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Comparison of performances of flexible sensors on foil and paper for efficient bacterial concentration measurement

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Comparison performances of flexible sensors on foil and paper for efficient bacterial concentration measurement

Abstract

Purpose – The purpose of this study was to develop flexible sensors for detection of different concentrations of bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in saline.

Design/methodology/approach – The sensors were fabricated using ink-jet printing technology and they consist of a pair of silver interdigitated electrodes printed on mechanically flexible substrates – foil and paper. In house measurement setup for testing and characterization of sensors has been developed. Structural, electrical and mechanical properties of flexible sensors have been compared.

Findings – The characteristics of sensor - the resonant frequency as a function of different concentrations of each bacteria are presented. The obtained results demonstrate different resonant frequencies for each dilution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in physiological saline.

Research limitations/implications – Both sensors showed accurate measurements of bacterial count, which can be achieved with detection of resonant frequency, and this is reflective of the number of bacterial cells within a sample.

Practical implications – The findings suggest that the newly developed method based on measuring resonant frequency, correspond well with bacterial cell count, thus, establishing a new proof-of-concept that such method can have significant applications in bacterial cell counting that are economic and easily maintained.

Social implications – Fast, cost-effective, accurate and non-invasive method for detection of different bacteria from saline.

What is original/value of paper – For the first time, comparison between performances of flexible sensors on foil and paper for bacteria detection is demonstrated. Almost linear dependence between shift of resonant frequency of developed sensors and concentration of bacteria has been obtained.

Keywords Flexible sensor, Kapton film, Paper, Nanoindentation, Bacteria concentration, Resonanat frequency

Paper type Research paper

1. Introduction

Bacterial infections represent a serious threat to human and one of the leading causes of death globally (Bellitti *et al.*, 2017, Sheybani and Shukla, 2016). Such infections can be water-borne, food-borne, air-borne, and environmentally borne or originate from surgical interventions, which were not completely sterile. Antibiotic resistance is a great problem in fighting against bacteria. One of the most important factors for effective antibacterial treatment is fast detection of the type of bacteria as well as its concentration. Two most common bacteria are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Pseudomonas aeruginosa* is a widely present in humans and highly resistant to antimicrobial drugs. It is Gram-negative, asporogen bacilli, strictly aerobic bacteria. *Pseudomonas aeruginosa* has become an important cause of Gram-negative infection (Sismaet *et al.*, 2017), and it is one of the most common pathogen isolated from patients who have been hospitalized longer than one week. *Staphylococcus aureus* (*S. aureus*) is like all staphylococci, Gram-positive bacteria of ball-shaped forms and build flocks, clusters, gatherings. Classical methods for bacteria detection such as polymerase chain reaction (PCR) (Cheng *et al.*, 2006), bacterial culture (Böcher *et al.*, 2008), or combination of PCR and cultivation techniques (Velusamy *et al.*, 2010) are time consuming, expensive and require trained and well-educated staff. Therefore, there is an increasing need for fast and reliable methods for detection of the most common bacteria. Alternative approach to above-mentioned traditional methods is development of various biosensors which do not require special sample preparation and which are cost-efficient and sensitive. They can implement different operational principles such as: optical (Cooper, 2002), mechanical (Arlett et al., 2011), thermal (Ramanathan and Danielsson, 2001), magnetoresistive (Oh *et al.*, 2013), potentiometric biosensors (Zelada-Guillen *et al.*, 2012), nanoparticle-based assays (Mocan *et. al.*, 2017), single-walled carbon nanotube-based biosensors (Oliver *et al.*, 2006). A very simple design of electrode configuration was used for monitor the growth of *Staphylococcus epidermidis* (Choi *et al.*, 2017). The biosensor which can detect *S. aureus* pathogen qualitatively by naked eye and quantitatively by using imaging software has been presented in (Suaifan *et al.*, 2017). A portable embedded system for bacterial concentration measurement that is suitable for in-situ measurements was reported in (Grossi et al., 2017). Sensor fabricated on FR4 and embedded in microfluidic structure for monitoring of *Pseudomonas aeruginosa* was presented in (Blakey *et al.*, 2013). The microfluidic-based electrochemical biosensor for detection of waterborne pathogens was studied in (Altintas *et al.*, 2018). Antibody-based assay for detecting pathogenic bacteria using fluorescence intensity readout was shown in (Heyduk and Heyduk, 2010). Additionally, flexible sensors are promising candidates for a new generation of personalized biomedical devices (Vilela *et al.*, 2016). Different types of papers can be used as substrates for electrochemical sensors (Cinti *et al.*, 2017), (Cinti *et al.*, 2017). However, none of these article compare sensors performance manufactured on different mechanically flexible substrates such as foils and papers, which can open new areas of their application, particularly for bacteria detection.

