Review

Candida biofilm formation on voice prostheses
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Laryngopharyngeal malignancy is treated with radiotherapy and/or surgery. When total laryngectomy is required, major laryngeal functions (phonation, airway control, swallowing and coughing) are affected. The insertion of a silicone rubber voice prosthesis in a surgically created tracheoesophageal puncture is the most effective method for voice rehabilitation. Silicone, as is the case with other synthetic materials such as polymethylmethacrylate, polyurethane, polyvinyl chloride, polypropylene and polystyrene, has the propensity to become rapidly colonized by micro-organisms (mainly Candida albicans) forming a biofilm, which leads to the failure of the devices. Silicone is used within voice prosthetic devices because of its flexible properties, which are essential for valve function. Valve failure, as well as compromising speech, may result in aspiration pneumonia, and repeated valve replacement may lead to either tract stenosis or insufficiency. Prevention and control of biofilm formation are therefore crucial for the lifespan of the prosthesis and promotion of tracheoesophageal tissue and lung health. To date, the mechanisms of biofilm formation on voice prostheses are not fully understood. Further studies are therefore required to identify factors influencing Candida biofilm formation. This review describes the factors known to influence biofilm formation on voice prostheses and current strategies employed to prolong their life by interfering with microbial colonization.

Introduction

Laryngopharyngeal malignancy is initially treated with radiotherapy or surgery. When these treatments fail or when the disease is in a more advanced stage, total laryngectomy (TL), separating the digestive and respiratory tracts with definitive tracheostoma to allow breathing, is required, which leads to loss of speech. As the voice is a basic human attribute, its loss is one of the most mutilating effects of TL. Surgical removal of the larynx therefore not only affects the major laryngeal functions (phonation, airway control, swallowing, effort closure during strenuous activity and coughing), but also leads to disfigurement and an increased risk of post-operative infection.

Three voice restoration methods after TL are currently available: artificial larynx voice, oesophageal voice and tracheoesophageal (shunt) voice. Since the introduction of tracheoesophageal (shunt) voice by Singer and Blom in 1978 (Singer & Blom, 1980), the insertion of a silicone rubber voice prosthesis (VP) in a surgically created tracheoesophageal puncture is the most valued and used method for voice rehabilitation after TL. A one-way valve shunt placed between the oesophagus and trachea allows air to pass from the trachea to the cervical oesophagus and pharynx, producing sounds that are further modulated to create speech. Various types of prosthetic devices have been developed: indwelling types (e.g. Groningen button, Provox2, Blom–Singer Classic) and non-indwelling types (e.g. Blom–Singer Low Pressure, Bivona valves). The indwelling types (or self-retaining devices) can be inserted at the time of laryngectomy or later as a secondary puncture, whereas the non-indwelling types have to be inserted after a true puncture has been developed (Blom, 1998).

VPs are usually made of silicone rubber due to its invaluable mechanical and moulding properties. However, silicone has the propensity to become rapidly colonized by micro-organisms (Busscher et al., 1997a) that form a biofilm in which the opportunistic pathogenic yeast Candida albicans is predominant (Bauters et al., 2002; Ell, 1996; Everaert et al., 1997). Fluids, food and saliva...
provide ideal conditions for the attachment and subsequent growth of yeasts and bacteria upon VP surfaces (Schwandt et al., 2005). The oesophageal part of the VP faces continuous mechanical, chemical and microbial influences, which lead to the gradual decline of the one-way valve function. Valve failure can promote oesophageal contents to leak into the trachea, resulting in an increased likelihood of aspiration pneumonia (Van Weissenbruch et al., 2002; Mahieu et al., 1999). Non-indwelling devices can be removed and replaced by the patient for cleansing, but numerous displacements and fistula formation have been reported (Andrews et al., 1996; Oosterhof et al., 2005; Rodrigues et al., 2007). As a result, VPs need to be replaced regularly and lifespan varies from several days to months or years (Ackerstaff et al., 1999). Non-indwelling devices can be replaced by a trained medical professional in an inpatient or outpatient setting. Frequent replacement throughout the patient’s life has both significant healthcare and cost implications; however, our current knowledge as to why such patient variation exists is limited.

