General Discussion
For some patients with a laryngeal or hypopharyngeal tumour the only chance upon survival is to undergo a laryngectomy. Since Billroth performed the first laryngectomy in 1873, major improvements have been established with regard to the quality of life of laryngectomized patients. However patients nowadays still are painfully aware that their appearance and voice will change. The vocal cords are removed leaving the patient with the inability to speak. It took until the late 19th century before the development in voice rehabilitation took a flight. Oesophageal speech and the electrolarynx were developed and in 1979 the first tracheoesophageal fistula with insertion of a silicone rubber voice prosthesis was described by Blom and Singer.1 Until now this is the preferred form of voice rehabilitation, because oesophageal speech is difficult to learn for most people and an electrolarynx gives a robotic voice. Different prostheses were designed after Blom and Singers first prosthesis. The Provox™2 voice prosthesis is the most widely used prosthesis in The Netherlands, followed by the Groningen Ultra Low Resistance prosthesis, both made of silicone rubber. A major drawback of the Groningen voice prosthesis was the retrograde insertion of the prosthesis which created much more discomfort to the patient when changing the voice prosthesis compared to the anterograde technique used with Provox™2 prostheses. This difference is resolved by the introduction of the frontloading system for the Groningen voice prostheses. Both frontloading systems are currently evaluated in a prospective multicentre trial. An important difference between these prostheses is the design of the valve. The Provox™2 voice prosthesis is a biflanged device which has a hinge valve molded into one piece with the prosthesis and supported by a radiopaque, fluoroplastic ring (Fig. 1a). The Groningen Ultra Low Resistance prosthesis has two flanges with a semicircular slit of 210° in the hat of the oesophageal flange (Fig. 1b).

Figure 1. A) Provox and B) Groningen Low Resistance voice prosthesis open valves.

However, both prostheses are in essence no more than one way valves, placed in a tracheoesophageal fistula, to prevent stenosis of the fistula and aspiration of food, drinks or
saliva from the oesophagus into the trachea. When the prosthesis would withstand time and the influences of saliva, food and drinks, this voice prosthesis could stay in situ, but as long as mankind uses prostheses for medical purposes, biofilm formation on these prostheses has been a troublesome fact. Biofilm formation on voice prostheses leads to dysfunction, resulting in the necessity to replace the prosthesis. In this thesis different strategies to reduce biofilm formation on the valve of voice prostheses are evaluated.

**METHODS TO EVALUATE BIOFILM FORMATION**

To evaluate strategies to decrease biofilm formation we used the so-called artificial throat, a model which was developed by Leunisse et al.\(^2\) and complemented by Elving et al.\(^3\) Every laboratory setup has its limitations compared to the in vivo situation and of course the artificial throat is no exception. Moreover creating a reliable experimental setup for biofilm formation and prevention on voice prostheses is very difficult because the variables between patients are multiple. Differences in composition of saliva, acid reflux and food habits for example, are not included in our experimental set up, but will create different biofilms. However including more variables will not necessarily improve the experimental setup as it is more difficult to differentiate what is influencing the results. Elving implied that an important step to improve the in vitro set up could be integrating leakage through the voice prosthesis. This can be done without changing the actual set up for biofilm formation in the artificial throat as described in chapter 2 and significant changes in leakage between voice prostheses with and without a biofilm were only seen for the Provox voice prostheses. Leakage characteristics of Groningen voice prostheses appeared not strongly influenced by the presence of a biofilm.

Biofilms have been described since Van Leeuwenhoek examined the plaque of his own teeth, but it took until 1978 before research in biofilm formation took a huge flight.\(^4\) Nowadays, we are becoming more and more aware of the complexity of biofilms. The artificial throat is an interesting instrument to study influences on voice prosthetic biofilms in vitro before testing in vivo. New ways to visualize biofilms, like fluorescence in situ hybridization (FISH), can also be used to label different micro-organisms in order to achieve a better understanding of the organization within a biofilm. It is interesting to look more closely to the biofilms again as they probably have changed over time as nowadays people are first treated with accelerated radiotherapy and surgery is performed in a later stage.
COATINGS

Different coatings are used on voice prostheses or other biomaterials to decrease biofilm formation. In literature different coatings on different biomaterials are described. In this thesis two positively charged quaternary ammonium silane coatings and antimicrobial peptides are used.

