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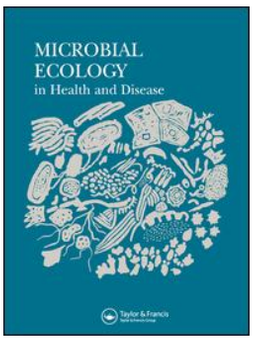

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Biofilm Development in Time on a Silicone Voice Prosthesis—A Case Study

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Voice prostheses from silicone elastomers become rapidly colonised by a mixed biofilm of bacteria and yeasts. In this study, microorganisms were isolated from biofilms on explanted prostheses after having been in place for various time intervals ranging from 1 to 67 d. The isolates were examined for their identity, adhesion to hexadecane and electrophoretic mobility. Bacteria from early (shorter than 8 d) and late (longer than 8 d) explants could not be classified according to their taxonomy, hydrophobicity or electrophoretic mobility. However, the yeasts clearly revealed a dominance of only hydrophilic *Candida albicans* isolates from early explants and only hydrophobic *C. tropicalis* isolates from late explants. These findings may be of significance for the development of strategies to control mixed biofilms on biomaterials.

KEY WORDS—Biomaterials; Silicone elastomers; Voice prostheses; Bacteria; Yeasts; Candida; Adhesion; Colonisation; Biofilms; Time study.

INTRODUCTION

Silicone voice prostheses are applied for speech rehabilitation in patients after laryngectomy.[3] They are implanted in a shunt between a tracheostoma, built for breathing and the upper digestive tract. This indicates that the device is located in a non-sterile, humid, nutrient-rich environment which will facilitate microbial adhesion to the biomaterial.[9] As a result, prostheses will be colonised by various microorganisms which will finally develop into a biofilm.[10] Furthermore, in the presence of yeasts, the silicone will even deteriorate.[11] As yet the heavy fouling of the silicone material cannot be controlled. If yeast growth and silicone deterioration is extended into the valve, being a crucial part for a proper functioning of the prosthesis, the only option available is to exchange the device.

The present lifetime of the prosthesis averages 3–4 mth, which is far from satisfactory. Therefore, strategies based on altering the adhesive properties of either the silicone or of the microorganisms should be designed, to achieve a less frequent exchange. After having described the electron-microscopic features of the biofilm on silicone voice prostheses,[10] and the material defects caused by yeasts,[11] this study was aimed at predicting a possible specific succession in the microbial colonisation of the implant which might be important for the final failure of the prostheses.

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MATERIALS AND METHODS

Voice prostheses

The voice prostheses used in the time study were so-called ESKA-Herrmann prostheses (ESKA Medical, Lübeck, Germany). This prosthesis is made of medical grade silicone with a metal core for stability. The L-shaped silicone tube has one retention flange on the tracheal side and one retention flange as well as a valve on the oesophageal side.[3] One male patient (65 yr) volunteered for this study and was willing to replace his prosthesis despite the absence of obvious failure features. The patient was edentulous and also wore a dental prosthesis. He had to undergo a laryngectomy 4 yr ago and normally the voice prostheses are exchanged every 1–2 mth. No special dietary instructions were given.

Isolation and growth of microorganisms

Each of the prostheses were removed and replaced by a new one by the patient himself after 1, 2, 4, 8, 18, 32 or 67 d and further processed within 1 h by a microbiologist. For transport, the prostheses were immersed in reduced transport fluid.[13] The microorganisms were detached from the prostheses by soft ultrasonic treatments using a water-bath (Bransonic 220, Branson, Shelton, CT, USA) for various time periods from 10 to 180 s and streaked on brain–heart infusion (BHI, Oxoid, Basingstoke, UK) bacto-agar (Difco, Detroit, MI, USA). The cultures were kept aerobically at 37°C and all distinguishable colonies were transferred onto fresh plates for subculture. The cultivable isolated microorganisms were grown on BHI agar for identification or in BHI broth for measurement of adhesive cell surface properties at 37°C on a rotary shaker (G76, New Brunswick, Edison, NY, USA) at 110 r.p.m.

Taxonomy

After microscopical examination and Gram-staining the microorganisms were identified. To identify the microbial isolates, the BIOLOG GP and GN test plates (Biolog INC, Hayward, CA, USA) were employed for bacteria, whereas identification of yeasts was done on the basis of API ID32C identification system (BioMérieux SA, Marcy-l'Etoile, France).

