

Silicone obturators and the bacterial flora in symptomatic nasal septal perforations

Anna Karin Hultström



**Department of Odontology/Dental Technician
Programme**
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To my family who never ceases to support and inspire

It has long been an axiom of mine that the little things are infinitely the most important (The Adventures of Sherlock Holmes 'A Case of Identity', sir Arthur Conan Doyle)

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Papers in the thesis

- I. Hulterström AK, Sellin M, Berggren D. The microbial flora in the nasal septum area prone to perforation. *APMIS* 2012; 120: 210-214.
- II. Hulterström AK, Sellin M, Berggren D. High prevalence of *S. aureus* around symptomatic perforations of the nasal septum. In manuscript /submitted.
- III. Hulterström AK, Sellin M, Berggren D. The effect of obturator treatment on the microbial flora surrounding symptomatic nasal septal perforations. In manuscript.
- IV. Hulterström AK, Berglund A, Ruyter IE. Wettability, water sorption and water solubility of seven silicone elastomers used for maxillofacial prostheses. *J Mater Sci: Mater Med* 2008; 19: 225-231.

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Abstract

Background A perforation in the nasal septum can cause symptoms such as bleeding, obstruction, crusts and pain, and can be a challenge to treat. Surgery is the treatment of choice, but disease, size of the perforation, or the patient's wish may contradict surgery. A custom-made silicone obturator is a successful treatment option, but little is known how this treatment affects the microbial flora. The purposes of this thesis were (i) to investigate the microbial flora around symptomatic nasal septal perforations before treatment, (ii) during and after a 12-month treatment period with a custom-made obturator, (iii) to compare the microbial flora around symptomatic perforations with the flora from the same area of the septum in healthy individuals, (iv) to investigate the microbial colonization of the silicone obturator, and (v) also to investigate the water sorption, solubility and if the wettability of silicones are affected by water. The hypotheses were (i) that the bacterial flora around symptomatic perforations would not differ from that found in healthy individuals, apart from a possible presence of *Helicobacter pylori*; (ii) the bacterial flora would change in composition during the course of treatment and that microorganisms and proteins could be seen on the surface of the silicone obturators; (iii) a material that has adsorbed water would also show an increase in wettability and the surface free energy of the material.

Methods Twenty-seven patients and 101 healthy individuals volunteered. Swabs were made around the rim of the perforation, or on the septum in the locus Kisselbach area in the healthy individuals. Bacteria and fungi were isolated and identified with standard laboratory techniques. A biopsy of the granulated tissue at the perforation was taken and cultivated for *Helicobacter pylori*. Swabs were also taken three, six and twelve months after inserting the obturator. The obturator was analysed after being used twelve months in the nose. Seven silicones were tested for water sorption and solubility according to ISO standards 1567:1999 and ISO 10477:2004. The change in wettability was examined by measuring the contact angle with a contact goniometer at various stages of the sorption/solubility test.

Results *Staphylococcus aureus* was present in 88% of the untreated patients. With treatment a significant reduction of *S. aureus* occurred to 54.5% ($p < 0.05$). In the healthy group *S. aureus* was present in 13% of the subjects. No *Helicobacter pylori* could be cultivated from the biopsies taken of the granulated tissue at the perforation. The flora round the untreated perforation was dominated by *S. aureus* with few other bacterial species

detected. In the healthy group there was a diversified flora with both aerobic and anaerobic bacteria. SEM revealed a rough surface on the silicone obturator and crazing of the silicone surrounding the pigment granules. Both bacteria and proteins could be seen on the obturators in SEM. *Candida albicans* was detected in one obturator, but not in the mucosal swab at the corresponding time. That patient had, however, been treated for *Candida* in the nose six months prior to the last visit in the study. Wettability was affected but did not increase with amount of adsorbed water. Some materials showed an increase and some a decrease in the surface-free energy. The tested addition silicones showed little sorption and solubility.

Conclusions The patients with symptomatic perforations of the nasal septum had a bacterial flora totally dominated by *S. aureus*. The massive presence of *S. aureus* around symptomatic perforations may have an impact on the persistence of the granulated and inflamed tissue present in symptomatic perforations, thus forming a vicious circle with bleeding and crustation.

S. aureus dominance in the mucosa surrounding symptomatic perforations was diminished by using a custom-made obturator. The microbial flora became more diversified with the treatment, although not resembling the flora in healthy individuals. The microbial flora of the obturators was similar, but not the same as the corresponding mucosal flora. The discovery of *Candida* in the obturator of a patient who had been treated for *Candida* in the nose six months earlier suggests that obturators need to be exchanged when fungal infections are being treated to prevent the fungus from re-infecting the patient at a later stage.

The silicone had a rough surface and a poor wettability, both aspects favours colonization of microorganisms. The silicone was negatively affected by the colouring pigments, this should be considered when colouring is not necessary. The slight, but existing solubility of silicones emphasises the importance of using medical grade silicones that are more purified than industrial silicones.

Abbreviations

CoNS	Coagulase-negative staphylococci
ENT	Ear-, Nose-, and Throat department
IRHD	International Rubber Hardness Degrees
LSR	Liquid Silicone Rubber
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	methicillin-sensitive <i>Staphylococcus aureus</i>
SEM	Scanning Electron Microscopy
TSB	Tryptic soy broth
WG	Wegener's granulomatosis

Enkel sammanfattning på svenska

Kort bakgrund

Strax under en procent av den svenska befolkningen har ett hål i nässkiljeväggen. De flesta har inga besvär av hålet, men de få som får besvär kan bli vårdkrävande. Nästäppa, svåra blödningar, smärta och krustabildningar är några av de vanligaste besvären. Besvären behandlas med återfuktande sprayer och salvor, samt kirurgi. Ibland kan man inte göra en kirurgisk slutning, hålet kan vara för stort, det kan finnas andra sjukdomar som gör att man inte vill göra ett operativt ingrepp, eller patienten vill inte opereras. I dessa fall kan man sluta hålet med hjälp av en knapp i silikon. Här i Umeå behandlar man med en specialanpassad knapp, dvs. en knapp som är tillverkad efter ett avtryck av hålet och som därför passar till enbart den patienten. Patienterna som får en sådan knapp upplever en förbättrad livskvalitet och det blir oftast en förbättring av de symptom som man sökt för. Frågan som vi har ställt oss är om bakteriefloran i näsan hos dessa patienter skiljer sig från floran hos friska personer, om bakteriefloran påverkas av knappen och om silikonet påverkas av en fuktig miljö.

Hur vi gjorde

Vi tog bakterieprov på 101 friska personer. Provet togs på det område av nässkiljeväggen där hål brukar kunna uppstå. Vi har också tagit bakterieprov på 27 patienter med hål på nässkiljeväggen och som var remitterade till Öron-, näs-, och halskliniken för behandling med en specialanpassad knapp. Bakterietestet på patienterna togs på slemhinnan runt hålet på nässkiljeväggen. När patienterna fått sin knapp kallades de tillbaka efter 3-, 6- och slutligen 12 månader på kontroll. Vid 3- och 6 månaderskontrollen togs ett nytt bakterieprov och sedan togs knappen ut, rengjordes och sattes tillbaka. Vid 12-månaderskontrollen togs ett sista bakterieprov, därefter ersattes den gamla knappen med en ny. Den gamla knappen analyserades för att se vilka bakterier som växte på den och hur ytan såg ut i svepelektron mikroskop. Vid första klinikbesöket togs också ett litet prov på slemhinnan vid hålet för att undersöka om magsårsbakterien *Helicobacter pylori* skulle kunna finnas där.

Sju silikonprover testades också för att se om de tar upp vatten och om de släpper ifrån sig någon beståndsdel. Vi testade också om vätskor som droppades på ytan av materialet blev mindre droppformade när silikonet hade legat i vatten ett tag (materialets vätkapacitet).

Vad vi fann

Det vi fann var att väldigt många, 88%, av de obehandlade patienterna hade en bakterie som heter *Staphylococcus aureus* och hela bakteriefloran dominerades av denna bakterie med endast några få andra påvisade bakterier. När patienterna behandlats med silikonknappen sjönk antalet patienter som hade *S. aureus* ner till 54,5%. I den friska gruppen var det 13% av personerna som hade *S. aureus* i näsan. Vi kunde inte få fram några *Helicobacter pylori* från nässlemhinnan. Elektronmikroskopin visade att silikonet hade en rå yta, vi kunde också se både bakterier och proteiner på ytan av knapparna. En jästsvamp *Candida albicans* hittades i en knapp, men inte i patientens bakterieprov på slemhinnan vid 12 månadskontrollen. Däremot hade patienten blivit behandlad för *Candida* i näsan sex månader tidigare. Materialens vätbarhet påverkades, men inte efter hur mycket vatten de tagit upp eller hur lång tid de legat i vatten utan en del material fick bättre vätbarhet och andra fick sämre vätbarhet. De testade additionssilikonerna förändrades väldigt lite i vatten, de tog upp lite vatten och de avgav också mycket litet substans.

