ELSEVIER

International Biodeterioration & Biodegradation (1994) 383–390

0964-8305/94/$7.00

0964-8305(94)00014-X

Short Communication

In Vitro Ingrowth of Yeasts into Medical Grade Silicone Rubber

H. J. Busscher,[a]* C. E. de Boer,[a] G. J. Verkerke,[b] R. Kalicharan,[c] H. K. Schutte[d] and H. C. van der Mei[a]

[a]Materia Technica; [b]Centre for Biomedical Technology; [c]Department of Cell Biology and Electronmicroscopy; [d]Ear, Nose and Throat Clinic, University of Groningen, Groningen, The Netherlands

(Final version received 29 September 1994)

ABSTRACT

Shunt-valves made of silicone rubber are placed between the trachea and the oesophagus in order to allow patients after laryngectomy to produce voice again. The oesophageal side of the shunt-valve, however, becomes rapidly colonized by a biofilm, consisting of bacteria and yeasts. Electron microscopy on explanted shunt-valves demonstrates that the yeasts show ingrowth into the silicone rubber. Surface treatment of the silicone rubber is often suggested to be a pathway to reduce adhesion and ingrowth of yeasts to prostheses. An effective development of such a treatment requires an in vitro *system by which similar defects as found* in vivo *can be created under laboratory conditions.* Candida tropicalis, *isolated from an explanted prosthesis, could be made to grow into the silicone rubber under laboratory conditions by the removal of all nutrients using a modified Robbins device. After the removal of all nutrients, the yeast cells adapted a filamentous growth form. Only filamentously growing yeasts appeared to be able to penetrate the silicone rubber.*

*To whom correspondence should be addressed at: Materia Technica, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.

INTRODUCTION

Surgical treatment of a malignant tumor of the larynx often requires total laryngectomy: the larynx must be removed, including the vocal cords and epiglottis. The top of the trachea is lead outside to an opening (stoma) in the neck, through which the patient can breath (see Fig. 1 for a schematic anatomy after laryngectomy). In addition, the surgeon inserts a shunt-valve, for instance the 'Groningen button' (see also Fig. 1), between the trachea and the oesophagus (Mahieu *et al.*, 1986). Some sort of voice can be reproduced by closing the stoma with a finger and forcing air through the valve into the oesophagus. The remaining muscular structures at the oesophageal entrance then function as pseudo vocal cords.

Although the exact reasons for selecting a particular material in many

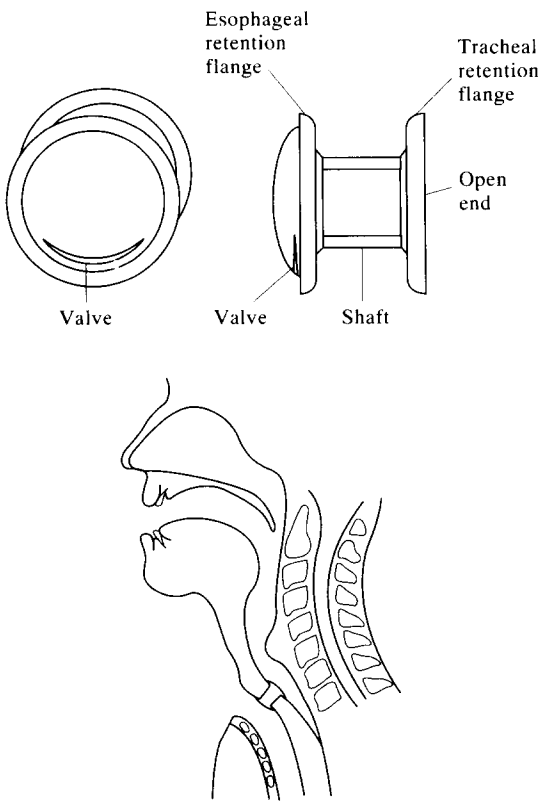

Fig. 1. Diagram of the 'Groningen button' shunt-valve (top) together with the anatomy after laryngectomy (bottom). After laryngectomy, the 'Groningen button' is placed between the trachea and the oesophagus.

