Laboratorinė medicina.
2014, t. 16, Nr. 4(64), p. 165–171.

Inhibition of Dental Biofilm Formation Using Antisense Oligonucleotide to *Streptococcus mutans* and *Streptococcus sobrinus* Glucosyltransferases

Povilas Kalesinskas[1, 2]
Tomas Kačergius[3]
Arvydas Ambrozaitis[1]
Vytautė Pečiulienė[2]
Ryo Jimbo[4]
Dan Ericson[5]

[1]*Clinic of Infectious and Chest Diseases, Dermatovenereology and Allergology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*
E-mail: p.kalesinskas@gmail.com

[2]*Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

[3]*Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

[4]*Department of Prosthodontics, Faculty of Odontology, Malmö University, Malmö, Sweden*

[5]*Department of Cariology, Faculty of Odontology, Malmö University, Malmö, Sweden*

Summary

Background. Dental caries remains one of the most prevalent human diseases due to uncontrolled increase of dietary sucrose consumption in modern society. Sucrose is metabolized by *Streptococcus mutans* and *Streptococcus sobrinus* to acids causing tooth decay. These streptococci produce glucosyltransferases (Gtfs) for synthesis of sticky glucan polymers from sucrose, which is important for biofilm formation on teeth. In order to reduce dental biofilm build-up and oral diseases it causes, one preventive measure could be blocking of Gtf synthesis.

Aim. This study aim was to test antisense phosphorothioate oligodeoxyribonucleotide (PS-ODN) targeting simultaneously *S. mutans gtfB*, *gtfC* and *S. sobrinus gtfI* mRNAs in order to inhibit biofilm formation *in vitro*.

Material and methods. Mixed culture of *S. mutans* and *S. sobrinus* bacteria were grown anaerobically on glass slides inserted vertically in 24-well cell culture plates containing Todd Hewitt broth with sucrose and sterile saliva under exposure to antisense or missense PS-ODNs at final concentration of 10 M. Untreated bacteria served as controls. After 24 h of incubation, glass slides were removed, air-dried and further used for quantitative evaluation of streptococci biofilm applying optical profilometry technique.

Results. It was found that antisense PS-ODN significantly reduced biofilm surface roughness and thickness of mixed *S. mutans* and *S. sobrinus* culture suppressing the biofilm development by 1.6-fold overall in comparison to untreated and missense PS-ODN-treated bacteria ($p<0.05$).

Conclusions. Data demonstrate that antisense PS-ODN considerably attenuate streptococci-induced biofilm build-up on glass slides, and might therefore significantly inhibit dental biofilm formation through simultaneous inactivation of bacterial *gtf* mRNAs.

Keywords: dental caries, *Streptococcus mutans*, *Streptococcus sobrinus*, biofilm, glucosyltransferase, antisense oligonucleotide.

INTRODUCTION

Recent epidemiological studies indicate a trend to global increase in tooth decay or dental caries despite the availability of fluoride toothpastes, water fluoridation, dental sealants, oral health educational programs and

various antiseptic mouth-rinses [1, 2]. This trend is mainly related to the increased intake of foods rich in carbohydrates and particularly sucrose, e. g., sweets, biscuits, snacks, sweet drinks [3]. Dietary sucrose is a nutrient for oral bacteria (mostly streptococci) that ferment it into organic acids [4]. Acids retained in bacterial deposits on the teeth can cause demineralization leading to cavitation. The bacterial metabolism of sucrose occurs in the structure called biofilm that is formed on tooth surfaces by oral bacteria [5]. Important structural and binding materials of biofilm are water-insoluble glucans synthesized from sucrose by several isoforms of glucosyltransferase (Gtf) enzyme present in the mutans group streptococci – *Streptococcus mutans* and *Streptococcus sobrinus*, colonizing human oral cavity [5, 6]. In this respect, *S. mutans* produces water-insoluble and partly water-soluble glucans by GtfB and GtfC enzymes, respectively, whereas *S. sobrinus* generates water-insoluble glucan employing GtfI enzyme [5]. Hence, it is important to design new pharmaceuticals in order to suppress dental biofilm formation through the mechanism involving the specific inhibition of water-insoluble glucans' synthesis.

Antisense technology provides the novel approach that can be applied for blocking the production of glucan polymers in *S. mutans* bacteria [7]. It is based on the use of chemically modified single-stranded nucleic acid molecules (usually 20 nt in length), which can selectively hybridize to their target complementary mRNA through Watson-Crick base pairing, and consequently leading to degradation of the bound mRNA by RNase H endonuclease, steric hindrance of ribosomal activity, inhibition of mRNA splicing or destabilization of pre-mRNA [8]. This results in specific down-regulation of the target gene and subsequent protein expression. A large number of studies have demonstrated the efficacy of antisense oligonucleotides for silencing of various genes in bacteria, including those responsible for antibiotic resistance [9]. Importantly, Guo et al. [7] showed that phosphorothioate oligodeoxyribonucleotides (PS-ODNs) designed to antisense *gtfB* mRNA in *S. mutans* reduces the expression and activity of Gtf enzyme as well as water-insoluble glucan production and biofilm formation. The PS-ODNs represent DNA molecules containing a phosphorothioate-modified backbone, where one of the non-bridging oxygen atoms in the phosphodiester linkage is replaced by a sulphur atom in order to increase the resistance to endo- and exonucleases [8]. Moreover, two other investigations performed by McLeod et al. [10] and Dufour et al. [11] revealed that the PS-ODNs can efficiently suppress the growth of *S. mutans* cells by blocking the expression of fructose-biphosphate aldolase and enoyl-CoA hydratase genes.

