Biofilm in urinary catheters - impacts on health care and methods for quantification

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Biofilm i urinkatetrar – inverkan på sjukvård och metoder för kvantifiering

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Abstract

Biofilm is an increasing problem in the healthcare and have in urinary catheters long been associated with nosocomial urinary tract infections. The infections caused in 2002 alone 13,000 deaths in the US and annual costs have been estimated to over $400 million. These costs are however most likely underestimated. The analysis of biofilm is important to aid the work on increasing patient safety and reducing the financial implications. A literature study was conducted in order to recommend a method for quantification that was fast, accurate and versatile. Methods used for biofilm quantification are primarily based upon light absorption, light scattering and changes in impedance. A few methods utilizing these properties are spectrophotometry, flow cytometry and coulter counters. Samples of biofilm are usually collected via traditional scraping with a sterile blade or with sonication (ultrasound). Flow cytometry was considered the superior method for quantification along with sonication for sample collection. The survey therefore came to the conclusion that biofilm sample collection should be done with sonication and analysis with flow cytometry.

Keywords: Biofilm, methods for quantification, urinary catheters, catheter associated urinary tract infections.
Sammanfattning


Nyckelord: Biofilm, metoder för kvantifiering, urinkatetrar, kateterrelaterade urinvägs-infektioner.
Nomenclature

CAUTI - Catheter Associated Urinary Tract Infection
UTI - Urinary Tract Infection
ICU - Intensive Care Unit
HAI - Hospital Acquired Infection
NMR - Nuclear Magnetic Resonance
CLSM - Confocal Laser Scanning Microscopy
ASTM - American Society for Testing and Materials
EIS - Electrochemical Impedance Spectroscopy
EPS - Extracellular Polymeric Substances
Preface

We would like to thank professor Jan van der Linden who has introduced this project to us and helped us with the work along the way, providing good feedback and keeping the enthusiasm high. We would also like to thank Mikael Charlés and Anders Hildeman at Observe Medical for their support, the medical student Anton Eklund and also professor Ute Römling.

This has been a fun and interesting project with many different tasks and problems, giving good insight in the modern medical care and closer collaboration with known medical companies. Together this has given us a greater understanding for the role of a medical engineer.

Edvin Kalmaru & Gustaf Lönn
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1. Introduction

Biofilm is a phenomena where a group of microorganisms creates a thin film of extracellular matrix in which they interact and communicate to create an optimum living environment. The non planktonic state makes them more resistant against both antibiotics and the hosts own defenses as well as mechanical forces. In health care, this creates major problems where it is believed that biofilm forming bacteria account for up to 80% of all bacterial infections (Römling, 2012).

Biofilm occurs after a sufficiently long time in all types of catheters in health care, and infection due to catheters are common. Today the problem is avoided by replacing the catheters within determined time intervals so that the expansion of the biofilm is limited. This type of preventive method is both labour intensive and require large amounts of disposable catheter products. Combined, this greatly affects the financial situation of the hospitals as well as the discomfort among patients (Foxman, 2002, Klevens et al., 2007, Gould et al., 2010). There are several methods capable of quantifying biofilm but none of these are non invasive and also require sample collection. Common methods is flow cytometry, coulter counter and impedance spectroscopy.

This work has been produced with close cooperation with Karolinska Institutet and Observe Medical AB, a company in the process of developing a new type of product used for urine measurements called Sippi. It is believed, however not proven, that Sippi can prevent and detect biofilm build up non-invasively.

The objective of this report was divided into three parts:

1) Survey biofilm occurrence in urinary catheters and its impact on health care.
2) How biofilm is/can be quantified.
3) To provide a basis for evaluation of Observe Medical's new method to measure and prevent biofilm in urinary catheters.

Hopefully the report will lead to a more qualitative and safe health care and reduce the complications related to biofilm in urinary catheters.