Vad betyder dessa fynd

Den bakterieflora som vi hittade hos de friska personerna, på det område av nässkiljeväggen där man oftast ser perforationer, påminde om den flora som rapporterats i litteraturen från bakterieprov tagna i näsborrarna, förutom mängden *S. aureus*. Det man skulle ha förväntat sig var att ca 27 % skulle ha haft *S. aureus* i näsan eftersom det är den siffra som man ser från undersökningar där bakterieprovet är taget i näsborrarna. Vi fann 13 %. Patienterna däremot hade mycket mer *S. aureus* än förväntat med sina 88 %. En sådan hög närvaro av denna bakterie i näsan har man rapporterat från ett par andra sjukdomstillstånd. Vad som skiljer dessa olika tillstånd från varandra är att har man ett hål i näsan så blir miljön torr, medan en hel nässkiljevägg ger en fuktig miljö. Vad dessa sjukdomstillstånd kan ha gemensamt är en försvagad slemhinnebarriär och även ett försvagat lokalt immunförsvar mot dessa bakterier.

Vi fann att den mikrobiella floran runt perforationerna förändrades under behandlingens gång, men kom aldrig att helt likna den friska bakteriefloran. Många av patienterna har andra sjukdomar som ger många sjukhusvistelser, dessutom så är några av de bakterier som vi fann ofta sedda i samband med biomaterial, dvs. material insatta i människa. Många av patienterna har troligen också blivit vana att smörja nashålorna med saltvattenspray eller sesamolja. Ett bakterieprov fångar bakteriefloran i ett ögonblick och en del av de bakterier vi sett kan vara bofasta, dvs. de växer och förökar sig i området, medan andra bakterier är på genomresa, ditförda av sprayer, fingrar och dylikt.

Vi fann också skillnader mellan de bakterier som vi kunde odla fram från slemhinnan hos patienterna och de bakterier som återfanns på de knappar som togs omhand efter 12 månader i näsan. Ytan på silikonet var rå. Knappen framställs i en pressform tillverkad av extra hårt gips. Silikon är bra på att återge den yta den härdat mot och i det här fallet har alltså silikonet återgett gipsytan. Det finns studier som visar att en rå yta gynnar bakteriernas fäste på ytan och silikonets dåliga förmåga att vätas (vatten pärlar sig på ytan) drar också till sig mikroorganismer.

En av de mikroorganismer som vi fann i obturatorn men inte på slemhinnan var jästsvampen *Candida albicans*, däremot hade patienten blivit behandlad mot denna svamp i näsan 6 månader tidigare. Det finns studier som visar att *Candida* kan växa in i silikon något som kan ge problem på sikt.

I elektronmikroskopet såg vi både bakterier och proteiner på ytan. Det hade vi väntat oss eftersom nassekret och saliv påminner mycket om varandra i sammansättning. Det har i flera studier visats att proteiner från saliv absorberas på ytan av tandemalj och fyllningsmaterial i munnen, därför förväntade vi oss ett liknande fenomen på våra silikonknappar. Man kunde också se att ytan på silikonet var krackelerad ovanför färgpigmentkornen.

Alla testade silikonerna hade en liten absorption och löslighet i vatten. Vätbarheten påverkades också, men mycket lite. En del av materialen fick en lätt ökning och några en lätt sänkning av vätbarheten.

De slutsatser som vi har dragit av studien

Patienterna med besvär från den perforerade nässkiljeväggen hade en bakterieflora som helt dominerades av *S. aureus*. Den massiva närvaron av *S. aureus* runt hål som ger besvär kan ha en betydelse för den inflammerade slemhinna som finns runt dessa hål. Detta kan då ge en ond cirkel med blödning och krustabildning.

Den dominans som *S. aureus* hade i slemhinnan runt de obehandlade hålen minskade när patienterna fick en specialanpassad knapp i hålet. Fler sorters mikroorganismer dök upp under behandlingens gång, men floran kom aldrig att helt likna den flora som sågs hos de friska individerna. Den mikrobiella floran på knapparna var liknande, men inte helt den samma som den som återfanns på slemhinnan. Upptäckten av *Candida* i en knapp som suttit i näsan på en patient som behandlats för just svampinfektion i näsan sex månader tidigare tyder på att man bör byta till en ny knapp när man behandlar mot svampinfektion. Detta för att förhindra svampen från att orsaka en ny infektion vid ett senare tillfälle.

Silikonet hade en rå yta och dålig vätbarhet, båda dessa egenskaper gynnar bakterieväxt. Silikonet påverkades negativt av färgpigment och detta bör beaktas när det inte är nödvändigt med färg. Den lilla, men existerande lösligheten hos silikonerna understryker vikten av att man använder medicinska silikonerna som har högre renhetsgrad än silikonerna ämnade för industriellt bruk.

Introduction

Prologue

How come it is that a dentist is writing a thesis about a small hole in the nose? Where such a story begins all depends on how far back you want to go in your narrative. I could start with my parents, schooling and all that, but that is too far back in the fogs of time to be of any real relevance to the story. I could also begin with getting married, and that would be a bit more relevant to the story, but I will start with approximately ten years ago. I had started my postgraduate career with a project solely oriented on medical silicones, but the whole project had ground to a halt when my first tutor retired from the faculty, and all my efforts were canalized into teaching. I was, however, still a postgraduate student and I was determined to follow through with my project one way or another. So I did work for the student union. This was when I met Diana. I already knew that Diana used medical silicones in the treatment of symptomatic perforations of the nasal septum, so it seemed natural to discuss this with her. From these first informal discussions a deeper desire to learn more about how the silicone would influence the nasal environment, and how that environment would influence the silicone grew, and suddenly I found myself with a totally new angle to my old theme for the thesis.

Perforations of the nasal septum

Slightly less than one per-cent of the Swedish population has a perforation of the nasal septum, an occurrence that is also reported from other parts of the world (Cogswell and Goodacre, 2000; Öberg et al., 2003).

Although many perforations are idiopathic, it can be a sign of Wegener's granulomatosis, lupus erythematosus, or some other medical condition. The most common known causes of nasal septal perforation are iatrogenic causes, such as complications after nasal septal surgery (Blind et al., 2009; Cogswell and Goodacre, 2000; Diamantopoulos and Jones, 2001; Kridel, 1999). Frequent use of astringent nose drops, cortico-steroids, or other foreign substances, can also result in perforation. Most perforations are asymptomatic, but some persons develop symptoms like crustation, bleeding, pain, obstruction, odour and alterations of smell (Cogswell and Goodacre, 2000; Kridel, 1999). Especially small perforations can also give rise to whistling sounds.

Most individuals with asymptomatic perforations are not aware that their nasal septum is perforated, and it is generally agreed upon in the healthcare

that this type of perforation will not need any treatment (Cogswell and Goodacre, 2000; Kridel, 1999).

A symptomatic perforation on the other hand is a challenge for the medicine, and can be treated conservatively or surgically. A conservative treatment can consist of nasal sprays or oils, with the purpose of softening the nasal crusting. It can also consist of closing the perforation with an obturator made of silicone. The surgical techniques are developing, but success-rate is still low with surgery, i.e. the majority of the perforations perforate again some time after surgery (Bateman and Woolford, 2003). The size of the perforation, underlying diseases, or the patient's wish may also exclude surgery as treatment option (Blind et al., 2009). A method for closing the perforation noninvasively is therefore desirable.

Nasal obturators

Most obturators are made of silicone, but there have been reports in the literature concerning obturators made of acrylic (Moergeli, 1982; Zaki, 1980). Nasal obturators made of silicone can be prefabricated or custom-made. Prefabricated obturators usually require some form of trimming to fit the perforation (Pallanch et al., 1982). This type of obturator cannot be removed and cleaned by the patient himself/herself, but requires medical help for cleaning and changing. The patient comfort and compliancy is also questionable (Luff et al., 2002). An alternative to the prefabricated obturators is the custom-made obturator that is made for a individual patient and can either be carved out by hand from a larger block of silicone using a template (Price et al., 2003), or made by using a lost-wax technique where an impression of the perforation is taken and a model of the perforation in dental stone is manufactured (Blind et al., 2009). A wax prototype of the obturator is then manufactured on the model of the perforation. A mould is produced from the wax prototype, and the obturator can then be fabricated in silicone. This is an obturator that the patient usually learns to handle by himself/herself, thus relieving the healthcare of the task of removing, cleaning and inserting obturators (Fig. 1). In Umeå, septal perforations have been treated for several years with custom-made obturators made with the lost-wax technique with good effects on bleeding, obstruction and crusting. They are especially effective on bleeding. It also improved the quality of life for the patients (Blind et al., 2009). However, it is not fully understood why such obturators are such a successful treatment.