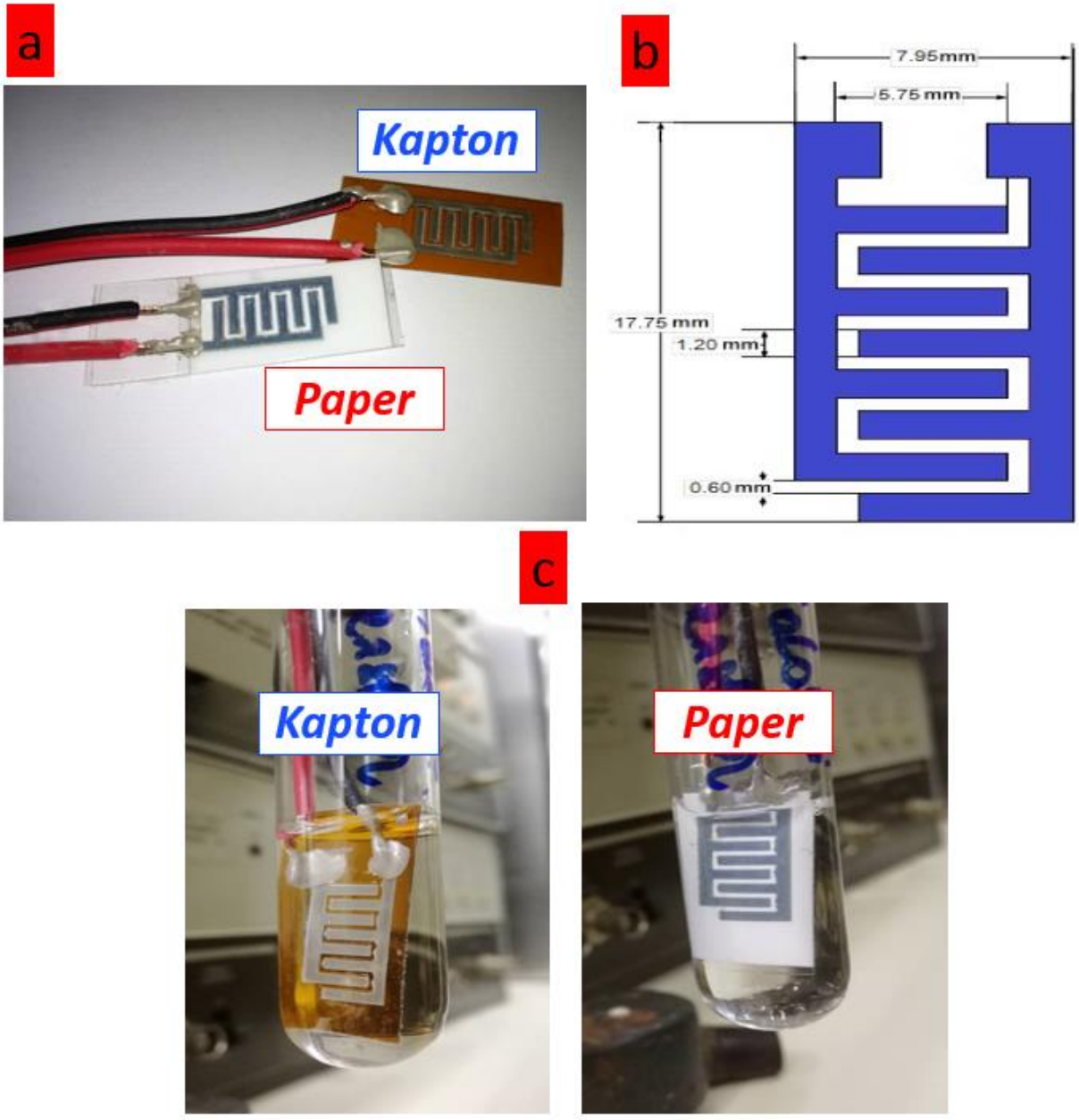
Accordingly, this paper investigates new technologies capable of providing more efficient, robust, selective and accurate systems for bacterial detection, qualification and quantification methods. Thus, this paper uses flexible sensors, for determination of concentration of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Two sensors, with interdigitated silver electrodes structure, were manufactured on foil and on paper. The structural, electrical and mechanical characterization of these sensors have been performed. Additionally, electrical impedance spectroscopy was used to measure resonant frequency of these sensors and correspondingly to detect exact concentration of bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