Therefore, in the present study, we aimed to test the PS-ODN targeting specifically and simultaneously *S. mutans gtfB, gtfC* and *S. sobrinus gtfI* mRNAs in order to inhibit biofilm formation in the mixed culture of mutans group streptococci.

MATERIAL AND METHODS

Bacterial strains and culture conditions

S. mutans UA159 (No. 700610) and *S. sobrinus* SL1 (No. 33478) strains, which are available through the American Type Culture Collection, were chosen for this experimental study because they preferably colonize humans. Stocks of these bacterial strains were kept in 10% skim milk (Difco) at -70 C. Before experiments, the bacteria were cultured in Todd Hewitt (TH) broth (Difco) containing 10% heat-inactivated horse serum (Gibco) in order to induce their competence state under anaerobic conditions (95% N_2 and 5% CO_2) at 37 C for 18 h. Purity of the cultures was checked on Mitis salivarius agar (Difco) and Columbia blood agar (E&O Laboratories).

Bioinformatical analysis and selection of target sequence

In order to find the appropriate target sequence, at first the conserved homologous regions among the *gtf* genes of oral streptococci were identified. For this purpose, it was primarily conducted a comparative analysis of amino acid sequences between *S. mutans* UA159 GtfB and other Gtf proteins present in oral streptococci. Using *S. mutans* UA159 GtfB protein as a query sequence, the BLAST (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov/Blast.cgi) search of homologs among all known proteins was performed. Then, the proteins with high identity amino acid sequences were collected and multiple sequence alignment produced employing MAFFT online server at the Max-Planck Institute for Development Biology (http://toolkit.tuebingen.mpg.de/mafft). It was determined the conserved amino acid sequence within the GtfB protein of *S. mutans* UA159, which is encoded by the respective region in *S. mutans* UA159 *gtfB* gene consisting of 26 nucleotide (nt) sequence: 5'-GTTAAGATTAAGCAATGGTCT GCCAA-3'. Applying such approach as described herein, it was identified that this segment of 26 nt has 100% homologous fragments in *S. mutans* UA159 *gtfC, S. criceti gtfI, S. dentirousetti gtfI, S. dentisuis gtfI* and *S. orisuis gtf* genes. Additionally, it was also found a highly similar sequence with only two mismatched nucleotides in *S. downei gtf* precursor and *S. sobrinus gtfI* genes.

PS-ODN optimization and preparation

For the PS-ODN optimization, the Primer3 service (http://primer3.ut.ee/) was used to analyze thermodynamics as well as other aspects of the heteroduplex forming, and then a complementary antisense sequence of 19 nt (PS-ODN1: 5'-GCAGACCATTGCTT AATCT-3') was picked out for the target region in genes and their encoding mRNAs of *S. mutans* UA159 *gtfB, gtfC* (GenBank accession No. AE014133) [100% complementarity] and *S. sobrinus* SL1 *gtfI* (GenBank accession No. D63570) [one mismatched nucleotide]. For a negative control purpose, the missense sequence of 19 nt (PS-ODN2: 5'-ACTCGTATGCTACAGCTAT-3') was designed, and differed from the antisense molecule by scrambling nucleotide sequence. These PS-ODNs were synthesized by the Metabion International AG (Germany) with all internucleotide linkages phosphorothioated. Stock solutions of the PS-ODNs were prepared in sterile nuclease-free distilled water (Thermo Fisher Scientific, Fermentas) and stored at -20 C until use.

Experimental design

For the experiments, optical density (OD) of the bacterial cultures was adjusted to 0.2 at 630 nm employing the microplate reader spectrophotometer (Dynex MRX). Prior to inoculation of the bacteria, 24-well flat-bottomed polystyrene cell culture plates (Sarstedt) were filled with the TH broth without 10% heat-inactivated horse serum, and then the PS-ODNs were added to the plate wells at the final concentration of 10 M either alone or in combination with the

transfection reagent composed of cationic polymer – TurboFect™ (TF) [Thermo Fisher Scientific, Fermentas] prepared according to the manufacturer's instructions. Afterwards, the sterile filtered human saliva was added to the plate wells at the final dilution of 1:10, and subsequently *S. mutans* and *S. sobrinus* cultures were mixed in equal parts and inoculated to the wells at the final dilution of 1:100. Immediately, the sterile glass slides of 1 mm thickness cut from standard microscope slides (76×26 mm; Thermo Fisher Scientific) were vertically inserted into wells, and the plates were incubated anaerobically at 37 C for additional 4 h. Afterwards, a sterile solution of sucrose was added to the appropriate wells at the final concentration of 1%, and the plates were incubated anaerobically at 37 C for another 20 h. Wells without bacterial cells were used as blank controls, and untreated *S. mutans* and *S. sobrinus* and TF-treated bacteria served as experimental controls. Following 24 h of the total incubation time, the glass slides were removed from wells, dried and further used for profilometric analysis of the mixed *S. mutans* and *S. sobrinus* biofilm.