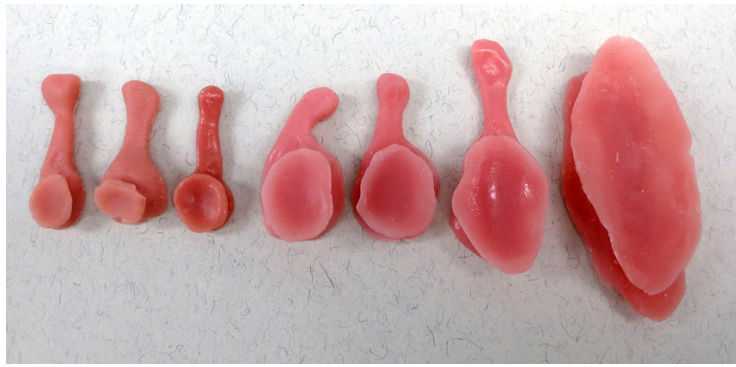


Fig. 1. Custom-made silicone obturators showing a wide variety of perforation sizes.

Silicones

Silicones are synthetic polymeric compounds that contain silicon together with carbon, hydrogen, oxygen and sometimes other elements.

Silicones have been used in medicine since the 1960s (Braley, 1970), and many uses have been found for the material since then (Fallahi et al., 2003). Silicones were considered to be inert when they were first introduced into healthcare, but it soon became obvious that the material is affected both physically and biologically over time (Aziz et al., 2003; Garrido et al., 1996; Hultström and Ruyter, 1999; Neu et al., 1993). Silicones can be divided into two categories, condensation and addition, according to the polymerization method. The condensation polymerisation is so-called because a by-product, usually an alcohol or water, is formed during the polymerisation. No by-products are formed during the addition polymerisation and the catalysts are usually peroxides or platinum. The peroxide-curing silicones require free radicals to initiate the polymerisation, whereas platinum is said to be a true catalyst, i.e. the platinum is not destroyed in the polymerisation process but remains unchanged, and this is called a hydrosilylation process (Brook, 2006; Mark et al., 1992). The first medical-grade silicones to be introduced were all condensation-type silicones (Braley, 2000), but today the majority of the medical-grade silicones are either platinum- or peroxide- curing (NuSil, 2011). Silicones can also have different consistencies, all from very runny to firm, and different degrees of hardness. Runny or past-like silicones are usually referred to as Liquid Silicone Rubbers (LSR) and are traditionally mixed by hand. More firm materials cannot be mixed by hand: a mill with steel cylinders is required. Hardness can be measured in International Rubber Hardness Degrees (IRHD) or shore degrees (ISO 48: 2007; ISO 7619-1: 2004). Hardness of a silicone is not depending on what firmness the base and catalyst materials have.

Silicones are used in a wide variety of fields, of which medicine is one. One must therefore make certain that the material used in a medical appliance is made with a material appropriate for the application. The medical silicones are either restricted or unrestricted. Restricted silicones are for external use or short-term implantation of 29 days or shorter, while the unrestricted silicones can be used for long-term implantation. The difference between industrial grade silicones and the medical grades is the level of purity. Under no circumstances may an industrial-grade silicone be used for medical purposes; this was stressed by Braley as early as 1964 (Braley, 2000).

Microbial flora of the nose

It is not known what influence the microbial flora has on perforations in the nasal septum, and there are few publications regarding the normal microbial flora in the nose (Gordts et al., 2000). The most common bacteria in cultivations from the nose are aerobic bacteria, but anaerobic bacteria can sometimes also be found (Araujo et al., 2003; Jousimies-Somer et al., 1989; Klossek et al., 1996). No studies have previously been made of the bacterial flora from the locus Kieselbach area. This makes it interesting to investigate this area in healthy individuals in order to have something to compare the flora surrounding symptomatic perforations with.

Staphylococcus aureus (S. aureus)

S. aureus is a part of the human commensal flora and it colonizes primarily the anterior nares but is also associated with other sites like the skin and pharynx (Wertheim et al., 2005). Around 27-30% of the population has *S. aureus* in their nares (Damm et al., 2004; Nilsson and Ripa, 2006; Wertheim et al., 2005). Figures as low as 8% have, however, been reported from the meatus media area (Gordts et al., 2000). Problems with multi-resistant *S. aureus* are at present frequently debated, and the cases of bacteraemia caused by *S. aureus*, both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA), are increasing in Europe (van Belkum, 2006). Second to coagulase-negative staphylococci (CoNS), *S. aureus* is the most prevalent organism causing intravascular device-associated bacteraemia (Wertheim et al., 2005). It is also a microorganism known to cause tissue destruction (Liang and Ji, 2007), and it has been associated with perforations of the nasal septum in persons with cocaine abuse (Simsek et al., 2006). In a couple of studies regarding *S. aureus* and Wegener's granulomatosis (WG) it was found that 72-74% of the patients carried the bacterium (Laudien et al., 2010; Stegeman et al., 1994). Stegeman et al. (1994) identified persistent carriers of *S. aureus* as being more prone to relapses of WG, and Laudien et al. found a correlation between the bacterium and endonasal activity of WG. *S. aureus* is also known to produce

exotoxins and recent studies have suggested that these exotoxins can act as superantigens and upregulate T lymphocytes to release inflammatory cytokines (Bernstein et al., 2011). Bernstein et al. (2011) have also found a high prevalence (87%) of *S. aureus* in association with nasal polyposis (Bernstein et al., 2011). When a population is monitored, three different carriage patterns of *S. aureus* in the nose emerge. Approximately 20% of a population are persistent carriers of *S. aureus*, 30% are intermittent carriers and about 50% are non-carriers. Persistent carriers are usually colonized with a single strain of the bacterium over time, while intermittent carriers may change strains. Also the load of *S. aureus* is usually higher in persistent carriers (Wertheim et al., 2005).

Helicobacter pylori

Helicobacter pylori are pathogens associated with ulcers in the stomach, with duodenum and gastric cancer and lymphomas. It has also been found in oesophagus, in the oral cavity and in the upper respiratory tract (Al-Hawajri et al., 2004; Cirak et al., 2003; Czesnikiewicz-Guzik et al., 2004; Dinis et al., 2005; Kignel et al., 2005; Koc et al., 2004; Özdek et al., 2003; Ylitalo, 2006). Since this bacterium is so closely associated with ulcers and have also been found in mucosa in the vicinity of locus KISS1, we decided to investigate if it could be found in the mucosa surrounding symptomatic septal perforations.

Biofilm

The natural state for bacteria is to form biofilms and only one per-cent of bacteria exists in a free-floating planktonic state. A biofilm is defined as a congregation of microorganisms surrounded by an extracellular, polymeric substance, which is self-produced (Keir et al., 2011).

The formation of a biofilm on the surface of a biomaterial is a complex development. In the mouth the saliva surrounds the teeth and biomaterials such as fillings and proteins from the saliva will adsorb on the surface of the hard substances (Everaert et al., 1998). Nose secretion, like saliva contains among other things also glycoproteins like mucins (Vanthanouvong and Roomans, 2004). The adsorption of mucins on the surface of a biomaterial is positively correlated to the formation of biofilm and will also make it more difficult to remove the biofilm (Li et al., 2012). It is therefore reasonable to assume that a similar process with adsorbed proteins on the surface of biomaterials can be seen in the nose. Bacteria interact with both the film of adsorbed proteins on the surface of the biomaterial and the biomaterial itself and it has been demonstrated that the microbial adhesion to a substrate is an interaction between hydrophobic and electrostatic forces between the different surfaces (Everaert et al., 1998). There are, however, several factors

that can complicate the interaction of hydrophobic and electrostatic forces. The film of adsorbed proteins, the surface free energy of the biomaterial, the strain of bacterium, and the interaction between different microorganisms are examples of known factors that may influence the adhesion (Li et al., 2012; Lina et al., 2003; Lydon et al., 1985; Muthukrishnan et al., 2011).