2. Methods

2.1 Sensors design and fabrication

The sensor is designed as an interdigitated capacitor (IDC) with eight "fingers". The overall dimension of the sensor's design is 17.75 mm × 7.95 mm. The length of each electrode is 6.75 mm, while the width and the gap are 1.20 mm and 0.60 mm, respectively. Dimensions of the sensor were developed based on previous studies (Simić *et al.*, 2017), (Stojanović *et al.*, 2010), (Figure 1).

Figure 1. Design of Kapton and Paper based sensors (a), their dimensions (b) and experimental set up (c).



The sensors were fabricated using ink-jet printing additive technological process, on two types of mechanically flexible substrates – paper and foil. Ink-jet printing was done on commercial paper from Felix Schoeller Group (https://www.felix-schoeller.com/) and Kapton polymide film, using Dimatix deposition material printer - DMP3000 (http://www.fujifilmusa.com/). The paper total thickness was 209 µm and thickness of upper gloss coating of paper, which is good for absorption of ink, was 50 µm. Total thickness of the Kapton film was 50 µm. Commercially available silver ink, UT Dots Silver NPs ink (https://www.utdots.com/) was used for printing conductive segments of IDC structure. Before printing, the stand (holder) on which the substrate was located, was heated to 50 °C. Sensors were printed and sintered at 120 °C for 45 min for structure on paper substrate and 220 °C for 30 min for structure on the Kapton film. The print resolution was 20 µm from the center to the center drops, which was calculated based on the measurement of the diameter of one drop of silver ink on the substrate which was around 32 µm. After printing, sensors was laminated with layer of plastic film to cover substrate and silver ink structure in order to avoid oxidation and aging degradation of fabricated sensors. The uncovered part was only gap between electrodes. This plastic film has two roles, one to prevent creation of short circuit between electrodes and the second one to prevent influence of immersing sensors (and their degradation) into liquid in test tube. After the fabrication, contacts on sensor were made and sensors were medically sterilized to avoid any possible impurities which could make influence on the measurements.

