Strategies to decrease biofilm formation on voice prostheses
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Summary
People who have had a laryngectomy because of a malignant tumor loose their vocal cords and the connection between lower and upper airway, therefore loosing their ability to speak. Voice rehabilitation is accomplished with tracheoesophageal shunt prostheses, in short voice prostheses. These biflanged silicone rubber tubes are placed in a tracheoesophageal fistula so air can flow from the trachea to the neopharynx, where the vibrating mucosa will produce a sound, enabling the patients to speak again. The oesophageal side of the tube is covered with a valve to prevent aspiration of fluids. The moist environment of the oesophagus and multiple nourishments make the valves of these prostheses prone for biofilm formation, which causes dysfunction of the valve. This results in a visit to the hospital to undergo an unpleasant replacement. The mean lifetime of a voice prosthesis is 3-6 months, however some patients need replacements every other week. Therefore finding (a) way(s) to decrease biofilm formation to increase in vivo lifetime of voice prostheses is the main aim of this thesis.

Although leakage through a voice prosthesis is the main reason for replacement of the prosthesis in the clinical setting, this has never been the subject of in vitro studies. Therefore in Chapter 2 a model comprised of an artificial throat equipped with a single prosthesis coupled to a water reservoir was developed to compare the in vitro leakage patterns of three commercially available voice prostheses, in absence or presence of a biofilm. In this study Blom Singer prostheses showed the lowest leakage followed by the Groningen Low Resistance. Provox®2 voice prostheses showed significantly the most leakage, which significantly decreased in presence of a biofilm. Regularly blowing air through the Provox®2 prostheses, to mimic oesophageal speech, significantly increased the leakage again. This is in line with clinical observations showing 76% replacements for Provox®2 compared to 57% replacements for Groningen Low Resistance due to leakage, out of 746 clinical replacements.

A letter to the editor send about this chapter elaborates about the assumed non clinical significance of this research because of the tight fit around the prostheses in the artificial throat, which causes a very small distortion enabling the prostheses to leak. The extreme differences in leakage patterns between the measured prostheses are irrelevant according to the author of the letter. In our opinion however, a non challenged voice prostheses will close perfectly as a column of water is put over them. This pressure helps the original elastic force from the silicone rubber to close the valve. In vivo closure is impeded by trapping food remnants, minor deformations due to uneven pressure exerted on the shaft by the fistula circumference, biofilm formation in the valve or deterioration of the elastic properties of the
silicone rubber by ingrowing biofilm. Therefore the extreme increase of leakage in the Provox®2 compared to the other voice prostheses, with a small distortion of the voice prostheses is relevant. This seems also to be acknowledged by Provox as they designed a voice prosthesis which overcomes this problem by using a magnet on the valve which enables closure in more challenged circumstances.

Biofilm integrity is ensured by extracellular polymeric substances (EPS). This statement is reinforced by the results described in Chapter 3. In this study synthetic salivary peptides, mucolytics and two different antiseptics (Chlorhexidine and Triclosan) were investigated on their effect on reduction and integrity of the biofilm on voice prostheses. The voice prostheses were incubated in an artificial throat model, allowing simultaneous adhesion and growth of yeast and bacteria. After initial biofilm formation they were dipped in the different substances, showing decreases in biofilm formation for the two positive controls and for N-acetylcysteine and ascorbic acid. However, significant decreases in airflow resistance was only seen in treatment with triclosan and N-acetylcysteine which also showed an absence of connecting slime threads on scanning electron micrographs which suggests the importance of EPS on the integrity of biofilm.

Antimicrobial peptides (AMPs) have shown in literature to be promising candidates for therapeutic drugs as they have a broad antimicrobial spectrum and development of antimicrobial resistance to AMPs is rare. In Chapter III dhvar5 showed a reduction of the voice prosthetic biofilm, but the application method is not applicable for patients. Therefore in Chapter 4 different AMPs were adsorbed in a single application on the valve of voice prostheses. Subsequently biofilms were grown on the voice prostheses in an artificial throat model. All the adsorbed AMPs showed a significant decrease in the yeast prevalence in the formed biofilms. In addition, LFampin 265-284 was the only AMP reducing bacterial prevalence. This lack of antibacterial efficacy of the other AMPs could be a result of their adsorption or the mixed species nature of the biofilms.

Other coatings, two quaternary ammonium silanes, were investigated in Chapter 5. One coating (trimethoxysilyl)-propyldimethyloctadecylammonium chloride] was applied through chemical bonding, while the other coating, Biocidal ZF was sprayed onto the silicone rubber surface. Both coatings were tested in the artificial throat showing significant reductions in numbers of viable yeast and bacteria compared to the control. This was confirmed using confocal laser scanning microscopy after live/dead staining of the biofilms. However the sprayed coating lost its stability within an hour, while the chemically bonded coating
appeared stable. Moreover in situ hybridization with fluorescently labeled oligonucleotide probes showed that yeasts expressed hyphae on the untreated and Biocidal ZF-coated prostheses but not on the QAS-coated prostheses. Whether this is a result of the positive QAS coating or is due to the reduced number of bacteria is currently unknown. This is the first report on the inhibitory effects of positively charged coatings on the viability of yeasts and bacteria in mixed biofilms.

The downside of the strategies to decrease biofilm formation described in the previous chapters are the extra steps and costs in the production process. Therefore in Chapter 6 the original Groningen ultra low resistance silicone rubber voice prosthesis was modified through the use of a different mold and liquid silicone rubber filling, which resulted in a decreased surface roughness. These smooth prostheses showed reduced biofilm formation in comparison with regular “Ultra Low Resistance” silicone rubber Groningen voice prostheses in vitro, without the need of major adjustments in the production process or coating of prostheses after production.

In Chapter 7, the general discussion, methods to evaluate biofilm formation, different coatings and modifications of prostheses are discussed. Furthermore suggestions for future research are made for these subjects.