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CASE REPORT

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Potential colonization of provox voice prosthesis by *Candida* spp. with no sign of failure for approximately 10 years exploitation time

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ABSTRACT

Vocal rehabilitation with implantation of voice prosthesis has been recognized as one of the most popular intervention for patients after total laryngectomy. The main threat to its users is leakage through the tracheo-esophageal fistula caused by prosthesis deformation and damage to the valve mechanism due to colonization with fungal or bacterial/fungal biofilms. The uniqueness of the described case represents the use of the same Provox voice prosthesis during the period of almost 10 years.

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KEYWORDS Biofilm; voice prosthesis; total laryngectomy; Candida spp

Introduction

Laryngeal cancer is diagnosed annually in about 14,000 people in the United States and about 40,000 people in Europe [1]. Total laryngectomy (TL) remains the treatment of choice for patients in advanced stages of this disease (\geq T3 in TNM8 staging system) and as salvage treatment in the case of recurrence after primary organ preservation treatment. It should be noted that these numbers are going to increase if we will consider TL cases due to a primary tumor located in the hypopharynx. Total laryngectomy results in loss of voice production that significantly excludes patients from social life. Vocal rehabilitation with the formation of a tracheo-esophageal (TE) fistula is performed in order to restore the connection between the airways (trachea) and the upper parts of the gastrointestinal tract (oral cavity, pharynx, esophagus), which were partly resected and readjusted in the course of the TL. This ground breaking method of surgical voice rehabilitation was first described by Polish professor Erwin Mozolewski in 1972; but, due to the economic situation in Poland at that time, was not adopted widely [2]. Since 1980, when Singer and Blom described the method of voice rehabilitation with the formation of a TE fistula and the use of their own voice prosthesis, this method has been widely used in practice [3]. From that time on, the method has been recognized and among others established as the method of choice due to the high success rates and superior quality of voice. [4]. The device used in this method is the voice prosthesis, which performs the function of a one-way valve, allowing air-flow from the trachea to the esophagus while preventing the passage of gastrointestinal content and saliva from the esophagus to the respiratory tract. In 1988, the silicone Provox type voice prosthesis was patented [5]. Since then, this type of prosthesis has been modified several times while the material from which it was made remains unchanged. For the voice prosthesis to be effective and safe for the patient, it is necessary to periodically check the condition of the fistula and the prosthesis and if necessary, replace the prosthesis. The main problem is a leakage. This can be transprosthetic or perprosthetic. It is usually caused by deformation and damage of the VP associated with biofilm formation [6-8]. Prosthesis destruction may also result in loss of voice emission due to blockage of the one-way valve. In all these situations, VP needs to be replaced. It has been reported that the average lifespan of a voice

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prosthesis among patients after TL ranges from 2 to 18 months. Moreover, among the treatment history only history of radiation therapy has small but statistically significant influence for the time of proper VP function. [9-11].

Case

A 69-year-old man presented to the Otolaryngology Head and Neck Surgery Outpatient Clinic in Holly-Cross Cancer Centre (Kielce, Poland) for choking while drinking liquids. The choking was caused by transprosthetic leakage through voice prosthesis. Patient's treatment history was shortly presented on the timeline (Figure 1). In 2001, the patient had undergone total laryngectomy, selective neck dissection on the right side (SND), and partial thyroid resection due to diagnosed squamous cell carcinoma of the larynx (stage T3N0M0). For a year, the patient used inefficient esophageal speech. In 2002, secondary surgical voice prosthesis implantation was performed and a Provox 2 8 mm voice prosthesis was inserted. The first replacement in 2004 was due to prosthesis dislocation of the esophageal flange and loss of voicing. Due to the thickening of the vocal fistula walls in the sagittal plane, the voice prosthesis was replaced with a longer one (12.5 mm). Over the next five years (2004-2009), the patient underwent eight voice prostheses replacements, the majority of which were due to transposition of the VP into the fistula canal (5), while the others were due to central leakage through the prosthesis (3). It is worth to noting that each time measurement of the fistula was performed and there were no doubts about the fitting of the VP.

In March 2007, the patient was diagnosed with a new tumor located in the floor of his mouth on the

left side (stage T2N0M0, squamous cell carcinoma G-2). The treatment was based on CO_2 laser tumor resection and sublingual salivary gland resection on the left side. The histopathology report confirmed complete surgical resection. However, after three years in November 2010, left-sided radical node dissection (RND) was done because of squamous cell carcinoma G3 metastases in the left lymphatic nodes of the neck (pN3b). The histopathology report confirmed complete surgical resection and then in December 2010, the patient underwent adjuvant radiotherapy to the lymph nodes of the neck on the right and left sides (50 and 60 Gy, respectively).

