Review

Candida biofilm formation on voice prostheses

Moira J. Talpaert,[1] Alistair Balfour,[2] Sarah Stevens,[3] Mark Baker,[4] Fritz A. Muhlschlegel[1,4] and Campbell W. Gourlay[1]

Correspondence
Fritz A. Muhlschlegel
F.A.Muhlschlegel@kent.ac.uk
Campbell W. Gourlay
C.W.Gourlay@kent.ac.uk

[1]Kent Fungal Group, School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK

[2]Ear, Nose and Throat Services, East Kent Hospitals University NHS Foundation Trust, The William Harvey Hospital, Kennington Road, Ashford TN24 0LZ, UK

[3]Macmillan Speech and Language Therapy Services, Kent and Canterbury Hospital, Ethelbert Road, Canterbury CT1 3NG, UK

[4]Clinical Microbiology Service, East Kent Hospitals University NHS Foundation Trust, The William Harvey Hospital, Kennington Road, Ashford TN24 0LZ, UK

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

Abbreviations: QAS, quaternary ammonium salt; TL, total laryngectomy; VP, voice prosthesis.

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.*, 1997a, b). VPs tend to shelter biofilms on their surface (Fig. 1) leading to the failure of the devices (Leunisse *et al.*, 2001; 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 removed and replaced by the patient for cleansing, but numerous displacements and fistula formation have been reported (Andrews *et al.*, 1987; Blom *et al.*, 1986; Manni & Van Den Broek, 1990; Van Weissenbruch *et al.*, 1997a, b). The indwelling devices require less maintenance, but daily cleansing with brushes or cotton is recommended. The indwelling types have self-retaining capacities with respect to the tracheoesophageal puncture and cannot be removed by the patient. Replacement can be performed by simple anterograde or retrograde routes, but necessitates 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.
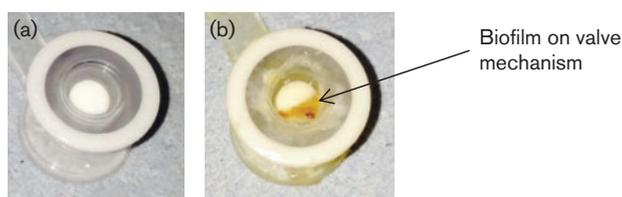

Fig. 1. Blom–Singer Classic indwelling voice prosthesis before insertion (a) and 3 months after insertion (b) in a laryngectomized patient.

Factors affecting biofilm formation upon VP

Saliva, food, drinks 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 exopolymeric 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
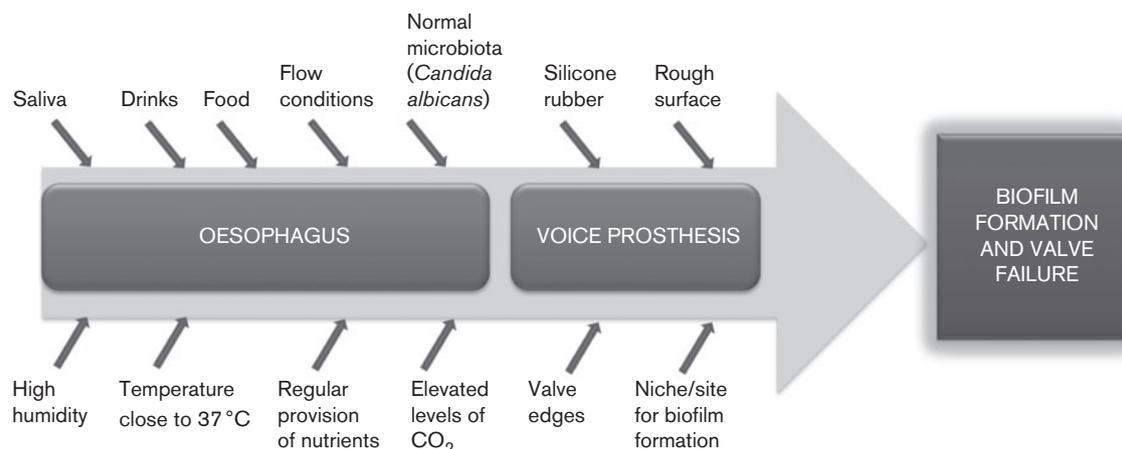

Fig. 2. Factors affecting biofilm formation on VPs that contribute to premature device failure. Device failure is associated with microbial colonization, with *C. albicans* the predominant organism. It is associated with salivary leakage, increased airflow resistance, compromised speech, increased risk 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; Nobile *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 CO_2 can act as an inducer of filamentation in *C. albicans* (Persi *et al.*, 1985). Detailed investigation found that an atmosphere of 5.5 % CO_2 induces hyphal and, to a lesser extent, pseudohyphal growth.