Antimicrobial peptides (AMPs) are natural defenses of the human species in the fight against bacteria and yeasts, as noted in many articles they are promising candidates for therapeutic drugs. In this thesis different antimicrobial peptides are tested on their activity on the voice prosthetic biofilm. Elving et al.\textsuperscript{5} showed a promising effect of the antimicrobial peptides dhvar 4 and dhvar 5 on single strain micro-organisms. However dhvar 4 did not show any effect when used on the mature voice prosthetic biofilm. Investigating the effect on younger biofilms can be interesting as it could be that reduced susceptibility develops only at a certain stage of biofilm maturation and that younger biofilms are not protected.\textsuperscript{6} Elving did not test these antimicrobial peptides on developing biofilms, because using these antimicrobial peptides from the start of the introduction of the prosthesis in the patient would mean frequent intake of the peptides demanding therapy loyalty of the patient. Adsorbing the peptides on the valve of the voice prostheses would overcome this problem. This was investigated in this thesis and achieved a significant decrease in yeast load, while the bacterial load was only significantly decreased for adsorbed LFampin 265-284. This antimicrobial peptide could be an interesting peptide to examine in an \textit{in vivo} setting. However at this moment we do not know if we can store the AMP-coated prostheses as we do not know how long the peptides stay active on the voice prostheses.

Polymer brushes are a different group of coatings which are described in literature. Cringus-Fundeanu described a polyacrylamide brush which showed a high reduction in microbial adhesion.\textsuperscript{7} The micro-organisms tested were \textit{Staphylococcus aureus}, \textit{Streptococcus salivarius} and \textit{Candida albicans}, all micro-organisms which are also seen in the biofilm on voice prostheses. This finding makes a polyacrylamide brush interesting in decreasing biofilm formation on these prostheses.

An important drawback of the use of coatings on biomaterials is the complexity of the final production process and the subsequent increasing costs.
MODIFIED PROSTHESES

Up to now in vivo research to decrease biofilm formation has led to an increase in costs due to a more complex production process or regular intake of medication for the patients. Decreasing biofilm formation, and therewith increasing in vivo lifetimes of voice prostheses without extra costs and discomfort to the patients, will be an important accomplishment in this line of research. In the last chapter of this thesis we produced such a modulated smooth voice prosthesis. The mold used for producing the voice prostheses was made smoother and a liquid silicone rubber was used, resulting in a smoother voice prosthetic surface. This prosthesis showed reduced biofilm formation in vitro compared to regular prostheses of the same type. Investigating the in vivo lifetime of these smoother prostheses will be a logical next step and is the topic of a next PhD project within UMCG.

Another modification of voice prostheses could be prostheses made of silicone rubber mixed with fluoride. Fluoride is known to decrease biofilm formation and a silicone rubber mixed with fluoride is commercially available therefore these prostheses could be made with little extra effort.

‘Prevention is better than cure’ and trying to influence biofilm formation from the onset is probably better than trying to eliminate a mature biofilm. However, as voice prostheses are situated in a moist environment surrounded by bacteria and yeast, total prevention will not be possible. Therefore, it is worthwhile to look at adjustments to the voice prostheses themselves so biofilm formation will have less effect on its function. Since the Groningen voice prostheses are used, the actual design has not been changed and changes in design may decrease the effect of biofilm formation on the closing mechanism of the valve.

The search for methods to prevent or decrease biofilm formation on medical implants is in progress and probably will be for a very long time. It will be a challenge to unravel the secrets of the micro eco system of the biofilm.
REFERENCES