It is worth noting that during the RND surgery and adjuvant radiotherapy, the same Provox 2 12.5 mm voice prostheses was in the vocal fistula (also in the irradiated field) and functioned correctly since last replacement in 2009. At that time, it was suggested to the patient to replace the VP with new one, but the patient did not consent.

To this day, the patient comes to follow-up visits to the Outpatient Clinic. At the same time, he uses the effective prosthetic voice.

A unique phenomenon in this case is the trouble-free, effective use of the same voice prosthesis for a period of almost 10 years (3,959 days). The prosthesis (Provox 2 12.5 mm) has been in use from 2009 until 2019 even during second primary cancer treatment. During regular inceptions features of the VP's surface biofilm coverage were reported. Despite VP's replacement recommendations the patient refused to exchange the prosthesis because of the reliability and effectiveness of the currently held prosthesis. The prosthesis was replaced only when transprosthetic leakage through the valve was identified in 2019.



2002: TE puncture with VP implantation

Figure 1. The timeline of patient's treatment history. Details in the text.

The patient's prosthesis was processed immediately after removal using microbiological examination and the AFM examination of the VP' s surface. During the prosthesis removing process made sure to not exceed the stresses and deformations of the prosthesis in a way greater than during everyday use, to prevent inadvisable damage.

In the next stage, the swab from the posterior wall of the oropharynx was collected by the physician.

According to the information from the interview, the patient had not been previously treated and did not have any chronic diseases. He also did not take any dietary supplements. Since the total laryngectomy, he has not smoked tobacco and consumes alcohol no more than several times a year. The patient assesses his level of oral hygiene as good, brushes his teeth twice a day. During the first months of using the prosthesis, he used the brush to clean the prosthesis's valve regularly, but after about 6 months he stopped cleaning. He has no difficulty swallowing and he usually eats three main meals per day. The patient's report shows that he consumes both meat and vegetables once a day and consumes dairy products twice daily. It is worth noting that the patient avoids eating sweets. The patient is 180 cm height and weighs 84 kg (BMI = 25,93).

Microbiological and AFM examinations

After removing, patient's prosthesis was transported in the sterile container with a sterile gauze soaked in saline. In the microbiological laboratory, part of it was immersed in thioglycolate broth and vortexed for 2-3 min. Then, $50 \,\mu$ L of the eluted material was transferred and seeded onto solid culture media (Sabouraud agar with antibiotics, Columbia agar with sheep's blood, Hemophilus selective agar, Mac Conkey agar – all from Thermo Fisher Scientific, MA,USA) and incubated. The throat swab was transported in the sterile microbial transport medium and then also seeded onto this same type of solid culture media and incubated. After incubation, the predominant bacteria and yeast were identified using routine laboratory procedures: the assessment of the morphology of colonies, Gram staining and the final biochemical identification. For the biochemical identification, the Vitek 2 automated system (bioMerieux) was used.

In the next step, a comparison between patient's prosthesis (Provox 2) and control prosthesis (Provox Vega) was assessed using AFM technics. Considering the technical description, there is no difference in the material of the Provox 2 and Provox Vega (both are made of medical silicone). The main difference between these two is only the shape of the prosthesis and the insertion set for the replacement procedure. However, even in the same model of prosthesis, its properties may differ in different zones. The comparison of the patient's voice prosthesis (panel B) and control one (panel A) is presented in Figure 2.

AFM experiments were made 5h after prosthesis removal. Prosthesis sections from the flanges in contact with the tissues were cut, glued to the Petri-Dishes (35 mm) and tested under wet conditions in distilled water. The cuts were made under sterile conditions using a scalpel. The topography of the VP polymer surface with biofilm, the surface after biofilm removal, and the surface of control samples (from manufacture new VP) were probe. Before measuring the polymer with biofilm structures samples were washed by triple immersion in distilled water to remove loose particles. In order to biofilm effect test on the polymer topography and elasticity, the samples were cleaned using an ultrasonic cleaner (30 min in room temperature). All samples' manipulations were performed carefully to prevent possible damage of the polymer structure. The assessment of the microbiological examination was presented in the Table 1.



Figure 2. Macroscopic view of voice prosthesis. Panel A and B shows a general view of the control Provox Vega prosthesis (Panel A) and pateint's Provox 2 prosthesis with biofilm formations respectively (Panel B). Flange A and B represent esophageal and tracheal parts respectively. Panel C shows pieces from each flange used for AFM measurements after biofilm removal.