More recent studies have shown that elevated levels of CO_2, 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; Klengel *et al.*, 2005). Our preliminary work shows that elevated levels of CO_2 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 prosthesis 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
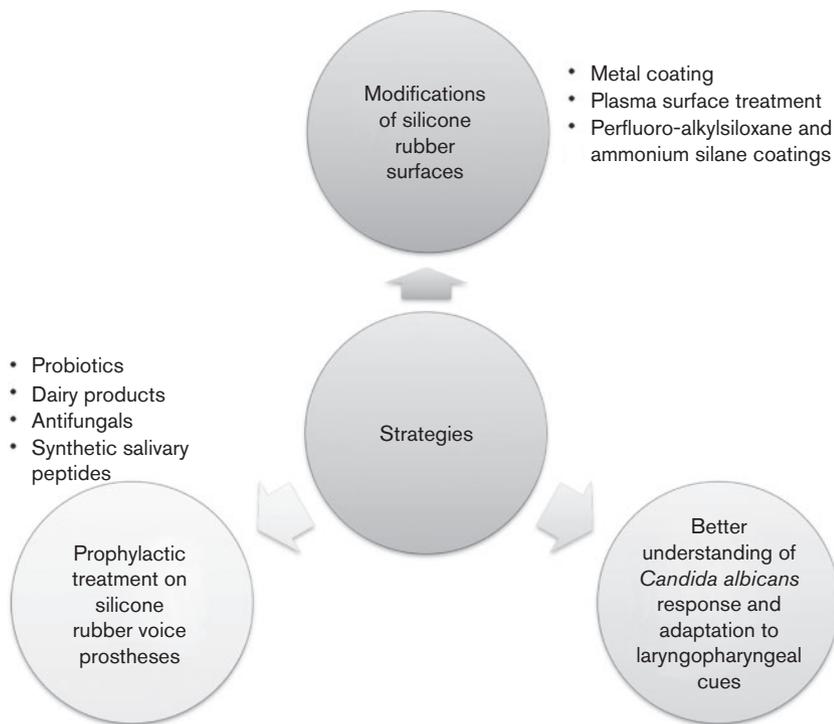
Fig. 3. Current strategies under research and development that may help prevent microbial biofilm formation on silicone rubber VPs and their subsequent failure.

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 metal 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 prosthesis (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 micro-organisms. 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 anti-microbial 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 polyethylenimine 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 polyethylenimine 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 dysfunctioning 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

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

Abbasi, F., Mirzadeh, H. & Katbab, A.-A. (2002). Bulk and surface modification of silicone rubber for biomedical applications. *Polym Int* **51**, 882–888.

Ackerstaff, A. H., Hilgers, F. J., Meeuwis, C. A., van der Velden, L. A., van den Hoogen, F. J., Marres, H. A., Vreeburg, G. C. & Manni, J. J. (1999). Multi-institutional assessment of the Provox 2 voice prosthesis. *Otolaryngol Head Neck Surg* **125**, 167–173.

Andrews, J. C., Mickel, R. A., Hanson, D. G., Monahan, G. P. & Ward, P. H. (1987). Major complications following tracheoesophageal puncture for voice rehabilitation. *Laryngoscope* **97**, 562–567.

Andriole, V. T. (1999). The 1998 Garrod Lecture. Current and future antifungal therapy: new targets for antifungal agents. *J Antimicrob Chemother* **44**, 151–162.

Arweiler-Harbeck, D., Sanders, A., Held, M., Jerman, M., Ehrich, H. & Jahnke, K. (2001). Does metal coating improve the durability of silicone voice prostheses? *Acta Otolaryngol* **121**, 643–646.