The report was primarily based upon studies on the U.S. health care and should therefor be seen as a limitation. Advanced microbiology of biofilm was also not considered to be in the scope of this report.
2. Background

2.1 Urinary Catheters

Urinary catheters are tubes used to drain the bladder from urine by being placed in the body. Evidence shows that catheterization was done in Egypt 3000 B.C. (Mueller and Sanborn, 1995). The early catheters were made out of natural materials such as wood and rolled up leaves. During the 18th century a new type of elastic catheter was introduced by Pierre-Joseph Desault, but it still had problems with its flexibility (Carr, 2000). Since then numerous scientist have developed different kinds of catheters and during the mid 20th century Dr. Frederick E. B. Foley designed the precursor of the indwelling urethral catheters used today (Carr, 2000).

Today, there are several different types of catheters made by various materials and sizes. The catheters are designed to be indwelling in the bladder to provide constant access, or be inserted intermittently when drainage is desired (MedlinePlus, 2011).

Indwelling Catheter are inserted either through the urethra or surgically through the abdominal wall, the latter referred to as suprapubic catheters (Gould et al., 2010, MedlinePlus, 2012). The catheter is kept in place by a balloon close to the catheter tip, which, when the catheter is positioned, is inflated with water. The indwelling catheter is either plugged on the outside to allow for intermittent bladder drainage or connected to a container outside the body where urine is collected. Indwelling catheters are mostly used for patients with urine leakage or patients dealing with problems to urinate (Gould et al., 2010, MedlinePlus, 2012).

Unlike the Indwelling catheter, the Condom Catheter is not placed inside the body. The Condom Catheter is a device made for men and as the name suggest it is similar to a condom. The appliance is rolled on the penis and connected to a tube that drains the bladder and like the Indwelling catheter, the urine is stored in a bag. The catheter needs to be replaced continuously every 48 hour (maximum) to minimize the risk of bacterial growth (Vitality-Medical).

The intermittent catheter is comparatively simple and can be inserted by the patient and the design varies according to the users gender and age (Porteous, 1986). Newman (2011) states that the intermittent catheter is the favorable choice for patient with bladder dysfunction and long term bladder emptying although it does not come without any risk for urinary tract infections or bleeding. There are hydrophilic PVC coated catheters which are created to be more smoothly inserted without external lubricant as well as non-coated catheters that have the ability to be reused (Newman and Willson, 2011). According to Newman (2011) the uncoated catheter is used most frequent due to its lower cost although the coated catheter seems to lead to less risk of urethral inflammation and irritation.
2.2 Biofilm

2.2.1 Definition and Properties
A biofilm is a surface on which microbial cells reside surrounded by their excreted extracellular matrix. This mainly consists of polysaccharides, but depending on the environment the extracellular substance may also consist of non-cellular material such as mineral crystals or blood components. In total, the extracellular matrix may contain as much as 50-90% of the organic carbon in the biofilm (Donlan, 2002). The biofilm is closely attached to the surface and the extracellular matrix hinders the process of removal (Donlan, 2002).

Bacteria living in biofilm experience advantages over their planktonic (freely suspended) counterparts. Their metabolic and reproduction rate is lower but their resistance against mechanical force and antimicrobials is increased substantially (Römling, 2012). According to Römling (2012) bacteria in biofilm can be as much as 1000 times less sensitive to antibiotics and also very troublesome for the human immune system to handle, especially in urinary catheters as the inside surface of the catheter cannot be accessed by the human immune system. Many types of antibiotics are capable of penetrating mature biofilms fully, inferring that the slower growth rate of bacteria in biofilm is probably one of the dominating factors regarding their increased antibiotic resistance (Trautner and Darouiche, 2004).

Transfer of extrachromosomal DNA/ plasmids occurs at a grater rate in biofilm compared with the planktonic state. This increased rate of gene transfer between the bacteria in biofilm is also closely related to antibiotic resistance and the biofilm is often regarded as a growing ground for multi-resistant bacteria (Gould et al., 2010, Trautner and Darouiche, 2004). To further improve the environment in the biofilm gene expression is regulated and attached bacteria are assigned to different tasks; such as production of extracellular matrix (Römling, 2012).