Adhesive cell surface properties

The adhesive properties of microorganisms are mainly determined by the hydrophobicity and charge characteristics of the cell surface.[8,14] For the measurement of both cell surface properties, microorganisms were grown in liquid media for 16 h, washed twice in 10 mM potassium phosphate buffer pH 7 and resuspended in this buffer to an optical density of 0·3–0·5 at 600 nm.

Microbial adhesion to hexadecane was chosen to probe for hydrophobicity.[12] After adding 150 µl hexadecane to 3 ml of the cell suspension and measuring the optical density, A_0, the two-phase system was vigorously vortexed (Genie, Winn, Tolbert, The Netherlands) twice for 30 s with a break of 15 s. Then, after 10 min, the optical density of the aqueous phase A was measured again at 600 nm to calculate the percentage adhesion to hexadecane using the formula:

$$\% \text{ adhesion to hexadecane} = (1 - A/A_0) \times 100$$

Electrophoretic mobilities were determined by using the Lazer Zee Meter 501 (PenKem, Bedford Hills, NY, USA). Six readings were taken per measurement at an applied electrical field of 150 V yielding an average standard deviation of $\pm 0{\cdot}14$ (10^{-8} m^2. V^{-1}. s^{-1}).

Scanning electron microscopy

The explanted voice prostheses were flushed with 6·8 per cent sucrose, 0·1 mol/l cacodylate buffer, pH 7·4 for 5 min. After fixation in 2 per cent glutardi-aldehyde, 0·1 mol/l cacodylate buffer, pH 7·4 for 2–24 h at 4°C, they were flushed a second time. For post-fixation the prostheses were slowly shaken in 1 per cent OsO_4, 0·1 mol/l cacodylate buffer at room temperature for 3 h. Dehydration involved the following rinsing procedure: 6·8 per cent sucrose, 0·1 mol/l cacodylate buffer 20 min; bidistilled water 3 × 10 min; ethanol series, 30 per cent, 50 per cent, 70 per cent, each 20 min; ethanol 100 per cent, 4 × 30 min. Then the specimens were critical point dried (CPD 020, Balzers, Liechtenstein) with CO_2 for 4 h, mounted on SEM stubs and sputtered (Sputtering Device, Balzers, Liechtenstein) with gold. SEM observations were made using an ISI-DS 130 (International Scientific Instrument, Japan).

RESULTS

A range of different bacterial and yeast strains could be isolated from each of the explanted voice prostheses after having been in place for 1 d up to 67 d as summarised in Table 1. Identification of the bacteria revealed mainly gram-positive cocci, but also some gram-positive rods and pleomorphic bacteria were recovered. Only three gram-negative bacteria were found. The yeast strains have been identified as *Candida albicans* and *C. tropicalis*. *C. albicans* was only isolated from the early (shorter than 8 d) explants, whereas only *C. tropicalis* was isolated from late (longer than 8 d) explants (see also Table 1).

The adhesion of bacterial strains to hexadecane (Figure 1) revealed that most of the isolates had a high affinity to the hydrocarbon, i.e. could be considered hydrophobic. Of the 28 bacterial strains tested, 16 had an affinity higher than 50 per cent. Only five strains showed an affinity lower than 25 per cent. The adhesion to hexadecane of the *C. albicans* and *C. tropicalis* strains was very different

Table 1. Bacterial and yeasts strains isolated from silicone voice prostheses explanted after various days of implantation

Prostheses in place	No. of isolates	Taxonomic identification	Code no.*
1 day	1	*Rothia dentocariosa*	1·5
	1	*Candida albicans*	1·3
	1	Unidentified cocci	1·1
	1	Unidentified pleomorph	1·4
2 days	3	*Streptococcus mitis*	2·3, 2·9, 2·10
	4	*C. albicans*	2·4, 2·5, 2·6, 2·7
	3	Unidentified cocci	2·1, 2·2, 2·8
4 days	1	*Aerococcus viridans*	4·3
	1	*Klebsiella pneumoniae*	4·6
	2	*Staphylococcus auricularis*	4·12, 4·14
	1	*C. albicans*	4·9
	2	*C. tropicalis*	4·2, 4·13
	3	Unidentified cocci	4·1, 4·7, 4·8
	2	Unidentified pleomorph	4·4, 4·11
8 days	1	*Bacillus coagulans*	8·7
	1	*S. sobrinus*	8·6
	3	*C. tropicalis*	8·1, 8·4, 8·5
	1	Unidentified cocci	8·3
	1	Unidentified pleomorph	8·2
18 days	1	*S. mitis*	18·5
	1	*C. tropicalis*	18·3
	2	Unidentified cocci	18·1, 18·2
31 days	1	*S. mitis*	31·4
	2	*C. tropicalis*	31·2, 31·3
	1	Unidentified cocci	31·1
67 days	1	*Micrococcus naucinus*	67·2
	1	*K. pneumoniae*	67·5
	1	*C. tropicalis*	67·1
	1	Unidentified cocci	67·4
	1	Unidentified rods	67·3

*Code numbers refer to those in Figures 1 and 2.