Optical profilometry

There were performed 6 measurements for evaluation of the biofilm roughness and 5 measurements for assessment of the biofilm thickness per slide halfway from bottom to top of the visible biofilm employing Sensofar PLu 2300 optical profilometer with 50× confocal objective. Data of the scanned and measured samples were further processed with Gwyddion programme (version 2.27, available at http://gwyddion.net) in order to quantify the biofilm surface's roughness parameters and its thickness reflecting a maturity of the formed biofilm. In addition, a Median filter (size of 10 pixels or 3 m) was selected to remove errors of form and waviness of the surface. For the quantitative evaluation of the biofilm surface's roughness, it was calculated one of the most critical surface's parameters – R_q (root mean square roughness) defining an average of the measured height deviations. The height of artificially produced vertical scratch on each slide covered with the bacteria was used for measurement and calculation of the biofilm thickness.

Evaluation of bacterial growth

S. mutans bacteria were used for the evaluation of bacterial growth in 24-well plates filled with TH broth, containing 10% heat-inactivated horse serum, without saliva and glass slides in the absence or presence of sucrose and PS-ODNs. The bacterial growth was monitored spectrophotometrically every 3 h interval after an initial 2 h of incubation until the time point of 14 h, and then at the time point of 24 h. For this purpose, OD of taken samples from the plate wells was measured at 630 nm employing microplate reader spectrophotometer (Dynex MRX). Also, the Gram staining was performed of the samples taken at time point of 24 h for the bacterial morphology evaluation using Leica DM500 light microscope.