2.2.2 Occurrence
Biofilm exists on almost every surface both in and outside healthcare. Common areas of occurrence are prosthetics, respiratory tubes and of course urinary catheters. The formation of biofilm is unavoidable despite todays advanced routines for hygiene and disinfection.

In urinary catheters, the first step in biofilm formation is the deposition of a film consisting of the hosts urinary components which includes proteins, electrolytes and other organic molecules (Donlan, 2002, Trautner and Darouiche, 2004). This may neutralize anti-adhesive properties of the catheter. Planktonic bacteria, reaching the catheter via both the intra- and extraluminal route, are then able to attach to the surface via electrostatic and hydrophobic interactions and with the use of flagella (Donlan, 2002, Trautner and Darouiche, 2004). As the catheter does not have any defense system of its own, the growth of the biofilm may progress rapidly.
2.3 Urinary Tract Infection

2.3.1 Definition and Incidence
Urinary tract infections (UTIs) are one of the most common bacterial infections. UTI is commonly defined as a presence of pathogens in the urinary tract. The severity of the infection "ranges from mild self-limiting to life-threatening systemic disease" (Chiu, 2013, p.1). The infection is often treatable with antibiotics, however the extensive use of antimicrobials has increased the number of infections caused by resistant organisms (Chiu, 2013).

Of all the hospital acquired infections (HAIs), UTIs is the largest group accounting for more than 30% (Klevens et al., 2007). The majority of these infections are caused by insertion of instruments in the urinary tract, such as catheters. The prevalence of catheter associated urinary tract infections (CAUTIs) vary between 3.1 to 7.5 CAUTIs per 1000 catheter days with the highest rates among patients in burn intensive care units (ICUs) (Gould et al., 2010). Overall it is estimated that between 60-80% of all bacterial infections are related to some form of biofilm forming bacteria (Römling, 2012).

CAUTIs are also associated to nosocomial bloodstream infections and Gould et al. (2010, p.23) states that "CAUTI is the leading cause of secondary nosocomial bloodstream infections; about 17% of hospital-acquired bacteriemias are from a urinary source, with an associated mortality of approximately 10%. In the nursing home netting, bacteriemias are most commonly caused by UTIs, the majority of which are catheter-related."

Compared with other HAIs in general the CAUTIs have a low morbidity and mortality, but the high prevalence of urinary catheters leads to a high incidence of infections. A survey by Klevens et al. (2007) of U.S. hospitals in 2002 revealed that UTIs where the largest group of HAIs with more than 560,000 infections reported. The estimated deaths related to the UTI where 13,000 corresponding to a mortality rate of 2.3% (Klevens et al., 2007, Gould et al., 2010).

The formation of biofilm inside urinary catheters occurs over time and poses a great challenge to the health care. Pathogens living inside the biofilm are not only more resistant to the hosts own defenses but also to a wide spectrum of antimicrobials. The antimicrobial resistance among the actual pathogens related to CAUTIs is an increasing problem. A quarter of all the isolated E.coli and one third of the isolated P. aeruginosa from CAUTI samples provided by U.S. hospitals have been reported to be resistant to fluoroquinolone (Gould et al., 2010). The multi-drug resistance (non susceptible to all antibiotics in four classes) among the pathogens causing UTI is also a growing issue, with rates up to 21% in some species (Gould et al., 2010). This makes the biofilm almost impossible to eradicate without removing the catheter and causes unnecessary use of antimicrobials (Gould et al., 2010).
2.3.2 Role of Biofilm in Pathogenesis of CAUTI
The use of a urinary catheter provides a surface of foreign material from the outside world to the normally sterile bladder. This surface will inevitably become colonized with microorganisms and pooled urine in the catheter or bladder will encourage bacterial reproduction (Trautner and Darouiche, 2004).

