

Antimicrobial activity of a novel silane coupling agent consisting of a quaternary ammonium salt using a polymicrobial biofilm model

Abstract

The purpose of this investigation was to evaluate the antimicrobial activity of a novel quaternary ammonium silane coupling agent, N-allyl-N-decyl-N-methyl-N-trimethoxysilylpropylammonium iodide (10-I), against early stage biofilms using a polymicrobial biofilm model that simulates oral plaque-like biofilm formation on a solid phase. Cover glasses were immersed in a sufficient volume of the 800-ppm solution of 10-I for 1 h to modify the surface. Aliquots of a mixture of stimulated saliva from a single subject and McBain medium were dispensed into polystyrene 24-well cell culture plates and then cultured at 37.0°C under anaerobic condition. After culturing, we calculated colony forming unit (CFU). CFU of the 10-I group was approximately 80% lower than that of the control group, demonstrating strong antimicrobial activity. Therefore, surface modification with 10-I is effective for treating oral indigenous bacteria-related dental diseases found in the elderly and immunocompromised individuals and possible systemic complications, such as aspiration pneumonia.

Keywords: silane coupling agent, quaternary ammonium salt, polymicrobial biofilm model

Volume 7 Issue 4 - 2017

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Received: May 20, 2017 | **Published:** May 30, 2017

Introduction

Because the Japanese population is rapidly aging, the involvement of oral indigenous bacteria in systemic diseases has been receiving particular attention in Japan. Although tooth loss rate has decreased because of increased awareness of oral care, more than 50 % of elderly individuals are still using dentures,^{1,2} indicating that oral care is not sufficient.³ The elderly and individuals requiring nursing care show significantly higher morbidity from chronic diseases, and they are more likely to develop multiple complications. The age-associated decline in immune function increases the susceptibility of the elderly to opportunistic infections caused even by generally nonpathogenic low-virulent microbes, leading to refractory infectious diseases, such as respiratory infection, caused by bacteria indigenous to the oral cavity.^{4,5} Moreover, dentures used by several elderly individuals facilitate the accumulation of plaque, called denture plaque, causing bacterial infections that can result in diseases, such as aspiration pneumonia and cerebrovascular disorders.⁶⁻⁹ Therefore, maintaining good oral hygiene not only contributes to oral comfort and the prevention of oral diseases but may also play a role in reducing the incidence of respiratory infections, such as aspiration pneumonia, which are the major causes of mortality among the elderly. We have developed various surface modifiers capable of reducing the surface free energy of tooth substances and dental restoration and prostheses materials, rendering them acid resistant. We have also studied their application for caries prevention and periodontal disease through the

suppression of adhesion and plaque formation and decalcification of dentine.¹⁰⁻¹³ Because the Japanese population is aging, development of an advanced surface modifier that not only prevents the adhesion and accumulation of plaque but also has antimicrobial activity is of urgent importance to actively kill the adhered microorganisms for the prevention of systemic complications. Quaternary ammonium salts, a class of commercially available antimicrobial agents sometimes referred to as invert soaps, display potent bactericidal and disinfecting effects with minimal irritation and low toxicity, and they are promising antimicrobial compounds because of their improved safety.¹⁴ Immobilization of an antimicrobial agent is the most common approach to prolong the duration of its antimicrobial effect; we chose an antimicrobial compound containing a silane coupling agent-derived molecular structure as the most promising and reliable method to achieve this purpose. We synthesized a new quaternary ammonium-based antibacterial silane coupling agent, N-allyl-N-decyl-N-methyl-N-trimethoxysilylpropylammonium iodide (10-I; Tokyo University of Science), to immobilize the antimicrobial activity on substrate surfaces.¹⁵ 10-I did not inhibit colony formation of the cells.¹⁶ Glass plate surfaces modified with 200 and 400 ppm of 10-I inhibited the growth of the following oral bacteria: *Actinomyces viscosus*, *Candida albicans*, *Fusobacterium nucleatum*, *Lactobacillus casei*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Staphylococcus aureus*, and *Streptococcus mutans*. *C. albicans* cultured in the presence of a glass plate treated with the 400-ppm 10-I showed strong growth inhibition.¹⁷ However, bacteria in the oral cavity include both

floating and surface-attached bacteria, and some studies have shown that biofilms comprising multiple surface-attached bacteria were one to two orders of magnitude more resistant to antibiotics than those comprising floating bacteria.^{18,19} Therefore, biofilm models that simulate the plaque environment should be used to accurately evaluate the antibacterial activity against bacteria in the oral cavity. In this study, we evaluated the antimicrobial activity of 10-I against early stage biofilms using a polymicrobial biofilm model,^{20,21} where oral plaque-like biofilms can be formed on a solid phase.

