration, as well as between the control group and xylitol at 20 % concentration, signifying that the results of the control xylitol concentration and 10 % as well as 20 % xylitol concentrations were significant (p<0.05). There was also a significant difference between xylitol at 5 % and xylitol at 10 %, as well as between xylitol at 5 % and xylitol at 20 % indicating that there is a significant difference in results between the 5 % xylitol concentration and 10 % as well as 20 % xylitol concentrations (p<0.05). Thus, 10 % xylitol concentration was further investigated for its antibacterial activity against the salivary model as it depicted the most inhibition of *S. mutans* growth.

Previous literature shows various studies on antimicrobial effects of xylitol against *S. mutans* as low as 0.01 % concentration of xylitol with the inhibition rates of 2.5 % [16] and as high as 20 % concentration of xylitol with the inhibition rates up to 76.4 % [17]. In this MIC test, *S. mutans* showed minimal susceptibility of 6% to the Mueller-Hinton (MH) broth medium of the xylitol concentration at 5 % after 12 h and 24 h (Figure 2). *S. mutans* exhibited a significant susceptibility 66 % to xylitol at a concentration of 5% and an almost complete inhibition of 90 % to the xylitol at a concentration of 10% at 12 h (p<0.05).

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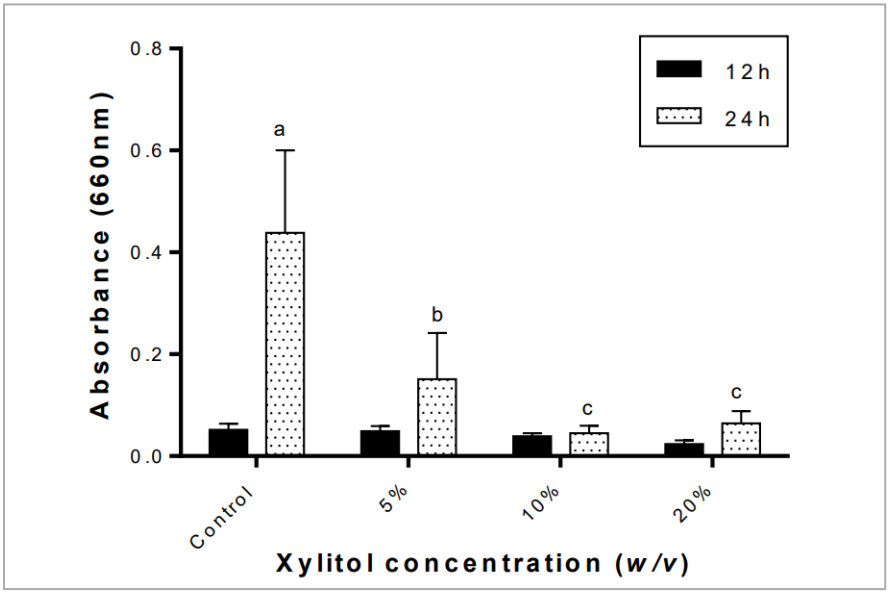

Figure 2: Effect of xylitol on the inhibition of *S. mutans.* Superscripts a, b, and c denotes the significance between the different groups of xylitol concentrations ($p < 0.05$)

All samples inhibited of *S. mutans'* growth compared to the control group and the effects seen were time dependent. As illustrated in Table 4, after the samples were incubated for 1 h, all of the samples resulted in inhibition of *S. mutans* growth. A further reduction of bacterial growth was observed after 4 h of incubation with an inhibitory percentage of 69.82 %. After 10 h of incubation, the optical density of the culture reached the lowest with an inhibitory percentage of 92.58 %, indicating xylitol to be more efficient in inhibiting the growth of *S. mutans* as time goes by. It is worthwhile to mention that OTF alone produce some inhibitory percentage against the growth of *S. mutans.* It could be contributed by the ingredients that made up the OTFs particularly the fish collagen (5g) used as it proven to have good antimicrobial properties against *S. mutans* [18].

According to Figure 3, there was no significant difference ($p > 0.05$) between the control group, OTFs, and OTFs with xylitol (1 h). However, there was a significant difference ($p<0.05$) between the control group, OTFs (10 h), OTFs with xylitol (1 h) and OTFs with xylitol (4 h) as well as between the control group, OTFs (10 h), OTFs with xylitol (1 h) and OTFs with xylitol (10 h). Therefore, it can be concluded that OTFs containing xylitol contribute to a decrease in absorbance over time, meaning that OTFs with xylitol have more significant inhibitory effect on *S. mutans*. In other words, the longer the duration of time, the lower the growth curve of the

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bacteria that justifies the promising use of the formulated OTFs containing xylitol with extended-release properties.