Topography of the control and patient's voice polymeric prostheses' surfaces (esophageal and tracheal flanges) shown in Figure 2 were recorded using an atomic force microscope NanoWizard 4 BioScience AFM (JPK Instruments, Bruker) equipped with a liquid cell setup. Triangular-shaped cantilevers (AppNano NITRA-TALL-V-G) characterized by a spring constant of 0.1 N/m were used. Due to the lateral forces during contact mode scanning, a force curves-based imaging mode (JPK QITM mode -Quantitative Imaging) was used with a resolution of 128 pixels per line in order to show characteristics of the prostheses' surfaces. Topography maps of sizes $20\,\mu\text{m}$ \times $20\,\mu\text{m}$ and $10\,\mu\text{m}$ \times $10\,\mu\text{m}$ were prepared. The QI topographical map also served as data for surface's adhesion forces examinations, average surface roughness values (Ra) examinations, and mechanical property measurements. Elastic modulus (i.e. the Young's modulus E) of the polymers were calculated based on force indentation curves from the QI mode and collected using the same type of cantilever but characterized by a spring constant of 0.46 N/m. Elasticity maps of sizes $10 \,\mu\text{m} \times 10 \,\mu\text{m}$ areas corresponding to a grid of 128×128 pixels were made. Elasticity maps were collected from various samples areas. Young's modulus was derived from the Hertz-Sneddon model applied to force-indentation curves [12].

Panels A and B of Figure 3 show topographies of control prosthetic esophageal and tracheal flanges from respectively. The surface is smooth without any cracks or scratches. However, porosity on the flange A is visible. Considering the construction of the prosthesis, both control prosthesis flanges are made of the same material, but their structure and properties may be different due to various conditions, such as cooling during production. Porosities may have arisen during the technological/production process. Adhesion forces from the two control prosthesis flanges are at a similar level (2.5 and 2.7 nN). This parameter did not affect the difference in biofilm-forming capability between two surfaces. The same finding applied to surface roughness, which was similar for both control flanges. Biofilm structures that formed on the flanges can be observed in panels C and D. A well-developed biofilm with an extensive extracellular matrix and cells (green arrows in the Figure 3) are visible. It should be noted that no significant differences in biofilm morphology were observed on either flange. Topographies of polymer surfaces after removal of biofilms from patient's are presented in panels E-H of Figure 3. Panels E and F represent flange A, and panels G and H represent flange B in various magnifications. Clear differences in morphology compared to control (new device, panel A and B) surfaces can be seen. Several cracks and deep grooves resulting from



Figure 3. The topography of the esophageal and the tracheal flange surfaces: panels A and B show the control (new) prosthesis, C and D show the prosthesis with biofilm; and panels E–H show the patient's prosthetic surfaces. Panel A shows topography of prosthesis flange A (esophageal flange) with average value of surface adhesion forces and roughness Ra. Panel B shows the topography of prosthesis flange B (tracheal flange) with an average value of surface adhesion forces and roughness Ra. Panel C shows topography of the biofilm formed on prosthesis flange A, and panel D shows topography of the biofilm formed on the prosthesis flange B. Panel E and F show damage to flange A after biofilm removal with average values of adhesion forces. Panel G and H show damages of the flange B after biofilm removing with average values of adhesion forces. The gray arrows indicate polymer cracks and fissures formed during the prosthesis exploitations. Green arrows indicate the formation of biofilms on the polymers.



Figure 4. Mechanical properties of the prostheses' surfaces. Panel A shows Young's modulus values distributions for the prosthesis flange esophageal flange (control and patient's samples). Panel B shows Young's modulus values distributions for the prosthesis flange tracheal flange (control and patient's device). Inside the panels mean values of Young's modulus are included.

 Table 1. Microorganism outgrowth from throat swab and prosthesis device.

	Throat swab		Patient's voice prosthesis	
Microorganism	Presence	Intensity of growth	Presence	Intensity of growth
Citrobacter braakii	Yes	+	Yes	+
Streptococcus spp.	Yes	+++	Yes	++
Candida krusei	Yes	+	Yes	+++
Candida albicans	No	-	Yes	+++

the prosthesis exploitation, polymer aging, and biofilm growth over the years are visible (grey arrows in the Figure 3). Biofilm formed a compact crust within the polymer material, especially in inner flange B (brown structure shows in Figure 2(C)). Since cracks can cause fluid leakage through the prosthesis, modification of polymeric material should be considered to prevent cracking during exploitation. A modification that gives the polymer anti-microbial properties would also be highly desirable as well. Adhesion forces acting between the tip and patient's prosthetic surface were smaller compared to the controls (Figure 3(F,H)). This difference indicates a change in the physicochemical properties of the prosthetic surface. The difference is most likely due to polymer aging. Local mechanical properties of the polymeric surfaces were quantified. Figure 4 shows Young's modulus value distributions of esophageal and tracheal flanges from control and patient's prostheses. Mean stiffness values are also presented. Different stiffness values for two flanges (esophageal and tracheal) from the control prosthesis were compared. As in the case of adhesion forces, stiffness could be caused by different cooling conditions of these elements during production. Comparison of control and patient's prostheses also revealed differences in material stiffness.

