Biofilm Formation In Vivo on Perfluoro-Alkylsiloxane–Modified Voice Prostheses

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Objective: To study the influence of perfluoroalkylsiloxane (PA) surface modification of silicone rubber voice prostheses on biofouling.

Design: Placebo-controlled clinical trial.

Setting: Tertiary referral center, with specialization in head and neck cancer treatment.

Patients: Eighteen consecutive patients with laryngectomies and experienced in the use of a voice prosthesis who visited the outpatient clinic for prosthesis replacement.

Material: Eighteen partially surface-modified voice prostheses (3 with short-chain PAs [1 fluorocarbon unit] and 15 with long-chain PAs [8 fluorocarbon units]) were inserted via the patients’ tracheoesophageal shunts and remained in place for 2 to 8 weeks.

Intervention: Replacement of the prostheses.

Main Outcome Measures: Evaluation of biofilm formation on short- and long-chain PA–modified and original silicone rubber surfaces on the esophageal side of the voice prosthesis.

Results: The planimetric biofilm scores of the surfaces of all 3 short-chain PA–treated voice prostheses indicated more biofouling on the treated surfaces than on the untreated surfaces of the same prostheses. For the long-chain PA–treated prostheses, the planimetric biofilm scores, as well as the numbers of colony-forming units per cm² for bacteria and yeasts, indicated less biofouling on the treated side than on the control side for 9 of the 13 prostheses that could be analyzed (2 were lost to analysis). Identical fungal strains, mainly Candida sp, were isolated from biofilms on each side of the esophageal flange.

Conclusions: Chemisorption of long-chain PAs by the silicone rubber used for voice prostheses reduces biofilm formation in vivo and therefore can be expected to prolong the life of these prostheses. Chemisorption of short-chain PAs by silicone rubber seems to have an adverse effect.

MATERIALS AND METHODS

“Ultra-Low Resistance” Groningen button voice prostheses (Medin Medical Instruments and Supplies) were used in this study. The silicone rubber was modified in a 2-step process, as schematically shown in Figure 1. One half of the esophageal flange of each voice prosthesis was oxidized in an argon-plasma treatment.17 In the second step, perfluoro-alkylvichlorosilanes (Fluka Chemie AG, Buchs, Switzerland) were chemisorbed onto the oxidized surfaces by immersion for 10 minutes in 0.5% perfluoro-alkylvichlorosilanes dissolved in perfluorohelpane, leaving one half of the esophageal flange untreated. Perfluorohelpane was chosen because it does not swell the silicone rubber. We used 2 different silanes with short (1 fluorocarbon unit) and long (8 fluorocarbon units) fluorocarbon chains, respectively. Silane-treated surfaces were washed with perfluorohelpane and absolute ethanal. Mean (SD) advancing water contact angles on the sides of the silicone rubber prostheses treated with chemisorbed short and long PA chains were 125 (5) and 140 (5) degrees, respectively,15 which is higher than on the untreated sides (115 ±3 degrees), signifying a higher degree of hydrophobicity of the treated surface. Chemisorption of PA chains did not adversely affect the biocompatibility of the silicone rubber, according to an approved agar diffusion test (BSC105/2; Bioscan BV, Bilthoven, the Netherlands). The airflow resistance of the partially modified Groningen button voice prostheses was tested as described previously18 and was identical to the airflow resistance of the original prostheses.

Following informed consent, each of 18 patients with laryngectomies and long-term experience with the use of a voice prosthesis was given a partially modified (by chemisorption of either short- or long-chain PAs) Groningen button voice prosthesis. All patients were randomly included in the study with no selection criteria other than that they required replacement of a prosthesis for medical reasons of leakage or increased airflow resistance. The tested voice prostheses were removed at the following regular outpatient clinic visit. After removal of the prosthesis from the tracheoesophageal shunt, biofilm formation on the modified and unmodified sides was compared by light microscopy, and a planimetric biofilm score was calculated as the percentage of the surface (of the esophageal flange) colonized by microorganisms. Subsequently, culture samples were taken and the prostheses prepared for scanning electron microscopy. Microbial compositions of the biofilms on both sides of the valve were compared by plating on brain-heart infusion and blood agar plates (OXOID, Basingstoke, England) and by culturing at 37°C under aerobic conditions.6 Also, the number of colony-forming units of bacteria and yeasts per unit area (cm²) was determined for each side of the prostheses as a second, quantitative measure for biofilm formation.

determined by the properties of the biomaterial’s surface. An alternative method to prevent or decrease biofilm formation might be to modify the silicone rubber surface. Recently, we have shown that the hydrophobicity of this surface influenced microbial adhesion in vitro14,15 and biofilm formation on voice prostheses in vivo, even after 4 weeks’ use.16,17 Further, chemisorption of PAs substantially reduced microbial adhesion to silicone rubber in vitro.15 Moreover, microorganisms were easily detached from silicone rubber surfaces with chemisorbed, long-chain PAs, making this modification promising for the preparation of low-fouling, silicone rubber voice prostheses.