Baillie, G. S. & Douglas, L. J. (1999). Role of dimorphism in the development of *Candida albicans* biofilms. *J Med Microbiol* **48**, 671–679.

Bauters, T. G. M., Moerman, M., Vermeersch, H. & Nelis, H. J. (2002). Colonization of voice prostheses by albicans and non-albicans *Candida* species. *Laryngoscope* **112**, 708–712.

Biswas, S., Van Dijck, P. & Datta, A. (2007). Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. *Microbiol Mol Biol Rev* **71**, 348–376.

Blom, E. D. (1998). Evolution of tracheoesophageal VPs. In *Tracheoesophageal Voice Restoration Following Total Laryngectomy*, pp. 1–8. Edited by E. D. Blom, M. I. Singer & R. C. Hamaker. San Diego: Singular Publishing Group.

Blom, E. D., Singer, M. I. & Hamaker, R. C. (1986). A prospective study of tracheoesophageal speech. *Arch Otolaryngol Head Neck Surg* **112**, 440–447.

Buijssen, K. J. D. A., Harmsen, H. J. M., van der Mei, H. C., Busscher, H. J. & van der Laan, B. F. A. M. (2007). Lactobacilli: important in biofilm formation on voice prostheses. *Otolaryngol Head Neck Surg* **137**, 505–507.

Buijssen, K. J. D. A., van der Laan, B. F. A. M., van der Mei, H. C., Atema-Smit, J., van den Huijssen, P., Busscher, H. J. & Harmsen, H. J. M. (2012). Composition and architecture of biofilms on used voice prostheses. *Head Neck* **34**, 863–871.

Busscher, H. J., Cowan, M. M. & van der Mei, H. C. (1992). On the relative importance of specific and non-specific approaches to oral microbial adhesion. *FEMS Microbiol Rev* **88**, 199–210.

Busscher, H. J., Geertsema-Doornbusch, G. I. & van der Mei, H. C. (1997a). Adhesion to silicone rubber of yeasts and bacteria isolated from voice prostheses: influence of salivary conditioning films. *J Biomed Mater Res* **34**, 201–209.

Busscher, H. J., van Hoogmoed, C. G., Geertsema-Doornbusch, G. I., van der Kuijl-Booij, M. & van der Mei, H. C. (1997b). *Streptococcus thermophilus* and its biosurfactants inhibit adhesion by *Candida* spp. on silicone rubber. *Appl Environ Microbiol* **63**, 3810–3817.

Busscher, H. J., Bruinsma, G., van Weissenbruch, R., Leunisse, C., van der Mei, H. C., Dijk, F. & Albers, F. W. (1998). The effect of buttermilk consumption on biofilm formation on silicone rubber voice prostheses in an artificial throat. *Eur Arch Otorhinolaryngol* **255**, 410–413.

Busscher, H. J., Free, R. H., Van Weissenbruch, R., Albers, F. W. & Van Der Mei, H. C. (2000). Preliminary observations on influence of dairy products on biofilm removal from silicone rubber voice prostheses in vitro. *J Dairy Sci* **83**, 641–647.

Cannon, R. D. & Chaffin, W. L. (1999). Oral colonization by *Candida albicans*. *Crit Rev Oral Biol Med* **10**, 359–383.

Cannon, R. D. & Chaffin, W. L. (2001). Colonization is a crucial factor in oral candidiasis. *J Dent Educ* **65**, 785–787.

Chaffin, W. L., López-Ribot, J. L., Casanova, M., Gozalbo, D. & Martínez, J. P. (1998). Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. *Microbiol Mol Biol Rev* **62**, 130–180.

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T. & Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol* **183**, 5385–5394.

Cottier, F., Raymond, M., Kurzai, O., Bolstad, M., Leewattanapasuk, W., Jiménez-López, C., Lorenz, M. C., Sanglard, D., Váchová, L. & other authors (2012). The bZIP transcription factor Rca1p is a central regulator of a novel CO_2 sensing pathway in yeast. *PLoS Pathog* **8**, e1002485.

De Prijck, K., De Smet, N., Coenye, T., Schacht, E. & Nelis, H. J. (2010). Prevention of *Candida albicans* biofilm formation by covalently bound dimethylaminoethylmethacrylate and polyethylenimine. *Mycopathologia* **170**, 213–221.