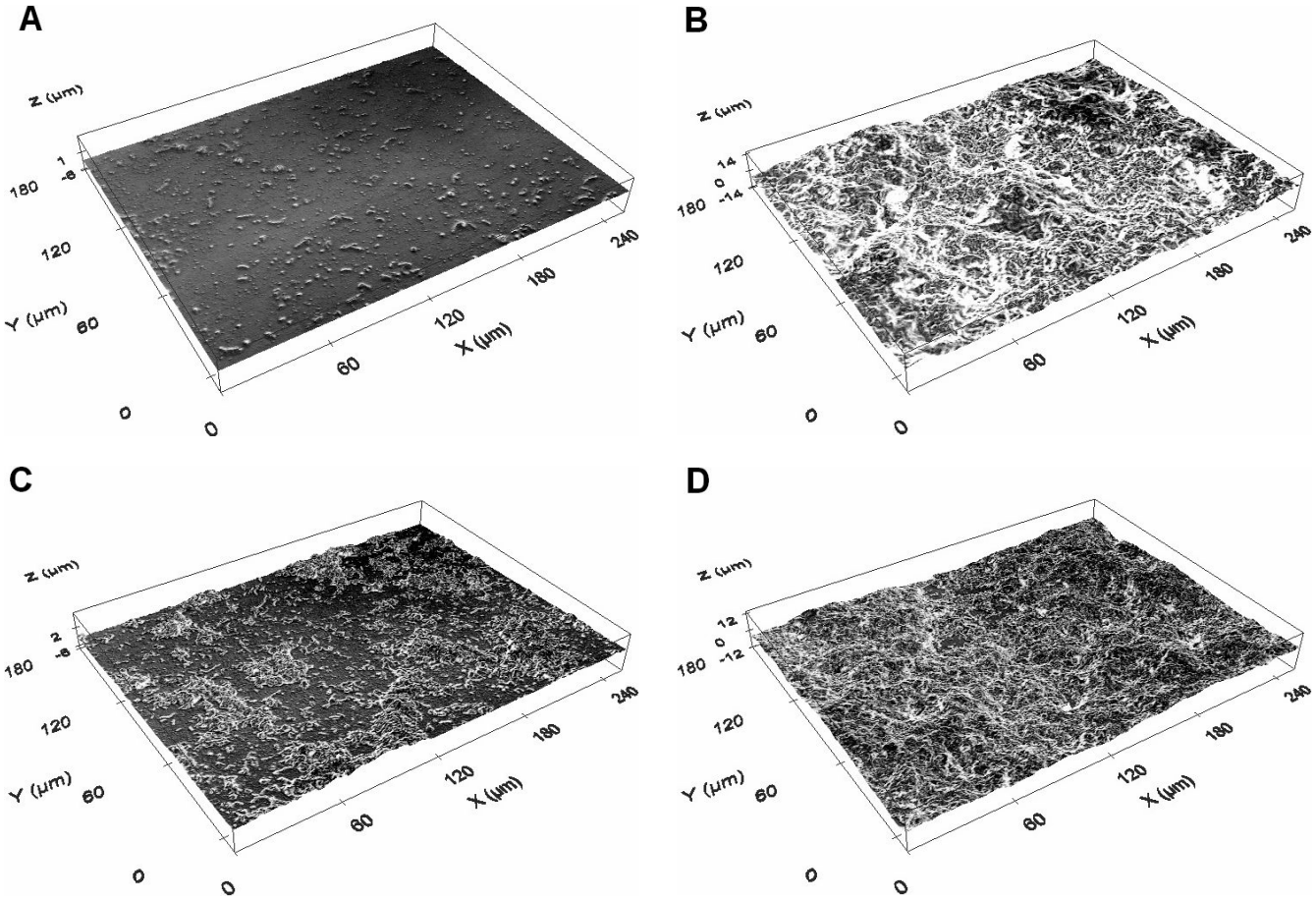

Fig. 1. **Optical profile of glass slides with the mixed *S. mutans* and *S. sobrinus* culture biofilm after 24 h of incubation under different treatments within Todd Hewitt broth, containing sterile saliva, in the absence or presence of 1% sucrose.**
A – glass slide surface with bacteria incubated without treatment with PS-ODNs in the absence of 1% sucrose; B – glass slide surface with bacteria incubated without treatment with PS-ODNs in the presence of 1% sucrose; C – biofilm under treatment with PS-ODN1 + TF (TurboFect™) in the presence of 1% sucrose; D – biofilm under treatment with PS-ODN2 + TF (TurboFect™) in the presence of 1% sucrose. Magnification, ×50.

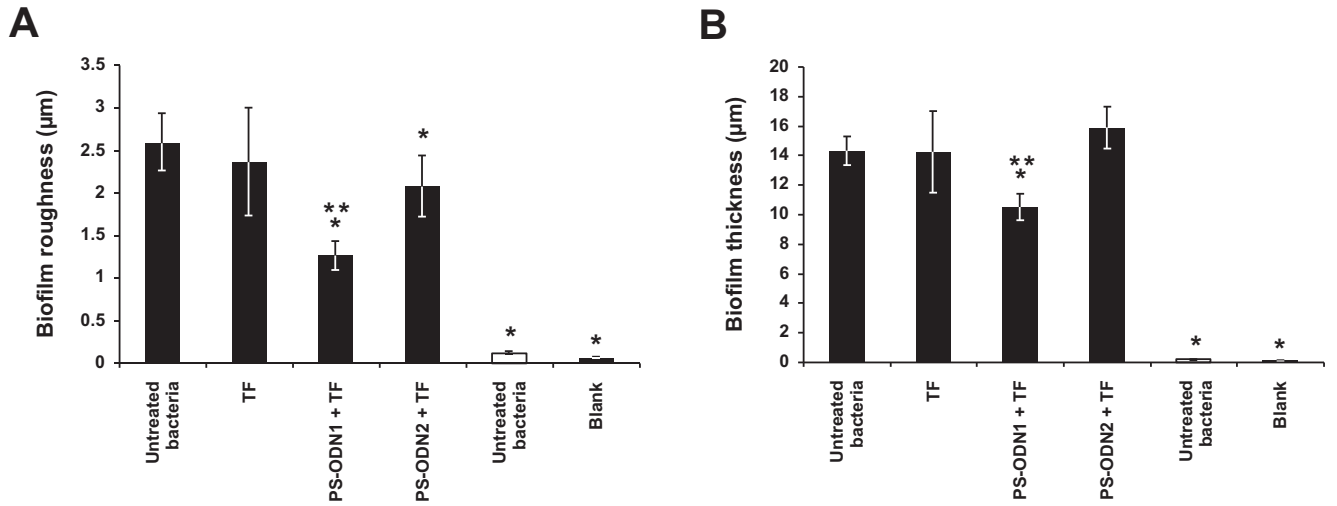

Fig. 2. **Quantities of the mixed *S. mutans* and *S. sobrinus* culture biofilm formed on the glass slide surface after 24 h of incubation under different treatments within Todd Hewitt broth, containing sterile saliva, in the absence (white bar) or presence (black bars) of 1% sucrose.**
A – biofilm surface's roughness (parameter R_q); B – biofilm thickness. Blank – glass slide surface after 24 h of incubation in TH broth, containing sterile saliva, in the presence of 1% sucrose, but without bacteria and treatments with PS-ODNs; untreated bacteria (white bar in A and B) – that are bacteria after 24 h of incubation in TH broth without 1% sucrose and treatments with PS-ODNs; untreated bacteria (black bar in A and B) – that are bacteria after 24 h of incubation in TH broth containing 1% sucrose, but without treatments with PS-ODNs. Data (n=6 for biofilm surface's roughness (parameter R_q); n=5 for biofilm thickness) are means±standard deviation.
*p<0.05 compared to the untreated bacteria (black bar), **p<0.05 compared to PS-ODN2 + TF (TurboFect™).

Statistical analysis

Data are expressed as means±standard deviation (SD). Statistical evaluation was performed using the One-Way ANOVA with LSD Post Hoc test of SPSS programme (version 20.0). A p value less than 0.05 was considered statistically significant.