Microbial composition of biofilms on voice prostheses

The range of micro-organisms that are most commonly involved in VP biofilms have been identified (Elving et al., 2002; Mahieu et al., 1986), with some able to penetrate the silicone rubber itself (Neu et al., 1993). Neu et al. (1993) reported that yeasts in a mixed biofilm were directly responsible for biodeterioration of silicone. The yeasts most routinely identified within VP biofilms are species of Candida: most notably Candida albicans, Candida tropicalis and Candida glabrata (Bauters et al., 2002; Neu et al., 1994a). Bacterial strains identified within VP biofilms are usually of oral origin and include Streptococcus spp., (Streptococcus mitis, Streptococcus sobrinus, Streptococcus salivarius), or are skin commensals, such as Staphylococcus spp. (Neu et al., 1994b). Buijssen et al. (2012) analysed 66 biofilms from several commonly used VP devices for their bacterial and fungal community population and reported the frequency with which micro-organisms were found as follows: C. albicans (77%), C. tropicalis (52%), Lactobacillus gasseri (96%), Lactobacillus fermentum (73%), Lactobacillus acidophilus (52%) and streptococci (61%). Lactobacilli were the predominant bacteria in voice prosthetic biofilms (Buijssen et al., 2007). Most prostheses showed a mature biofilm with lactobacilli and Candida as major constituents.

In a further study, conducted in India, Sayed et al. (2012) analysed 22 patients’ implanted VPs that had malfunctioned and required removal as a result of the attachment and growth of micro-organisms. The authors found mixed growth (both yeast and bacteria) in 19 of the 22 cultures (86.4%); a single yeast species was present in 13 cultures (59.1%) and multiple yeasts species were found in 6 (27.3%). The remaining three cultures (13.6%) yielded bacteria only. The most common yeast isolated was C. albicans, which was present in 15 cultures (68.2% of cultures); in nine cultures (40.9%), it was the only yeast isolated. Other isolated yeasts included C. tropicalis and C. glabrata. The most common bacterium identified in this study was Pseudomonas aeruginosa, which was present in 14 cultures (63.6%); in eight cultures (36.4%), it was the only bacterium isolated. Other bacteria identified included Staphylococcus aureus (31.8%), Klebsiella oxytoca (27.3%), Klebsiella pneumoniae (9.1%), and non-fermenting Gram-negative species (9.1%). The mean lifetime of the prostheses was 201 days. This study, the first of its kind in India, revealed that the microbial picture was different from that found in previously reported studies of European populations. The authors presumed the differences were attributable to different lifestyles and dietary habits. In India, consumption of dairy products in the form of yoghurt and buttermilk is widely prevalent. Consumption of yoghurt might reduce biofilm formation on indwelling prostheses, due to the presence of Streptococcus thermophilus and Lactobacillus (Busscher et al., 1997b; Havenaar & Huis in’t Veld, 1992). Buttermilk is rich in lactoferrin, which has bactericidal and fungicidal activity against a number of oral pathogenic organisms, including C. albicans, Aggregatibacter actinomycetemcomitans and Streptococcus mutans (Holmes et al., 2012; Kalmar & Arnold, 1988; Soukka et al., 1991, 1992). This may explain the relatively prolonged lifetime of the prostheses and the varied microbial colonization in this study.

It is most probably the case that multiple factors such as biofilm formation (influenced by patient diet, lifestyle and VP care), mechanical deterioration and inflammation of the surrounding tissue due to secondary colonizers of the biofilm such as staphylococci and enterobacteria can all contribute to VP failure over time. This complexity may account for the variable nature of the time it takes for devices to fail from patient to patient.

