COMPARISON OF BIOFILM FORMATION ON NEW PHONAX AND PROVOX 2 VOICE PROSTHESES—A PILOT STUDY

Matthias Leonhard, MD,1 Doris Moser, MD,2 Adrian Reumueller,1 Gudrun Mancusi, MD,1 Wolfgang Bigenzahn, MD,1 Berit Schneider-Stickler, MD1

1Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria.
E-mail: matthias.leonhard@meduniwien.ac.at
2Department of Cranio-Maxillofacial and Oral Surgery, Medical University of Vienna, Vienna, Austria

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Abstract: Background. In voice rehabilitation for laryngectomized patients, voice prosthetic biofilm formation is still an unsolved problem. Design and materials of voice prostheses have been altered by manufacturers to improve function and extend the lifetime of devices. The goal of the study was to investigate biofilm formation on Provox 2 and Phonax, recently introduced voice prostheses made of thermoplastic polyurethane.

Methods. Five laryngectomized patients were equipped with both Phonax and Provox 2 voice prostheses. Microbial colonization was analyzed using standard microbiological methods. Biofilm formation and material infiltration were illustrated using scanning electron microscopy, fluorescence microscopy, and thin-section light microscopy.

Results. Although no differences in quality or quantity of microbial colonization were assessed, microscopic imaging revealed differences in material surfaces, biofilm composition, and infiltration morphologies; the polyurethane material seems to destabilize biofilm architecture by inhibition of hyphal Candida growth forms.

Conclusions. Polyurethane material for voice prostheses seems to reduce biofilm stability and infiltrative processes.


Keywords: biofilm adhesion; Candida albicans; polyurethane; silicone; voice prostheses

In laryngectomized patients, voice prosthetic biofilm formation is a well-studied but still unsolved problem. Microbial deposits on the valve lead not only to aspiration of nutritional components and saliva but also to luminal obstruction with speaking difficulties. In this case, replacement of the malfunctioning prostheses is necessary, but usually has to be performed by a physician. Thus, the short lifetime of the device causes discomfort for the patient.

Various attempts have been made to extend device lifetime and to improve material resistance of voice prostheses to yeasts and bacteria: surface treatments with active agents, alteration of surface properties by laser, and admixture of silver oxide into the silicone material.1–6 Various valve designs, such as the hinged valve flap (Provox 1, Provox 2, Provox Activevalve), the slit valve type (Groningen Button), the tripod-ball valve type (Voicemaster), and even valveless design (Nijdam) have been used in prosthesis construction. However, the vulnerability of the valve mechanism to biofilm colonization still
remains unsolved. Recently, the Phonax voice prosthesis by Heimomed (Kerpen, Germany) was introduced and licensed for voice rehabilitation of laryngectomized patients. The hinged valve flap design of Phonax bears resemblance to that of Provox 2 (Atos Medical, Höryby, Sweden). However, Phonax is manufactured of thermoplastic polyurethane (TPU), a polymer material considered to be more resistant to fungal colonization, whereas Provox 2 is made of medical-grade silicone.7–9 Provox 2 is the most commonly used prosthesis in voice rehabilitation of laryngectomized patients in Europe; recent multicenter studies revealed a median lifetime of 92 to 135 days.10–13

Surface biofilm formation evolves in phases. In the beginning, microorganisms from the trachea and the upper airway loosely adhere to the polymer surface of the voice prosthesis. Then the microbes multiply and form a continuous growing biofilm layer. Local nutritional and oxygen gradients within the biofilm provide living conditions for more sophisticated microbial species and bring forward the growth of complex symbiotic microecosystems.14 This microbial community encases itself in a protective extracellular polysaccharide matrix (EPS), which reduces the impact of environmental fluctuations in moisture, pH value, and drug therapy on the microbial population.15 Mature biofilms consist of multiple cell layers that can partly detach and disseminate onto new surfaces.16 Candida species are most often isolated from voice prostheses. They possess specific skills to colonize and to cause deterioration of medical-grade silicone.17,18 The tight bonding of biofilm deposits to surfaces of voice prostheses withstands even daily cleaning procedures (manual brushing) by the patient. The growth of biofilm deposits is accompanied with initial deterioration of the smooth polymer surface and consecutive infiltration. Biofilm-resistant elastomers, such as TPU in Phonax, might play a key role in exceeding device lifetimes of voice prostheses. More data on the process of material disintegration itself are still needed to establish comparable criteria for evaluation of polymer biofilm resistance.