RESULTS

Effect of the antisense oligonucleotide on formation of streptococci biofilm

Analysis of the glass slides' surfaces with the mixed *S. mutans* and *S. sobrinus* culture biofilms applying technique of the optical profilometry revealed that the presence of 1% sucrose in TH broth with saliva considerably increased biofilm surface's roughness parameter – R_q as well as biofilm thickness in comparison to the untreated bacteria growing without sucrose (p<0.05) (Fig. 1A and B; Fig. 2). However, the antisense PS-ODN1 in combination with TurboFect™ reagent decreased biofilm surface's roughness (R_q) of the mixed *S. mutans* and *S. sobrinus* cultures by 2-fold compared to the untreated bacteria as well as by 1.6-fold compared to the bacteria exposed to missense PS-ODN2 in combination with TurboFect™ reagent within TH broth containing 1% sucrose and saliva (p<0.05) (Fig. 1B–D and Fig. 2A). Moreover, the antisense PS-ODN1 in combination with TurboFect™ reagent reduced biofilm thickness of the mixed *S. mutans* and *S. sobrinus* cultures by 1.4-fold compared to the untreated bacteria as well as by 1.5-fold compared to the bacteria exposed to missense PS-ODN2 in combination with TurboFect™ reagent within TH broth containing 1% sucrose and saliva (p<0.05) (Fig. 1B–D and Fig. 2B). The exposures of streptococci to PS-ODN1 and PS-ODN2 without TurboFect™ reagent resulted in 1.83±0.43 m and 1.83±0.76 m for the biofilm surface's roughness parameter R_q, respectively. Similarly, the treatments with PS-ODN1 and PS-ODN2 without TurboFect™ reagent produced values of 10.25±5.73 m and 9.87±4.34 m for the biofilm thickness, respectively. As shown in Fig. 1B, 1D and Fig. 2, the transfection reagent TurboFect™ alone and even combined with missense oligonucleotide (PS-ODN2) had minimal effect on the streptococci biofilm formation only in terms of the biofilm surface's roughness parameter R_q.

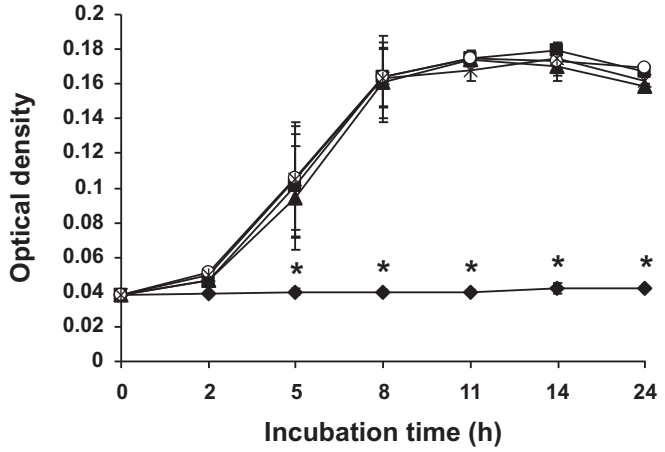

Fig. 3. **Optical densities of *S. mutans* bacteria growing in TH broth, containing 10% heat-inactivated horse serum, without saliva and sucrose under different treatments during 24 h of the total incubation time.**
Symbols: ◆, blank; ■, untreated bacteria; ▲, TF (TurboFect™); ○, PS-ODN1 + TF (TurboFect™); ✳, PS-ODN2 + TF (TurboFect™). Data (n=2) are means±standard deviation. *p<0.05 compared to the untreated bacteria.