Dijk, F., Westerhof, M., Busscher, H. J., van Luyn, M. J. & van Der Mei, H. C. (2000). *In vitro* formation of oropharyngeal biofilms on silicone rubber treated with a palladium/tin salt mixture. *J Biomed Mater Res* **51**, 408–412.

Donlan, R. M. & Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* **15**, 167–193.

Ell, S. R. (1996). *Candida* 'the cancer of silastic'. *J Laryngol Otol* **110**, 240–242.

Elving, G. J., van der Mei, H. C., Busscher, H. J., van Nieuw Amerongen, A., Veerman, E. C. I., van Weissenbruch, R. & Albers, F. W. J. (2000). Antimicrobial activity of synthetic salivary peptides against voice prosthetic microorganisms. *Laryngoscope* **110**, 321–324.

Elving, G. J., van der Mei, H. C., Busscher, H. J., van Weissenbruch, R. & Albers, F. W. J. (2002). Comparison of the microbial composition of voice prosthesis biofilms from patients requiring frequent versus infrequent replacement. *Ann Otol Rhinol Laryngol* **111**, 200–203.

Everaert, E. P., Mahieu, H. F., Wong Chung, R. P., Verkerke, G. J., van der Mei, H. C. & Busscher, H. J. (1997). A new method for *in vivo* evaluation of biofilms on surface-modified silicone rubber voice prostheses. *Eur Arch Otorhinolaryngol* **254**, 261–263.

Everaert, E. P., van de Belt-Gritter, B., van der Mei, H. C., Busscher, H. J., Verkerke, G. J., Dijk, F., Mahieu, H. F. & Reitsma, A. (1998a). *In vitro* and *in vivo* microbial adhesion and growth on argon plasma-treated silicone rubber voice prostheses. *J Mater Sci Mater Med* **9**, 147–157.

Everaert, E. P. J. M., van der Mei, H. C. & Busscher, H. J. (1998b). Adhesion of yeasts and bacteria to fluoro-alkylsiloxane layers chemisorbed on silicone rubber. *Colloids Surf B Biointerfaces* **10**, 179–190.

Everaert, E. P. J. M., Mahieu, H. F., van de Belt-Gritter, B., Peeters, A. J. G. E., Verkerke, G. J., van der Mei, H. C. & Busscher, H. J. (1999). Biofilm formation in vivo on perfluoro-alkylsiloxane-modified voice prostheses. *Otolaryngol Head Neck Surg* **125**, 1329–1332.

Free, R. H., Van der Mei, H. C., Dijk, F., Van Weissenbruch, R., Busscher, H. J. & Albers, F. W. J. (2000). Biofilm formation on voice prostheses: influence of dairy products in vitro. *Acta Oto-laryngol* **120**, 92–99.

Free, R. H., Busscher, H. J., Elving, G. J., van der Mei, H. C., van Weissenbruch, R. & Albers, F. W. (2001). Biofilm formation on voice prostheses: *in vitro* influence of probiotics. *Ann Otol Rhinol Laryngol* **110**, 946–951.

Fu, Y., Ibrahim, A. S., Sheppard, D. C., Chen, Y.-C., French, S. W., Cutler, J. E., Filler, S. G. & Edwards, J. E., Jr (2002). *Candida albicans* Als1p: an adhesin that is a downstream effector of the EFG1 filamentation pathway. *Mol Microbiol* **44**, 61–72.

Gottenbos, B., Grijpma, D. W., van der Mei, H. C., Feijen, J. & Busscher, H. J. (2001). Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* **48**, 7–13.

Gottenbos, B., van der Mei, H. C., Klatter, F., Nieuwenhuis, P. & Busscher, H. J. (2002). In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. *Biomaterials* **23**, 1417–1423.

Gow, N. A. R., Brown, A. J. P. & Odds, F. C. (2002). Fungal morphogenesis and host invasion. *Curr Opin Microbiol* **5**, 366–371.

Hall, R. A., De Sordi, L., Maccallum, D. M., Topal, H., Eaton, R., Bloor, J. W., Robinson, G. K., Levin, L. R., Buck, J. & other authors (2010). CO_2 acts as a signalling molecule in populations of the fungal pathogen *Candida albicans*. *PLoS Pathog* **6**, e1001193.