Since biofilm is the predominant mode of growth in aquatic ecosystems, and together with the susceptibility of the inert catheter material to microbial colonization, biofilm plays a central role in the pathogenesis of CAUTI. According to the Catheter guideline (Gould et al., 2010), more detailed knowledge of the pathogenesis is unknown and the report urges the need for further research in the area. Also, the detachment of bacteria from the biofilm will result in pathogens in the urine (bacteriuria) (Trautner and Darouiche, 2004).

2.3.3 Financial Implications
The financial impacts of nosocomial urinary tract infections, caused mainly by catheterization and the formation of biofilm, is substantial. A study by Saint (2000) estimated the increased costs at the University of Michigan Health System by hospital-acquired symptomatic UTI to a minimum of $676 per infection and increased the length of stay by one to two days. Urinary catheter related nosocomial bacteremia (bacteria in the blood) is estimated to cost $2836 per treatment and causes in average 17 days longer hospital stay. However these costs are most likely to be underestimated (Saint, 2000). In a later report, Foxman (2002) calculated the financial implications of nosocomial UTI in the US to an estimated annual cost of $424-451 million with UTI related bacteremia excluded.
3. Method

The primary method for the background research, and enquiry on how to measure biofilm, was a literature study consisting of scientific articles and papers from various databases accessed from KTH library search engine *KTH Primo*. The articles were mainly downloaded in an electronic format from databases and printed if found necessary. Content from multiple articles regarding the same subjects were compared to ensure good scientific quality. Used search words were; biofilm*, catheters*, spectrophotometry*, flow cytometry*, coulter counter*, confocal laser scanning microscope*, sonication*, financial implications*, urine, UTI, applications, bacteria, pathogenesis, quantification, cell count, matrix, impedance spectroscopy.

The different techniques for quantification of biofilm where evaluated primarily upon these criteria to provide an adequate conclusion.

- **Speed**; Quick analysis where rated higher compared to slow.
- **Accuracy**; High accuracy where rated higher compared to poor.
- **Versatility**; Higher ratings for methods with capabilities of measuring more than one parameter.
- **Availability**; Higher ratings for commercially available methods.
- **Human labour intensity**; Highly automated methods yielded higher rating.

*Sippi* and its current issues were evaluated according to information provided by *Observe Medical*. Using the extensive background research and product information, suggestions for evaluation experiments and improvements were presented.

A close collaboration and contact was kept with project supervisor Jan van der Linden and medical student Anton Eklund as well as with Mikael Charlés at *Observe Medical Nordic AB*. The contact has mainly been through phone calls, email and meetings, in which the previous work and future plans for the project have been discussed and evaluated. Contact was also kept with Ute Römling, expert on biofilm and professor at the Institution of microbiology at *Karolinska Institutet*, to provide additional feedback along the process.
4. Result

The following result was obtained and divided into sample collection and measuring methods of biofilm.

4.1 Sample collecting methods

4.1.1 Scraping
Scraping is a method to collect samples of biofilm. The method involves the use of a sharp sterile blade to scrape parts of the biofilm from a surface. Scraped samples are collected in a vessel for further examination and counting, using for example one of the methods mentioned below (Bjerkan et al., 2009).

4.1.2 Sonication
Sonication is a common method used for retrieving and dissolving biofilm. The method has been proven by Bjerkan et al. (2009) to be more effective than traditional scraping on metal surfaces and the basic idea is to use ultrasound waves with frequencies from 20kHz to 10MHz to rattle particles, for example bacteria in a solution or biofilm.

The ultrasound transducer transforms electrical energy to mechanical waves by the piezoelectric effect. The high frequency waves create a phenomenon called inertial cavitation, causing small expanding gas bubbles in the solution which erupt and generate small shockwaves. These shockwaves separate the cells in the sample, creating a homogenous solution (Suslick, 1990). At lower ultrasound frequencies the acoustic cycle is longer, giving the cavitation bubbles longer time to form and allows them to collapse with greater energy and vice versa (Joyce et al., 2011). That is one reason why lower frequencies tend to reduce the viability more among the bacteria compared with high frequencies at the same acoustic intensity (Joyce et al., 2011). When the sample has been sonicated and fully dissolved, the idea is to count the cells and in that way quantify the biofilm (Jost et al., 2014). Cell counting can be done using various methods, for example a hemocytometer, spectrophotometry, coulter counter and/or flow cytometry.
4.2 Measuring methods