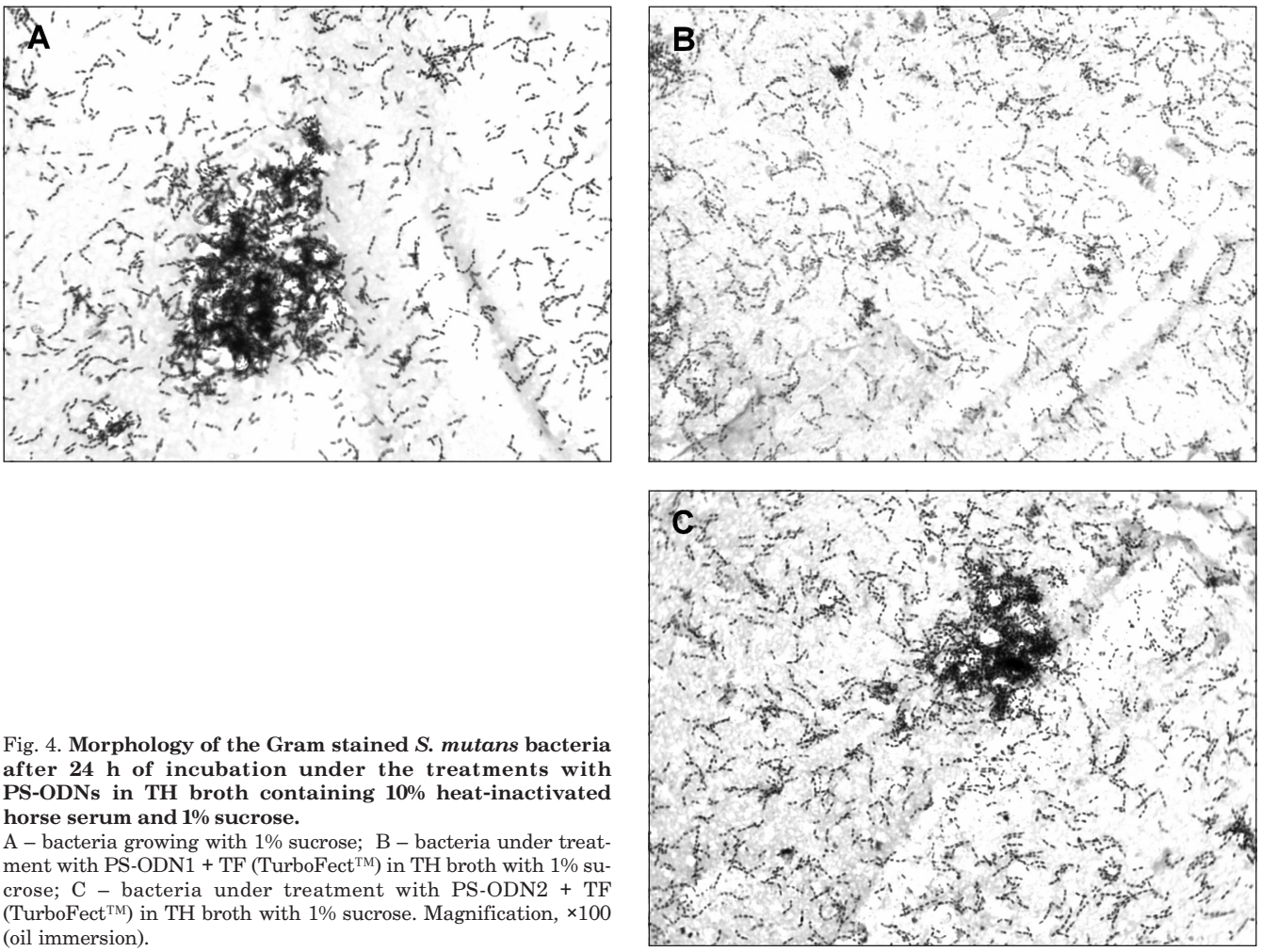

Fig. 4. **Morphology of the Gram stained *S. mutans* bacteria after 24 h of incubation under the treatments with PS-ODNs in TH broth containing 10% heat-inactivated horse serum and 1% sucrose.**
A – bacteria growing with 1% sucrose; B – bacteria under treatment with PS-ODN1 + TF (TurboFect™) in TH broth with 1% sucrose; C – bacteria under treatment with PS-ODN2 + TF (TurboFect™) in TH broth with 1% sucrose. Magnification, ×100 (oil immersion).

Effect of the antisense oligonucleotide on streptococci growth

Spectrophotometric monitoring of the untreated *S. mutans* cultures in TH broth, containing 10% heat-inactivated horse serum, without sucrose revealed normal bacterial growth during 24 h of the total incubation time (Fig. 3). According to the measurement data, growth of the untreated bacteria peaked at 14 h, where optical density (OD) value was 0.18±0.001. The treatments of *S. mutans* with TurboFect™ reagent alone, antisense PS-ODN1 and missense PS-ODN2 in combination with TurboFect™ reagent did not significantly reduce the bacterial growth as compared to the untreated bacteria (p>0.05) at all time points during 24 h incubation period (Fig. 3). However, the addition of sucrose resulted in a gradual and substantial increase of OD values over the incubation time reaching peak at 11 h, where OD value was 0.3±0.019 for the untreated bacteria (data not shown). On the other hand, Gram staining of samples demonstrated that the exposure of *S. mutans* to antisense PS-ODN1 combined with TurboFect™ reagent led to decrease of the bacterial aggregation in TH broth with 1% sucrose as compared with the untreated bacteria and bacteria treated with the missense PS-ODN2 in combination with TurboFect™ reagent at the time point of 24 h (Fig. 4).

DISCUSSION

This study demonstrated the capacity of antisense oligonucleotide targeting specifically *S. mutans gtfB*, *gtfC* and *S. sobrinus gtfI* mRNAs to suppress biofilm formation by these streptococci *in vitro* conditions. A most likely mechanism of action is that one antisense oligonucleotide of the same sequence can simultaneously down-regulate the expression of three target genes (glucosyltransferases) in the oral streptococci leading to reduction of the biofilm development on solid surface (i. e. glass). In this aspect, our investigation essentially differs from the study performed by Guo et al. [7], where the researchers used antisense oligonucleotides for inhibition of one target function only – *gtfB* mRNA in *S. mutans* bacteria. On the basis of results reported by Guo et al. [7], we selected 10 M as the final concentration of PS-ODNs for treatment of streptococci, and also applied the similar transfection reagent (TurboFect™) consisting of cationic polymer in order to facilitate penetration of the PS-ODNs to the bacterial cells. The cationic polymer forms complexes with PS-ODNs via electrostatic interaction between negatively charged oligonucleotide molecules and positively charged reagent molecules [7, 12]. Since such complexes maintain positive charge, therefore they can bind to the negatively charged bacterial cell wall, and then can be taken up by the cells. Indeed, a significant reduction of the biofilm quantity parameters under exposure to the test antisense oligonucleotide (PS-ODN1) in combination with TurboFect™ reagent strongly supports this mechanism of oligonucleotide transfer to bacteria. Moreover, since the treatments of bacteria with antisense (PS-ODN1) and missense (PS-ODN2) oligonucleotides without TurboFect™ reagent generated approximately the same values for biofilm quantity parameters, hence it indicates that the transfection reagent is necessary for