Havenaar, R. & Huis in't Veld, M. J. H. (1992). Probiotics: a general view. In *The Lactic Acid Bacteria in Health & Disease*, vol. 1, pp. 151–170. Edited by B. J. B. Wood. Amsterdam: Elsevier Applied Science Publishers.

Hawser, S. P. & Douglas, L. J. (1994). Biofilm formation by *Candida* species on the surface of catheter materials in vitro. *Infect Immun* **62**, 915–921.

Hawser, S. P., Baillie, G. S. & Douglas, L. J. (1998). Production of extracellular matrix by *Candida albicans* biofilms. *J Med Microbiol* **47**, 253–256.

Helmerhorst, E. J., Reijnders, I. M., van't Hof, W., Simoons-Smit, I., Veerman, E. C. & Amerongen, A. V. (1999). Amphotericin B- and fluconazole-resistant *Candida* spp., *Aspergillus fumigatus*, and other newly emerging pathogenic fungi are susceptible to basic antifungal peptides. *Antimicrob Agents Chemother* **43**, 702–704.

Holmes, A. R., Cannon, R. D. & Jenkinson, H. F. (1995). Interactions of *Candida albicans* with bacteria and salivary molecules in oral biofilms. *J Ind Microbiol* **15**, 208–213.

Holmes, A. R., Chong, K., Rodrigues, E., Cannon, R. D., Carpenter, E., Ruske, D. R. & Dawes, P. J. D. (2012). Yeast colonization of voice prostheses: pilot study investigating effect of a bovine milk product containing anti-*Candida albicans* immunoglobulin A antibodies on yeast colonization and valve leakage. *Ann Otol Rhinol Laryngol* **121**, 61–66.

Kalmar, J. R. & Arnold, R. R. (1988). Killing of *Actinobacillus actinomycetemcomitans* by human lactoferrin. *Infect Immun* **56**, 2552–2557.

Kenawy, el-R., Abdel-Hay, F. I., el-Raheem, A., el-Shanshoury, R. & el-Newehy, M. H. (1998). Biologically active polymers: synthesis and antimicrobial activity of modified glycidyl methacrylate polymers having a quaternary ammonium and phosphonium groups. *J Control Release* **50**, 145–152.

Klengel, T., Liang, W.-J., Chaloupka, J., Ruoff, C., Schröppel, K., Naglik, J. R., Eckert, S. E., Mogensen, E. G., Haynes, K. & other authors (2005). Fungal adenylyl cyclase integrates CO_2 sensing with cAMP signaling and virulence. *Curr Biol* **15**, 2021–2026.

Kress, P., Schäfer, P. & Schwerdtfeger, F. P. (2006). [Clinical use of a voice prosthesis with a flap valve containing silver oxide (Blom-Singer Advantage), biofilm formation, in-situ lifetime and indication]. *Laryngorhinootologie* **85**, 893–896 (in German).

Kuhn, D. M., Chandra, J., Mukherjee, P. K. & Ghannoum, M. A. (2002). Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. *Infect Immun* **70**, 878–888.

Kumamoto, C. A. (2002). *Candida* biofilms. *Curr Opin Microbiol* **5**, 608–611.

Leunisse, C., van Weissenbruch, R., Busscher, H. J., van der Mei, H. C., Dijk, F. & Albers, F. W. J. (2001). Biofilm formation and design features of indwelling silicone rubber tracheoesophageal voice prostheses–an electron microscopical study. *J Biomed Mater Res* **58**, 556–563.

Li, X., Yan, Z. & Xu, J. (2003). Quantitative variation of biofilms among strains in natural populations of *Candida albicans*. *Microbiology* **149**, 353–362.

Lorenz, M. C., Bender, J. A. & Fink, G. R. (2004). Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot Cell* **3**, 1076–1087.

Mahieu, H. F., van Saene, H. K., Rosingh, H. J. & Schutte, H. K. (1986). *Candida* vegetations on silicone voice prostheses. *Arch Otolaryngol Head Neck Surg* **112**, 321–325.

