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Biofunctionalization Layer and Bacteria Cover Properties Impact on Microfabricated Planar Resonant *E. Coli* Bacteria Microwave Biosensor Response

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Abstract

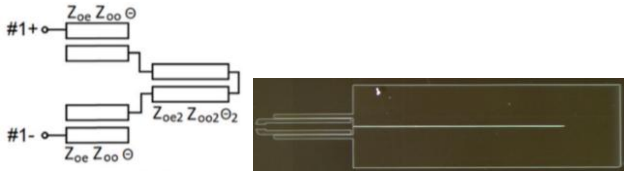
This paper presents an analysis of biological layers properties covering a bacteria-selective microwave biosensor to provide an insight on phenomena driving its electrical response. A differentially-fed coupled-line section resonant sensor operating at 4.7 GHz is used as a test vehicle to establish thickness and dielectric properties of a biofunctionalization layer required to obtain selective bonding of specific bacteria to sensor`s surface. The influence on various concentrations of bacteria Escherichia coli on the frequency response of the biosensor is investigated and discussed. The frequency upshift behavior of the sensor when exposed to bacteria is explained so that sensitivity optimized geometries can be identified towards development of high-performance sensors.

1. Introduction

Some of bacteria such as Escherichia coli (E. coli) are commonly found in the human and animal body and are harmless [1]. Nevertheless, there are some strains of E. coli which are dangerous due to the toxins they produce and they cause a wide variety of diseases, some of them leading even to death. Thus, a fast detection of pathogen and introduction of appropriate treatment is of great important. Conventional methods, which are currently used for E.coli detection are culture- and colony-counting methods, which require stationary laboratories with appropriately trained personnel [2]. Other methods, such as polymer chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) allow to reduce detection time from a few days to several hours, however, require appropriate sample preparation and expensive instrumentation [3]. Taking all of this into account, there is a need for novel methods allowing for fast and accurate detection of specific pathogens. Microwave biosensors are solutions, which could be used for that purpose [4]-[8]. In such case, the sensing element is composed of appropriately constructed microwave circuit and selective biorecognition layer covering its surface which can catch only specific biomolecule [4]-[9]. Special care needs to be taken during realization of both, to ensure high sensitivity and selectivity on a specific bacteria type.

Among microwave sensors [4]-[8], [10]-[13] the resonant ones feature greatest sensitivity on the Material-Under-Test (MUT) parameters change (here biosample). They exhibit a strong field – matter interaction due to resonant nature while sensing principle is based on detecting the resonant frequency detuning and quality factor variation, which are reasonably simple to measure.

This paper presents an analysis of biological layers properties on an example of a microwave biosensor for E. Coli detection in which selectivity on the specific bacteria is obtained by utilization of polyclonal anti-E.coli antibody [9]. The sensor is of resonant nature with a peak located in the vicinity of 4.7 GHz and so the results are po The thickness and permittivity of the biosensitive layer are determined based on the analysis of frequency characteristics' changes of a measured batch of end-shorted coupled-line sensors [5] compared against full wave electromagnetic (EM) simulations. Moreover, the influence of various bacteria concentrations on the resonant frequency and quality factor changes of said sensor is investigated and widely discussed. Finally, bacteria permittivity is assessed and phenomena governing the sensor response is explained considering an equivalent three-shell bacteria model. All the above enables to draw some conclusion towards development of sensitivity optimized microwave biosensors.



(a) (b)

Figure 1. Schematic diagram (a) and photograph (b) of a differentially-fed end-shorted coupled-line microwave biosensor [5] used as a test vehicle in this study.