Surface-free energy

A low surface-free energy of biomaterials promotes human cells, bacteria and fungi to grow and a biofilm starts to form on the surface of a biomaterial as soon as it is introduced into the human body (Lydon et al., 1985; Waters et al., 1999). A hydrophobic material has a low surface-free energy and a poor wettability (Good, 1993). The interaction between hydrophobic and electrostatic forces is a complicated interaction and this can be demonstrated by two different studies. Rodrigues et al. treated a silicone surface with rhamnolipid biosurfactants that produces a less hydrophobic surface, and Everaert et al. treated a silicone surface with fluoro-alkylsiloxane that produces a more hydrophobic surface than the untreated silicone. Both studies could then show that adherence of microorganisms to the treated surface was significantly reduced compared to the untreated silicone surface (Everaert et al., 1998; Rodrigues et al., 2006).

Silicones are hydrophobic materials which mean that their wettability is poor. They are also easily colonized by microorganisms, and one explanation, given by Rodrigues et al. is that water is easily removed from the hydrophobic silicone surface, giving the bacteria a good access to the surface, thus facilitating the adhesion (Rodrigues et al., 2006).

Aims of the study

- To investigate the microbial flora of healthy individuals in the area where nasal septal perforations most commonly occur (Paper I).
- To investigate the microbial flora around symptomatic perforations of the nasal septum. The hypothesis was that the bacterial flora around symptomatic perforations would not differ from that found in healthy individuals, apart from a possible presence of *Helicobacter pylori* (Paper II).
- To investigate the microbial flora around symptomatic nasal septal perforations during and after a 12-month treatment period with a custom-made silicone obturator. The hypotheses were that the bacterial flora would change in composition during the course of treatment, and that microorganisms and proteins could be seen on the surface of the silicone obturators (Paper III).
- To study wettability, water sorption and solubility of commercially available silicone elastomers used for maxillofacial prostheses. The hypothesis was that a material that has adsorbed water would also show an increase in wettability and surface free energy of the material (Paper IV).

Materials and methods

Healthy population and patients

For paper I healthy students and staff at the police education programme were asked to volunteer for a bacterial swab in the locus Kasselbachii area on the nasal septum. In total 101 individuals that included 48 women and 53 men with a mean age of 28.2 and 29.4 years respectively, volunteered to participate. In papers II and III patients referred to the Ear-, Nose-, and Throat (ENT) department in Umea, Sweden with untreated, symptomatic perforations of their nasal septa, and planned for treatment with an obturator were asked to participate. Twenty-seven patients agreed to participate. Twelve patients were followed for three months and eleven patients were followed through six months as well as for the entire test period of 12 months. Nine of the 27 patients (two men and 7 women with a mean age of 49 and 48 respectively) have dropped out of the study and reasons for that can be seen in tab.1. The remaining patients who have not been followed through three to twelve months are new in the study and had not reached those control points when these data were compiled. Ten obturators were collected and analysed. In paper II the results from the bacteriological swabs from 25 patients were recorded and in paper III the data from 18 patients were used. In tab. 1 a consecutive compilation of the patients and data concerning the patients has been made. Of the 25 patients in paper II 13 were women (mean age 48.6) and 12 were men (mean age 46; in paper III there were eight women (mean age 49.9) and ten men (mean age 45.8). The mean diameter of the perforation for the patients remaining in the study was 13 mm with a median value of 12 mm, and the mean diameter of the perforation for the dropouts were 12 mm with a median of 12 mm. The majority of the dropout patients have continued to wear the obturator, but either wanted treatment closer to home or quickly learned to handle the obturator themselves. Only two patients felt uncomfortable with the device.

Tab. 1. A table of the patients in paper II and III in consecutive order; their symptoms; diseases, medication and trauma or surgery to the nose; size of perforation at the start of treatment; reason for not staying in the study; and paper(s) the patients' data are used. Dropout patients marked with grey.

Cases, sex (age) at inclusion	Symptoms (-, +, ++, +++) ¹ Crust obstruction bleed	Trauma, surgery, disease, medicines	Perf. size (mm)	Using obturator after 12 months, and reasons for not staying in the study	Paper
1 M (61)	++ ++ +	Septoplasty 1970s and post op. infection. Diabetes.	11.6	Using obturator	II, III
2 M (22)	+ ++ +	Trauma to the nose a few years ago, not treated at hospital	18.0	Using obturator	II, III
3 F (27)	++ ++ +	Ulcerous colitis, frequent sinusitis	14.1	Using obturator	II, III
4 M (24)	++ ++ +	Rhinitis medicamentosa, over consumption of astringent nose drops	16.4	Using obturator	II, III
5 F (44)	++ + ++	Spondylosis	*2	Took out obturator after 2 months and not continued wearing it	II
6 M (55)	+ + ++	Multiple sclerosis, Rheumatoid arthritis, anticoagulation drugs, nasal corticosteroids	10.2	Using obturator	II, III
7 F (58)	++ ++ ++	Asthma, fractured nose 3 times, deviated nose and obstructed, nasal corticosteroids	11.6	Using obturator	II, III

8 M (57)	+	+	+	Trauma against nose, septoplasty	15.4	Using obturator, but needed new obturator after 3 m. handles obturator self	II
9 M (66)	++	+	+		10.2	Using obturator, handles self	II, III
10 M (41)	++	++	+	Nasal corticosteroids	9.6	Using obturator, wanted treatment at hospital closer to home	II
11 M (28)	++	++	+	Mb Crohn, nasal and general corticosteroids	12.4	Using obturator	II, III
12 F (36)	+	-	-		9.2	Using obturator. Wanted to be without obturator during summer but returned for new in autumn	II
13 M (48)	+++	+	+	Asthma, over- consumption of astringent nose drops	16.4	Using obturator. Handles obturator self	II, III
14 M (58)	++	++	+		14.3	Using obturator	II, III
15 F (30)	++	++	++		14.4	Was not comfortable with obturator	II
16 F (49)	++	+	++		11.1	Using obturator, but wanted treatment at hospital nearer	II

				to home	
17 F (64)	++ + ++		15.2	Using obturator	II, III
18 F (88)	+++ ++ ++		17.6	Using obturator, but wanted treatment at hospital nearer to home	II
19 F (51)	+ + +	Asthma, perforation caused by trauma (a dog claw)	13.7	Not using obturator, did not feel comfortable with treatment	II
20 F (39)	+ ++ +	Over consumption of astringent nose drops	9.8	Probably using obturator, learned very quickly to handle obturator, saw no point in continuing visiting hospital	II
21 F (58)	+ + +	Sarcoidosis, anticoagulation drugs	11.4	Not finished study period	II, III
22 M (50)	- + -	Trauma and septoplasty. Perforation known complication after surgery	9.9	Not finished study period	II, III
23 F (52)	++ + -		21.8	Not finished study period	II, III
24 M (46)	++ + ++		12.6	Not finished study period	II, III
25	- - ++	over consumption of	6.8	Not finished	II, III

F (36)		astrigent nose drops, nasal corticosteroids		study period	
26 F (45)	++ + +	Asthma, nasal corticosteroids	11.5	Not finished study period	III
27 F (59)	++ ++ +	Nasal trauma. Diabetes, MB Bechterew	14.2	Not finished study period	III

12

1

Crusts: + some irritating crusts, ++ large problems with crusts that all the time needs removal, +++ completely obstructing crusts on one side or both.

Obstruction: + moderate obstruction, ++ troublesome obstruction that for example hinders sleep, +++ almost completely obstructed on one or both sides, mouth-breathing during longer periods.

Bleeding: + small but frequent bleeding and crusts with blood, ++ often larger bleeding but can be managed at home, +++ large bleeding that demands hospital care.

2 The size of this perforation is unknown. All perforations were measured on their dental stone casts and that cast was missing.

All sampling was made in the left nostril. A sterile speculum was used to allow access to the area and the bacteriological swab was made with a cotton swab moistened with sterile sodium chloride solution. The swab was made on the mucosa in the locus Kisselbach area on the healthy individuals, and around the rim of the perforation on the patients. After sampling, the swabs were inserted into the Copan swab transport system with Amies gel agar with charcoal (Copan, Italy) and taken to the bacteriological laboratory where they were cultivated on different media (tab. 2).

Tab 2. Materials used in cultivating bacterial swabs.

