Textronics Interdigitate Electrodes for \textit{Staphylococcus Aureus} bacteria detecting

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Abstract. The aim of the paper is to investigate the changes in electrical parameters of the interdigitated electrodes (IDEs) due to the development of \textit{Staphylococcus aureus}. More precisely, the article presents the results of electrode’s resistance, capacitance and inductance changes in the function of bacteria density. The electrodes are made in the process of physical vacuum deposition on composite textile substrates. This allows to apply them in textronic applications. Changes in the electrical parameters of the produced IDEs were observed during microbial cell culture growing. The results of the research have demonstrated that the bacteria density influences the electrical parameters of the electrode. The greatest changes in these parameters are observed at the frequencies of 100 and 120 Hz after the day of inoculating staphylococcal cells into the medium, where the measuring electrodes were placed.

1. Introduction

The presence of many bacteria is common. The size of bacterial cells ranges from 0.2 μm to 750 μm [1], making them not visible without a microscope. Bacteria differ not only in size but also in shape of individual patogen (spherical, rod-shaped or spiral) as well the shape of loosely connected colonies that constitute characteristic spatial systems. They can be found in many environments such as soil, water in natural reservoirs, the digestive tract of humans and animals, and sewage. Many types of bacteria have been used for thousands of years in fermenting and rotting a variety of foods. In the organisms of humans and animals, they correspond, among others, for digesting food that contributes to enabling or facilitating nutrition. Thanks to bacteria, it is possible to produce various substances necessary for the functioning of the ecosystem, including vitamins. Among the 5·10\textsuperscript{30} bacteria found on Earth [2], many can have a negative impact on the functioning of organisms, among other causing health problems. Each species of bacteria produces specific effects in contact with the human body. The presence of \textit{Staphylococcus} or \textit{Streptococcus} in the human body can cause skin infections, meningitis, pneumonia, and may even result in a very violent immune system response, i.e., sepsis. As a result of the latter complication, the muscles around the blood vessels may sag, which may result in death [3]. For this
reason, the development of methods of detecting bacteria is crucial in the prevention of bacterial
diseases. The timely initiation of appropriate antimicrobial therapy is essential to achieving the best
possible results.

Over the past century, extensive methods of detecting bacteria have been developed, including
traditional breeding methods, immunological techniques, molecular biology techniques, and biosensors.

In all the above-mentioned cases, it is important to develop sensors with high sensitivity to detect
microbes, preferably in real-time.

In addition to the challenges of correct operation, engineers developing sensors also face
requirements for geometric dimensions due to the possibility of their use in miniaturized systems, as
well as the possibility of their implementation in clothing. From this point of view, it is important to
develop textronic structures that can be used in wearable electronics systems.

The most promising laboratory devices are components constructed in the laboratory-on-a-chip LoC
system as well as systems for microprocessor analysis (μTAS). They belong to the group of the most
promising systems used in the production and development of automated biosensors, with a short
response time to the occurrence of microorganisms [4,5]. The main element of the LoC system is the
biosensor, which consists of an element recognizing microorganisms and a reading system [6]. The
identifying elements can be, for example, antibodies, bacteriocins, antimicrobial peptides or
bacteriophages, which are used to transform a given biological phenomenon, such as bacterial
multiplication into chemical or physical variability [7-10]. A given biological phenomenon can be any
unique feature of a bacterium or the presence of a specific factor in a particular microorganism [10].
This biological material should serve to recognize the target molecule and generate detectable signals.
Recognition elements can cause mechanical, electrochemical, acoustic and also optical changes, which
are then detected, amplified and finally measured by physicochemical transducers placed in reading
systems. These changes are proportional to the concentration of the analyte in the volume of which the
sensor is located. Biosensors' efficiency depends on their limit of detection, response time to biological
changes, and their dynamic range. The existing sensors are built based on glass or silicon [11-15]. There
are no reports in the literature on sensors for detecting bacteria produced on a textile basis. This article
aims to investigate the changes in interdigitated electrodes’ parameters produced by physical vacuum
deposition on a flexible composite textile substrate in the presence of Staphylococcus aureus. Applying
these electrodes to clothing or medical devices may allow the detection of this microorganism on the
skin.