Manni, J. J. & Van Den Broek, P. (1990). Surgical and prosthesis-related complications using the Groningen button voice prosthesis. *Clin Otolaryngol Allied Sci* **15**, 515–523.

Millsap, K. W., Bos, R., van der Mei, H. C. & Busscher, H. J. (1999). Adhesion and surface-aggregation of *Candida albicans* from saliva on acrylic surfaces with adhering bacteria as studied in a parallel plate flow chamber. *Antonie van Leeuwenhoek* **75**, 351–359.

Millsap, K. W., Bos, R., van der Mei, H. C. & Busscher, H. J. (2001). Adhesive interactions between voice prosthetic yeast and bacteria on silicone rubber in the absence and presence of saliva. *Antonie van Leeuwenhoek* **79**, 337–343.

Neu, T. R., Van der Mei, H. C., Busscher, H. J., Dijk, F. & Verkerke, G. J. (1993). Biodeterioration of medical-grade silicone rubber used for voice prostheses: a SEM study. *Biomaterials* **14**, 459–464.

Neu, T. R., De Boer, C. E., Verkerke, G. J., Schutte, H. K., Rakhorst, G., Van Der Meil, H. C. & Busscher, H. J. (1994a). Biofilm development in time on a silicone voice prosthesis – a case study. *Microb Ecol Health Dis* **7**, 27–33.

Neu, T. R., Verkerke, G. J., Herrmann, I. F., Schutte, H. K., Van der Mei, H. C. & Busscher, H. J. (1994b). Microflora on explanted silicone rubber voice prostheses: taxonomy, hydrophobicity and electrophoretic mobility. *J Appl Bacteriol* **76**, 521–528.

Nobile, C. J., Schneider, H. A., Nett, J. E., Sheppard, D. C., Filler, S. G., Andes, D. R. & Mitchell, A. P. (2008). Complementary adhesin function in *C. albicans* biofilm formation. *Curr Biol* **18**, 1017–1024.

Noble, S. M., French, S., Kohn, L. A., Chen, V. & Johnson, A. D. (2010). Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat Genet* **42**, 590–598.

O'Sullivan, J. M., Jenkinson, H. F. & Cannon, R. D. (2000). Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. *Microbiology* **146**, 41–48.

Oosterhof, J. J., Elving, G. J., Stokroos, I., van Nieuw Amerongen, A., van der Mei, H. C., Busscher, H. J., van Weissenbruch, R. & Albers, F. W. (2003). The influence of antimicrobial peptides and mucolytics on the integrity of biofilms consisting of bacteria and yeasts as affecting voice prosthetic air flow resistances. *Biofouling* **19**, 347–353.

Oosterhof, J. J. H., van der Mei, H. C., Busscher, H. J., Free, R. H., Kaper, H. J., van Weissenbruch, R. & Albers, F. W. J. (2005). In vitro leakage susceptibility of tracheoesophageal shunt prostheses in the absence and presence of a biofilm. *J Biomed Mater Res B Appl Biomater* **73B**, 23–28.

Oosterhof, J. J. H., Buijssen, K. J. D. A., Busscher, H. J., van der Laan, B. F. A. M. & van der Mei, H. C. (2006). Effects of quaternary ammonium silane coatings on mixed fungal and bacterial biofilms on tracheoesophageal shunt prostheses. *Appl Environ Microbiol* **72**, 3673–3677.

Pérez-Giraldo, C., Rodríguez-Benito, A., Morán, F. J., Hurtado, C., Blanco, M. T. & Gómez-García, A. C. (1997). Influence of N-acetylcysteine on the formation of biofilm by Staphylococcus epidermidis. *J Antimicrob Chemother* **39**, 643–646.

Persi, M. A., Burnham, J. C. & Duhring, J. L. (1985). Effects of carbon dioxide and pH on adhesion of *Candida albicans* to vaginal epithelial cells. *Infect Immun* **50**, 82–90.

Quirynen, M., Marechal, M., Busscher, H. J., Weerkamp, A. H., Arends, J., Darius, P. L. & van Steenberghe, D. (1989). The influence of surface free-energy on planimetric plaque growth in man. *J Dent Res* **68**, 796–799.

Radford, D. R., Sweet, S. P., Challacombe, S. J. & Walter, J. D. (1998). Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent* **26**, 577–583.