Culture		Manufacturer	Incubation
Blood agar	Columbia Agar Base supplemented with 5% horse blood	Acumedia, Lancing, MI, USA	35-37°C 4 days with daily inspections
Chocolate agar	Difco GC Medium Agar Base (BD), with 1% Iso VitaleX (BD), 1% purified haemoglobin (O)	Becton, Dickinson and company (BD), Sparks, MD, USA Oxoid (O), Basingstoke, UK	5-10% CO ₂ at 35-37°C 4 days with daily inspections
Fastidious anaerobe agar	With 5% defibrinated, lysed, horse blood	LabM, Bury, UK	Incubated for 10 days in a Concept 400 anaerobic chamber (Ruskin Technology LTD, Pencoed, UK)
5 mL Tryptic soy broth		Acumedia, Lancing, MI, USA	4 days with daily inspections
Sabouraud dextrose agar	With chloramphenicol (50mg/L) and gentamicin (50mg/L)	Becton, Dickinson and company (BD), Sparks, MD, USA	10 days

Tryptic soy broth (TSB) with visible growth was sub-cultured if agar plates from the corresponding sample were culture negative. Pure cultures were isolated from each of the different colony morphologies for later identification.

Identification of bacterial isolates

Bacterial isolates were presumptively classified based on colony morphology, Gram-stain results and by standard laboratory techniques (Murray et al., 2003).

The identification process of Gram-positive and negative cocci are illustrated in fig. 2-3. *S. Aureus* isolates were screened for methicillin-resistance by examining growth on MRSA select agar (BioRad, Marnes La Coquette, France). Gram-positive irregular aerobic rods were identified according to colony morphology. Two types of colonies could be observed. After 1-2 days of incubation large, white colonies were visible and after 2-3 days small greyish colonies appeared. A random subset of the two groups were tested for lipophilism by looking for enhanced growth in media containing 1% Tween 80 (Merck, Darmstadt, Germany) (Funke et al., 1997). They were then identified to the species-level using the API-Coryne biochemical panel (BioMerieux, Marcy l'Etoile, France) and 16S rDNA sequencing. Anaerobic Gram-positive irregular rods were tested for susceptibility to metronidazole. Anaerobic, Gram-positive, irregular rods resistant to metronidazole, were identified as *Propionibacterium* spp.

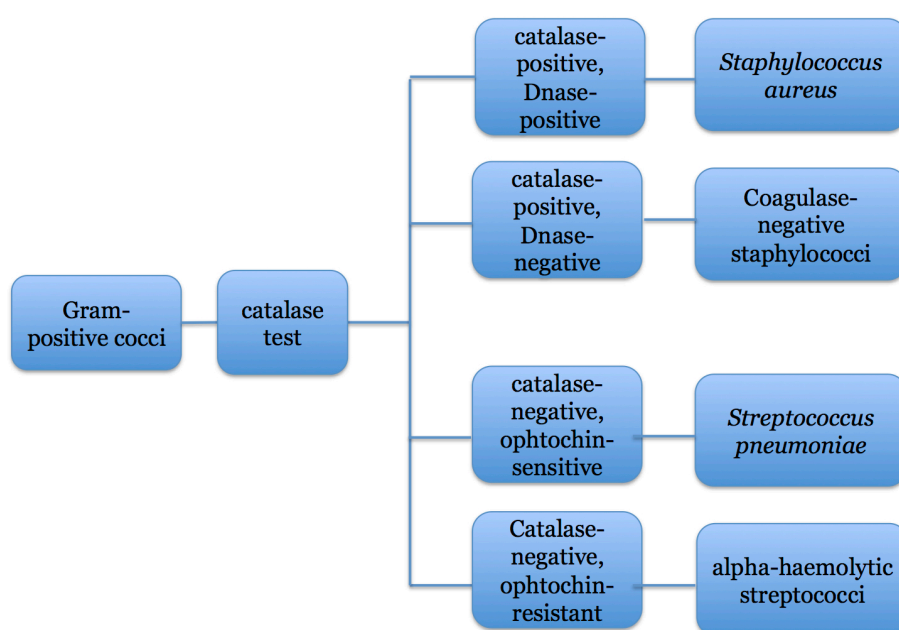


Fig. 2. Identification of Gram-positive cocci.

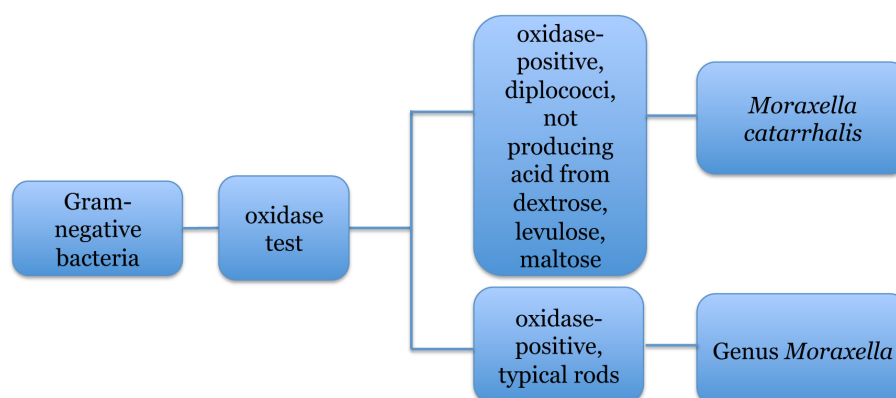


Fig. 3. Identification of Gram-negative bacteria.

16S rDNA sequencing

The 16S rDNA gene was analysed on a subset of the larger bacterial groups to support the presumptive classification. Five isolates each of lipophilic, non-lipophilic and anaerobic Gram-positive rods, and three probable *Moraxella*-isolates were included. 16S rRNA genes were amplified by PCR using primer SSU1 (Mariani et al., 1996; Fredricks and Relman, 1998). The PCR products were analysed by using SSU1 as primer and the Taq DyeDeoxy terminator cycle sequencing kit and an ABI PRISM 3700 DNA sequencer according to the manufacturers instructions (Applied Biosystems, Foster City, CA, USA). The 16S rDNA sequences were compared with known sequences in the GenBank/EMBL databases by using the BLASTN 3.2 algorithm (Altschul et al., 1990).

Patient treatment

An impression in alginate of the perforation was made at the patients' first visit to the ENT department, after the swab had been made. An obturator was fabricated in a medical grade milled silicone (Versasil, Nusil Silicone Technology, Polymer Systems Technology Ltd, High Wycombe, Bucks, UK) with the lost wax technique after the impression. A biopsy of the mucosa surrounding the perforation was also taken for cultivation of *Helicobacter pylori*, but when the first fifteen biopsy results had come back negative for *H. pylori*, this procedure was stopped for ethical reasons.

The patients were called back for follow-up visits at three, six and twelve months after the obturator had been inserted. At the three- and six-month visits, a swab was made, through the left nostril, of the mucosa surrounding

the perforation. The obturator was then removed, cleaned and reinserted. The twelve-month visit concluded the study. The obturator was removed and a new obturator inserted, after the swab had been made. The old obturator was then halved, and one half was sent for bacteriological analysis undergoing the same types of cultivation and identification as the bacteriological swabs. The other half was fixated in glutaraldehyde for later examination in a scanning electron microscope (SEM).

Scanning Electron Microscopy

In addition to the fixated halved obturators, three sterilized unused obturators and *in vitro* cultivated bacterial film of cocci (*Staphylococcus aureus* CCUG 1800) cultivated on two different medical grade silicones was examined in a SEM. The unused obturators and the *in vitro* cultivated films were made of the same two different silicones, one LSR silicone (Med 4930, Nasil Silicone Technology, Polymer Systems Technology Ltd, High Wycombe, Bucks, UK) and one milled silicone (Versasil, Nasil Silicone Technology, Polymer Systems Technology Ltd, High Wycombe, Bucks, UK). The catalyst and base of the LSR silicone for the obturators were mixed together in two different ways - the traditional hand mixing, and by speedmixer™ DAC150FVZ-K (Siemens, Synergy Devices Ltd, High Wycombe, Bucks, UK).

Material testing

The wettability, water sorption and solubility were tested on seven silicone elastomers commonly used in maxillofacial prostheses during the 1990s (table 3).

Water sorption and solubility were tested according to ISO standards 1567:1999 and 10477:2004.

Wettability was tested with a contact angle goniometer (Ramé-Hart Inc., Mountain Lakes, NJ, USA) by measuring the contact angle of five different liquids (tab. 4) on the surface of the specimens. Advancing and retreating angles were determined and the surface free energy was calculated according to the equations given in fig. 4. An apolar liquid, in this case di-iodmethane, is needed to determine the Lifshitz-van der Waals component of the surface free energy. At least two polar liquids are needed to determine the acid and base components of the surface-free energy and water must be one of these liquids.