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**Fig. 1.** Blom–Singer Classic indwelling voice prosthesis before insertion (a) and 3 months after insertion (b) in a laryngectomized patient.
Factors affecting biofilm formation upon VP

Saliva, food, and oropharyngeal microflora contribute to valve failure and frequent exchange of VP. High humidity, temperature close to 37 °C, and regular provision of nutrients make the oesophagus an ideal environment for microbial growth. Moreover, the organisms colonizing the silicone rubber are largely inaccessible to host defence mechanisms and antimicrobials, which can explain why VPs become colonized with a multispecies biofilm. Candida species are found in the normal microbiota of humans, which facilitates their encounter with implanted biomaterials and host surfaces. C. albicans adheres to a range of salivary pellicle components, including proline-rich proteins and statherin, and the adhesion of C. albicans to saliva-coated surfaces is an important early step in its colonization of the oral cavity (Cannon & Chaffin, 2001; Holmes et al., 1995; O’Sullivan et al., 2000). The sheer forces associated with saliva flow may also favour the development of VP biofilms. For example, C. albicans biofilms formed under static conditions are found to contain small amounts of exopolymeryc material (Hawser et al., 1998), while cells incubated with shaking during biofilm formation produce far larger amounts (Hawser et al., 1998). Bacterial biofilms also form more readily and are stronger when the cells are growing in high shear conditions (Donlan & Costerton, 2002). Changes in oxygen or nutrient delivery or the effects of shear may be responsible for these phenomena.

VP biofilms most commonly contain fungal and bacterial organisms, but is the order of colonization or microbial composition an important factor? On most prostheses studied by Buijssen et al. (2012), bacteria were mostly located near the outermost surface of the biofilm and yeasts were predominantly located near or in the silicone rubber, demonstrating in-growing of yeast colonies. It has been suggested that the adhesion of bacteria to VPs is a prerequisite for subsequent colonization by yeasts (Neu et al., 1994a). Such a sequence in colonization has also been described for denture stomatitis (Radford et al., 1999). Millsap et al. (2001) studied in vitro adhesion of yeasts suspended in saliva to silicone rubber with and without adhering bacteria. In this study C. albicans and C. tropicalis were found to adhere in higher numbers to silicone rubber when adhering Rothia dentocariosa or Streptococcus aureus was present. Adhesive interactions between bacteria and yeast strains could therefore play a crucial role in the development of a biofilm. Leunisse et al. (2001) studied the susceptibility of various design features for biofilm formation in an artificial throat and concluded that both Groningen button and Provox2 VPs have specific sites that are more likely to be colonized but that, in both cases, the valve edges are the crucial site for biofilm formation. It also has been suggested, less surprisingly perhaps, that yeasts adhere more easily and more strongly to rough as opposed to smooth surfaces (Radford et al., 1998). Factors that have been shown to, or are hypothesized to, affect biofilm formation on VPs are outlined within Fig. 2.

**Formation and structure of Candida biofilms**

As C. albicans is the most commonly found fungal organisms within VP biofilms and is also the most common human fungal pathogen, a number of studies have focused upon factors that influence its ability to colonize surfaces and medical devices. C. albicans grows as a budding yeast, within a pseudohyphal state, or switches to a filamentous form via the extension of hyphae. C. albicans is able to undergo morphological transitions between growth as yeast and filamentous forms (hyphae and pseudohyphae), and this morphological plasticity is influenced by numerous environmental signals (Biswas et al., 2007). To colonize any surface, fungal cells must first adhere to it. The initial