The goal of this study was the examination of biofilm formation on Phonax and Provox 2 voice prostheses using standard microbiological and imaging techniques (scanning electron microscopy, fluorescence microscopy, and thin-section light microscopy).

MATERIALS AND METHODS

Overview of Phonax and Provox 2 Voice Prostheses. Construction details of both Phonax and Provox 2 are illustrated schematically in Figure 1. Both types have a similar hinged valve flap mechanism, but they differ in design and material features. The thin esophageal flange of the Phonax is affixed in an angled position to the corpus to retreat during anterograde implantation. Its flexibility allows simple insertion into the tracheoesophageal shunt and does not require a loading tube to fold and fit the flange through the fistula like the Provox 2 system. The hinged valve flap is pressed open by increased air pressure on the tracheal side and retracts to closed position by elasticity of the hinge. The valve niche is formed by a circular hood and provides space for the valve flap movement. This hood is shaped semicircular on the

![FIGURE 1. Construction details of Phonax and Provox 2 voice prostheses.](image)
Provox 2 and is localized on the opposite side of the hinge. The main difference is the prosthesis material: whereas the medical grade silicone of Provox 2 appears smooth and transparent, Phonax is made of TPU with increased opacity and surface roughness. Original surface structures of unused voice prostheses of each type should be assessed by examination with scanning electron microscopy complying with identical preparation methods.

Patients. In our department, the Provox 2 has been the favored prosthesis type in the past decade. In cases with difficult local situation of the tracheoesophageal fistula or discomfort with Provox 2, patients are offered other prosthesis types as an individual solution. With its introduction, Phonax was offered to the patients as an alternative replacement. To collect comparable data on device lifetimes, 5 laryngectomized patients (mean age, 63.2 ± 3.7 years; Table 1), who had been using Provox 2 on average 5 years and agreed to be equipped with the Phonax, were included in the study. Patients 2 and 3 were already using other voice prostheses prior to Provox 2. The patients were selected with respect to consideration of regular previous replacement intervals (mean, 114 days over the past 5 years) and stable microbial spectra. All patients reported good verbal communication skills after prosthesis implantation. Prosthesis cleaning was performed by manual brushing at least once a day. Patients visited our office because of prosthetic leakage and aspiration. For this study, Provox 2 prostheses were replaced by Phonax of the same size (either 6 or 8 mm). On follow-up, they were again replaced by Provox 2. Both prosthesis types were collected and processed by the following methods.

Qualitative and Quantitative Analysis of Microbial Colonization. The withdrawn prostheses were cut in half under sterile conditions. One half
was vortexed in 10 mL of phosphate-buffered solution (PBS) for 30 seconds. Dilution series of the rinsing fluids were plated out onto tryptic soy agar (TSA) for quantitative analysis and on selective growth agars (Sabouraud agar, McConkey agar, Columbia 5% agar, Columbia colistinalidixic acid agar (Columbia CNA), S. aureus ID agar (SAID), Trimethoprim agar (TMP), Cetrimide agar, CHROMagar Candida) for qualitative analysis. After incubation for 24 or 48 hours, respectively, the grown colonies were counted and identified according to the diagnostic protocol of each agar. Inaccurate results were processed further using the Vitek 2 system (BioMérieux Inc., Durham, NC). The vortexed prosthesis half was then prepared for examination with fluorescence or light microscopy.

**Imaging Techniques for Capturing Biofilm Formation and Material Alterations**

**Scanning Electron Micrography.** The second prosthesis half was retained in a solution of 2.5% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer (pH 7.0) for 24 hours, dehydrated in a series of ethanol concentrations (70%, 80%, 96%, and 100%, 1 hour each), and stored dry in an exsiccator with silicagel. The surface was sputtered with gold (Spatter Coater: SC502, Polaron, Fisons Instruments, Surface Science Division, Cambridge, UK), and examined by scanning electron microscopy (JSM 6310, JEOL Ltd., Tokyo, Japan).