2. Microfabricated Biosensor

A schematic diagram and a photograph of the microwave biosensor used in this this analysis as a test vehicle are presented in Fig. 1 while all its details are elaborated in [5]. The sensor is composed of two coupled-line sections at the input terminal allowing for introduction of differential excitation to the circuit and one end-shorted coupled-line section. The most sensitive part here is the slot between the end-shorted coupled lines thanks to differential feeding providing a favorable electric field distribution with its maxima being along the slot [13]. Importantly, the distance between the coupled lines is desired to be of the same order

as the bacteria size for high sensitivity however is limited the used manufacturing technology, here the slot is 10 μm wide. Sensor`s resonance that is being used for detection is in the vicinity of 4.7 GHz.

A batch composed of 17 units was manufactured in a single run through a standard microelectronic process based on silicon technology. The sensors were measured using the Agilent PNA N5224A vector network analyser (VNA) in combination with on-wafer GSSG (Ground-Signal-Signal-Ground) probes from Cascade Microtech, Inc. and the results are presented in Fig. 2a. As seen, a good repeatability of the utilized manufacturing process is obtained. To obtain a microwave sensor sensitive to a specific type of bacteria e.g., *E. coli*, the sensor surface needs to be covered with a biological layer, which ensure sensor's selectivity. One of the method, briefly described in [9] utilize polyclonal anti-E.coli antibody for this purpose. In order to determine the parameters of such layer i.e., thickness h_{bio}, permittivity ε_{rbio} and loss tangent $tan\delta_{bio}$, the whole batch was biofunctionalized. The measurement results obtained after biofunctionalization process are presented in Fig. 2b. A significant, yet incoherent detuning of resonant frequenc is observed across the units. Such wide spread of the resonances after covering the sensor with bio-layer can be caused by different thicknesses h_{bio} and/or different complex permittivity values of the layer (ε_{rbio} and $tan\delta_{bio}$).

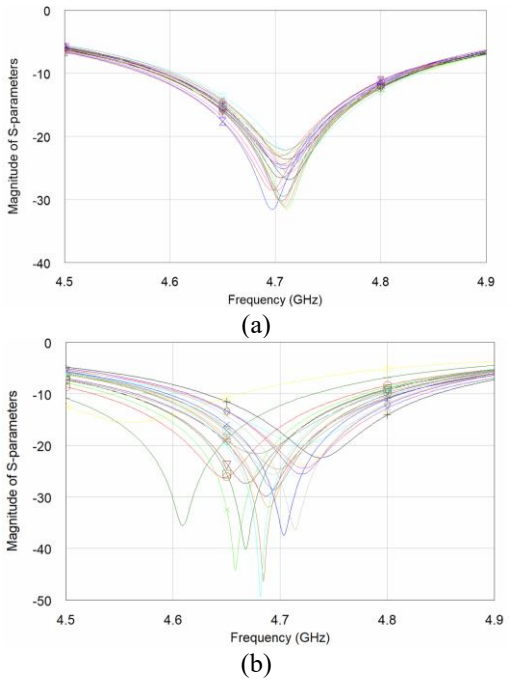


Figure 2. Measured magnitude of Reflection coefficient of the manufactured 17 sensor units prior (a) and post (b) biofunctionalization process.

3. Extraction of Biofunctionalization Layer Thickness and Dielectric Properties

To estimate the values of h_{bio}, ε_{rbio} and $tan\delta_{bio}$ an electromagnetic model (EM) of the sensor was created and following assumptions were made:

- Since a strict protocol is used (the same chemicals for biofunctionalization of all of sensor units) [9] the biofunctionalization layer complex permittivity should be stable for all of the units.
- The resonant frequency change spread is caused by various biofunctionalization layer thickness.
- Thickness of the bio-recognition layer based on the Scanning Electron Microscopy images [9] is estimated to be below 250 nm.

Based on the performed full-wave EM simulations carried out using the *AWR Design Environment* software for various biofunctionalization layer thicknesses and complex permittivity values it was concluded that the biofunctionalization layer is low-loss, but feature high permittivity. The reason behind this conclusion is general increase of the quality factor of the sensors. The comparison of the measurement results and EM model are shown in Fig. 3. The estimated parameters of the biofunctionalization layer are following: ε_{rbio} between 65 and 80, $tan\delta_{bio}$ about 0.01, h_{bio} between 10 and 250 nm.