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**Fig. 2.** Factors affecting biofilm formation on VPs that contribute to premature device failure. Device failure is associated with microbial colonization, with C. albicans the predominant organism. It is associated with salivary leakage, increased airflow resistance, compromised speech, increased risk of aspiration pneumonia, reduced quality of life and increased healthcare costs.
attachment of *Candida* cells to biomaterials is mediated both by non-specific factors (cell surface hydrophobicity and electrostatic forces) and by specific adhesins on the fungal surface recognizing ligands in the conditioning films, such as serum proteins (fibrinogen and fibronectin) and salivary factors (Chaffin *et al.*, 1998). Importantly, biofilm formation correlates with cell surface hydrophobicity (Li *et al.*, 2003). Additionally, *Candida* cells can also co-aggregate and/or bind to bacteria, which may aid in biofilm formation (Cannon & Chaffin, 1999; Holmes *et al.*, 1995; Millsap *et al.*, 1999, 2001). The initial focal attachment of individual cells to a substratum is followed by cell division, proliferation and biofilm development. Hawser & Douglas (1994) showed the initial attachment of yeast cells is followed by germ tube formation within 3 to 6 h. Following this, after 24 to 48 h of incubation, the fully mature *C. albicans* biofilms consist of a dense network of yeasts, hyphae and pseudohyphae, and extracellular polymeric material is visible on the surfaces of some of these morphological forms. Similar observations were made by Ramage *et al.* (2001). Mature *Candida* biofilms exhibit a complex three-dimensional structure and display extensive spatial heterogeneity (Chandra *et al.*, 2001; Hawser & Douglas, 1994; Hawser *et al.*, 1998; Kumamoto, 2002). This structural complexity is thought to represent the optimal spatial arrangement to facilitate the influx of nutrients, the disposal of waste products, and the establishment of micro-niches throughout the biofilm. The overall architecture of the biofilm may vary, depending on the substrate on which it is formed and its growth conditions (Chandra *et al.*, 2001). Moreover, different strains of *C. albicans* and different *Candida* spp. differ in their capacities to form biofilms (Hawser & Douglas, 1994; Kuhn *et al.*, 2002; Li *et al.*, 2003). Several artificial biofilm systems have been developed to study *C. albicans* biofilm formation. Organisms were allowed to adhere to various materials such as catheter or denture material and then incubated in a growth medium. *C. albicans* biofilms formed in this way were typically composed of a layer of cells in the yeast form adhering to the surface and, above this, a layer of filamentous cells in the hyphal form surrounded by extensive exopolymeric matrix (Baillie & Douglas, 1999; Chandra *et al.*, 2001). A morphological mutant unable to form hyphae formed a dense biofilm composed of yeast cells, whereas a mutant that produced only filamentous cells formed a hyphal biofilm (Baillie & Douglas, 1999). Therefore, biofilms can be composed of either morphological type, or both, depending upon the conditions, and it would appear that neither morphological cell type is absolutely essential for biofilm formation. Baillie & Douglas (1999), however, demonstrated that hyphae are essential elements for providing the structural integrity and the multi-layered architecture characteristic of mature/fully developed biofilms. The authors also reported that filamentous forms were the most important factor in the three-dimensional architecture, with yeast cells located in the basal layer.

*C. albicans* morphogenetic conversions (the reversible switching between yeast and filamentous forms) are important for multiple aspects of *C. albicans* biology, pathogenicity and biofilm development. Morphogenesis is considered a key virulence trait of the fungus, since strains locked in a given morphology exhibit attenuated virulence (Noble *et al.*, 2010; Saville *et al.*, 2003). Hyphal growth and the coordinated expression of hypha-specific genes are also important for virulence, as they promote attachment to biotic and abiotic surfaces (Fu *et al.*, 2002; Noble *et al.*, 2008), tissue invasion (Gow *et al.*, 2002) and escape from phagocytic immune cells (Lorenz *et al.*, 2004). Growth in the yeast morphology is thought to be important for dispersion *in vitro* (Uppuluri *et al.*, 2010) and may thus be significant during disseminated disease. Despite the research conducted to date, the exact mechanism of biofilm formation on VPs is not yet fully understood. Further studies are required to identify factors influencing *Candida* biofilm formation, which leads to malfunctioning of VPs. It has been demonstrated that CO2 can act as an inducer of filamentation in *C. albicans* (Persi *et al.*, 1985). Detailed investigation found that an atmosphere of 5.5% CO2 induces hyphal and, to a lesser extent, pseudohyphal growth.