2.2 Characterization methods

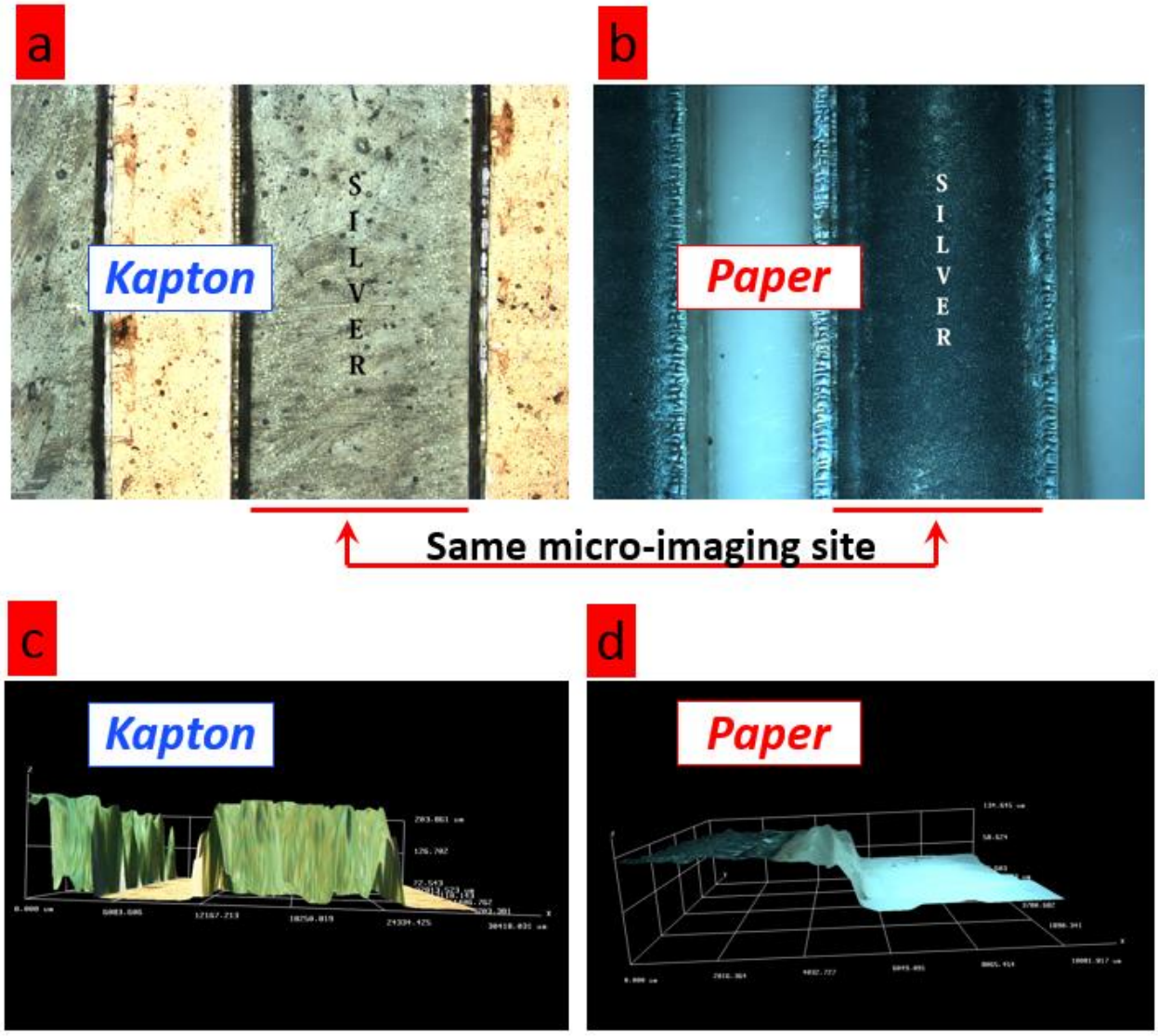
The following instruments have been used for sensors characterization: (1) For structural characterization - 3D Optical Profilometer, Huvitz microscope with Panasis software, (2) For mechanical characterization - Nanoindenter G200, which uses the Berkovich diamond indenter with a face angle of 65.27°, and (3) For electrical impedance spectroscopy - Impedance Gain-Phase Analyzer HP4194A, which covers frequency range from 100 Hz to 40 MHz.

3. Results and Discussion

3.1 Profilometeric analysis

Profilometer analysis was carried out in order to discover structural characteristics and the degree of roughness of sensors surface, which is very important parameter from the point of view of successful ink-jet printing as well as potential application of the sensors. Profilometer light microscope was used to display the 2D and 3D surface profile of the sample. With Profilometer microscope it can be seen the exact structure of surface, because it measures great number of surface pictures from bottom to top and creates one picture in 2D and total picture in 3D. Profilometar 2D results and 3D results are displayed in Figure 2.

Figure 2 Micrographic profilometeric imaging of the sensors fabricated on Kapton foil (a) and paper (b) and their 3D profilometeric imaging of sensors fabricated on Kapton foil (a & c) and paper (b & d).