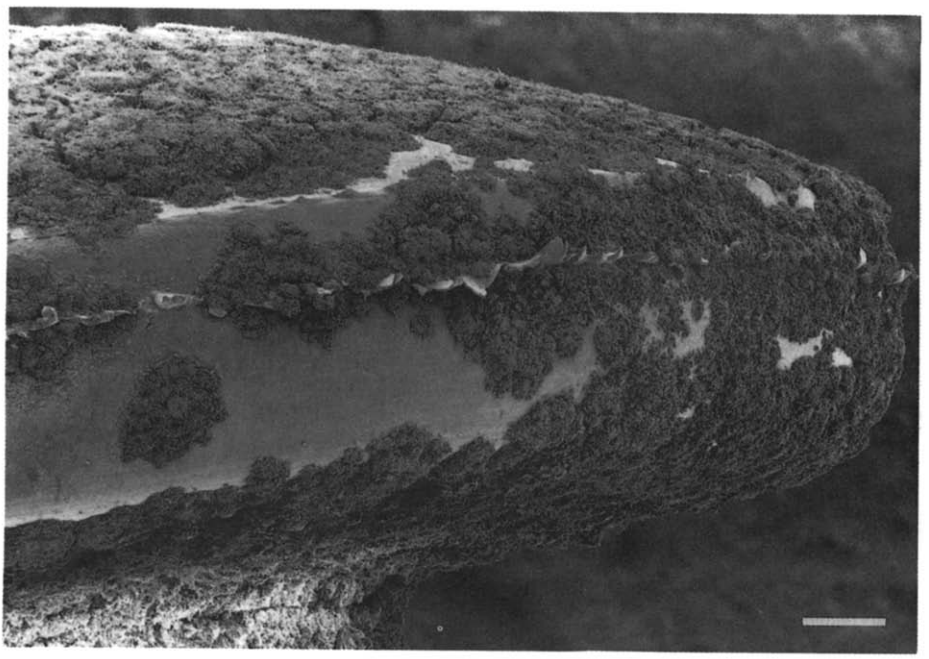

Fig. 2. Scanning electron micrograph of an explanted 'Groningen button' shunt-valve (implantation time 40 days), showing a thick biofilm on the valve side. *Method.* After explantation, the explant was immediately transferred into reduced transport fluid, subsequently flushed with 6·8% sucrose and 0·1 M cacodylate buffer (pH 7·4), fixed in 2% glutaraldehyde and 0·1 M cacodylate buffer and flushed again. Post-fixation was done in 1% OsO_4 and 0·1 M cacodylate buffer. Dehydration was then carried out in a graded ethanol series prior to critical point drying. Finally, for scanning electron microscopy (a Jeol-SEM, acceleration voltage 15–25 kV), 15 nm gold was sputtered on the samples (see Neu *et al.*, 1993, for details). The bar equals 600 μm.

biomedical applications are not really known, the choice is mostly determined by its mechanical properties and ease of manufacturing. The interfacial properties of a biomaterial are, however, of equal importance, being the connection between a biomedical implant and its biological environment. Many biomedical implants become colonized by an infectious biofilm (Gristina, 1987) which eventually necessitates removal of the implant. The oesophageal side of the 'Groningen button' shunt-valve is located in a non-sterile environment and therefore rapidly collects a thick biofilm (Izdebski *et al.*, 1987), consisting of a variety of bacteria and yeasts (see Fig. 2). Clinically, fouled shunt-valves are replaced when patients complain about leakage of food and liquid or, though less often, increased air flow resistance (Hilgers & Schouwenburg, 1990). On average, prosthe-