More recent studies have shown that elevated levels of CO2, as found in exhaled breath, can trigger a morphological switch in *C. albicans* and that this is important for infection and virulence (Cottier *et al.*, 2012; Hall *et al.*, 2010; Klenel *et al.*, 2005). Our preliminary work shows that elevated levels of CO2 also promote biofilm formation upon VP surfaces (our unpublished results) leading to the possibility that this is a factor that may influence colonization rates in patients fitted with prosthetic devices following larynx removal.

**Strategies for the prevention of microbial biofilm formation on VPs**

Microbial colonization and biofilm formation can lead to salivary leakage through the prosthetic valve, salivary leakage around the prosthesis, deterioration of the prosthesis, and increased airflow resistance due to valve mechanism blocking. Valve failure, as well as compromising speech, may result in aspiration pneumonia, and repeated valve replacement may lead to either tract stenosis or insufficiency. Prevention and control of biofilm formation will therefore be beneficial not only for the lifespan of the prosthesis, but also for general patient health. A number of different approaches have been suggested to inhibit or minimize biofilm formation. A summary of these approaches can be found in Fig. 3.

**Modifications of silicone rubber surfaces**

Different approaches have been undertaken to modify the silicone rubber surface as a strategy to inhibit biofilm formation. Although VPs will become covered by a
conditioning film of adsorbed salivary components prior to adhesion of bacteria or yeasts, experiments in the human oral cavity have demonstrated that the properties of this conditioning film are determined by the material itself (Busscher et al., 1992, 1997a). Biofilm formation may therefore be influenced by adjusting the properties of the VPs material, by surface modification, and thus a number of strategies have been employed.

Metal coating techniques. Arweiler-Harbeck et al. (2001) attempted to create a Candida-resistant surface by gold or titanium coating of silicone VPs using a new method of surface modification by anodic vacuum arc coating. No functional change in the properties of the prostheses and no difference in the quality of speech were reported by patients as a result of metal coating (Arweiler-Harbeck et al., 2001). Further studies are needed to show that metal coating with gold or titanium leads to a retardation of Candida growth. Dijk et al. (2000) treated Groningen button VPs with a colloidal palladium/tin solution to form a thin metallic coat which was reported to lead to a significant biofilm reduction when compared with untreated prostheses.

The Blom–Singer Advantage voice prosthesis is a device with a flap valve containing 7% silver oxide in the silicone matrix. Kress et al. (2006) reported that the mean device lifetime increased from 36 days (median 36 days) to 110 days (median 87 days) with the use of the Blom–Singer Advantage prosthesis, compared with other types of indwelling voice prostheses (Provox2, Blom–Singer Indwelling).

Plasma surface treatment. Polymeric surfaces can be modified by a glow discharge plasma treatment, in which a non-polymer-forming plasma (i.e. plasma of argon, oxygen or nitrogen) is used. Plasma treatment modifies the composition and structure of a few molecular layers at the surface of the material without affecting the bulk properties (Abbasi et al., 2002). Everaert et al. (1998a) investigated the effects of repeated argon plasma treatment of medical grade hydrophobic silicone rubber on in vitro adhesion and growth of selected bacteria and yeasts isolated from VPs, as well as in vivo biofilm formation. The results obtained for in vitro experiments showed a reduction in microbial adhesion and growth on silicone rubber. However, in vivo biofilm formation on silicone rubber VPs was enhanced by hydrophilizing the silicone rubber surface. It is not clear at this stage why such contradictory results were obtained for in vitro and in vivo experiments, and the use of this technology as a useful approach to extending VP lifespan requires further validation.

Perfluoro-alkylsiloxane surface treatment. Everaert et al. (1998a) demonstrated that biofilm formation on VPs surfaces in vivo is governed by hydrophobicity. Therefore, the improved antifouling performance of VPs may be achieved by increasing the hydrophobicity of the silicone rubber, such as by adsorption of fluorocarbons to the surface. Fluorocarbon surfaces are slightly more hydrophobic than silicone rubber and were reported to retard the accumulation of dental plaque during 9 days of exposure to the dynamic conditions of the human oral
cavity (Quirynen et al., 1989). Additionally, Everaert et al. (1999) prepared reactive surfaces by argon plasma glow discharge prior to anchoring fluoro-alkyltrichlorosilanes. Long chain fluoro-alkylsiloxanes chemisorbed to silicone rubber were found to reduce microbial adhesion and to increase the percentage of detachment of adhering microorganisms. Finally, Everaert et al. (1998b) also reported significant reductions over an evaluation period of approximately 2–8 weeks when using chemisorption of long (eight fluorocarbon units) perfluoro-alkylsiloxane polymer chains due to the high hydrophobicity and mobility of these chains.