It is the aim of this article to compare the biofilm formation on silicone rubber Groningen button voice prostheses in vivo, with and without chemisorbed PAs.

RESULTS

After evaluation of the first 3 prostheses with the short PAs (1 fluorocarbon unit), it became obvious that the surface-modified part of the esophageal flange attracted approximately twice the amount of biofilm as the untreated part. Therefore, it was decided to stop the experiment with the short PAs. Figure 2 shows a scanning electron micrograph of the esophageal flange of a partially long-chain PA surface-
modified Groningen button prosthesis after 4 weeks of use. On the modified side, long PA chains (8 fluorocarbon units) had been chemisorbed to the silicone rubber. Fewer microcolonies formed on the modified side than on the original silicone rubber side of the prosthesis.

The Table summarizes the planimetric biofilm scores and the numbers of CFUs per unit area cm² on each side of the voice prostheses after chemisorption of the long-chain PAs. One voice prosthesis was lost to microbial analysis because the patient did not show up for routine follow-up but had the experimental voice prosthesis changed outside of office hours, 6 months after it had been inserted. Another prosthesis is still in place in a patient who chose to keep it; this prosthesis has now been situated for more than a year. Chemisorption of PAs by bacteria and yeasts yields the typical detrimental effects on prosthesis life because these prostheses were only partly modified.

The results of this study indicate that chemisorption of long-chain PAs on silicone rubber yields reduced biofilm formation with possibly increased effects when longer fluorocarbon chains are used. The effects of the surface modification are obvious from the number of CFUs isolated per unit area cm² and from the planimetric biofilm scores. The planimetric biofilm score does not differentiate between thick and thin biofilms once the score has reached 100% and a single film of adhering microorganisms has formed. Therefore, the planimetric biofilm score is a measure of the extension of biofilm over the esophageal side of the prosthesis and may have great relevance in predicting the occurrence of valve failure during use.

The reduction of biofilm formation on the long-chain PA–modified silicone rubber is a result of a variety of factors. First, the increased hydrophobicity of surface-modified silicone rubber contributes to the reduction in biofilm formation, as also demonstrated for dental plaque formation in the oral cavity. The reduction in dental plaque formation through increased substratum hydrophobicity involves bacteria more than yeasts. This is in line with the present results, demonstrating reductions in bacterial colonization of treated voice prostheses by several orders of magnitude. In comparison, reductions in colonization by yeasts were minor (Table), although in 3 of the 13 patients, no yeasts were isolated from the modified sides. In vitro studies have indicated, however, that only mixed colonization of silicone rubber by bacteria and yeasts yields the typical detrimental ingrowth of microcolonies causing dysfunction of prostheses. For the present application, increased hydrophobicity due to fluoridation of the silicone rubber alone

<table>
<thead>
<tr>
<th>Patient No. / Implantation Time, wk</th>
<th>Untreated Side</th>
<th>Perfluoro-Alkylsiloxane–Treated Side</th>
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<tr>
<td></td>
<td>Planimetric Biofilm Score, %</td>
<td>Bacteria, CFUs/cm²</td>
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<tr>
<td>1/4</td>
<td>5</td>
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<td>10</td>
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is not sufficient protection against bacterial colonization; chemisorption of short-chain PAs does not show a favorable effect on biofilm formation. We have proposed that chemisorbed PA chains on silicone rubber form a dendritic wedge structure\textsuperscript{15} with a high degree of freedom of the chemisorbed chains. The favorable effects of chemisorbed long-chain PAs compared with short-chain PAs can be attributed to a higher mobility of the chemisorbed polymer chains, in a sense repelling the biofilm. Hence it is anticipated that the use of even longer fluorocarbon chains may show even greater beneficial effects.

CONCLUSIONS

This study demonstrates that biofilm formation on silicone rubber Groningen button voice prostheses over an evaluation period of approximately 2 to 8 weeks can be reduced by chemisorption of long (8 fluorocarbon units) PA polymer chains owing to the high hydrophobicity and mobility of these chemisorbed chains. Whether the average life of indwelling voice prostheses can also be prolonged by the modification studied here remains to be determined.

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REFERENCES