penetration of the antisense oligonucleotide into bacterial cells in order to reach the target mRNAs. In addition, the finding that missense oligonucleotide (PS-ODN2) in combination with TurboFect™ reagent did not affect the biofilm formation highlights that the activity of test antisense oligonucleotide (PS-ODN1) was very specific, i. e. the selective inhibition of *S. mutans gtfB*, *gtfC* and *S. sobrinus gtfI* mRNA functions resulting in the decreased biofilm production. The latter fact suggests a suppression of water-insoluble glucans' synthesis since these polymers are essential for the biofilm development, and no glucans leads to no biofilm formation [6, 13]. It should be noted that, in the presence of sucrose, *S. mutans* and *S. sobrinus* adhesion to solid surfaces (e. g., glass, tooth enamel) is mainly dependent on the activity of glucosyltransferases, especially those synthesizing water-insoluble glucans [13–15]. This was evidently demonstrated by Ooshima et al. [13] using *S. mutans* GtfB/C-deficient mutant strains – their ability to adhere to glass surface was significantly reduced in spite of the presence of sucrose within medium. Therefore, in the context of these studies, we can state that the test antisense oligonucleotide (PS-ODN1) exhibited highly specific effect on the biofilm formation, probably by simultaneous inactivation of *S. mutans gtfB*, *gtfC* and *S. sobrinus gtfI* mRNAs. Importantly, this inhibitory effect occurred in the medium containing human saliva and sucrose that in part imitated natural microenvironment of oral cavity.

Furthermore, we also performed an additional experiment in order to find out whether the PS-ODNs affect the bacterial growth. For this purpose, the growth of *S. mutans* bacteria was monitored spectrophotometrically in the medium with or without sucrose under exposures to the PS-ODN1 and PS-ODN2 conjugated to TurboFect™ reagent. In the medium without sucrose, these oligonucleotides did not attenuate *S. mutans* growth as compared to the unexposed bacteria indicating that the PS-ODNs conjugated to TurboFect™ reagent did not affect the bacterial viability, and thus they are not bacteriostatic or bactericidal. This would infer a low interferences with the oral ecosystem, as no species would be eliminated. However, according to Gram staining results, in the medium with sucrose, the exposure of *S. mutans* to test antisense oligonucleotide (PS-ODN1) in combination with TurboFect™ reagent caused a substantial decrease in bacterial aggregation as compared to the unexposed bacteria, but not viability, whereas missense oligonucleotide (PS-ODN2) did not possess such effect. Since the aggregation or clustering of *S. mutans* bacteria are also dependent on the water-insoluble glucans produced from sucrose due to enzymatic activities of GtfB and GtfC as indicated in review by Koo et al. [6], therefore the latter finding supports and corresponds to the main results outlined herein proving that the test antisense oligonucleotide (PS-ODN1) suppressed selectively *S. mutans gtfB* and *gtfC* mRNAs.

CONCLUSIONS

In accordance with the presented results, we conclude that the phosphorothioate oligodeoxyribonucleotide designed to antisense simultaneously *S. mutans gtfB*, *gtfC* and *S. sobrinus gtfI* mRNAs inhibits biofilm formation by these streptococci via the specific inactivation of their respective *gtf* mRNAs function. From the data of additional experiment, we also conclude that this phosphorothioate oligodeoxyribonucleotide reduces aggregation of the streptococci without bacterial viability or affecting the oral ecology. Taking together, these results are important for the development of novel pharmaceuticals aiming to prevent caries through the suppression of dental biofilm formation.

ACKNOWLEDGEMENTS

We would like to thank Dr. Albertas Timinskas (Institute of Biotechnology, Vilnius University) who helped in bioinformatical analysis of the selected glucosyltransferase targets and design of antisense oligonucleotide. The study was funded by a grant "VFT0: Prevention of Caries No. VO_MAH530310" from the IKS Lund University, and Vilnius University, which supported Povilas Kalesinskas scientific work by a grant "ES BIOMEDOKT No. 300949173" from the European Union structural funds. ◆

Gauta: 2014 11 20
Priimta spaudai: 2014 12 30

Santrauka

DANTŲ BIOPLĖVĖS FORMAVIMOSI SLOPINIMAS NAUDOJANT PRIEŠPRASMINĮ OLIGONUKLEOTIDĄ PRIEŠ *STREPTOCOCCUS MUTANS* IR *STREPTOCOCCUS SOBRINUS* GLIUKOZILTRANSFERAZES

Povilas Kalesinskas, Tomas Kačergius, Arvydas Ambrozaitis, Vytautė Pečiulienė, Ryo Jimbo, Dan Ericson

Įvadas. Dantų ėduonis išlieka viena iš labiausiai paplitusių žmogaus ligų dėl nekontroliuojamai padidėjusio maisto sacharozės vartojimo šiuolaikinėje visuomenėje. Sacharozę metabolizuoja *Streptococcus mutans* ir *Streptococcus sobrinus* iki rūgščių sukeldami dantų irimą. Šie streptokokai gamina gliukoziltransferazes (Gtf), reikalingas lipnių glukano polimerų sintezei iš sacharozės, o tai yra svarbu bioplėvės formavimuisi ant dantų. Siekiant sumažinti dantų bioplėvės susidarymą ir jos sukeliamas burnos ligas, viena iš prevencijos priemonių galėtų būti Gtf sintezės slopinimas.