Covalently coupled quaternary ammonium silane coatings. Another strategy to prevent VPs microbial colonization is by functionalization of the silicone rubber surface with quaternary ammonium groups, widely known as disinfectants. Poly(methacrylates) with methyl or ethyl quaternary ammonium chloride side groups showed antimicrobial activity against Gram-negative strains, although staphylococci were little affected by these polymers (Gottenbos et al., 2001; Kenawy et al., 1998). Gottenbos et al. (2002) determined the antimicrobial activity of 3-(trimethoxysilyl) propyldimethyloctadecylammonium chloride [quaternary ammonium salt (QAS)] coating on silicone rubber. Antimicrobial activity of QAS-coated silicone rubber was demonstrated both in vitro and in vivo. The positively charged QAS coating affects the viability of Gram-negative bacteria as well as of Gram-positive bacteria in single-strain bacterial biofilms. The application of positively charged biomaterial surfaces to prevent infection is unusual as current research has been mainly focused on designing non-adhesive surfaces. Positively charged surfaces are strongly adhesive to the negatively charged bacteria; however, the positive charge inhibited biofilm progression from the initial adhesion stage toward the growth stage since immobilized QAS molecules interact with the cell membranes of adhering bacteria, presumably causing membrane leakage and cell death. Oosterhof et al. (2006) demonstrated that QAS also reduces the number of viable bacteria and yeasts in mixed biofilms. Upon incubation in an artificial throat model, allowing simultaneous adhesion and growth of yeast and bacteria, all coated prostheses showed significant reductions in the numbers of viable yeast and bacteria compared with those for silicone rubber controls, as confirmed using confocal laser scanning microscopy after live/dead staining of the biofilms. This study demonstrates for the first time that the viability of both yeasts and bacteria in mixed biofilms is affected by positively charged QAS coatings on silicone rubber. Because QAS coatings are non-toxic, clinical application could increase the useful lifetime of tracheoesophageal shunt prostheses by decreasing biofilm mass. In a recent study, De Prijck et al. (2010) aimed to produce a surface capable of inhibiting C. albicans biofilm formation by dimethylaminoethylmethacrylate and polyethyleneimine coating the surface of polydimethylsiloxane (silicone) or polymethylmethacrylate. Physico-chemical characterization of the grafted surfaces was carried out and their effect on C. albicans cell numbers was assessed using a modified Robbins device to grow the biofilms. Covalently bound quaternized poly(dimethylaminoethylmethacrylate) and quaternized polyethyleneimine inhibited biofilm growth, with reductions of up to 92%.

Prophylactic treatment of silicone rubber voice prostheses

Another approach to prevent biofilm formation and prolong the lifetime of VPs is to inhibit the adhesion and the growth of C. albicans and other micro-organisms. The following strategies have been explored.

Use of probiotics. Lactobacilli, lactococci, enterococci and streptococci are known to have probiotic effects.

Some strains are able to release biosurfactants, while others are known to have antimycotic effects, or produce lactic acid or hydrogen peroxide. Free et al. (2001) assessed the influence of probiotic bacteria (L. lactis 53 and Streptococcus thermophilus B) on biofilm formation on both Groningen and Provox VPs in an artificial throat model. These studies found that both strains affected biofilm formation and could increase VP lifetime.