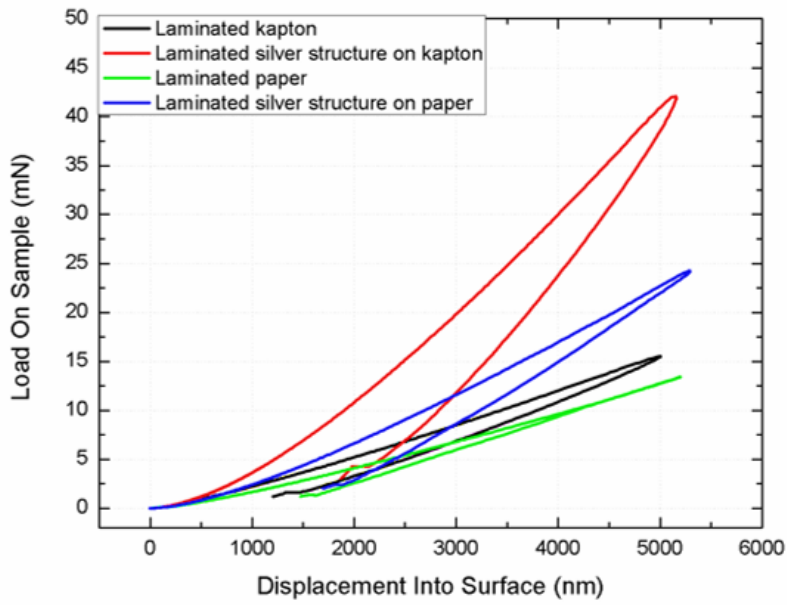


A layer of printed silver ink on the Kapton and paper is uniform and that the edges are made without spraying silver ink drops around. Moreover, it can be seen that laminated layer covers the printed structure on the Kapton and paper, leaving the non-covered part between the fingers of the IDC capacitor to be exposed to the influence of different bacteria. It can be seen that thickness of laminated layer is about 130 µm. Thickness of whole laminated sensor on Kapton is 316 µm and thickness of laminated sensor on paper is 480 µm, which confirms that laminated layer from top and bottom side is 130 µm (Figures 1 and 2).

3.2 Mechanical characterization

Foil and paper as substrates have enabled application of our sensors at different surfaces, in particular, due to their mechanical flexibility. Bearing in mind that the sensors will be exposed to various mechanical stresses during application, especially in curved and conformal shapes, it is necessary to determine their mechanical characteristics. For mechanical characterization it was used nanoindentation measurements by means of NanoIndenter G200 instrument, equipped with a Berkovich three-sided pyramidal diamond tip. A pre-set depth (maximum of 5 µm) has been applied to this pyramidal indenter in contact with the laminated surface layer. The indentation cycle is set, time to load was set to 10 s, while peak hold time was set to 1 s. Examination of samples were performed at room temperature and Poisson's ratio for paper was set to be 0.33. The indents were located 250 µm apart to avoid the influence from adjacent impressions. Nanoindentation tests were multiple, at least 9 indentations were made, to ensure measurement repeatability for the mechanical properties of analyzed samples. Figure 3 shows mean value of load-displacement curves measured on laminated Kapton and laminated silver structure on the sensor fabricated on Kapton foil.

Figure 3. Load-displacement profile for Kapton and paper based sensors without or with silver cover.