Table 4: The mean inhibitory percentage of Oral Thin Films (OTFs) containing xylitol on *S. mutans* at different disintegration time

Sample	Mean Absorbance (660 nm)	Standard Deviation	Inhibitory Percentage (%)
Control	0.6485	0.0624	
OTFs (10 h)	0.5812	0.0279	10.10
OTFs +Xyl (1 h)	0.5328	0.0371	16.58
OTFs +Xyl (4 h)	0.1961	0.0184	69.82
OTFs +Xyl (10 h)	0.0488	0.0268	92.58

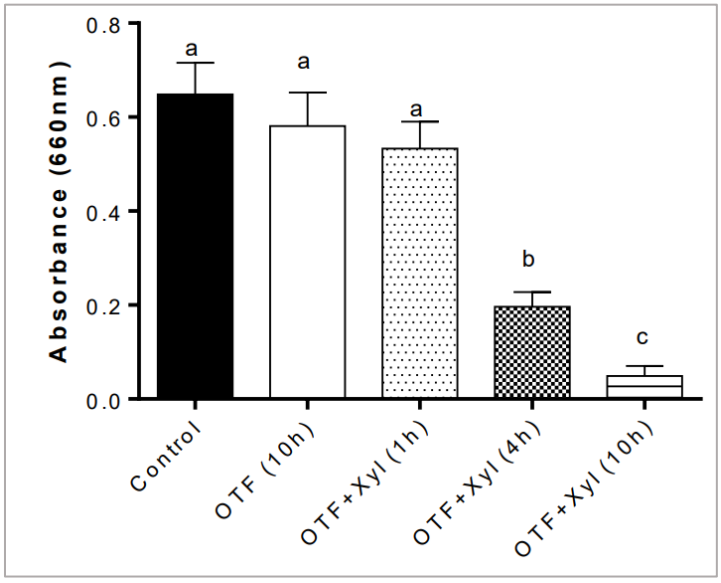

Figure 3: Inhibitory effect of Oral Thin Films (OTFs) containing xylitol on *S. mutans* at different time. Superscripts a, b, and c denotes the significance between the different xylitol-containing oral thin films (OTFs) and their inhibitory effect on *S. mutans* (p<0.05)

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This analysis demonstrates that oral thin films can indeed be utilized as a transport or medium for xylitol to prevent dental caries. A similar study conducted by Shubha, et al. [19] incorporated *Emblica officinalis* into slow releasing mouth dissolving films. The results showed that the extended local bioavailability of the films allowed for good antibacterial activity. Thus, this study further supports the potential of xylitol incorporated in OTFs as a means of preventing dental caries. OTFs containing xylitol may find application in preventive dentistry either alone or in combination with other biomaterials. For example, it can be placed with a thin layer on the inner surface of the night guards to be worn by the patient with high dental caries risk during nighttime. It can also be placed with a thin layer in dental braces to be worn by a patient during the daytime to decrease the risk of getting dental caries.

Therefore, there is a prospect of using OTFs incorporating xylitol as an alternative source for preventive dentistry. However, due to our low budget and time constraints in this study, further studies are needed to check the physical and chemical properties of OTFs and viability studies to validate the findings of this study further, as this concept is still new, and little is known regarding its applications. Additionally, further studies could be conducted to investigate OTFs with xylitol incubated at longer hours to see if there is a further decrease in bacterial growth or a limited time before the effects of the OTFs wear off.

CONCLUSION

In conclusion, oral thin films containing xylitol with extended released properties were developed and successfully disintegrated in the simulated salivary fluid. Through this study, oral thin films have been determined as a viable alternative for delivering xylitol to the oral cavity. This sugar substitute is known to have good antibacterial effects on oral health as well as anti-caries properties. It was found that the same applies to xylitol incorporated in OTFs. A 10% xylitol concentration incorporated in OTFs was found to have the most inhibitory effect on the growth of *S. mutans*. Therefore, a relationship was established that when using 10% xylitol concentration, the slow-releasing properties of the OTFs was found to be directly proportional in the inhibition of the growth of *S. mutans* over time. This result implied that OTFs containing xylitol may have a beneficial influence on the prevention and treatment of dental caries.

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AUTHOR'S CONTRIBUTION

Elizabeth Teng Yi Ern and Hee Xixian Khoo wrote methodology, performed the analysis and prepared the original draft. Muhamad Fareez Ismail provide the conceptualization, methodology, performed the analysis and anchored the review & editing.

CONFLICT OF INTEREST STATEMENT

The authors declared that there is no conflict of interest.

REFERENCES

[1] Reddy, K. S. (2016). Global Burden of Disease Study 2015 provides GPS for global health 2030. *The Lancet*, *388*(10053), 1448-1449. https://doi.org/10.1016/S0140-6736(16)31743-3

[2] GBD 2017 Oral Disorders Collaborators, Bernabe, E., Marcenes, W., Hernandez, C. R., Bailey, J., Abreu, L. G., ... & Kassebaum, N. J. (2020). Global, regional, and national levels and trends in burden of oral conditions from 1990 to 2017: a systematic analysis for the global burden of disease 2017 study. *Journal of Dental Research*, *99*(4), 362-373. https://doi.org/10.1177%2F0022034520908533

[3] Bader, O., Schaller, M., Klein, S., Kukula, J., Haack, K., Mühlschlegel, F., Hans Christian Korting, H.C., Schäfer, W., & Hube, B. (2001). The KEX2 gene of Candida glabrata is required for cell surface integrity. *Molecular Microbiology*, *41*(6), 1431-1444. https://doi.org/10.1046/j.1365-2958.2001.02614.x

[4] Bilbilova, E. Z. (2020). Dietary Factors, Salivary Parameters, and Dental Caries. In *Dental Caries* (pp. 1-18). IntechOpen.