There is still a lot to learn about biofilm (Bakke et al., 2001), how it engenders and progresses, and how to measure the biofilm with a method that is accurate, fast and non-labour intensive. One way to monitor the occurrence and proliferation of biofilm is via optical instruments where by the risk to affect the sample is reduced (Bakke et al., 2001). Another way could be by dividing the sample and count the individual cells by using different kinds of automated methods (Jost et al., 2014). Today there is no proven non invasive method for biofilm quantification in urinary catheters.

4.2.1 Optical measurement

Bakke et al. (2001) designed an experiment where they measured the optical density, thickness and biomass of a biofilm (Bakke et al., 2001). The optical measurement uses the light absorption properties and the fact that it is proportional to bacterial concentration. Light is transmitted from a probe and passes through the sample containing biofilm. While passing through the sample, light scatters, gets absorbed and shifts in wavelength due to the sample characteristic. This information is processed and used to determine the concentration of the biofilm and its optical density. The volumetric densities of the cells are settled by obtaining the thickness and mass of the biofilm. The thickness of biofilm is determined by looking at the sample with a microscope. Thereafter samples of the biofilm are collected and put in formaldehyde solution and the biofilm cell carbon and biomass can be calculated (Bakke et al., 2001).

4.2.2 Impedance Spectroscopy

Another approach to monitor biofilm is with the help of impedance using the fact that biofilm work as electrical insulator (Munoz-Berbel et al., 2006). One method, created by Munoz-Berbel et al. (2006) used this property by letting biofilm grow on a electrode chip. The chip was immersed in a vessel containing the bacteria Pseudomonas aeruginosa and the growth of biofilm affected the chips impedance. These properties were recorded and gave information about the biofilms formation process. Another independent yet similar experiment made by Leonardo Pires (2013) also used impedance to monitor biofilms growth. Pires used two sets of electrochemical impedance spectroscopy (EIS) electrodes; one set that was used as a reference and the other with applied biofilm. Just as in Munoz-Berbels experiment, the biofilm altered the electrodes surface impedance and these values were compared with the reference values. Pires also used amperometric sensors to observe the biofilms respiratory activity and combined these two measurements to monitor the biofilms growth.
4.2.3 CLSM and NMR
Garny et al. (2010) combined confocal laser scanning microscopy (CLSM) and nuclear magnetic resonance spectroscopy (NMR) to get information about the biofilms composition and dynamics. With the CLSM Garny got high resolution images of the biofilm using laser with wavelength around 500 nm and from which a 3D visualization was created. Samples of the biofilm are then put in a Nuclear Magnetic Resonance (NMR) spectrometer and T1-pictures are obtained with the nuclear magnetic resonance spectra of the biofilm.

4.2.6 Hemocytometer
The hemocytometer is a slide with a marked grid where a cell culture is placed and the cells are then counted manually with the help of the grid using a microscope (AmritaUniversity, 2014).

4.2.7 Spectrophotometry
In spectrophotometry the sample’s light absorbing characteristic is used, called turbid. A power source emits light with a predefined wavelength using a monochromator and a small slit. The filtered light passes the sample and is detected. With Lambert-Beers law and the information about the samples turbid the specimens cell concentration is calculated. There are two different categories of spectrophotometers, Single Beam Spectrophotometer and Double Beam Spectrometers operating with wavelength between 185 - 400 nm (UV-spectrophotometer) and 700-1500 nm (IR-spectrophotometer). In the Single Beam Spectrophotometer all of the light passes through the specimen compared with the Double Beam in which one beam is used as reference value. The drawback of spectrophotometry is the fact that the method does not count the cells directly but uses the change in the lights wavelength, however it is widely used method due to its speed and since it is quite inexpensive. (Kamentsky et al., 1965, Vo, 2014)

4.2.8 Coulter Counter Principle
The coulter counter is a sensing system used to count and quantize particle mass with impedance measurements. It is effective on particles between 0,4µm to 1600µm and can be applied on different types of cells and abiotic particles such as minerals, metals etc. The method is also quick and modern machines have the capacity to count and size up to 10,000 p/sec with good accuracy (Graham, 2003, Coulter, 2014).