Materials and methods

Materials

Preparation of modifier solutions: The silane coupling agent 10-I, containing a quaternary ammonium structure,¹⁵ was used in this study. Each modifier solution was prepared at 800 ppm with ethanol (EtOH). The chemical formulas of the modifiers are shown in the Table 1.

Table 1 Chemical formula and code of the surfactant used in this study

Formula	Code
$\left[\begin{array}{c} \text{CH}_2 = \text{CHCH}_2 - \text{N}^{\oplus}(\text{CH}_2)_{10}\text{H} \\ \text{CH}_3 \\ \text{Si}(\text{OCH}_3)_2 \end{array} \right] \text{I}^-$	10-I

Preparation of specimens: Cover glasses (ϕ 12 mm; thickness: 0.15 mm; Menzel, Braunschweig, Germany) were immersed in 1-mol/L aqueous solutions of sodium hydroxide first and successively immersed in hydrochloric acid for one day each to remove contaminants on the surface; they were then immersed in a sufficient volume of the 800-ppm solution of 10-I for 1 h to modify the surface. The cover glasses were then washed with EtOH to remove the physically adsorbed excess modifier, allowed to dry naturally, and subjected to autoclave sterilization. The control specimens were prepared by treating cover glasses in the same manner as for test specimens; however, EtOH was used instead of the 10-I solution.

Preparation of saliva samples: Saliva was collected from a healthy adult with natural dentition who had not taken any antibiotics in the previous three months and did not have dental caries or periodontal disease. The subject was not allowed to clean the oral cavity for 24 h or to eat or drink for 2 h before the sampling of saliva. Saliva was collected following stimulation by chewing Parafilm, and it was cooled on ice after collection. The collected saliva sample was immediately diluted to 70 % with sterile glycerin and stored at -80°C . The use of human saliva was approved by the Research Ethics Committee of Kanagawa Dental University (Approval Number: 206).

Methods

Antibacterial effects against polymicrobial biofilms: The polymicrobial biofilm was prepared as reported by Exterkate et al.¹⁸ Briefly, the stimulated saliva sample from a single subject was diluted 50-fold with McBain medium²² (pH 7.5) to which sucrose was added at a final concentration of 0.2 %; the mixture was dispensed in 1.5-mL wells of polystyrene 24-well cell culture plates; the plates were then incubated at 37°C under anaerobic condition of 10 % CO_2 ,

10 % H_2 , and 80 % N_2 for 10 h to allow biofilm formation.

Colony forming unit (CFU) counts: After culturing, the specimens were washed with cysteine peptone water (CPW) three times, transferred into 2 mL of phosphate buffered saline, and sonicated for 90 s to detach bacterial cells from the specimens. Each bacterial solution was then serially diluted with CPW, and 50- μL aliquots were seeded into tryptic soy blood agar plates. The plates were incubated at 37°C under anaerobic condition of 10 % CO_2 , 10 % H_2 , and 80 % N_2 for 3 days, and the viable bacteria in each culture were then counted.

Results

CFU counts in polymicrobial biofilms are shown in the Figure 1. CFU in the 10-I group was 3.4×10^4 CFU/disk and that in the control group was 16.8×10^4 CFU/disk, indicating an 80% reduction; hence, this demonstrated strong antimicrobial activity.

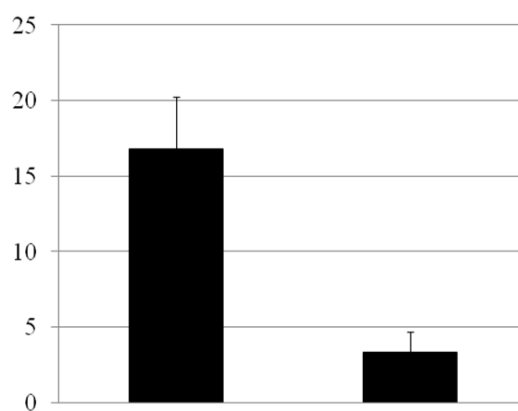


Figure 1 Colony forming unit of polymicrobial biofilms on the glass surface modified with 10-I.

