Chewing Gum with Added Chitosan Reduces the Number of Cariogenic Bacteria Colonies in Human Saliva

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Abstract

In our study, we compared the numbers of cariogenic bacteria colonies in the saliva from subjects before and after 14 days of chewing gum with added chitosan. The control group chewed sugar-free gum with no chitosan. Chewing chitosan-containing gum significantly reduced the numbers of colonies of both Streptococcus mutans (MS) and Lactobacillus (LB) compared to chitosan-free gum, suggesting that chitosan-enriched gum may have an anticariogenic effect which is independent of beneficial effects of chewing any gum.

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Introduction

Dental caries is a complex and multifactorial disease. Even though the contemporary approach classifies it as a non-communicable disease, caries is admittedly a biofilm-mediated condition[1,2]. Dental plaque, an oral biofilm, is composed of several bacterial species which contribute to the natural relationship between the resident microflora and the host[3,4]. Caries occurs when the dental plaque microbiota is imbalanced allowing the increase of acidogenic and aciduric bacteria. Such a shift disturbs the repeated cycles of demineralization and remineralization on tooth surface, and a caries lesion may develop[4]. Especially mutans streptococci (*Streptococcus mutans* (MS), *Streptococcus sorbinus*) and lactobacilli (LB) can quickly metabolize carbohydrates to acids causing the fall in pH to a level sufficient to initiate the demineralization of tooth hard tissues. Cariogenic microflora is also acid tolerant and able to polymerize the intracellular and extracellular polysaccharides[4]. Recent studies showed that non-mutans streptococci may also metabolize sugars to acids to a level capable of enamel demineralizing, but the diversity of microflora in acidic dental plaque is limited[5]. MS- or LB-dominated microbiome is significant for advanced stages of caries[4].

The basic method for reducing the amount of oral bacteria is a mechanical plaque removal[6]. Antimicrobial agents act as adjuncts to mechanical plaque control by interfering bacterial metabolism, disturbing biofilm and inhibiting biofilm formation. They may have bacteriostatic or bactericidal properties with broad or narrow spectrum[7,8]. Several agents are used for chemical plaque control: fluorides, bisbiguanides (chlorhexidine), cetylpyridinium chloride, phenols (Triclosan**),** enzymes, essential oils, metal salts, plant extracts, surfactants[8]. Antimicrobial agents can be delivered as toothpastes, mouthrinse, gels, varnishes. A beneficial effect of xylitol sugar-free chewing gum on plaque composition, caries indices and parameters of gingivitis was proved[9-13]. A regular consumption of such gum may reduce the cost of dental treatment worldwide[14,15]. Chewing gum was also successfully used as a vehicle to deliver agents like casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), tea polyphenol, magnolia bark extract for their anti-plaque properties[16-18].

Recently several studies focused on the possibility to use a chemical compound called chitosan in caries prevention due to its natural origin, biocompatibility, non-toxicity and bactericidal properties[19-26]. Chitosan with a chemical formula $(C_6H_{11}O_4N)_n$ is a natural biopolymer derived from the shells of shrimps

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and other crustaceans. It is obtained by deacetylation of chitin. After cellulose, chitin is the second most common organic polymer in the world [19]. The mechanism of action of chitosan is different from the one acting in ingredients used so far on an industrial scale. Chitosan is a cationic biomaterial so it adheres to the wall and the membrane of cellular bacterium and disturbs its structure, which results in a direct bacteriostatic and bactericidal effect[22,27].

Tarsi et al.[28,29] found that MS adsorption to the hydroxyapatite was inhibited in the presence of low-molecular-weight chitosan. They suggested to use this agent in toothpastes, mouthrinses and chewing gum. Fujiwara, et al.[30] were the first to show that chitosan dissolved in water inhibits the growth and the development of MS bacterial colonies. Chen and Chung[31] also proved that chitosan dissolved in water and used for mouthwash had an inhibitory effect on MS. Chewing chitosan-containing gum resulted in a significant decrease of the number of MS lasting up to 1h after the use[27]. Neilands et al.[32] found that chitosan nanoparticles may influence the acid tolerance response of MS. Chitosan was also successfully used in complex formulations together with other active components like silver nanoparticles, fluorides, propolis, amorphous calcium phosphate, copper, Pam_3CSK_4, monophosphoryl lipid A, Mentha piperita essential oils, and biosurfactant[33-40].

The aim of the study was to determine whether the addition of chitosan to chewing gum had an antibacterial effect against *Streptococcus mutans* (MS) and *lactobacilli* (LB) in the saliva of healthy volunteers.