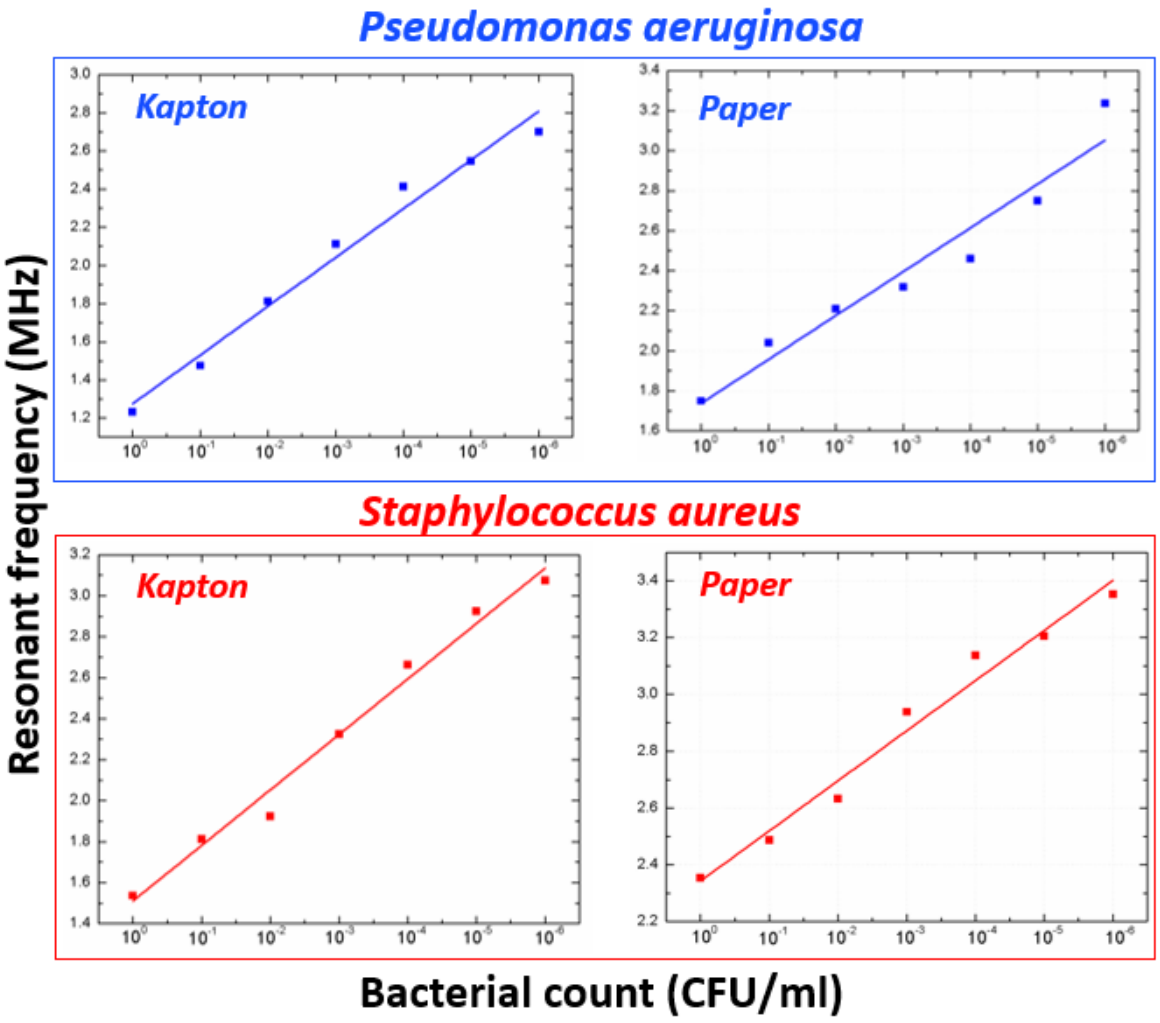
In loading depth at maximum pre-set depth of 5 µm, penetration load is 16 mN for a laminated layer on Kapton film and 43 mN for a laminated layer on silver IDC structure. For laminated layer on the paper load is 14 mN and 25 mN for laminated layer on silver structure on the paper. The curve demonstrates a smooth shape, and no pop-in could be detected. It can be confirmed that Kapton film, paper substrate and silver layer on both have not been reached (the thickness of laminated layer is 130 µm). In both curves it can be seen a viscoelastic return of the samples during the hold load period of 1 s after unloading. Viscoelastic return is smaller on laminated layer on Kapton film. Based on obtained results, it can be concluded that laminated layer on Kapton film has smooth morphology and structure compared with the part of laminated layer, which are on silver structure, because it needed the smallest load to reach the pre-set depth of 5 µm. The same is with laminated layer on paper comparing to laminated layer on silver. By comparing the sensor on the Kapton film and the paper, it can be concluded that paper sensor has the soften structure, because Kapton sensor is thinner than the paper sensor for 150 µm and lamination was better on Kapton film.