The particles to be measured are suspended in a low concentration electrolyte and focused by dielectrophoretic forces through a narrow channel often known as the ”sensing zone” (Wu et al., 2010). The sensing zone basically consists of two electrodes separated by the electrolyte medium. In the presence of a constant electrical field between the electrodes, the particles passing through briefly change the conductivity (proportionally to their volume) which is then measured as a voltage or current pulse. If the particle density (such as in cells) is assumed to be constant, the signal is also proportional to particle mass (Graham, 2003, Coulter, 2014).

To obtain optimal accuracy, the size of the aperture is chosen with respect to the particles measured. A 30µm channel may for example measure particles from 0,6µm to 18µm in size, with the lower limit restricted by electronic noise. If the particle size is spread over a wider range, the method allows the use of two or more ”sensing zones” and by combining the results, a better size distribution of the particles is provided (Graham, 2003, Coulter, 2014).
When working with cells, coulter counters require a rather large sample volume (>5ml) and high particle concentration (>10^4/ml) (Wu et al., 2010). However, too large concentrations may give an increase in "double coincidences", when two particles are detected as one. Although the coulter counter is an effective method to quantify volume and size, it can not discriminate between two different type of particles with the same volume and conductive properties. For instance it cannot separate two different species of bacteria as long as they have the same volume. In a recent study (Zhang et al., 2013), sophisticated coulter counters are used in a method to measure cell count and biomass as well as the extracellular polymer distribution in detached biofilms. The results were validated by direct counts and crystal violet staining assay and showed that the coulter counter was an effective method to measure the contents in biofilm (Zhang et al., 2013).


4.2.9 Flow Cytometry
The flow cytometer uses the light scattering properties of cells to quantify their size, inner structure and concentration. A liquid solution containing cells or particles are forced one by one through the cytometer with the help of hydrodynamic focusing. The cells passing through the cytometer are irradiated with monochromatic light and the forward scatter, proportional to the cell size, is detected by a photodetector. Scatter is transferred to a voltage pulse and the magnitude of the signal acquired from the detector is also proportional to cell size, with larger cells yielding a higher voltage. The basic setup can measure both total cell count and size effectively (Givan, 2001, Jahan-Tigh, Ryan et al. 2012, TheFlowCytometryNetwork, 2011).

The granularity and inner structural complexity of the cells scatter the light to the sides and adding an extra side detector allows these properties to be measured and used for additional sorting. All particles, both biotic and abiotic, scatters light as they pass through the laser beam. Separating different particles with similar scatter properties is therefore difficult in the basic setup. To differentiate particles, the flow cytometer can use various fluorescence substances, often attached to cells using different antibodies. The fluorescence is excited by the laser and emits light with wavelengths characteristic for the substance and the energy of the laser. Using different filters and mirrors the light is directed to a photodetector with sensitivity corresponding to a certain wavelength (Givan, 2001, Jahan-Tigh, Ryan et al. 2012, TheFlowCytometryNetwork, 2011).

The flow cytometer is a multi-parameter analysis method with high speed and the ability to detect single cells with high accuracy. Accuracy is highly dependent on the desired speed of the analysis and higher speed results in a lower accuracy. Accuracy of >99% can be achieved using sufficiently low speed (Givan, 2001). However the method involves complex computer required analysis of the data obtained and is also constricted to liquid samples only (Wang et al., 2010, Jahan-Tigh, Ryan et al. 2012, TheFlowCytometryNetwork, 2011).
4.2.10 Observe Medical

In their urine output measurement system, *Sippi*, *Observe Medical* uses a new automated electronic method to measure the flow of urine in a catheter. Urine from the patient fills a small plastic reservoir and the capacitive *Sippsense* sensor continuously measures the urine level.