(Figure 2). *C. albicans* had a very low affinity, whereas *C. tropicalis* had a high affinity for the hydrocarbon.

The electrophoretic mobilities (see Figure 1) of the bacterial strains isolated from early explants did not show any significant differences as compared to those of the bacterial strains from late explants. The values measured were all negative and ranged from $-0{\cdot}7$ to $-4{\cdot}0$ ($10^{-8}\,m^2.V^{-1}.s^{-1}$). Similarly, the electrophoretic mobilities of the yeast strains (see Figure 2) did not reveal differences when comparing the values for early and late colonisers. However, as compared to the electrophoretic mobilities of bacteria, the yeast strains appear to be slightly more positively charged.

Occasionally scanning electron micrographs were made from some of the explanted prostheses to visualise the microorganisms on the silicone surface. In Figure 3 an example is given showing the biofilm consisting of cocci and yeasts as well as the deteriorated silicone elastomer for an explant having been in place only 8 d.

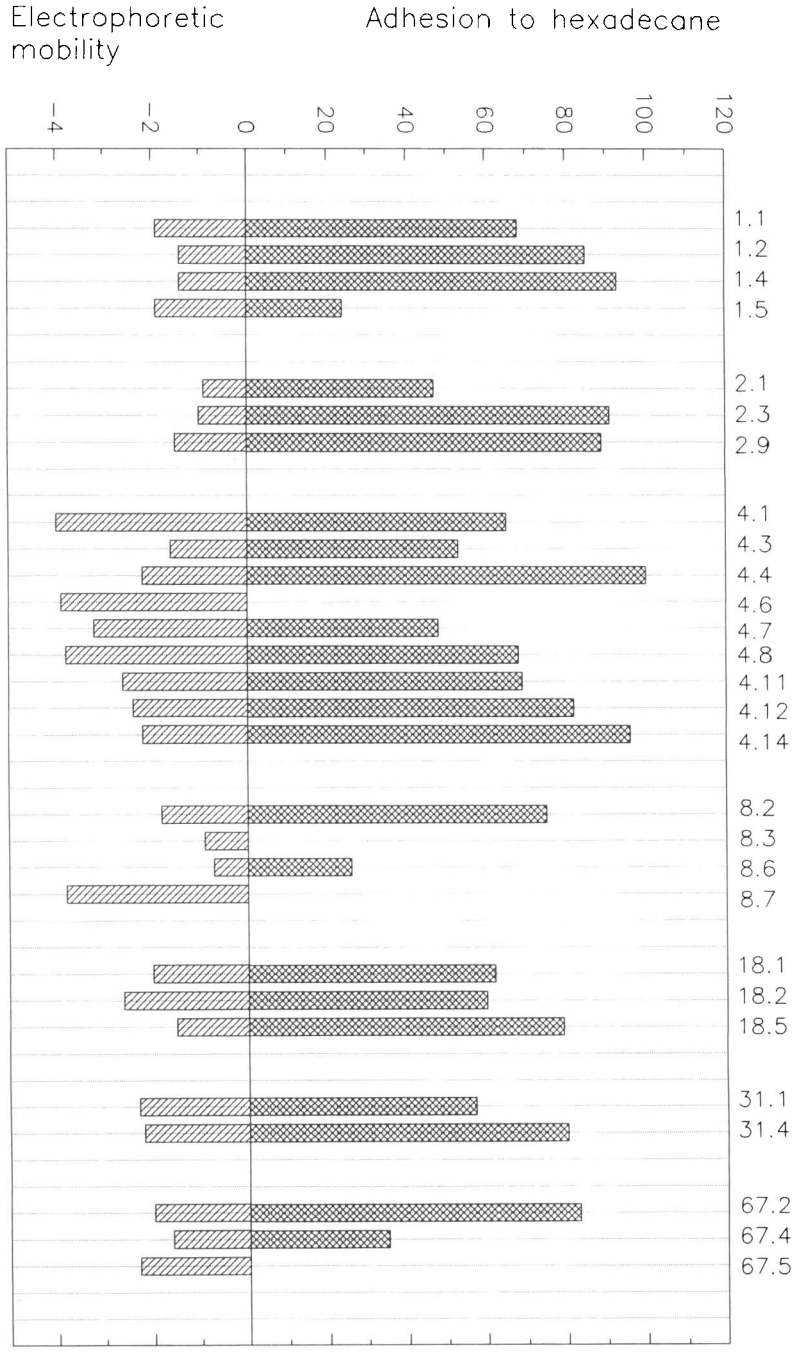