Effects of dairy products. Patient support groups for laryngectomy patients have suggested that buttermilk consumption prolongs the clinical usefulness of indwelling silicone rubber VPs and resolves early leakage of dysfunctions valves (Busscher et al., 1998, 2000; Free et al., 2000; van der Mei et al., 1999, 2000). Busscher et al. (1998) simulated the consumption of buttermilk in an artificial throat model and found that it almost completely prevented biofilm formation. Buttermilk is a mildly acidic dairy product with a pH of 4.5 due to the presence of lactic acid produced by L. lactis and Streptococcus cremoris, and contains a number of enzymes in addition to having a high calcium content (110–120 mg per 100 g). L. lactis strains are known to release antimycotic substances, while the proteins present in buttermilk include casein, lactoglobulin, and immunoglobulins, which may have detergent properties. Therefore buttermilk contributes to the referred prevention of biofilm formation. Free et al. (2000) demonstrated that it is feasible to formulate a dairy product based on probiotics that will strongly inhibit biofilm formation on VPs.

Use of antifungal agents. Several antifungal drugs, and their effects on the lifetime of VPs, have been studied (Ackerstaff et al., 1999; Andriole, 1999; Bauters et al., 2002; Mahieu et al., 1986; Van Weissenbruch et al., 1997a, b). Oropharyngeal yeast decontamination using amphotericin B lozenges and buccal adhesive slow-release tablets containing miconazole nitrate has been applied by otolaryngologists to increase the lifetime of VPs (Mahieu et al., 1986; Van Weissenbruch et al., 1997a, b). In studies

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with Groningen button VPs, the successful decontamination of the oropharynx with amphotericin B lozenges (10 mg) four times daily was also associated with a prolonged device life and lower airflow resistances (Mahieu et al., 1986). However, one of the drawbacks found in using this agent is the need for daily applications, leading to poor compliance by the patients. Also, liposomal preparations of amphotericin B were described as substantially less toxic and more effective; clinical trials, however, have yet to be carried out. Van Weissenbruch et al. (1997a, b) conducted a double-blind randomized trial among 36 laryngectomees to assess the influence of a buccal bioadhesive slow-release tablet (10 mg) containing miconazole nitrate on the lifetime of the Provox VP. All patients colonized with Candida strains and treated with miconazole showed a significant decrease of colonization at the end of the study. The airflow resistances were remarkably higher in the placebo group after 2 months of follow-up. No local or systemic adverse reactions to miconazole were observed. Patient compliance was acceptable according to regular miconazole determination in saliva samples. The prostheses’ lifetime was significantly longer in patients treated with miconazole even after 1 year of follow-up. The use of a buccal bioadhesive slow-release tablet containing an antimycotic agent proves to be an adequate method of preventing fungal colonization and deterioration of silicone VPs. Unfortunately, these buccal bioadhesive miconazole tablets have recently been discontinued by the drug company. Common otolaryngologists’ practice to prevent microbial colonization of VPs is oropharyngeal yeast decontamination by using antifungal agents. However, there is little evidence that the use of antifungal agents will prolong the lifetime of VPs. Furthermore, prophylactic use of antifungal agents could contribute to the development of resistant strains.