Discussion

In several countries, including Japan, cleaning of the oral cavity in the elderly and in individuals requiring nursing care has received less attention, and various infectious diseases in this population have been attributed to oral indigenous bacteria.²³ Therefore, oral care is a key preventive measure for not only aspiration pneumonia but also for other infectious diseases. Cleanliness and amenities have increased over the recent years as the quality of life has improved, and antibacterial agents are now used in various types of commercial products. Although antibacterial products are attractive for the control of microbial contamination, their use is subject to limitations because of short durations of antibacterial effect because of the elution or volatilization of the ingredients and metal ions from the material surface and their own toxicity. Recently, the photocatalytic generation of reactive oxygen species by titanium oxide has been used for organic matter decomposition, antibacterial activity, pigment degradation, deodorants, and other uses.²⁴ Titanium oxide has been demonstrated to have antimicrobial activity against bacterial species similar to that in the present study, including *P. gingivalis*, *S. mutans*, and *C. albicans*. However, its oral applications are limited because light irradiation is required as a catalyst.^{25–28} Previous studies have shown that the surface treatment of dentures with modifiers containing a fluoroalkyl group²⁹ reduced the adhesion of *C. albicans*, but physical properties were problematic.³⁰ Immobilization of antibacterial agents on the surface

of substrates is a possible approach to address these problems. These antibacterial agents are called as immobilized antibacterial agents that act upon contact with microorganisms. Their advantages include the absence of environmental residues, continuous microbial treatment, and long-term use and reuse after regeneration.³¹ In this study, 10-I was tested for antimicrobial activity against early stage biofilms using a polymicrobial biofilm model in which the bacterial plaque environment in the oral cavity can be simulated. The number of viable bacterial cells on the 10-I-modified substrate surface was reduced by approximately 80% as compared with that of the control group, demonstrating the strong antimicrobial activity of the 10-I-modified surface. The bacterial surface is slightly negatively charged because of proteins and sugar chains on the cell membrane. Positively charged quaternary ammonium salts, such as 10-I, are considered to exert antibacterial activity by electrostatically attracting negatively charged bacteria and creating nonuniform charge distributions on the cell membrane of bacteria adjacent to the quaternary ammonium salt; the result is the gradual weakening and destruction of the cell membrane from the opposite side of adhesion [32]. Although the number of bacteria in the oral cavity is temporarily reduced by oral care, it gradually increases and regains the original state over time. Therefore, maintaining good health of the oral cavity is difficult. 10-I used in the present study can be used as a surface modifier or a cleaner for dental restorations and prostheses as well as for tooth surfaces. The results of the present study demonstrated the potential of 10-I as a surface modifier in reducing oral microorganism adhesion to the surface of the substrate as well as its contribution to the maintenance of oral hygiene; also, they suggested the possibility of using 10-I as an immobilized antibacterial agent to suppress or prevent systemic infectious diseases in addition to the local infections caused by microorganisms in the oral cavity. Furthermore, this surface modifier may also be helpful in post-disaster oral health management.

Conclusion

In this study, we evaluated the antibacterial activity of 10-I against early stage biofilms using a polymicrobial biofilm model in which oral plaque-like biofilms can be formed on a solid phase. The result indicated that polymicrobial biofilm formation was strongly inhibited by 10-I-modified glass plates. This result suggests that the surface modification with 10-I is effective for the suppression or prevention of not only oral indigenous bacteria-related dental diseases observed in the elderly and immunocompromised individuals but also of possible systemic complications, such as aspiration pneumonia.

Funding

None.

Acknowledgments

A part of this study was supported by the Strategic Research Infrastructure Development Program for Private Universities (2012-2014) and a Grant-in-Aid for Scientific Research (B) (23792257) from the Ministry of Education, Culture, Sport, Science, and Technology.

Conflicts of interests

The author declares that there is no conflict of interest.

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