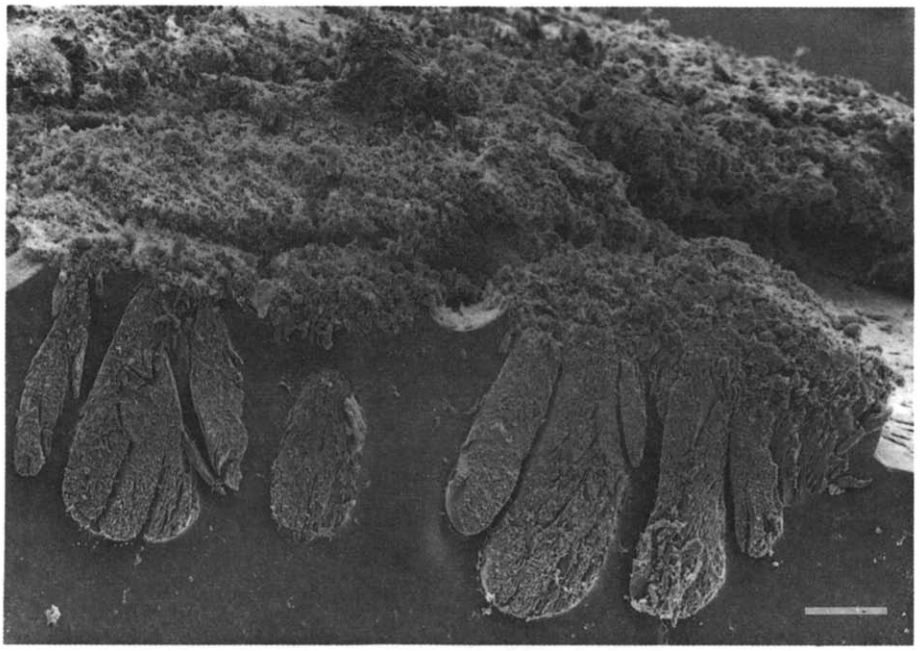

Fig. 3. Scanning electron micrograph of a sectioned, explanted 'Groningen button' shunt-valve (implantation time 434 days), showing ingrowth of yeasts into the silicone rubber (filled 'bag-like' defects). *Method.* See Fig. 2. The bar equals 100 μm.

ses remain implanted for three months and necessary frequent exchanges burden the patients.

Explanted 'Groningen buttons' did not only show biofilm formation on the implants (Neu *et al.*, 1992), but also ingrowth of yeasts (Neu *et al.*, 1993), presumably *C. tropicalis* (Neu *et al.*, 1994), into the silicone rubber (see Fig. 3). The mechanisms of ingrowth have only been speculated upon. Mechanical effects have been ruled out on the basis of the rather snug fit between the borders of the ingrowing colonies and the silicone rubber. Possibly, ingrowth is a result of enzymatic degradation of the silicone rubber, the products of which may be used as nutrients or the yeasts use the silicone rubber directly as a nutrient source.

A pathway to prolong the lifetime of shunt-valves is to develop a coating or surface modification for silicone rubber, discouraging biofilm formation and ingrowth as observed *in vivo*. As one of the first steps along this pathway, we set out to design an experimental set-up in which the *in vivo* fouling phenomena could be simulated on medical-grade silicone rubber samples.

MATERIALS AND METHODS

A modified Robbins device (Costerton *et al.*, 1986), in which 10 silicone rubber (Silastic®, medical grade ETR elastomer Q7-4750, Dow Corning) samples (6·3 mm diameter, thickness 1·0 mm) could be simultaneously inserted was used. First, the device was inoculated with an overnight culture of *C. tropicalis* GB 9/9, a clinical isolate from an explanted Groningen button. In one set of experiments, the device was then perfused for 12 days with defined growth medium (glucose 7·5 g litre^{-1}, $(NH_4)_2SO_4$ 3·5 g litre^{-1}, L-asparagine 1·5 g litre^{-1}, L-histidine 10 mg litre^{-1}, DL-methionine 20 mg litre^{-1}, DL-tryptophane 20 mg litre^{-1}, KH_2PO_4 1 g litre^{-1}, $MgSO_4 \cdot 7H_2O$ 500 mg litre^{-1}, NaCl 500 mg litre^{-1}, $CaCl_2 \cdot 2H_2O$ 500 mg litre^{-1}, yeast extract 100 mg litre^{-1}, H_3BO_3 500 μg litre^{-1}, $ZnSO_4 \cdot 7H_2O$ 400 μg litre^{-1}, Fe(III)Cl_3 120 μg litre^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 200 μg litre^{-1}, KI 100 μg litre^{-1}, $CuSO_4 \cdot 5H_2O$ 40 μg litre^{-1})