**Use of synthetic salivary peptides.** Surgical therapy, radiation therapy and the ageing process itself result in salivary dysfunction. The low salivary secretion reduces the amount of histatins in saliva, yielding better chances for opportunistic micro-organisms such as C. albicans, as histatins contain fungicidal activity (Tenovuo, 1998). Synthetic salivary peptides are promising antimicrobial agents. They possess bactericidal and fungicidal activities and have not been associated with the development of microbial resistance (Helmerhorst et al., 1999). Helmerhorst et al. (1999) reported antifungal peptides, including human salivary histatin 5, a designed histatin analogue designated dhvar4, and a peptide from frog skin, PGLa, that are active against amphotericin B-resistant C. albicans, C. krusei and Aspergillus fumigatus strains and against a fluconazole-resistant C. glabrata strain. In addition, Elving et al. (2000) studied the antimicrobial activity of different synthetic salivary peptides derived from histatin against a variety of oropharyngeal micro-organisms isolated from explanted VPs. Histatin analogues designated dhvar4 and dhvar5 were the only synthetic peptides with an antimicrobial spectrum broad enough to cover the variety of oropharyngeal micro-organisms found on VPs. Oosterhof et al. (2003) carried out experiments in an artificial throat to determine the effectiveness of dhvar4 and dhvar5 on oropharyngeal biofilm formation. The dhvar4 treatment had no effect on mixed biofilms, while dhvar5 significantly reduced the number of both bacteria and yeasts in mixed biofilms. However, this reduction was not accompanied by a reduction in airflow resistance, suggesting that the integrity of the biofilm was not affected. This may be due to the remaining exopolysaccharide and connecting slime threads within the biofilm, as the integrity of a biofilm is determined by the exopolysaccharide matrix rather than by the number of organisms within. The authors concluded that the integrity of voice prosthetic biofilms is not principally determined by the number of organisms in the biofilm, but is determined to a much greater extent by exopolysaccharide production, which glues the biofilm together. This was confirmed by the observation that treatment with the mucolytic N-acetylcysteine did result in a decrease in airflow resistance. Pérez-Giraldo et al. (1997) studied the influence of various concentrations of N-acetylcysteine on the formation of biofilms of different strains of Staphylococcus epidermidis and found a dose-related decrease in biofilm and slime formation. Schwandt et al. (2004) inoculated Groningen button and Provox2 VPs with a mixture of bacteria and yeasts. On Groningen button VPs N-acetylcysteine reduced the amount of bacteria and yeasts to 2.4% (P<0.01) and 55% (P<0.15) of the control value, respectively. On Provox2 VPs, a reduction in the amount of bacteria was also observed, but there was no reduction in the amount of yeasts. Although the effect of N-acetylcysteine was less pronounced than that observed on Groningen button VPs, it reduced the amount of bacteria to 31% (P<0.01) of the control value. In contrast, the prevalence of yeasts in the biofilm was nearly tripled by N-acetylcysteine. The different results obtained with Groningen button and Provox2 highlight the difficulty in obtaining a unifying treatment procedure that will extend the lifespan of all VP devices. However, the beneficial effect of N-acetylcysteine on bacterial biofilm disrupting the integrity of VPs biofilms, together with the convenience and safety of the product in long-term use (it can be swallowed and used over a long period without adverse effects), makes N-acetylcysteine a potential medicine for the prevention of biofilm formation on VPs in laryngectomized patients.

**Conclusion**

The insertion of silicone rubber VPs in a tracheoesophageal puncture is generally considered to be the ‘gold standard’ for speech rehabilitation of laryngectomized patients. These implants need to be replaced when leakage through or around the prosthesis occurs, or when it becomes difficult to produce tracheoesophageal speech due to increased airflow resistance. A continuous exposure to saliva, food, drinks and oropharyngeal microflora contributes to rapid colonization of the prostheses by biofilms of mixed bacteria and yeast strains, leading to failure and
frequent replacement. Achieving an antifouling improvement for the silicone rubber material by the development of new biomaterials or new antimicrobial agents is highly desirable as a means to reduce patient discomfort, the chance of pulmonary infection and the cost of post-operative care. In this review we have summarized the different approaches described to date that may provide a route to achieve this goal and outlined some of the significant difficulties that are associated with a one-size-fits-all treatment approach. When designing new biomaterials, inhibition of microbial adhesion and growth should be achieved by changing the physico-chemical properties of the biomaterial surface or by covalently binding antimicrobial agents to the biomaterial surface. Techniques used to modify silicone rubber surfaces and prophylactic treatments for silicone rubber VPs have shown varying effects but remain an attractive proposition. As antimicrobial resistance is a growing source of concern, the development of novel alternative prophylactic and therapeutic agents, including probiotics and other surface-active compounds such as biosurfactants, has been explored as it is also an exciting possibility. Lastly, to fully appreciate the complexities of biofilm formation upon VPs, we must obtain a better understanding of the cell biology of C. albicans, its interactions with other colonizing microorganisms and how this pathogen responds and adapts to laryngopharyngeal cues.

References