Figure 1. Adhesion to hexadecane (%) and electrophoretic mobility (10^{-8} $m^2 . V^{-1} . s^{-1}$) of bacterial strains isolated from explanted silicone voice prostheses after having been in place for various times. The code numbers given on the right hand side of the figure correspond with those in Table 1

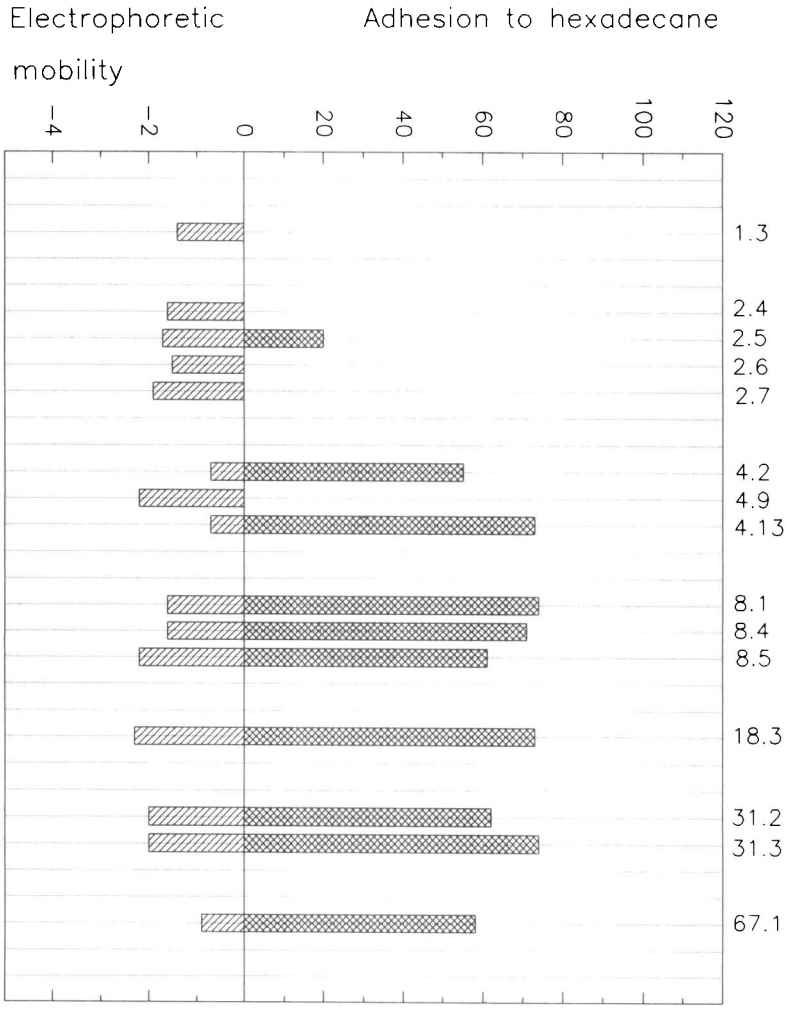

Figure 2. Adhesion to hexadecane (%) and electrophoretic mobility (10^{-8} $m^2 . V^{-1} . s^{-1}$) of yeast strains isolated from explanted silicone voice prostheses after having been in place for various times. The code numbers given on the right hand side of the figure correspond with those in Table 1

DISCUSSION

Voice prostheses made from silicone elastomers become rapidly colonised by microorganisms leading to the frequent exchange of the implants.[4,5,10,11] Although yeast strains have been suggested to be the causative organisms for the failure of the implants,[4,5,11] the development of biofilms in time on silicone voice prostheses has never been studied due to the necessary frequent exchanges of the implants. For the present study, we were fortunate to find one volunteer willing to participate in such a time study.