3.3 Sensors application for bacterial assessment

Impedance spectroscopy can be successfully used for detection of bacteria cells (Varshney and Li, 2009). In this study, measurements were performed using in-house built set up. The built-in system consists of a sterile test tube filled with different concentration of measured bacteria, test tube holder and HP4194A Impedance/gain-phase analyzer connected to PC. The sensor is immersed inside of solution in test tube and connected to test fixture of the Impedance analyzer.

As testing material human isolate of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from materials which is routinely arrived for treatment in the Centre for Microbiology, Institute of Public Health of Vojvodina is used. From colonies of 24-hour cultures on blood agar (HiMedia, India) for mentioned bacteria, in sterile tubes, suspensions with density 0.5 McFarland (McF) were made in 4.5 ml of physiological saline using EUCAST standard (The European Committee on Antimicrobial Susceptibility Testing, Version 6.0, 2016). Furthermore, this suspension was diluted to the 6 concentrations in range of $10^{-1} \div 10^{-6}$ from initial concentration (*Pseudomonas aeruginosa* and *Staphylococcus aureus* suspensions with density 0.5 McF in 4.5 ml of physiological saline). All measurements were performed in sterile conditions at the room temperature. Figure 4 displays the test results of the resonant frequency versus the concentration of *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively, for all measured dilution for sensor on the Kapton film (foil) and the paper.

Figure 4. Resonant frequency-bacterial count profile of the both sensors on the Kapton file and the paper**.**



Each dilution has its own resonant frequency which makes it easily recognizable one from each other. As a reference pure saline was measured, and it has been found that the resonant frequency of saline was 3.543 MHz for the Kapton film sensor and 4.35 MHz for the paper. In this way the concentration of *Pseudomonas aeruginosa* and *Staphylococcus aureus* presence can be easily differentiated and an adequate treatment can be applied.

Table I. Resonant frequency of dilutions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in physiological saline for sensor on the Kapton film and on the paper

Dilution	Resonant frequency for sensor on Kapton film (MHz)		Resonant frequency for sensor on paper (MHz)	
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
e^{0}	1.232	1.537	1.752	2.354
e^{-1}	1.475	1.812	2.041	2.487
e^{-2}	1.812	1.925	2.221	2.632
e^{-3}	2.112	2.112	2.327	2.938
e^{-4}	2.413	2.325	2.465	3.137
e^{-5}	2.547	2.925	2.753	3.205
e^{-6}	2.701	3.075	3.237	3.352

From Table I can be seen that each bacteria dilution has its own resonant frequency value which makes them easily recognizable with proposed sensors. Following the values from the Table I, it can be seen than resonant frequency of both, diluted *Pseudomonas aeruginosa* and *Staphylococcus aureus* increases as the concentration of bacteria in solution decreases. Bacteria presence between the fingers of IDC electrode structures modifies capacitance. Actually, with increasing of degree of dilution in test tube, the concentration of bacteria is decreased and consequently the number of particles between fingers of capacitor. When permittivity decreases, the total capacitance measured at the terminals using the impedance analyzer also decreases. It is known that capacitance and resonant frequency have indirect dependence and accordingly if capacitance decreases, resonant frequency will have an increase, as Figure 4 confirms explained behavior. The same behavior, e.g. an increase in permittivity and capacitance was reported also in study (Ong *et al.*, 2002), where LC sensor was applied for monitoring of bacteria growth or in the paper (Jing *et al.*, 2010) where concentration *of S. aureus* was measured in milk. The sensors presented in our paper are cost-effective, which open possibility that they can be used on a disposable basis as well as they can be applied by untrained persons which can wider their application fields in biomedical sector.

4. Conclusion

Two sensors manufactured on foil and paper as flexible substrates for detection of both Gram-positive and Gram-negative bacteria were developed and tested as new methods for bacterial quantification and qualification methods. We demonstrated that electrical impedance spectroscopy can be used as a non-destructive fast technique for determining concentration of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in solution, by means of proposed capacitive ink-jet printed sensors. The overall goal of these study is to develop efficient sensors for detection of these bacteria in human saliva in dentistry. The presented sensors can be used for quick point-of-care diagnostics because it simplifies the detection of numerous pathogens.

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