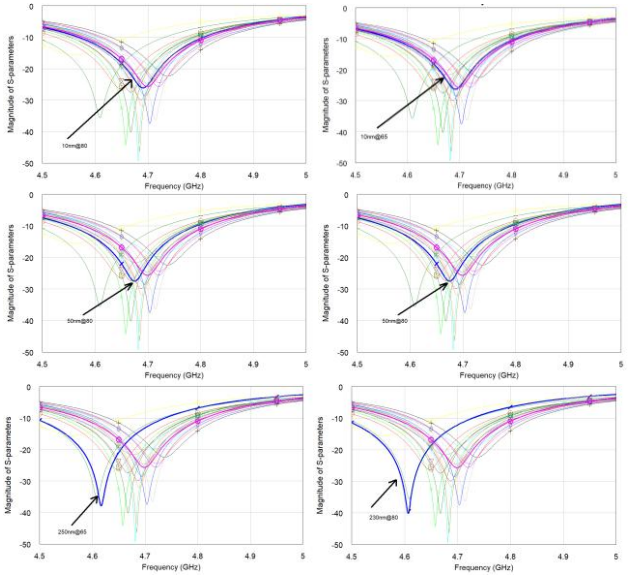


Figure 3. Comparison between magnitude of Reflection coefficient for sensors covered with biofunctionalization layer obtained from measurements and EM model (solid thick line). Various biofunctionalization layer thicknesses and permittivity values were simulated: h_{bio} = 230 nm, ε_{rbio} 80; h_{bio} = 250 nm, ε_{rbio} = 65; h_{bio} = 50 nm, ε_{rbio} = 80; h_{bio} = 50 nm, ε_{rbio} = 80; h_{bio} = 10 nm, ε_{rbio} = 65; h_{bio} = 10 nm, ε_{rbio} = 80 (from left to right, from top to bottom).

4. Influence of E. Coli Bacteria Binding on S-Parameters of the Biofunctionalized Sensors

To assess the influence of bacteria binding to the biofunctionalized sensors surface on its reflection coefficient, the experimental data based study was conducted. In [5] the sensors` batch was divided into 6 groups i.e., five groups were covered with *E. coli* at

concentrations of 10^3, 10^5, 10^7, 10^9, 10^{11} CFU/ml (Colony Forming Units) (3 units for each concentration) and one group for negative control covered with LACTO - *L. rhamnosus* LOCK 0919 at concentration of 10^{11} CFU/ml (2 units). The measurement results being resonance frequency detuning and quality factor change are listed in Table 1.

Table 1. Influence of various concentrations of *E. coli* and negative control using *L. rhamnosus* on the resonant frequency change f_0 and quality factor change Q.

Unit no.	Bact. Conc.	Biofunc.-Bact. Bind. Δf_0 [MHz]	Biofunc.-Bact. Bind. ΔQ	|mean(ΔQ)|	|mean(Δf_0)| [MHz]
1	10^3 E. coli	-67	844.62	267.75	27.70
2		22	8.50		
3		-38	-49.86		
4	10^5 E. coli	4	9.45	3.5	49
5		-75	82.10		
6		-76	-81.02		
7	10^7 E. coli	-3	1273.90	579.34	37.33
8		-36	145.46		
9		-46	318.66		
10	10^9 E. coli	-75	506.13	207.96	41.33
11		-23	93.44		
12		-26	24.32		
13	10^{11} E. coli	-10	319.05	748.71	78
14		-54	2014.45		
15		-170	-87.37		
16	10^{11} L. rhamnos	-3	-31.43	33.50	0.50
17		4	-35.57		

Interestingly, for all of the concentrations of *E.coli* except 10^5 CFU/ml a monotonic resonant frequency change can be observed, whereas there is almost no change of resonant frequency for negative control. This is an important aspect proving high selectivity of the designed biosensor on a specific *E. coli* bacteria. Moreover, based on the quality factor change obtained for 10^5 CFU/ml of *E. coli* this concentration can be excluded from further analysis, since the observed |mean(ΔQ)| significantly differs from the results obtained for the rest of the test set. Based on the above, the detection and determination of the bacteria concentration must not only consider the resonant frequency detuning but also quality factor variation with respect response prior to bacteria exposition. Summary of the obtained results for a set of *E. coli* concentrations is shown in Fig. 5.