**Fluorescence Microscopy.** Fluorescence microscopy was used to visualize biofilm deposits. The fluorescent properties of organic material were enhanced by sample preparation in 2% glutaraldehyde for 24 hours. Examination under ultraviolet light increased the contrast between biofilm structures and the nonfluorescent polymer materials, showing both superficially adhered deposits and material invasions.

**Thin-section Light Microscopy.** One Phonax prosthesis and 1 Provox 2 prosthesis with comparable biofilm adhesions were embedded in Technovit 7200 VLC (Heraeus-Kulzer, Hanau, Germany) and prepared as thin sections for light microscopy.

**FIGURE 2.** Macroscopic views of withdrawn Phonax and Provox 2 voice prostheses.
microscopy (Nikon Eclipse E800, Nikon Imaging Inc., Tokyo, Japan). Thionin staining (1% thionin acetate solution [Sigma–Aldrich, St. Louis, MO], 45 minutes) was used to highlight organic material.

**RESULTS**

**Qualitative and Quantitative Analysis of Microbial Colonization.** Microbiological results and colonization patterns for all prostheses examined are shown in Table 2. Microbial concentration of the rinsing solutions averaged $2.9 \times 10^6$ colony forming units per milliliter (cfu/mL). Phonax prostheses showed a slightly elevated mean microbial concentration of $3.1 \times 10^6$ cfu/mL and a mean device lifetime of 77.8 days, shorter than that of Provox 2 ($2.7 \times 10^6$ cfu/mL, 80.6 days). Candida species were identified on all prostheses ($n = 10$), with Candida albicans being the most frequent subspecies ($n = 9$). Most often isolated bacteria were Staphylococcus aureus ($n = 7$) and Streptococcus oralis ($n = 6$). No affinity of specific microbial species to any of the 2 polymer materials was observed.

**Macroscopic View on Microbial Colonization.** Macroscopic comparison of the prostheses indicated more biofilm masses and mucus secrete adhesion to the surface of Phonax. The esophageal surfaces of both prosthesis types—in particular the esophageal flange, the valve flap, and the valve seating—were predominantly affected by biofilm infestation. Colonization patterns ranged from single scattered biofilm deposits to extended covers of the esophageal valve. Thin continuous biofilm covers were observed more often on Phonax prostheses, whereas bulged solitary deposits were encountered more often on Provox 2 (Figures 2A–2D). Removal of biofilm deposits by careful scratching was not possible without damaging the polymer surface.

**Microscopic Examination of Native Polymer Surfaces.** Surfaces of the unused voice prostheses, examined by scanning electron microscopy, are shown in Figure 3. The TPU surface of Phonax is characterized by asperities, whereas the medical-grade silicone of Provox 2 appears smooth, with minor elongated embossments. Remnants of the manufacturing process, such as irregular polymer flashes on the outer diameters of the flanges, were found on both prosthesis types.

**Microscopic Examination of Biofilm Formation**

*Scanning Electron Microscopy.* All prostheses were examined by scanning electron microscopy. An
overview of colonization patterns on the esophageal surfaces of both prosthesis types is presented in Figure 4. The roughened TPU material of Phonax is covered by an evenly continuous layer of EPS matrix, apparently contouring the material surface beneath. Transitions between material and biofilm surfaces appear seamless. The flanges of the prostheses shown in Figures 4A, 4G, and 4I were clipped to assess multiple material samples with