Tab. 3. Silicone elastomers tested in paper IV.

No	Type	Brand name	Code	Manufacturer
1	Condensation	Cosmesil Regular	CR	Principality Medical Ltd., Rogerstone Newport, Wales, UK
2	Condensation	Cosmesil High Compliance	CH	Principality Medical Ltd., Rogerstone Newport, Wales, UK
3	Condensation	R&S 330 T-RTV ³	RS	Ringsted & Semler, Copenhagen, Denmark
4	Addition	Wacker RTV-ME 625	RT	Wacker Chemie GmbH, Munich, Germany
5	Addition	A-2186	A2	Factor II, Lakeside, Arizona, USA
6	Addition	LSR 30-10:1	LS	Applied Silicone Corp. Ventura, California, USA
7	Addition	SEN 240	SE	Shin-Etsu Silicones Europe B.V, AV Almere, Netherlands

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Tab. 4. Liquids and their polarity used in paper IV to examine surface free energy of the materials.

	Liquid	Polarity
1.	Di-iodmethane	Apolar
2.	Chemically pure water	Polar
3.	Ethylene glycol	Polar
4.	Formamide	Polar
5.	Glycerol	Polar

³ Synonymous with Wacker M-539, Wacker Chemie GmbH, Munich, Germany

$$\gamma_s^{LW} = \gamma_1^{LW} \text{apolar liquid} \frac{(1 + \cos \Theta)^2}{4}$$

$$\gamma_s = \gamma_s^{LW} + \gamma_s^{AB}$$

$$\gamma_s^{AB} = 2 \sqrt{\gamma_s^{\oplus} \gamma_s^{\ominus}}$$

$$\sqrt{\gamma_s^{\oplus}} = \frac{AF - BC}{CF - DE} \quad \sqrt{\gamma_s^{\ominus}} = \frac{BC - AE}{CF - DE}$$

$$A = \gamma_2 (1 + \cos \Theta) - 2 \sqrt{\gamma_s^{LW} \gamma_2^{LW}}$$

$$B = \gamma_3 (1 + \cos \Theta) - 2 \sqrt{\gamma_s^{LW} \gamma_3^{LW}}$$

$$C = 2\sqrt{\gamma_2^{\ominus}} \quad D = 2\sqrt{\gamma_2^{\oplus}}$$

$$E = 2\sqrt{\gamma_3^{\ominus}} \quad F = 2\sqrt{\gamma_3^{\oplus}}$$

γ^{LW} = Lifshitz-van der Waals component of γ

γ^{\oplus} = (Lewis) acid component of γ

γ^{\ominus} = (Lewis) base component of γ

Fig. 4. Equations used to calculate surface free energy of the different materials in paper IV.

Water was used as liquid 2 in pair with one other polar liquid in these equations, and water was also used to calculate the coefficients A, C and D, while each of the other polar liquids were used to calculate B, E and F. The wettability measurements were made with newly made specimens (start), when the specimens had been dried to a constant weight (conditioned), after three days in water, and on water-saturated specimens.

Statistical analysis

Statistical analysis was made using SPSS 19 (paper III) and SPSS 11 (paper IV) for Macintosh using paired t-tests and Bonferroni ANOVA.

Results

Bacterial flora in healthy population and patients

There was a distinct difference in the bacterial nasal flora between the healthy individuals and the patients with symptomatic septal perforations (Fig. 5).

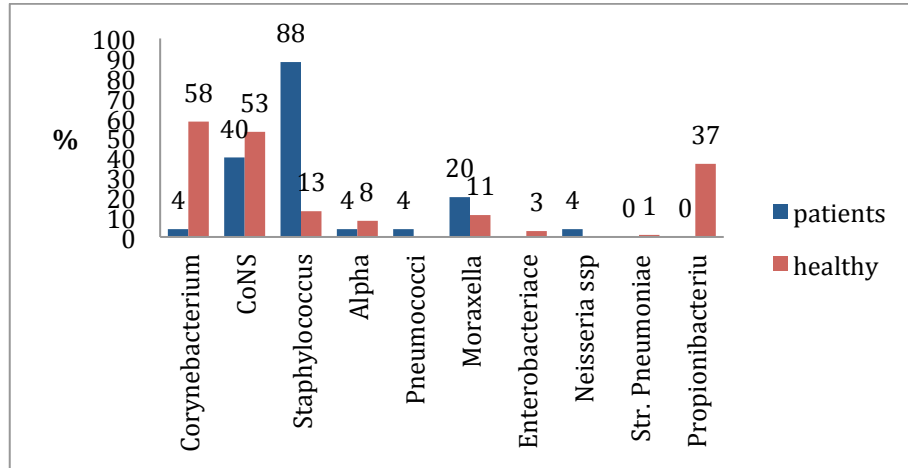


Fig. 5. The bacterial flora in the locus Kisselbachi area in patients with symptomatic septal perforations before treatment, n= 25 (patients) and in healthy individuals, n= 101 (healthy).

The anaerobic *Propionibacterium* could be found in the healthy group, but was not seen in the patient material. The proportion between the different species was also more balanced in the healthy individuals. *S. aureus* could be identified from 13% of the healthy group, whereas, 88% of untreated patients carried the bacterium in the nasal mucosa.

H. pylori was not detected from the mucosal biopsies from the patient group.

Bacterial flora in patients during treatment

There was a significant reduction in the nasal presence of *S. aureus* both after six months and after twelve months compared to the start in the group of patients studied ($p < 0.05$). The microbial flora also became more diversified.

The microbial flora on the obturators differed slightly from that found on the patients mucosa at the end of the test-period (tab. 5). Fungi were found in two obturators, *Candida albicans* and a *Chaetomium* species. One patient, the same whose obturator later contained the fungus in question, had *Candida albicans* in the nasal mucosa surrounding the perforation at

the six-month visit and was treated for that at that time (fig. 6). At the last visit in the study no fungi could be found in the mucosal swab.

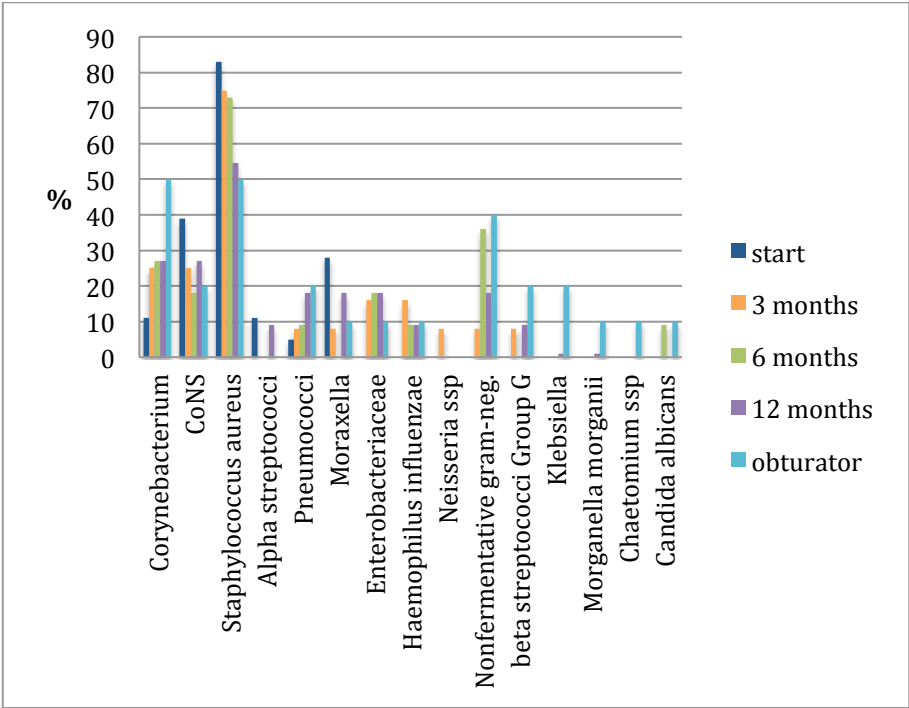


Fig. 6. The microbial flora surrounding symptomatic nasal septal perforations before treatment, n=18 (start), after three (n=12), six (n=11) and twelve months (n=11) of treatment, and on obturators used for twelve months (n=10).

Tab. 5. The distribution of bacteria between patients and their obturators. . 'P' = patient and 'O' = obturator. No 1-10 are the patients the bacterial swabs and obturators refer to. *4, a-n are the different bacterial species and are defined in the footnote at the bottom of the page.

