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CMOS Compatible Anodic Al_2O_3 Based Sensors for Bacteria Detection.

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Abstract

Rapid, real-time detection of pathogenic microorganisms is an emerging evolving field of research, especially for microorganisms that pose a major threat to public health. Alumina covered interdigitated capacitive microsensors were previously designed in our laboratory for DNA hybridization electrical detection (LOD of 30 nM target DNA). The device is constructed with standard CMOS materials. We show here that when coated with an appropriate anti- Staphylococcus aureus monoclonal antibody (MoAb), this device also permits to specifically detect this bacteria. The binding of bacteria to the microsensors induce a significant capacitance shift that is proportional to the amount of immobilized bacteria, thus enabling a possible quantitative analysis.

Keywords: Biosensor, aluminum oxide, bacteria

1. Introduction

The rapid detection of microorganisms, such as Staphyloccocus aureus responsible for 35% of mortality associated to a bacterial infection, is a major goal of the identification methods actually in development. Most of them, expensive and time consuming, are based on enzymatic or polymerase chain reaction (PCR) tests. New sensor based methods are also in development such as optical scatter light sensors [1], impedimetric systems [2] [3], resonance, microbalances, cantilevers [4], electrochemical [5], piezoelectrical systems [6] carbon nanotubes [7], and Au electrodes [8] [9], Table 1. All those methods seem to be time consuming, to require sophisticated systems or to be colony growth dependent. It is not easy to compare their sensitivities. When expressed in g/ ml, these can vary with bacteria species and sizes. Besides this, there is no direct correlation between volume concentrations and actual sensor bound bacteria. Possibly best performance is found in [6] with a detection level down to 1 cell/ 100 ml.
Anodization has been proposed in [10] to form a protective dense Al_2O_3 passivation layer allowing the use of Al interdigitated electrodes for chemical and biological measurements in solution avoiding Al deterioration by chemical erosion [11]. A thin passivation layer (i.e. 50-100 nm) is required to enable target molecules capacitive detection. Our interdigitated (ID) capacitors system would be helpful to circumvent the cited two major problems. Target is to detect specifically and immediately the presence of about 100 bacteria/ml, as found in infected blood sample.

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Table 1. Bacteria detection recent publication examples.

Reference	Principle	Bacteria	Concentration
[1]	Scatter light at 635 nm, label free	Escherichia, Salmonella, Listeria, Staphylococcus, and Vibrio	1 cfu/25 g
[2]	Impedimetric Solid-medium-integrated biosensor	B. subtilis, Tryptic, Soy Broth, P. aeruginosa, Luria Broth and S. aureus	100 counts
[3]	Impedance spectroscopy	E. Coli	10^5 cell/ml
[4]	Plasmon resonance (SPR), quartz crystal microbalance (QCM) and cantilever-based	Salmonella, L. monocytogenes, Streptococcus mutans, Bacillus cereus, Campylobacter and E. coli	SPR 0.06 pg, 12.5 µg/ml QCM 1 ng, 100 ng/ml Cantilever 100 cfu/ml
[5]	Electrochemical PCR sensing	Legionella pneumophila	10 genomes
[6]	Nano-Ag at TiO_2-coated piezoelectric quartz crystal (PQC)	E. coli	1 cell/100 ml
[7]	Carbon nanotube field effect transistors	Salmonella Infantis	100 cfu/ml
[8]	Au interdigitated electrodes, EIS	Salmonella Typhimurium	$3.45x10^6$ cfu/ml
[9]	Au electrodes arrays, current	E. Coli	1.26 pA/bacteria (0.5 V)

Cfu = colony forming unit, a measurement of viable bacterial or fungal numbers

2. Fabrication and Methods

2.1.Construction of the interdigitated capacitors

Our biochips are fabricated over $10^{15}cm^{-3}$ boron doped silicon <100> wafers. After standard cleaning, 400 nm wet thermal silicon oxide is grown at 1000°C. The interdigitated Al fingers are patterned on top of the oxide by lift-off process involving positive photo-resist deposition, optical mask photo-definition, etch of illuminated resist, 500 nm thick evaporated Al and removal of remaining resist and Al residues. An 80 nm thick aluminum oxide layer was formed over the aluminum fingers by electrochemical anodization. It is done in an electrochemical cell at 3 mA/cm^2 and using an ammonium pentaborate in ethyleneglycol based solution [11]. The wafer backside is covered with Al to allow the control of electrical effects in the bulk silicon substrate. The biochips are then diced into dies of 3 x 3 mm^2. The sensors are located at the center area, each ID capacitor is 200 x 200 μm^2, and has its connecting pads on the sides (Fig. 1a).