In this study, the presence of microorganisms originating from the oral and skin microbiota could be shown. By comparing the bacterial species, their adhesion to hexadecane and their electrophoretic mobility, no clear classification could be established between early and late colonisers. However, the yeasts showed a clear distinction between *C. albicans* and *C. tropicalis* strains on the basis of their hydrophobicities, with *C. albicans* being more hydrophilic and *C. tropicalis* being more hydrophobic, as has been found before.[2,6,7] It is striking that hydrophilic *C. albicans* was only isolated from the early explants, whereas more hydro-

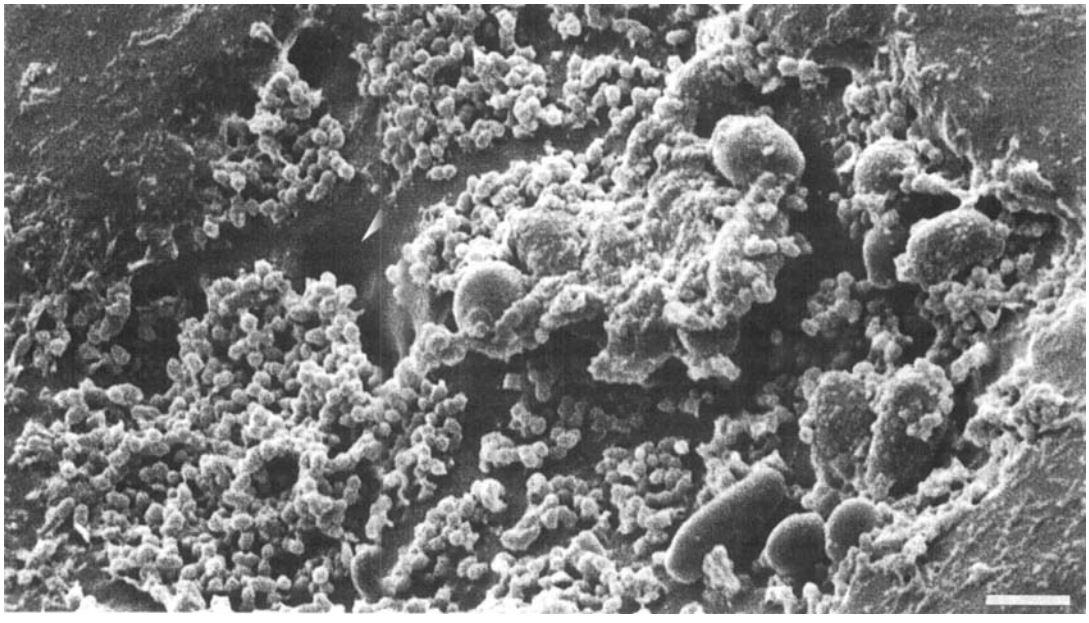

Figure 3. Scanning electron micrograph of the mixed biofilm on an explanted silicone voice prosthesis after 8 d implantation. The biofilm shows cocci and yeasts as well as the deteriorated silicone surface (arrow) (scale bar = 3 μm)

phobic *C. tropicalis* was only found in the later explants.

Nevertheless, it is difficult to understand the dominance of hydrophilic *C. albicans* cells in the early stages of colonisation as the hydrophobic silicone elastomer should facilitate the adhesion of hydrophobic rather than hydrophilic microorganisms.[1] To resolve this obvious contradiction it has been argued that colonisation by *C. albicans* is supported by a conditioning film, possibly produced by pioneering, adherent, hydrophobic bacteria analogous to colonisation succession of bacteria and yeasts in denture stomatitis.[15,16] Such a proposal would involve a change of the surface properties of the silicone material towards becoming more hydrophilic due to the presence of bacteria and their extracellular products, for example, by adsorbed biosurfactants.

In conclusion, a dominance of hydrophilic *C. albicans* in early and hydrophobic *C. tropicalis* in late stages of colonisation of silicone elastomers was found, together with a ubiquitous presence of bacteria in a study on the development of microbial biofilms on voice prostheses over time. It is suggested that bacterial colonisation is a prerequisite for the adhesion of more harmful yeast strains and that *C. tropicalis* is tentatively identified as the main causative organism for the failure of the implant as this organism was only isolated from late explants. This observation may have practical implications for the control of mixed biofilms on a variety of biomaterials, especially silicone elastomers.

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