Early in the development of *Sippi* it was found that the signal from *Sippsense* degenerated with time. *Observe Medical* concluded that this phenomenon could be due to a film build up in the measurement chamber formed after repeated depletions of urine. Biofilm has been shown to reduce capacitance in experiments (Kim et al., 2011), and the signal degeneration in *Sippi* is believed to be related to biofilm growth. However, it has not yet been shown that the *Sippsense* actually measures biofilm or if the signal degeneration is due to another phenomena. As the signals lower threshold is reached, the system notifies medical staff and encourages them to replace the disposable unit and thereby theoretically prevents UTIs.

To reduce signal degeneration a small pill containing *Sippcoat*, a silicone-based oil, has been added to the measurement chamber. When urine enters the chamber the pill is dissolved and its content spread on the chambers inside surface. The addition of *Sippcoat* lessened the signal degradation, but whether it reduces any biofilm buildup has not yet been proven. The lessened signal degeneration enables the biosensor to acquire more accurate readings on the patient’s flow of urine as well as increasing the service lifetime of the product (ObserveMedical, 2013). Data from compared runs using the same urine along with data showing individual patient differences in signal degeneration (Hildeman, 2013) is presented in appendix A.

Figure 1 shows a setup of two *Sippi* from *Observe Medical’s* laboratory in Gothenborg (Kalmaru, 2014).
5. Discussion

The report indicated that buildup of biofilm in urinary catheters is a pressing concern due to its relation to UTIs and great cost of these infections. Removal and quantification of biofilm is essential to future research and patient safety and there are several methods available for invasive biofilm quantification. However a proven non-invasive method is requested.

5.1 Evaluation of methods for quantification

The criterion used to evaluate the different techniques were chosen to deliver the most suitable method for quantification. Speed, versatility, availability and labour intensity were mainly chosen in order to minimize the economic costs as a fast, versatile and highly available method could save precious time and be of greater use. High accuracy was needed to produce the qualitative measurements required for scientific analysis. The actual cost of acquiring the equipment was not taken in consideration during the evaluation.

Bjerkan et al. (2009) stated that sonication gave a sample with higher number of profound bacteria compared with scraping. The method can also easily be automated so that the efficiency can be kept high and remain non labour intensive. Sonication could also be quite conveniently performed straight into Observe Medicals product *Sippi* with some minor tuning of the products container by mounting a socket for the probe. As the container is divided into different chambers, sonication may have to be conducted several times. An alternative is to lower the whole container in a sonication bath, however this may reduce the effectiveness of the ultrasound on the inside surface.

The choice of ultrasound frequencies in sonication affect the viability of the cells in the biofilm. If the viability is crucial, higher frequencies are recommended. However, many bacteria found in urinary catheter biofilms are pathogens and should be treated with caution in order to reduce the spread of infections. Using a lower ultrasound frequency will reduce the risk of samples spreading infections.

The aim was to find a method to count the cells that was accurate, versatile, quick but at the same time not too complicated and labour intensive. The optical method might give accurate numbers but it involves too many steps along the way. Using impedance spectroscopy as Munoz-Berbel and Pires is too time-consuming but might work in the future after it has been more developed. Counting the cells with hemocytometer was deemed too labour intensive. The preferred alternatives seemed to be the coulter counter and the flow cytometer: both which are more available than CLSM and NMR and are more versatile and accurate than the spectrophotometer that measures the samples light characteristic and not the individual cells.
The coulter counter is fast and can accurately count and size particles in a wide range. It is also not as complicated, and thus, expensive as the flow cytometer. The method is well-proven and is considered as a standard in automated cell counting, sizing and sorting with a standardized test method published by the ASTM. As the method is based upon impedance measurements it can only distinguish the difference in size/volume, hence limiting the versatility of the analysis. However if the main interest is not primarily the bacteria, the coulter counter would be the most preferable method as it has been proven to quantify both cells as well as extracellular polymeric substances (EPS) and aggregates in detached biofilm.