2. Materials and Methods

2.1. Staphylococcus aureus

Staphylococcus aureus is a bacterium causing many diseases in humans. This bacterium produces
enterotoxin, resistant even to high temperatures. Staphylococcus aureus toxin poisoning can be as
dangerous as the infection with the bacterium itself. It is a rapidly spreading pathogen that can spread
through contact with infected objects, contact with the host, or droplets. It colonizes the mucous
membranes and skin.

Staphylococcal cells are arranged in irregular clusters resembling grapes, each spherical with a
diameter of 0.8-1 µm. In natural environments and in liquid media, they often appear singly, in the form
of split, quadruple or short chains [16].

The optimal growth temperature for staphylococcus is 30 – 37 °C while maintaining a neutral pH.
The pathogen thrives in aerobic conditions but also does well in anaerobic environments. It is
characterized by high biochemical activity thanks to the enzymes produced by them. It is resistant to
drying out. The characteristics of Staphylococcus allow it to survive for many weeks outside the system,
especially in the presence of protein, e.g. in dried pus on bedding, in dust, and other places where the
sun rays do not reach [17,18].
Detection of *Staphylococcus*, as well as other bacteria, is possible using the optical technique of the McFarland scale. The procedure for determining the correlation between the concentration of bacteria and the optical density on this scale was described by Mysłowska and Bucla-Śladowka in [19]. Using this technique, the concentration of microorganisms in the conducted tests was also determined. *Staphylococcus aureus* ATCC 25923 from the American Type Culture Collection ATCC - Manassas, Virginia was used as a pathogen to conduct research.

### 2.2. IDEs electrodes

The detection of live bacteria is desirable over a wide range of different concentrations. In food and drinking water, the detected bacterial concentrations should be less than 1 CFU/ml, while in wastewater treatment processes, the concentration values can be hundreds of CFU/ml [20]. Currently used methods of bacteria detection in solutions are based on the detection of colonies or single cells present in a selective medium using a microscope [21], automated flow cytometry, or optical detection using fluorescence [22]. Microbial detection methods also include sensors based on the measurement of the impedance of two electrodes immersed in a solution. Changes in the conductivity of the culture medium [23] can be equated with the presence of bacteria. Cells captured on the electrode surface will slightly change the electrode impedance [24]. Due to their low cost and ease of implementation, this type of sensors can be used in a wide range [25]. Optical or impedance tomography can also be used to detect microorganisms [26, 27], however, these methods are more expensive.

In the research presented in this article, the electrodes were made on a Cordura composite substrate. It is a wear-resistant material made of a polyamide layer covered with a polyurethane layer. Silver IDEs were deposited on the selected substrate in the physical vacuum deposition process. The process took place in the Pfeiffer Vacuum 250 chamber, after obtaining an initial vacuum of $5 \cdot 10^{-5}$ mbar. The 99.99% pure silver was placed in a tungsten boat and then evaporated for 5 minutes. In order to obtain the appropriate shape of the electrodes, masks protecting part of the substrate were used. The electrodes are supplied with test leads with the use of an electrically conductive adhesive from Amepox. The electrical and parasitic properties of the produced electrodes [28, 29] were used the research.

The created IDEs electrodes are shown in Figure 2.
2.3. Measurement Set

Measurements were made in direct measurement with the CEM DT-9935 impedance meter, taking care of the power quality [30, 31]. The values of electrical parameters differ from the temperature of the environment in which the sensor is located. The thin-film sensor are particularly sensitive [32]. Therefore, all measurements were made at a temperature of 23 °C, without the presence of a magnetic field [33].