Darbo tikslas – išbandyti priešprasminį fosforotioato oligodeoksiribonukleotidą (FT-ODN), veikiantį tuo pačiu metu *S. mutans gtfB*, *gtfC* ir *S. sobrinus gtfI* iRNR, siekiant nuslopinti bioplėvės formavimąsi *in vitro*.

Medžiaga ir metodai. Mišri *S. mutans* ir *S. sobrinus* bakterijų kultūra buvo auginama anaerobinėmis sąlygomis ant objektinių stikliukų, įdėtų vertikaliai į 24 šulinėlių plokštelę, pripildytą *Todd Hewitt* buljono su sacharoze ir steriliomis seilėmis, esant 10 M galutinės koncentracijos priešprasminio arba išmaišytos sekos FT-ODN poveikiui. Nepaveiktos bakterijos pasirinktos kontrolei. Po 24 val. inkubacijos objektiniai stikliukai buvo ištraukti, išdžiovinti ore ir toliau panaudoti kiekybiniam streptokokų bioplėvės įvertinimui taikant optinės profilometrijos metodiką.

Rezultatai. Nustatyta, kad priešprasminis FT-ODN labai sumažino mišrių *S. mutans* ir *S. sobrinus* kultūrų bioplėvės šiurkštumą ir storį, užslopindamas bioplėvės susidarymą 1,6 karto, palyginti su nepaveiktomis ir išmaišytos sekos FT-ODN paveiktomis bakterijomis (p<0,05).

Išvados. Duomenys rodo, kad priešprasminis FT-ODN labai susilpnina streptokokų sukeltą bioplėvės susidarymą ant objektinių stikliukų ir todėl galbūt galėtų smarkiai slopinti dantų bioplėvės formavimąsi vienu metu inaktyvindamas bakterijų *gtf* iRNR.

Reikšminiai žodžiai: dantų ėduonis, *Streptococcus mutans*, *Streptococcus sobrinus*, bioplėvė, gliukoziltransferazė, priešprasminis oligonukleotidas.

LITERATURE

1. Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990–2010: a systematic analysis. J Dent Res 2013; 92: 592–7.
2. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent 2009; 22: 3–8.
3. Anderson CA, Curzon ME, Van Loveren C, Tatsi C, Duggal MS. Sucrose and dental caries: a review of the evidence. Obes Rev 2009; 10(Suppl. 1): 41–54.
4. Decker EM, Klein C, Schwindt D, von Ohle C. Metabolic activity of *Streptococcus mutans* biofilms and gene expression during exposure to xylitol and sucrose. Int J Oral Sci 2014; doi: 10.1038/ijos.2014.38 (Epub ahead of print).
5. Nishimura J, Saito T, Yoneyama H, Bai LL, Okumura K, Isogai E. Biofilm formation by *Streptococcus mutans* and related bacteria. Adv Microbiol 2012; 2: 208–15.
6. Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res 2013; 92: 1065–73.
7. Guo QY, Xiao G, Li R, Guan SU, Zhu XL, Wu JZ. Treatment of *Streptococcus mutans* with antisense oligodeoxyribonucleotides to *gtfB* mRNA inhibits GtfB expression and function. FEMS Microbiol Lett 2006; 264: 8–14.
8. Deleavey GF, Damha MJ. Designing chemically modified oligonucleotides for targeted gene silencing. Chem Biol 2012; 19: 937–54.
9. Bai H, Xue X, Hou Z, Zhou Y, Meng J, Luo X. Antisense antibiotics: a brief review of novel target discovery and delivery. Curr Drug Discov Technol 2010; 7: 76–85.
10. McLeod FS, Simmonds RS. Penicilin facilitates the entry of antisense constructs into *Streptococcus mutans*. FEMS Microbiol Lett 2013; 349: 25–31.
11. Dufour M, McLeod FS, Simmonds RS. Zoocin A facilitates the entry of antisense constructs into *Streptococcus mutans*. FEMS Microbiol Lett 2011; 317: 93–9.
12. Zhu L, Mahato RI. Lipid and polymeric carrier-mediated nucleic acid delivery. Expert Opin Drug Deliv 2010; 7: 1209–26.
13. Ooshima T, Matsumura M, Hoshino T, Kawabata S, Sobue S, Fujiwara T. Contributions of three glucosyltransferases to sucrose-dependent adherence of *Streptococcus mutans*. J Dent Res 2001; 80: 1672–7.
14. Söderling EM, Hietala-Lenkkeri AM. Xylitol and erythritol decrease adherence of polysaccharide-producing oral streptococci. Curr Microbiol 2010; 60: 25–9.
15. Zhao W, Li W, Lin J, Chen Z, Yu D. Effect of sucrose-dependent adhesion and glucosyltransferase expression of *S. mutans* in children with severe early-childhood caries (S-ECC). Nutrients 2014; 6: 3572–86.