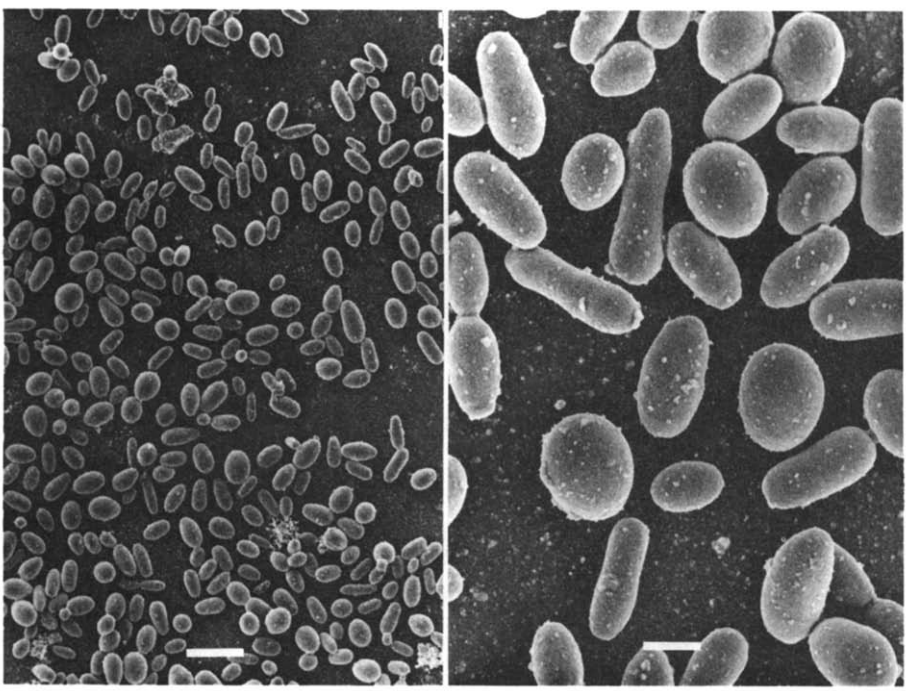

Fig. 4. Scanning electron micrograph of silicone rubber, exposed to *C. tropicalis* under constant nutrient conditions in a modified Robbins device, showing vegetatively growing yeast cells, appearing not to be capable of penetrating the material. The sample was removed from the device after 12 days, i.e. after perfusion of the device for 12 days with growth medium. *Method.* See Fig. 2. The bar equals 10 μm for the low and 2·5 μm for the high magnification micrograph.

and samples were removed every 1–2 days for electron microscopy. In another set of experiments, the device was first perfused for 6 days with growth medium and subsequently, also for 6 days, with phosphate buffered saline, in order to mimic the dynamic nutrient conditions (varying availability of nutrients) occurring *in vivo*. The temperature of the device was maintained at 34–37°C during all experiments.

RESULTS AND DISCUSSION

In both sets of experiments, samples appeared to be covered within 1–2 days with a thick biofilm, as could be observed with the naked eye. During preparation for electron microscopy, however, the major part of the biofilm detached, leaving only those organisms in direct contact with the silicone rubber.