*4	1		2		3		4		5		6		7		8		9		10	
	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O
a	-	x	-	-	x	x	x	-	-	-	-	-	-	-	-	-	x	-	x	
b	-	x	x	-	-	-	x	-	-	-	x	-	-	-	x	-	-	x	-	x
c	x	x	x	-	-	-	-	-	-	-	-	-	x	x	x	x	x	x	x	x
d	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
e	-	-	-	-	x	x	-	-	-	-	x	x	-	-	-	-	-	-	-	-
f	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
g	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	-
h	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
i	x	-	-	x	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	x
j	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	x	-	-	-
k	-	-	x	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
l	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-
m	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-
n	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-

4

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- 4 a) *Corynebacterium*
 b) CoNS
 c) *S. Aureus*
 d) alpha streptococci
 e) Pneumococci
 f) Moraxella
 g) Enterobacteriaceae
 h) *Haemophilus influenzae*
 i) non-fermenting Gram-negative rods
 j) beta streptococci
 k) Klebsiella
 l) Morganella
 m) *Chaetomium* ssp
 n) *Candida albicans*

Scanning Electron Microscopy

The surfaces of the unused obturators were rough (fig. 7). In vivo cultivation of cocci on the two different silicones (Versasil and MED 4930) gave no visible difference in growth (fig. 8). The method of mixing the LSR silicone, however, gave a visible difference under the electron microscope (fig. 9). In SEM a striation after the repetitive folding of the material done with the hand mixing technique was clearly visible also bleeding of unbound silicone oil from the pigment-carrier was visible, drawn out by the vacuum in the microscope. Pigment granules could also be seen in the materials, but speed mixing reduced the number of visible granules (fig. 10). Both cocci and rod-shaped bacteria could be seen growing on the surface of used obturators. Proteins could also be seen as thread-like and popcorn-like structures. In obturators stored longer in fixating fluid, the protein matrix became more visible (Fig. 11).

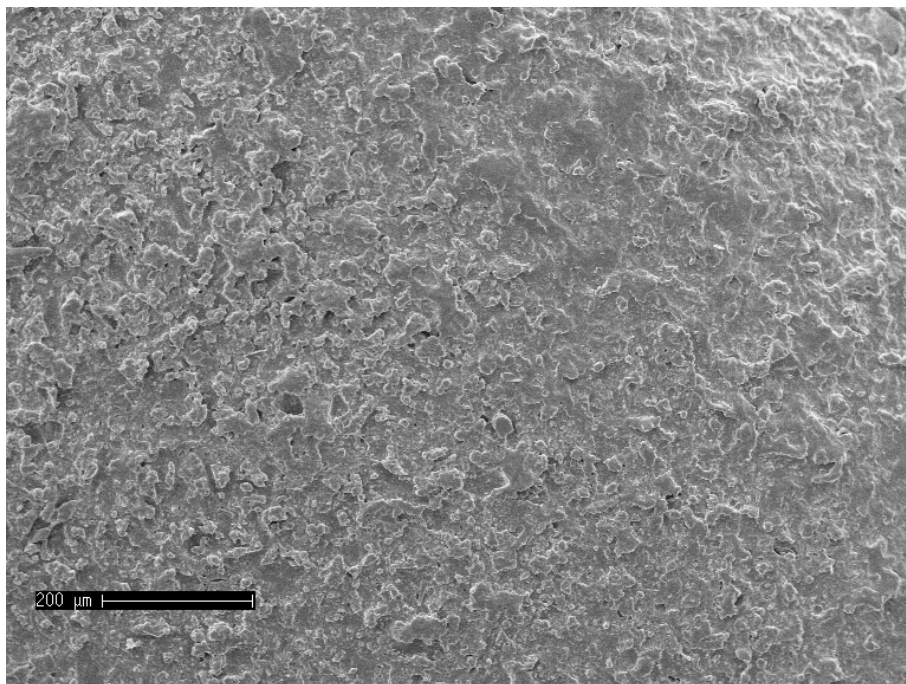


Fig. 7. SEM image of the surface of an unused obturator made of Versasil. Magnification 100.

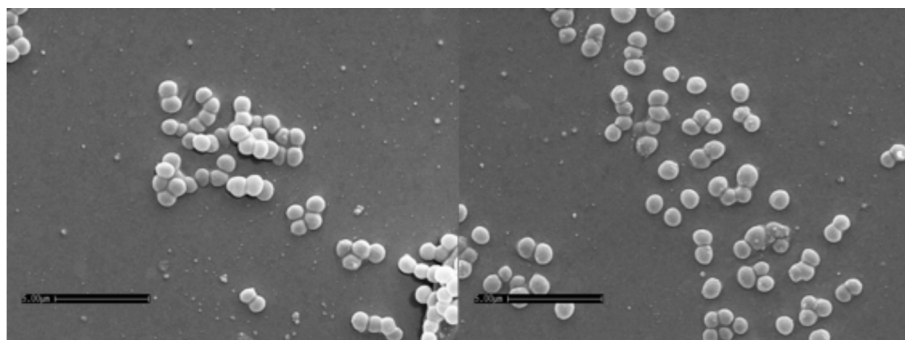


Fig 8 LSR silicone MED 4930 to the left and the milled silicone Versasil to the right. Magnification 5000.

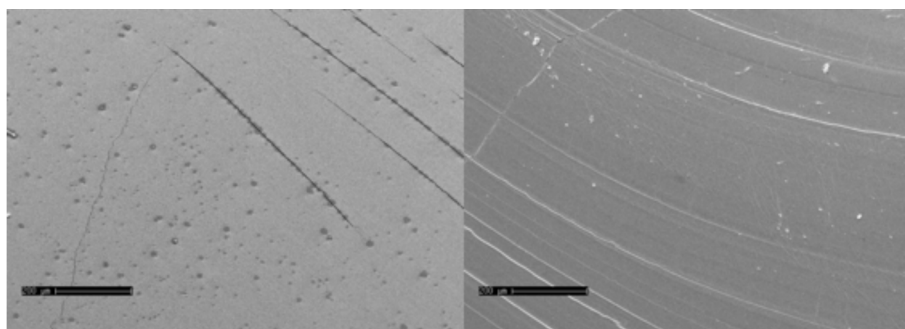


Fig. 9. LSR silicone MED 4930 mixed by hand to the left and mixed in Speedmixer to the right. Magnification 100.

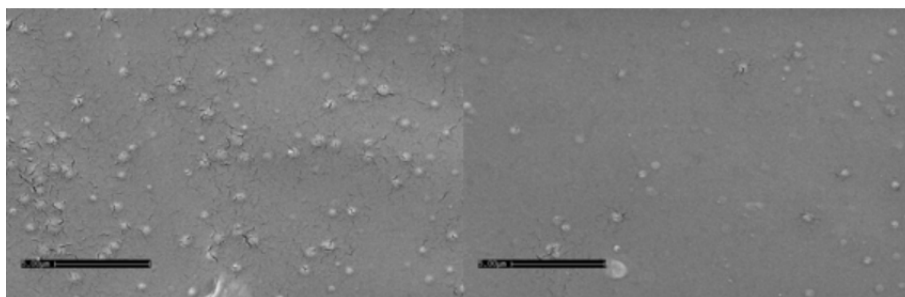


Fig. 10. Pigment granules in Milled silicone to the left and Speed mixed silicone to the right. Magnification 5000.

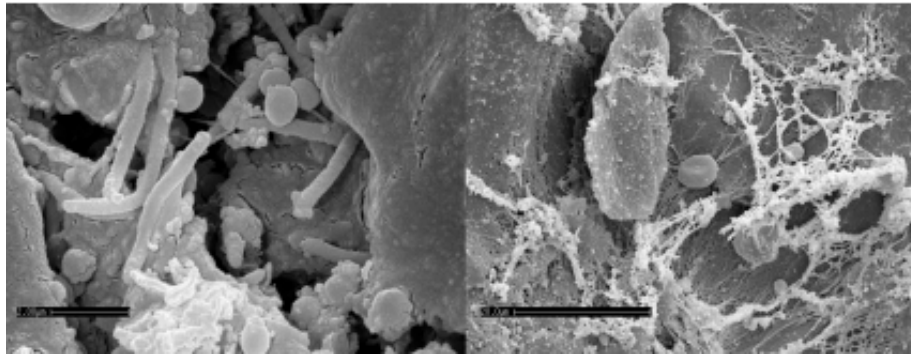


Fig. 11. To the left cocci and rod shaped bacteria can be seen on the surface of a used obturator (x 10,000). The small granule-like structures in the center of the bottom part of the picture are proteins. To the right the protein layer on the surface of a used obturator is more visible. The structure to the left of the erythrocyte is an artefact from the preparation of the specimen. Magnification left picture x 10 000, right picture 1000.