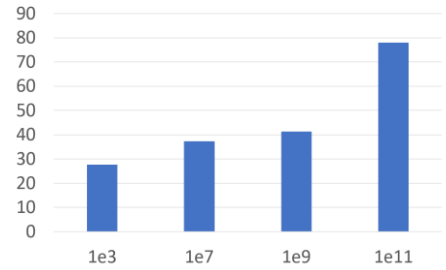
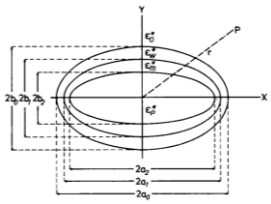


Figure 4. Resonant frequency change (MHz) as a function of *E. coli* bacteria concentration (in CFU/ml).

5. Impact of E. Coli Bacteria Properties

The *E. coli* bacterium cell has a rod shape with a diameter of 0.68 μm and length of 2.79 μm [14]. Its electrical properties can evaluated based on a three-shell structure model shown in Fig. 4 composed of protoplasm, membrane and cell wall. Along, the relative permittivity values and bulk conductivity [15] are listed. The model is defined at lower frequencies around 1 MHz, thus the dielectric properties of *E. coli* at higher frequencies might deviate from the quasi-static ones. However, for further measurement results assessment the above are sufficient.



(a)

	Relative permittivity	Bulk conductivity (Sm^{-1})
Protoplasm	67	0.17
Membrane	9.5	5e−8
Cell wall	60	0.73

(b)

Figure 5. Three-shell model of an *E. coli* bacteria [15] (a) with relative permittivity and bulk conductivity values: ε_p^* complex dielectric constant of protoplasm, ε_m^* complex dielectric constant of ε_w^* complex dielectric constant of cell wall (b).

Generally, after bacteria binding process the resonant frequency is shifted towards higher frequencies. This behavior is in opposite to what is observed after biofunctionalization procedure thus cannot be related to complex permittivity change of the layer covering the sensor containing bacteria (shifts of resonance frequency peaks should be towards the lower frequencies). On the other hand, a plausible explanation is that the phenomenon is related to the sensor geometry change after bacteria binding procedure. The distance between coupled lines of the sensor is 10 μm, whereas a single E. coli bacterium length is 2.79 μm. Bacteria in general have tendency to form colonies and so they can accumulate in the region of coupled-line sensor end-short (in microscale this is a shallow well with three walls), shortening the resonator and hence shifting its frequency of operation towards the higher frequency range. It is possible considering that the E. coli membrane, according to the model shown in Fig. 5 is highly conductive. The obtained results show, that change of the sensor geometry could have greater impact on the resonance frequency shift, than the complex permittivity change introduced by bacteria, which according to [14] and [15] should feature similar permittivity as the biofunctionalization layer.

6. Conclusion

The above presented study and described observations provide an inside on the bacteria sensing phenomena using microwave biosensors. This in turn can be applied towards development of high-performance and highly sensitive optimized structures. In general, a sensor design featuring geometry, which can be modulated by accumulated bacteria in certain area(s) is expected to feature high

probability of bacteria detection and so high sensitivity and resolution as well as low detection threshold. When fabricated using a low-cost at high-volume microfabrication technology on inorganic substrate that provides sufficient geometrical resolution, such biosensors might become replacement for the current bacteria detection techniques.

6. Acknowledgements

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