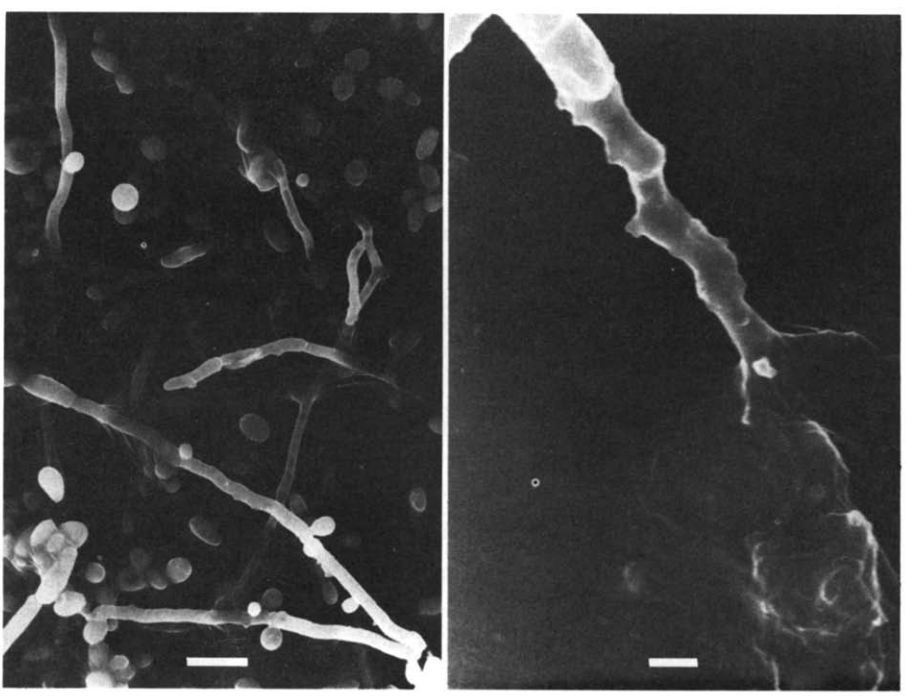

Fig. 5. Scanning electron micrograph of silicone rubber, exposed to *C. tropicalis* under dynamic nutrient conditions in a modified Robbins device showing filamentous yeasts penetrating the material. The sample was removed from the device after 12 days, i.e. after perfusion of the device for 6 days with growth medium and for 6 days with phosphate buffered saline. *Method.* See Fig. 2. The bar equals 10 μm for the low and 2·5 μm for the high magnification micrograph.

When perfusing continuously with growth medium, the yeasts adapted their vegetative growth form and no ingrowth into the silicone rubber was seen, even not after 12 days (Fig. 4.). Removal of all nutrients, in the second set of experiments, induced a change in growth form of the yeasts and a major part of the organisms adapted a filamentous growth form. Filamentously growing yeasts also grew into the silicone rubber (Fig. 5), presumably as the onset of the development of the type of defects observed *in vivo*. Note (Fig. 5) that at the point of entry of a yeast cell, there is an extremely smooth transition between the organisms and the material. No indication was found that the yeasts especially sought for rugosities or pores (if existing) on the silicone rubber in order to enter the material.

CONCLUSION

Silicones and silicone rubber have long been considered as inert biomaterials. Recently, this notion has been questioned (Press *et al.*, 1992; Fisher, 1992) due to the problems arising with silicone-filled breast implants. Also in dental materials science, it has been frequently observed that silicone rubber denture liners are apt to colonization and degradation by yeasts (Gettleman *et al.*, 1983). This study shows that *in vivo* fouling of silicone rubber as seen clinically on voice prostheses, can be simulated in a modified Robbins device. Penetration of the silicone rubber is induced by removal of all nutrients, causing *C. tropicalis* cells to adapt a filamentous growth mode, in which they penetrate the material. The methodology developed here may be useful for several other biomedical applications of silicone rubber, where non-adhesive variants or coatings need to be tested *in vitro* before clinical trials.

REFERENCES

Costerton, J. F., Nickel, J. C. & Ladd, T. I. (1986). Suitable methods for the comparative study of free-living and surface-associated bacterial populations. In *Bacteria in Nature*, eds. J. S. Poindexter & E. R. Leadbetter, Vol. 2, Plenum Press, New York, pp. 49–84.

Fisher, J. C. (1992). The silicone controversy — when will science prevail. *N. Engl. J. Med.*, **326,** 1696–8.

Gettleman, L., Fisher, D. J. & Farris, C. (1983). Self-sanitizing soft denture liners: paradoxical results. *J. Biomed. Mater. Res.*, **17,** 731–4.

Gristina, A. G. (1987). Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science*, **237,** 1588–95.

Hilgers, F. J. M. & Schouwenburg, P. F. (1990). A new low-resistance, self-retaining prosthesis (Provox^{TM} for voice rehabilitation after total laryngectomy. *Laryngoscope*, **100,** 1202–7.

Izdebski, K., Ross, J. C. & Lee, S. (1987). Fungal colonization of tracheoesophageal voice prostheses. *Laryngoscope*, **97,** 594–7.

Mahieu, H. F., Van Saene, H. K. F., Rosingh, H. J. & Schutte, H. K. (1986). *Candida* vegetations on silicone voice prostheses. *Archs Otolaryng.*, **112,** 321–5.

Neu, T. R., Dijk, F., Verkerke, G. J., Van der Mei, H. C. & Busscher, H. J. (1992). Scanning electron microscope study of biofilms on silicone voice prostheses. *Cells Materials*, **2,** 261–9.

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,** 459–64.

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

Press, R. I., Peebles, C. L., Kumagai, Y., Ochs, R. L. & Tan, E. M. (1992). Antinuclear auto-antibodies in woman with silicone breast implants. *The Lancet*, **340,** 1304–7.