[5] Segal, G., & Ron, E. Z. (1998). Regulation of heat-shock response in bacteria. *Annals of the New York Academy of Sciences*, *851*(1), 147-151.

[6] Yadav, K., Prakash, S., Khanal, S., & Singh, J. K. (2015). Prevalence of dental caries among adolescence of Dhanusha district, Nepal. *Janaki Medical College Journal of Medical Science*, *3*(2), 29-37.

[7] Babaeekhou, L., Mehrizi, A. A., & Ghane, M. (2020). Streptococcus mutans, sugar consumption, and oral hygiene: Which one has more effect on decayed, missing, and filled teeth (DMFT) score in Iranian adults?. *Dental Research Journal*, *17*(2), 134.

[8] Nur, A.J., Siti, S.D.A., Alya, N.A.K., Che, A.I.C.M., & Yahya, M. F. Z. R. (2020). Anti-biofilm potential and mode of action of malaysian plant species: a review. *Science Letters*, *14*(2), 34-46. https://doi.org/10.24191/sl.v14i2.9541

[9] Bolin, D. A. K., Bolin, A., & Koch, G. (1996). Children's dental health in Europe: caries experience of 5-and 12-year-old children from eight EU countries. *International Journal*

https://scilett-fsg.uitm.edu.my/
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of Paediatric Dentistry, *6*(3), 155-162. https://doi.org/10.1111/j.1365-263X.1996.tb00234.x

[10] Davies, G. N. (1998). Early childhood caries—a synopsis. *Community Dentistry and Oral Epidemiology*, *26*(S1), 106-116. https://doi.org/10.1111/j.1600-0528.1998.tb02102.x

[11] Nayak, P. A., Nayak, U. A., & Khandelwal, V. (2014). The effect of xylitol on dental caries and oral flora. *Clinical, Cosmetic and Investigational Dentistry*, *6*, 89. https://dx.doi.org/10.2147%2FCCIDE.S55761

[12] Zhan, L., Featherstone, J. D. B., Lo, J., Krupansky, C., Hoang, N., DenBesten, P., & Huynh, T. (2012). Clinical efficacy and effects of xylitol wipes on bacterial virulence. *Advances in Dental Research*, *24*(2), 117-122. https://doi.org/10.1177%2F0022034512449835

[13] Ahmed, A., Dachang, W., Lei, Z., Jianjun, L., Juanjuan, Q., & Yi, X. (2014). Effect of Lactobacillus species on Streptococcus mutans biofilm formation. *Pakistan Journal Of Pharmaceutical Sciences*, *27*.

[14] Pati, F., Dhara, S., & Adhikari, B. (2010, April). Fish collagen: A potential material for biomedical application. In *2010 IEEE Students Technology Symposium (TechSym)* (pp. 34-38). IEEE. https://doi.org/10.1109/TECHSYM.2010.5469184

[15] Mahboob, M. B. H., Riaz, T., Jamshaid, M., Bashir, I., & Zulfiqar, S. (2016). Oral films: A comprehensive review. *International Current Pharmaceutical Journal*, *5*(12), 111-117.

[16] Kościelniak, D., Gregorczyk-Maga, I., Jurczak, A., Staszczyk, M., Kołodziej, I., Magacz, M., Kędziora, K., Papież, M., Vyhouskaya, P., Jamka-Kasprzyk, M., & Krzyściak, W. (2019). Low concentration of xylitol improves children tooth protection against" Streptococcus mutans" biofilm formation. *Oral Health and Care*, *4*. https://doi.org/10.15761/OHC.1000160

[17] ElSalhy, M., Zahid, I. S., & Honkala, E. (2012). Effects of xylitol mouthrinse on Streptococcus mutans. *Journal of Dentistry*, *40*(12), 1151-1154. https://doi.org/10.1016/j.jdent.2012.08.014

[18] Shalaby, M., Agwa, M., Saeed, H., Khedr, S. M., Morsy, O., & El-Demellawy, M. A. (2020). Fish scale collagen preparation, characterization and its application in wound healing. *Journal of Polymers and the Environment*, *28*(1), 166-178. https://doi.org/10.1007/s10924-019-01594-w

[19] Shubha, P., Namratha, K., Vicas, C. S., Byrappa, K., Gurupadaiah, B. M., Rashmi, N. G., & Shinde, C. G. (2015). Formulation and evaluation of slow releasing mouth dissolving films from Emblica officinalis fruit for prevention of dental caries. *Journal of Chemical and Pharmaceutical Research*, 7(7), 950-960.