Materials and methods

The study was conducted on 60 voluntary dentistry students aged 22-23 (mean age of 22.5). The study protocol was approved by the Bioethical Committee of the Medical University of Bialystok, Poland. The inclusion criteria for the subjects were general good condition and written consent to participate in the study. The exclusion criteria were allergy to chitosan, chitin or crustaceans, significant reduction of salivary secretion due to local factors (e.g. tumours or traumatic injuries of salivary glands) or co-existing systemic diseases, general and local antibiotic therapy in the last 3 months, diseases and dysfunctions in the temporomandibular joint.

Participants were randomly divided into two groups (30 people each): chitosan group - was recommended to chew gum with chitosan during the period of the study and control group - recommended to chew sugar-free chewing gum during the testing period.

In both groups, subjects were instructed to chew gum 3 times a day for about 30 minutes after the main meals for a period of 14 days. Participants from the study group were explained how to prepare self-made chewing gum with chitosan. Guidelines for the production of chitosan gum were as follows: thoroughly disperse two sugar-free capsules to a homogeneous consistency, open the capsule with chitosan powder (Chitosan 0.18 g, TIENS®), pour out the powder onto the gum, chew prepared gum for 30 minutes. Subjects from the control group were supplied with the commercially available sugar-free Wrigley gum (ORBIT SPEARMINT, 35 g Wrigley Comp.). During the entire period of study, the subjects were allowed to conduct their normal oral hygiene practices (brushing and flossing), but without using any antibacterial mouthwash.

The CRT® bacteria tests (IvoclarVivadent, Madrid, Spain) were used for the microbiological examination of saliva for the presence of MS and LB according to the manufacturer's guidelines. Stimulated saliva samples were collected two times, at the baseline and after 14 days of using gum, by the chewing paraffin cube and spitting out into disposable cups. The saliva samples were collected after the end of student's morning classes. The minimum time from last meal and oral hygiene procedures was 4 hours. Before collecting the samples, the subjects were asked to rinse the mouth with water for 1 minute. The saliva collection lasted until at least 2 ml were obtained. A portion of saliva was collected from the obtained material by a disposable pipette and put into thoroughly wetted agar mediums of the kits included in the CRT® tests. The sealed agar vials were placed vertically in an incubator at 37°C for 48 hours. After this time, the density of colony-forming units (CFU/ml saliva) of MS and LB was visually compared with a pattern attached by the manufacturer, following the recommendations given in the instruction manual. The follow-up was done after 2 weeks. The saliva samples were collected and CRT® tests were conducted according to the procedures described above.

The colony forming unit (CFU/ml saliva) density for both salivary MS and LB at the baseline and at the follow-up was compared between study groups and within each study group. For the statistical analysis, three classes of bacteria quantity were featured. The following thresholds were established: no colonies, $< 10^5$ CFU/ml saliva and $\geq 10^5$ CFU/ml saliva. Statistica 10.0 (StatSoft Inc., 2011) was used for the statistical analysis. The chi-square and the Wilcoxon paired tests were used. The significance level $\alpha = 0.05$ was assumed. The results were presented in a tabular form.

Results

	Baseline		Follow-up	
	Chitosan (**a**) N (%)	Control (**b**) N (%)	Chitosan (**c**) N (%)	Control (**d**) N (%)
No colonies	4 (13.3)	2 (6.7)	25 (83.3)	3 (10.0)
$< 10^5$	17 (56.7)	10 (33.3)	4 (13.3)	14 (46.7)
$\geq 10^5$	9 (30.0)	18 (60.0)	1 (3.3)	13 (44.3)
	***ab** p=0.064; ***cd** p<0.001, ****ac** p<0.001, ****bd** p=0.119			

Table 1. Results of the CRT ® test for MS at the baseline and at the follow-up. (*Chi^2 test, **Wilcoxon test).

	Baseline		Follow-up	
	Chitosan (**a**) N (%)	Control (**b**) N (%)	Chitosan (**c**) N (%)	Control (**d**) N (%)
No colonies	3 (10.0)	5 (16.7)	19 (63.4)	8 (26.7)
$< 10^5$	15 (50.0)	17 (56.7)	7 (23.3)	10 (33.3)
$\geq 10^5$	12 (40.0)	8 (26.7)	4 (13.3)	12 (40.0)
	***ab** p=0.490; ***cd** p<0.01, ****ac** p<0.001, ****bd** p=0.629			

Table 2. Results of the CRT ® test for LB at the baseline and at the follow up. (*Chi^2 test, **Wilcoxon test).