Distributions of stiffness values for used prosthesis indicate a local decrease in polymer stiffness in addition to nano-scale stiffeners, possibly due to the local incorporation of biofilm structures into the polymer. A large number of low values of the elastic modulus are present; however, very rigid points increase the mean stiffness value for the prosthesis after biofilm removal. After averaging the results of Young's modulus measurements, the patient's prosthesis has significantly higher stiffness compared to the control one. Changes in local physicochemical properties, including mechanical and structural heterogeneity, may act as areas of stress accumulation and cause cracking of the polymers during exploitation of the prostheses, as shown in Figure 3. The success of long-term use of this type of prosthesis may be associated with the lack of short-term structural and surface changes of the prosthesis from the action of the biological environment, including the action of the formed biofilm and daily mechanical and thermal exploitation.

The assessment of the following measurements was presented shortly in the Table 2.

Discussion

The uniqueness of the described case is a matter not only of many years of the voice prosthesis use without signs of prosthesis damage but also its effective phonation. This exceptionally long time of prosthesis exploration is very intriguing, but the factors that made possible to use the voice prosthesis for a period, calculated as 39 times longer than the average replacement periods are unclear [9]. Biofilm formation on prosthesis surface should be indicated as a major factor damaging the material from which the prosthesis is made. However, the host tissue response to

	Control VP	Patient's VP
Topography	smooth, no cracks or scratches	several cracks, deep grooves
Adhesion forces Stiffness	higher Iower	lower higher
Biofilm	no	yes. no difference in morphology between pharyngeal and tracheal phalange

presence of prosthesis should be considered as well. Because voice prostheses in patients after laryngectomy are placed in not sterile niche, microorganisms rapidly colonize them and biofilm is formed [13,14]. Interestingly, infections of surrounding tissues are relatively rare. Similarly, in our patient, no clinical signs of infection (as symptoms of local inflammation reaction) were found in the area where the prosthesis was placed. A local infectious complication, for example, aspiration pneumonia or systemic infections has also not been reported despite the presence of material potentially infectious (colonized voice prosthesis).

The source of microorganisms forming biofilm on the voice prosthesis is the patient's oropharyngeal mucosa. In the case described above, the prosthesis biofilm consists of two species of Candida and additionally Streptococcus spp. and Gram-negative aerobic rods - Citrobacter braakii which are respectively, a natural and colonizing oropharyngeal microbiota. Some researchers have shown a relationship between species composition and the quantity and quality of biofilm formed on the surface of a voice prosthesis made of medical silicone. Lactobacillus casei was demonstrated as a factor reducing the percentage hyphal formation in Candida biofilms and having favorable effects on the lifespan of voice prostheses. The relationship with short clinical lifespan of voice prostheses was observed when Candida grown in combination with Rothia dentocariosa [15]. None of these microorganisms has been identified in our patient. On the other hand, some publications show that there is no direct relationship to anaerobic bacteria [16]. The question of whether microbial composition and their diversity has an impact on the prosthesis lifespan remains open and requires further studies.

There is no relationship between the lifespan of the prosthesis and factors as patient's age, comorbidities, level of education and history of treatment [17]. However, it should pay attention to the issue of adjuvant radiotherapy or radio-chemotherapy as an

indirect factor in the formation of biofilms due to the changes the conditions of the mucous membranes of the mouth and throat, and thus interference of the microbial flora and the possibility of a local immune response of the mucous membrane [18]. Only consuming large quantities of dairy products has a statistically significant effect on the extension of the intervals [10]. This case also confirms this relationship. This phenomenon is explained by the presence of both lactoferrin in addition to *Streptococcus thermophilus* and *Lactobacillus* in dairy products (especially fermented), the presence of which inhibits the growth of fungi and some bacteria.

Other probable factors influencing the very long lifespan of the voice prosthesis in our patient could be: patient's lifestyle (e.g. no smoking, no invasive infections), general good condition, and good mouth hygiene. The confirmation of this hypothesis requires further studies.

It is worth to notice that adhesion forces were higher on the surface of the control prosthesis than in patient's one after removing biofilm. Due to character of this study as a case report only one failure VP and only one brand new VP as a control were examined. Results should be developed in the future study in more numerous (statistically significant) groups.

In conclusion, the factors that determine the lifespan of voice prostheses are not fully understood. Undeniably, the formation of a fungal-bacterial biofilm is a key element in this respect. Further analysis of this process is important to describe the way to extend individual time of voice prosthesis use. It is necessary to improve technological processes and develop new material to eliminate imperfections of the polymer and reduce the susceptibility to aging and limit the impact of biofilm development through additives. For example, by the addition of nanosystems or chemical compounds, which can reduce the capability of microorganisms to form biofilm on the surface, that accelerates polymer degradation.

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Disclosure statement

The authors declare that they have no competing interests.

Informed consent statement

Written informed consent for publication of their details was obtained from the patient.

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