The flow cytometer offers a fast, accurate and more versatile analysis compared to the coulter counter. This means that not only cell count and cell size could be measured but also the cell structure and receptiveness to different antibodies, which could be used for species determination. Although the method requires more sample preparation and involves complicated data analysis, the increased amount of measurement data acquired makes the flow cytometer a superior choice for fast identification and quantification of bacteria in biofilm.

5.2 Limitations
This report focuses on the bacteria when it comes to quantification of biofilm and the methods presented have been evaluated accordingly. Presented data related to urinary tract infections, antimicrobial resistance and financial implications are acquired from studies in the U.S. health care system. However this was not considered a drawback since the U.S. healthcare system is one of the largest in the world and the amount of previous work available was vast. Many of its institutions are also setting world wide standards in health care and life science. The work is also limited to a literature study and the theoretical findings need to be evaluated with physical experiments.

5.3 Further work
This work should be continued with further studies about the properties of biofilm followed by an evaluation experiment using sonication and flow cytometry, to quantify the bacteria in dissolved biofilm. A suitable method for benchmarking would be the hemocytometer.

5.4 Observe Medical
The non-invasive Sippsense method for biofilm quantification developed by Observe Medical needs thorough evaluation to prove its effectiveness and whether it actually quantifies biofilm or not. A first step could be to conduct a small study consisting of only a few Sippi units draining the same urine. The signal degeneration would be allowed to proceed to certain levels whereafter quantification of the units surface content may detect any correlation between the amount of biofilm present and signal degeneration.

An initial evaluation of the effectiveness of Sippcoat could be done by pairing several Sippi units two and two (with and without Sippcoat) and draining the same urine. The signal in the units without Sippcoat should be allowed to degenerate fully to maximize potential biofilm buildup before quantification of the paired units surface contents is performed. Fewer cells in the units treated with Sippcoat may indicate that the Sippcoat has biofilm reducing properties and that more thorough experiments are needed for further evaluation.
Needed is also a more profound study about the product *Sippi* and how it can be developed to give a more precise reading on the assumed biofilm growth rate. As the performance of the current biosensor may be sufficient to provide such accurate readings needed, only development of additional software would be necessary.

Another suggestion for further research is if it is possible to dissolve urinary components or filter urine before it enters the measurement chamber in *Sippi*. This might eliminate the problem of other substances affecting the signal such as bilirubin or residues from patients suffering from hemolysis.

Data obtained from *Observe Medical* showed that there are big individual differences between patients regarding the *Sippi* service lifetime. Unfortunately no record of these patients clinical condition was kept. A proposal for further studies is therefore too investigate whether the rate of biofilm buildup in *Sippi* can be related to the clinical condition of the patients being catheterized. A first step could be to compare the biofilm buildup between patients in the ICUs at *Karolinska Sjukhuset Solna*, as many of the patients are catheterized. It would also be interesting to compare biofilm buildup among patients with weaker immune system, such as elderly in retirement homes. The weaker defense may allow biofilm growth to progress more rapidly and thus generating a more rapid acquisition of data.

### 5.5 Conclusion

Biofilm in urinary catheters is today unavoidable and threatens patient safety and affects hospital finances. Quantification of biofilm is crucial for further research and several invasive methods are able to measure biofilm. *Observe Medicals* non-invasive biosensor has not yet been shown to measure biofilm and additional studies are needed. Considering the findings in this report, the use of sonication and flow cytometry is suggested for use in further studies.
6. References


Appendix A

Compared runs using the same urine. The purple line shows degeneration of the signal (Hildeman, 2013).

With *Sippcoat*:

![Graph showing degradation with Sippcoat](image1)

Without *Sippcoat*:

![Graph showing degradation without Sippcoat](image2)
Graphs showing individual differences (Hildeman, 2013):