Table 1 and 2 present the results of the CRT ® tests. At the baseline, there were no statistically significant differences in the distribution of subjects between study and control groups according to the class of MS and LB bacteria quantity. After two weeks of chewing chitosan gum, a reduction in the number of subjects with the presence of MS and L in both $< 10^5$ and $\geq 10^5$ CFU/ ml classes was observed (Wilcoxon test, p<0.001). The number of subjects with no MS colonies increased from 4 (13.3%) to 25 (83.3%) for MS and from 3 (10%) to 19 (63.4%) for LB. In the control group, there were no statistical differences, however, the number of subject with the highest class ($\geq 10^5$) of MS decreased from 18 (60%) to 13 (44.3%) individuals. An increase in the number of subjects with the highest rates of LB was observed in this group, respectively 8 (26.8%) persons at the baseline and 12 (40%) at the follow-up. The Chi^2 test proved that chewing chitosan gum was sufficient to reduce the MS and LB amounts in saliva as compared to the sugar-free gum (p<0.001 for MS, p<0.01 for L).

Discussion

Chewing gum is recommended as a method of maintaining oral hygiene in a situation where it is not possible to conduct normal hygienic procedures. The masticatory stimulation due to chewing gum increases the salivary flow rate[41]. The salivation is also increased by ingredients like sweeteners (xylitol, sorbitol) and flavours added to the gum[42,43]. Stimulated saliva modifies the cariogenic process in several ways[44] , the increased amount of saliva causes a dilution of sugars and a reduction of its concentration. At the same time, it induces the reflex of swallowing saliva, which removes some amount of sugars and bacteria. It also has buffering properties thanks to the two carbonate and phosphate ions. This allows neutralising the acid pH in the oral cavity to values that do not endanger the hard dental tissues. Due to the presence of calcium, phosphorus and fluoride ions, the enamel resistance is increased. Saliva also contains antibacterial agents that modify adhesion, metabolism and microbial growth. The addition of substances with bactericidal and bacteriostatic activity increases the anti-carious action of chewing gum[13,42,44-46].

The present study showed that chewing gum supplemented with chitosan resulted in a significant decrease in the number of MS and LB CFU, therefore the use of chitosan may have a beneficial effect on oral health. These results are in compliance with the study of Hayashi et al. [27]. They found that chewing chitosan gum for 5 minutes was sufficient to reduce the number of MS for at least one hour. On that basis, they concluded that chitosan should have bactericidal and bacteriostatic properties. Some in vitro studies also proved the antibacterial effect of chitosan against *S. mutans* and *S. sobrinus*, *S. sobrinus, S. sanguis* and *S. salivarius, Lactobacilli brevis, Candida albicans*[32,36,47-49] .

Costa et al. found that a chitosan mouthwash had a higher antimicrobial activity than a commercial mouthwash. Dentifrices supplemented with chitosan may be useful for

patients with a high risk of dental caries[50].

In contrast, no significant reduction in the amount of cariogenic bacteria in saliva in the control group was noticed. A positive change in the distribution of persons between classes of bacteria quantity was observed for MS, but not at a statistically significant level. For LB the number of individuals with ≥ 10^5 CFU was higher at the follow-up than at the baseline by 13.3%. These results are difficult to explain. The use of xylitol and sorbitol containing gum in caries prevention has been widely evaluated. Its positive effect on enamel remineralization, reduction in caries increase, decrease in total salivary bacteria quantity was proved[11,13,46,51-53]. Xylitol was shown to inhibit the growth of MS[44]. Also the level of salivary lactobacilli was reduced in a longitudinal study on children using xylitol-containing chewing-gum[54]. On the other hand, Soderling et al. found that the salivary MS levels of the mothers of young children remained high despite a regular use of xylitol gum, but it influenced the risk of a mother-child transmission of MS [55]. It was suggested that the antibacterial effect of xylitol was dose-dependent[56] and probably the dose in gum used in this study was too small to induce a significant effect.

The limitation of present study is a short time of the observation. Longitudinal studies are necessary to establish whether the antibacterial effect of chitosan gum is permanent and whether it can prevent the development of carious lesions.

Conclusions

The present *in vivo* study showed the inhibitory effect of chitosan on MS and LB in the saliva of healthy volunteers. Chewing chitosan supplemented gum potentially plays a role as a caries-preventive factor.

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Declaration of Interest

The authors report no conflict of interest.

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