We used the meter with the ES51919 / ES51920 chipset. It allows measuring the inductance, capacitance, parasitic resistances, dissipation factor (D), Q factor (Q), and impedance phase angle automatically or manually. It is also possible to determine the equivalent resistance of the equivalent circuit of a series or parallel capacitor and coil. It is also possible to select test frequencies of 100 Hz / 120 Hz / 1 kHz / 10 kHz / 100 kHz depending on the type of device under test.

Due to the capacitive nature of the circuit, the authors used the measurement at alternating current to obtain the value of the real part (resultant resistance) and the reactance (imaginary part) of the thin-film structure. Due to the occurrence of equivalent resistance values $R_s$ (for the series circuit) and $R_p$ (for the parallel circuit), it is necessary to select the method for the dominant size of the RLC equivalent circuit in the equivalent capacitor or inductor circuit. In the case of tested structures, the quantity sensitive to bacterial concentration changes is the capacity, the measurement for a serial equivalent scheme was used.

3. Results and Discussion

The experiments were conducted according to the methods introduced in Section 2. The optical density of the solution was checked in parallel to the performed tests. On this basis, the presence of live *Staphylococcal* cultures was confirmed. The results of the observations are presented in Fig. 3. The obtained shape of the curve proves that the tests were carried out during the growth phase of the number of bacteria (0 to 20h), during which bacteria divide very quickly. The shape of the chart above 20h is consistent with phase III of cell culture [19]. It is a stationary phase, characterized by a constant number of cells in culture due to the decreasing number of nutrients and the increasing amount of metabolites in the medium.
The electrodes were placed in a suspension in which *Staphylococcal* cells were grown. The basic electrical parameters of the produced IDEs were measured at certain time intervals. Changes in the real part of impedance as a function of frequency and depending on the time of appearance of the first microbial cells in the solution are shown in Fig. 4. Adding the first bacterial cells to the clean medium causes a slight decrease of this parameter. The transition between phase II and III in the *Staphylococcus* breeding cycle increases its value to a maximum after 21 hours. With time, the value of the real component decreases. This tendency is visible regardless of the frequency of the signal used.

During the conducted works, the inductance of the electrodes was also measured. It can be seen from the diagram in Fig. 5 that the inductance of such a system should only be determined for frequencies 100 and 120 Hz. The inductance decreases with time, and its maximum value is also reached 28 hours after the start of the experiment.
The electrode capacitance was also tested as another basic parameter of electrical circuits. Due to the method of producing the electrodes, it is not only the capacity of the electroconductive layer itself but the entire system of electrodes and the space between them [28, 29]. In this case, no monotonic tendency of the capacitance value changes as a function of the duration of the experiment was observed. Large values of the capacity were read immediately after inoculation of the medium with bacterial cells (Fig. 6), and then a decrease in the capacity was observed, and after 50h the capacity values increased again, reaching the maximum value. Also, in the case of this parameter, the most significant changes could be observed when the electrodes were supplied with a signal of 100 and 120 Hz.

4. Conclusions
The findings presented in the paper can significantly contribute to the new methods of bacteria detecting in the area where the flexible substrate can be used, such as in the textile industry and telemedicine.
The study presented in the paper addresses the problem of detecting *Staphylococcus aureus* using interdigitated electrodes produced on a textile composite substrate that can be used in wearable electronics. Changes in the basic electrical parameters of the constructed electrodes as a function of either the frequency of the measurement signal or the duration of the experiment were observed. Changes in the real part of impedance as a function of frequency result from the skin effect of the measurement current for the thin-film structure [28]. Hence, it is necessary to estimate the frequency of the measurement signal at which the highest sensitivity of the measurement method can be obtained. For the considered structure of the measuring electrode, the authors estimate the optimal frequency of the measuring signal at 100 - 120 Hz.

Based on the conducted research, it can be concluded that changes in the capacity and part of the real impedance less than a day after the bacterial colony inoculation indicate the presence of the microorganism in the suspension.

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