Radford, D. R., Challacombe, S. J. & Walter, J. D. (1999). Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. *Crit Rev Oral Biol Med* **10**, 99–116.

Ramage, G., Vandewalle, K., Wickes, B. L. & López-Ribot, J. L. (2001). Characteristics of biofilm formation by *Candida albicans*. *Rev Iberoam Micol* **18**, 163–170.

Rodrigues, L., Banat, I. M., Teixeira, J. & Oliveira, R. (2007). Strategies for the prevention of microbial biofilm formation on silicone rubber voice prostheses. *J Biomed Mater Res B Appl Biomater* **81B**, 358–370.

Saville, S. P., Lazzell, A. L., Monteagudo, C. & Lopez-Ribot, J. L. (2003). Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell* **2**, 1053–1060.

Sayed, S. I., Kazi, R., Sengupta, S., Chowdhari, A. & Jagade, M. (2012). Microbial colonization of Blom–Singer indwelling voice prostheses in laryngectomized patients: a perspective from India. *Ear Nose Throat J* **91**, E19–E22.

Schwandt, L. Q., Van Weissenbruch, R., Stokroos, I., Van der Mei, H. C., Busscher, H. J. & Albers, F. W. J. (2004). Prevention of biofilm formation by dairy products and N-acetylcysteine on voice prostheses in an artificial throat. *Acta Otolaryngol* **124**, 726–731.

Schwandt, L. Q., van Weissenbruch, R., van der Mei, H. C., Busscher, H. J. & Albers, F. W. J. (2005). Effect of dairy products on the lifetime of Provox2 voice prostheses in vitro and in vivo. *Head Neck* **27**, 471–477.

Singer, M. I. & Blom, E. D. (1980). An endoscopic technique for restoration of voice after laryngectomy. *Ann Otol Rhinol Laryngol* **89**, 529–533.

Soukka, T., Lumikari, M. & Tenovuo, J. (1991). Combined inhibitory effect of lactoferrin and lactoperoxidase system on the viability of *Streptococcus mutans*, serotype c. *Scand J Dent Res* **99**, 390–396.

Soukka, T., Tenovuo, J. & Lenander-Lumikari, M. (1992). Fungicidal effect of human lactoferrin against *Candida albicans*. *FEMS Microbiol Lett* **90**, 223–228.

Tenovuo, J. (1998). Antimicrobial function of human saliva – how important is it for oral health? *Acta Odontol Scand* **56**, 250–256.

Uppuluri, P., Chaturvedi, A. K., Srinivasan, A., Banerjee, M., Ramasubramaniam, A. K., Köhler, J. R., Kadosh, D. & Lopez-Ribot, J. L. (2010). Dispersion as an important step in the *Candida albicans* biofilm developmental cycle. *PLoS Pathog* **6**, e1000828.

van der Mei, H. C., van de Belt-Gritter, B., van Weissenbruch, R., Dijk, F., Albers, F. W. J. & Busscher, H. J. (1999). Effect of consumption of dairy products with probiotic bacteria on biofilm formation on silicone rubber implant surfaces in an artificial throat. *Food Bioproducts Processing* **77**, 156–158.

van der Mei, H. C., Free, R. H., Elving, G. J., Van Weissenbruch, R., Albers, F. W. & Busscher, H. J. (2000). Effect of probiotic bacteria on prevalence of yeasts in oropharyngeal biofilms on silicone rubber voice prostheses *in vitro*. *J Med Microbiol* **49**, 713–718.

Van Weissenbruch, R., Albers, F. W., Bouckaert, S., Nelis, H. J., Criel, G., Remon, J. P. & Sulter, A. M. (1997a). Deterioration of the Provox™ silicone tracheoesophageal voice prosthesis: microbial aspects and structural changes. *Acta Oto-laryngol* **117**, 452–458.

Van Weissenbruch, R., Bouckaert, S., Remon, J. P., Nelis, H. J., Aerts, R. & Albers, F. W. (1997b). Chemoprophylaxis of fungal deterioration of the Provox silicone tracheoesophageal prosthesis in postlaryngectomy patients. *Ann Otol Rhinol Laryngol* **106**, 329–337.