FIGURE 4. Scanning electron microscopy of Phonax and Provox 2 voice prostheses (esophageal sites, top-down). Biofilm formation on Phonax (made of polyurethane) resembled continuous thin layers covering the esophageal flange and hood (A, E). Scattered circumscibed biofilm deposits were identified on Provox 2 (made of medical-grade silicone; B, D, F). Cracks in biofilm covers are caused by dehydration during sample preparation (B). Arrows indicate sites of surface bulging on the esophageal flange of Phonax (G, I).
sagittal-section planes to screen for material infiltration. Material flashes located on the distal perimeters of the flanges are visible remnants from manufacture by injection die molding (Figure 3A, marked by arrow, and Figures 4A, 4C, and 4G). The esophageal surfaces of Provox 2 showed insular biofilm deposits adhering to the smooth prosthesis material (Figures 4B and 4F). Their circumscribed character allows a clear distinction between biofilm and silicone. Cracks in the biofilm surface are caused by sample dehydration, which is an essential step for scanning electron microscopy (Figure 4B). Detailed imaging of the adhered deposits revealed differences in biofilm composition on the polymer surfaces (see Figure 5). Deposits on Provox 2 consisted of typical biofilm structures with bacteria and both budded and filamentous growth forms of Candida species. Fungal hyphae appeared interwoven with EPS matrix and bacterial aggregations forming complex 3-dimensional structures (Figure 5B). Biofilm on the surface of Phonax was characterized by a 2-dimensional layer of crowded microbes attached to the polymer material (Figure 5A). Cell boundaries submerged in an amorphous mass of EPS. No signs of stabilizing filamentous growth were detected.

All withdrawn prostheses were microscopically screened for biofilm infiltration. On 6 prostheses (3 Phonax, 3 Provox 2) with minimum in vivo periods of 56 days (Phonax) and 60 days (Provox 2), respectively, biofilm ingrowth was detected. Surface deteriorations appeared as minor material defects located close to the polymer surface. They were mainly found in proximity to or underneath adhering biofilm deposits. On Phonax, dense microbial colonies advanced from the surface into material fissures (Figure 6A). On Provox 2, the smooth material surface was locally disrupted by ingrowing colonies. Excavations appeared filled with microbial content that anchored larger biofilm deposits adhering to the prosthesis surface. Infiltrations of greater depth resembled voluminous “baglike” microbial deposits inside the silicone (Figure 6B).

Fluorescence Microscopy. Fluorescence microscopy proved to be a fast and simple method to contrast between polymer material and organic matter. No hyphal infiltration, but sharp-edged transitions between ingrowing spherical deposits and the prosthesis materials were visible. The TPU material was less infiltrated in depth than the medical-grade silicone, which showed voluminous “baglike” deposits with expanding growth character inside the valve flaps (Figures 6C and 6D). These infiltrations seemed to locally deform the valve flap of Provox 2.

Thin-section Light Microscopy. Ingrowing fungal hyphae were identified in thin-section light microscopy. They were located underneath superficially adhering biofilm deposits. Solitary violet-stained hyphae penetrated “rootlike” into the silicone material of the Provox 2. Hyphal invasion was also found on Phonax, although to a
lesser degree and material depth (Figures 6E and 6F).

**Material Form Stability.** Deformation of functional prosthesis parts was found in 3 Phonax prostheses: the esophageal flange of Phonax retrieved from patient 1 appeared macroscopically warped and discolored after 147 days in situ (Figure 2C). Phonax of patients 4 and 5 showed surface bulges on the esophageal flanges already after 11 and 56 days in situ, respectively, which might have been caused by biodeterioration processes (Figures 4G and 4I, marked by arrows).

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**DISCUSSION**

Voice prostheses have become standard in voice rehabilitation of laryngectomized patients.\(^{19,20}\) However, valve function is regularly impaired as a result of adhering biofilm deposits, thus leading to aspiration through the prosthesis and making periodic replacements by a physician necessary. A long device lifetime and uncomplicated replacement procedures are essential to improve quality of life by reducing discomfort arising from frequent hospital visits. Various valve designs, selected materials (silicone, polyurethane, titan, teflon, silver oxide admixtures), and surface modifications have been incorporated into voice prosthesis design to improve...
function and device lifetime. Phonax (Heimomed) is a voice prosthesis manufactured of TPU. This thermoplastic elastomer is reported to be more resistant to biofilm infestation. The present pilot study investigated in vivo biofilm formation on Phonax and Provox 2 in 5 laryngectomized patients, with special emphasis on reciprocal effects between biofilm and the different polymer materials.