Material testing

There were some sorption and solubility in all materials, although the addition silicones were less affected than the condensation silicones (tab. 6). Surface-free energy was little affected by water sorption in all the materials (tab. 7).

Tab. 6. Mean values and standard deviation (SD) of sorption and solubility for the seven silicone elastomers tested.

Material	Sorption	sd sorption		Solubility	sd solubility
CR	208.44	7.77		13.75	0.66
CH	5.51	0.61		2.83	0.18
RS	57.46	2.13		25.95	4.03
RT	4.34	0.81		1.51	0.30
SE	1.34	0.34		-0.13	6.78
LS	3.47	0.46		1.10	0.26
A2	3.84	0.57		3.50	0.43

Tab. 7. Mean values and (standard deviation, sd) of surface free energy at various stages of water sorption/solubility test of the seven silicone elastomers tested.

Material	Start (sd)	Condition (sd)	3-days (sd)	Saturated (sd)
CR	36.2 (10.6)	25.1 (6.2)	26.6 (2.7)	26.3 (2.7)
CH	26.6 (6.7)	24.9 (3.4)	25.6 (4.4)	25.4 (2.6)
RS	27.4 (2.8)	30.8 (5.8)	32.1 (6.8)	29.4 (4.9)
RT	24.9 (7.7)	19.4 (3.7)	23.9 (3.0)	34.5 (9.4)
SE	16.5 (2.4)	20.2 (4.3)	17.1 (2.9)	21.6 (5.0)
LS	22.7 (5.7)	20.4 (6.4)	20.3 (8.8)	23.0 (5.3)
A2	26.3 (4.4)	20.0 (1.9)	22.1 (1.7)	25.1 (4.1)

Discussion

The normal nasal flora in the locus Kesselbach area resembled the flora reported from the outer nares. Apart from *S. aureus* where the 13% of our healthy group is far from the expected 27% that most authors agree is the most likely carriage rate in the nares in a healthy population (Wertheim et al., 2005). The patient group, however, had a very high yield of *S. aureus*, thus falsifying our hypothesis that the microbial flora of the two investigated groups of individuals would not differ from each other.

This elevated prevalence of *S. aureus* has been reported from patients with Wegener's granulomatosis (WG) and with nasal polyposis (Bernstein et al., 2011; Laudien et al., 2010; Stegeman et al., 1994). The environment in a nose where the septum is perforated is dry, whereas an intact septum provides a humid atmosphere (Lindemann et al., 2001 a and b). In three different ailments with different conditions in the nose nasal septal perforation (dry), nasal polyposis (obstruction and secretion) and WG (obstruction, granulation) an increased presence of *S. aureus* has been seen. A common denominator between these ailments could be a compromised mucosal barrier, thus facilitating for *S. aureus* to settle and proliferate. There is also evidence in the literature suggesting that a defective local immune response to the presence of these bacteria could be responsible for the colonization of *S. aureus* (Wormald and Foreman, 2012).

Helicobacter pylori was not found in our patient material, thus falsifying the hypothesis that this bacterium would be present. The biopsies from the mucosa with granulated tissue were handled in the same manner as stomach biopsies. This seemed at the time to be a suitable method; however, not using PCR is a weakness in our study design.

The microbial flora surrounding the perforation changed during the test-period, thus confirming the hypothesis that this would be seen. *S. aureus* dominance was reduced and twice as many species of bacteria could be seen after twelve months of obturator treatment compared to the untreated microbial flora. It has been shown that a surgical closure of a perforated nasal septum will raise the humidity and temperature in the nose and it is reasonable to assume that also a closure with an obturator would give the same effect (Lindemann et al., 2001 b).

The microbial flora after the end of the treatment period did not fully resemble the flora seen in the healthy test-group. Several of the patients have other diseases exposing them to frequent hospital visits. The obturator itself could also attract some groups of bacteria since biomaterials and implants are associated with infections around biomaterials and implants (Gaynes et al., 2005; Looney et al., 2009; Montanaro et al., 2011; Warren, 2001). As a swab captures a single moment, there might be both transient and resident

bacteria caught in the swab. This is a group of patients that probably are accustomed to use saline and or sesame oil sprays in the nose. Especially *Stenotrophomonas maltophilia* is a bacterium that thrives in humid and aquatic milieus and thus could be a transient from a humidifying or emollient nasal spray (Looney et al., 2009).

There were also differences between the flora found on the mucosa and what could be cultivated directly from the obturators. The surface of the obturators was rough. They are made in moulds made from dental stone, which is a hard gypsum product. Silicone is good at reproducing surfaces and it is also used as impression materials. The surface of the stone moulds are treated with a separation medium, in this case first a medical grade water based Teflon followed by a medical grade high temperature wax (Medimold, Polymed ltd, Cardiff, Wales, UK). This treatment of the stone surface blocks some of the surface roughness, but obviously not all as the SEM image showed a rough silicone surface. A rough surface promotes bacterial adhesion, and the hydrophobic nature of the silicone also attracts microorganisms (Mei et al., 2011; Rodrigues et al., 2006).

The most noteworthy of the microorganisms found on one of the obturators was the fungus *Candida albicans*. This was not seen in the mucosal swab at the corresponding time, i.e. the last swab of the test period, but it was found after six months wearing the obturator. Treatment against the fungus was made at that time, but the same obturator was used the whole test period through. *Candida* infection in close proximity to silicones is a problem since it has been shown that the yeast can grow into the material (Everaert et al., 1998; Taylor et al., 2003).

The SEM images supported the hypothesis that we would see bacteria and proteins on the surface of the silicone obturators. This was expected since the nasal secretion has a composition that resembles the saliva and proteins from the saliva readily adsorbs to surfaces in the oral cavity (Everaert et al., 1998; Vanthanouvong and Roomans, 2004). Furthermore, the SEM also revealed differences between the mixing techniques of the LSR silicone. When mixing together catalyst and base materials of a LSR silicone by hand, a technique where a repeated folding of the material is adopted. After several foldings the material appears to be homogenous to the naked eye, but the SEM revealed a clear striation after the folding, something that was not visible in the material mixed in the Speedmixer™ (Fig. 9). The tested materials were coloured with pigments of iron oxide, the same colouring that the obturators have. This is done for two reasons, so they will blend into the nasal cavity, and for the patient to see them better if dropped. The silicone in itself is more or less colourless. The pigments are suspended in a non-active silicone oil, i.e. the oil will not chemically bond with the silicone base. In fig. 9 it is clearly shown that this pigment carrier is unbound within the silicone

as the vacuum of the SEM drew the unbound oil to the surface of the hand mixed specimen. Both hand mixed and milled silicone exhibited plenty of pigment granules, but speed mixing seemed to reduce the size of at least some of the pigment granules. Crazing of the surface of the silicone directly above the granules could also be seen as something that indicates a chemical reaction (Fig. 9). This needs to be further investigated. The tradition to pigment the silicone should also be reconsidered when colouring is not necessary.

The hypothesis that an increase in wettability would be seen when the silicones had adsorbed water had to be rejected. Some increase in wettability could be seen, but not according to theory. The tested addition silicones had little sorption and solubility, but as some solubility could be seen it is important to use medical grade silicones in appliances that are used on or in human bodies, something that Braley pointed out as early as 1964 (Braley, 2000).

Conclusions

The patients with symptomatic perforations of the nasal septum had a bacterial flora totally dominated by *S. aureus*. The massive presence of *S. aureus* around symptomatic perforations may have an impact on the persistence of the granulated and inflamed tissue present in symptomatic perforations, thus forming a vicious circle with bleeding and crustation.

S. aureus dominance in the mucosa surrounding symptomatic perforations was diminished by using a custom-made obturator. The microbial flora became more diversified with the treatment, although not fully resembling the flora in healthy individuals. The microbial flora of the obturator was similar, but not the same as the mucosal flora. The discovery of *Candida* in the obturator of a patient who had been treated for *Candida* in the nose six months earlier suggests that obturators need to be exchanged when fungal infections are being treated to prevent the fungus from re-infecting the patient at a later stage.

The silicone had a rough surface and a poor wettability, both aspects favouring colonization of microorganisms. The silicone was negatively affected by the colouring pigments, this should be considered when colouring is not necessary. The slight, but existing solubility of silicones emphasises the importance of using medical grade silicones that are more purified than industrial silicones.

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