In this study, prostheses remained in situ until leakage was reported by the patients. All prostheses were examined not only with standard microbiological methods, but also with microscopic imaging techniques after withdrawal. Microbiological findings are consistent with recent data and confirm Candida species and bacteria originating from the oropharyngeal space as the main biofilm-forming colonizers of the esophageal valve surfaces. The microbiological spectrum and quantity proved to be similar both on Phonax and on Provox 2, which indicates no specific microbial affinities toward TPU or medical grade silicone. However, scanning electron microscopy of the unused prostheses revealed an increased surface roughness of TPU material, which seemed to facilitate initial microbial in vivo adhesion. This might explain the continuous thin microbial coating of the esophageal surfaces of the Phonax, whereas the smooth silicone surface of Provox 2 restraints initial biofilm formation to corners and niches of the valve ring and the valve flap. Upon withdrawal, the Phonax prosthesis was coated with more loosely attached mucus of low viscosity. Provox 2 showed less mucus, but solid and circumscribed biofilm deposits continuously spreading in diameter and increasing number according to in vivo time. These macroscopically visible differences in biofilm configuration might be ascribed to alterations in biofilm architecture, as illustrated in the results of scanning electron microscopy: the plane biofilm structure on TPU is characterized by an amorphous EPS matrix and the absence of germ tubes. The hyphal growth form of Candida species seems to enhance biofilm stability by complex cellular netting inside the EPS matrix and was found constantly in biofilms on Provox 2 prostheses. A similar impact of surface-modified polyurethane material on Candida biofilms has also been reported by Chandra et al in an in vitro assay.

Various explanations on how biofilm is able to cause deterioration of polymer material have been discussed, but the exact processes involved remain unclear. A combination of lytic processes and extraction of soluble material compounds by microbial agents may lead to focal embrittlement and structural damage of the polymers. These areas and fissures might be subject to further active microbial attack in the form of hyphal advance or expansive growing microbial deposits. To fully illustrate morphologies of material damages, examination by scanning electron microscopy was completed by fluorescence microscopy and light microscopy. The revealed morphologies comply with forms of microbial intrusion into silicone that were described and classified earlier by Neu et al on Groningen Buttons and by van Weissenbruch et al on Provox 1. On Provox 2, biofilm infiltration occurred mainly on the esophageal surface of the valve flap. Infiltrations resembled multiple baglike deposits excavating and expanding into the silicone. Voluminous and compact deposits anchored the superficial biofilm layer, explaining its tight bonding to the prosthesis. The TPU of Phonax showed ingrown microbial deposits of comparably smaller dimension and depth, which indicates improved material resistance to biofilm infiltration. This resistance might also be associated with the described absence of hyphal growth forms on TPU. As illustrated in Figure 6F, a direct infiltration of germ tubes into the silicone material of Provox 2 was found, whereas no similar equivalent was detected in TPU. The deformation of the esophageal flange of Phonax in patient 1 after 147 days in situ (Figure 2C) might be attributable to less form stability of the TPU material. Another explanation is a passive adaption of the flange to surrounding mucosal structures of the fistula. The bulges located on the flanges (Figures 4G and 4I, marked by arrows) could be caused by local moisture expansion of the deteriorated polymer material or may be the image of a circumscribed lifting of the biofilm layer. Further investigation on long-term material stability of Phonax under biofilm exposure is required to evaluate the observed material alterations.

The impact of the found differences between biofilm formation on Phonax and Provox 2 on clinical practice was not investigated in this study, although device lifetimes and microbial loads were revealed to be similar for both prostheses types tested. The use of multiple examination methods restricted the number of prostheses included in the study. However, the findings might contribute to interpretation of
future results of clinical trials of Phonax on larger scales.

CONCLUSIONS

In summary, the Phonax voice prosthesis shows qualitative and quantitative microbial colonization similar to that of Provox 2. The TPU surface structure seems to facilitate biofilm adherence, but the material also seems to inhibit filamentous growth of Candida species. Compared with biofilms on Provox 2, this might lead to less-stable biofilm configuration, less hyphal infiltration, and smaller subsurface biofilm deposits. The presented results with Phonax indicate that TPU reduces the vulnerability of voice prostheses to biofilm damage, although the impact on clinical practice and in vivo device lifetimes still needs to be verified on a larger scale. Further in vitro and in vivo investigation on biofilm formation, composition, and stability on TPU are required.

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