Table 2 and Fig. 1 depict our ID capacitor design. 4 different fingers configurations (denoted a to d) are integrated with varying electrodes widths and spacings (either 2, 5 and 10 µm width with 2 µm spacing, or 2 µm width with 4 µm spacing). Si dies are finally packaged and each sensor capacitance is calibrated using an LCR meter at frequencies from 1 kHz to 1 MHz.

Using a model based on work in [12], and [13], the electrical behavior of the interdigitated micro-arrays in the presence of conductive particles, is predicted (Fig. 1c). The parasitic capacitance C_{board} due to silicon oxide and substrate dominates at zero densities. After particles addition (silver grains in [10], bacteria in the present paper), the low particle resistance (Z) short-circuits the gaps between the electrode fingers and hence couples them in AC measurements through the Al_2O_3 layer capacitance C_{mox}. This induces an increase of the measured capacitance and a well defined measurement range from a minimum C_{board} to a maximum of half of C_{mox}.

The sensors are finally coated with the MoAb by an overnight incubation at 4°C in a MoAb borate containing buffer (10 µg/ml final concentration).

2.2.Antibody-based specific pathogen detection

A drop of bacterial sample is spread over the sensor surface previously rinsed with phosphate-buffered saline (PBS). The samples consist of a liquid culture (Luria Broth medium) of Staphylococus aureus (positive sample) or Staphylococcus epidermidis (negative sample) that is not recognized by the MoAb. Each culture is performed until the end of the exponential phase growth of the bacteria. After 5 minutes incubation, the sensor is rinsed 3 times with PBS, 3 times with distilled water and slightly dried at room temperature during 10 minutes. One sensor is shown in Fig. 1d after bacteria immobilization.

A first test is performed, on both positive and negative samples, using the 4 cited fingers configurations and reproduced 4 times using the (2, 2) µm system (sensor "a").

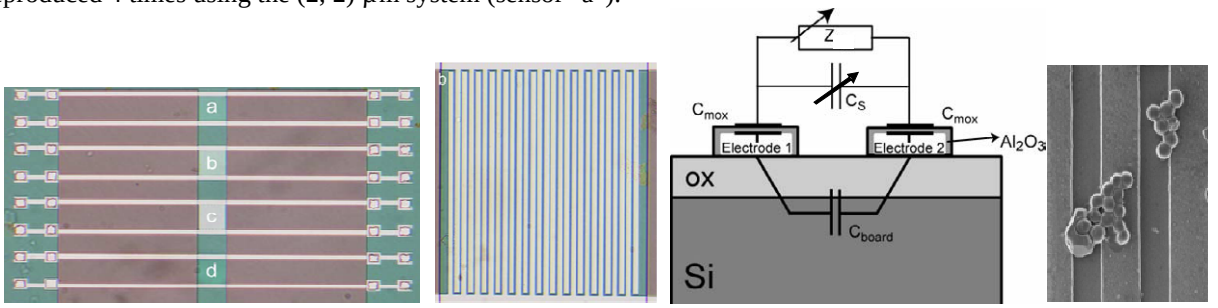

Fig. 1. (a) Interdigitated capacitors configuration; (b) Close-up on capacitor "b". c) Equivalent circuit, d) SEM picture showing a few Staphylococcus aureus fixed over the surface of the sensor fingers.

Table 2. Finger's configuration used for the IDF device.

ID	Finger width (µm)	Inter-finger distance (µm)
Sensor "a"	2	2
Sensor "b"	5	2
Sensor "c"	10	2
Sensor "d"	2	4

3.Results and Discussion

Different device configurations are tested and results are summarized in Fig. 2. All devices show a significant capacitance change after the specific bacteria immobilization, as well as a significant difference between the positive vs. the negative samples. The number of bacteria immobilized on each sensor configuration is also determined by scanning electron microscopy (SEM). Their correspondent capacitance shifts are given in Table 3.

It is found that capacitance sensitivity per bound bacteria depends on sensor configuration, such as finger width and spacing. As low as only 30 bacteria deposited on the surface is enough to have a detection, with a contribution of around 0.009 to 0.02 pF by bacteria. This is a qualitative agreement with our model considering that half of C_{mox} per unit area is about 0.02 pF/µm^2. This indicates that the sensitivity of our sensor could be improved by optimizing the geometrical parameters of the interdigitated capacitor. Part of the capacitance contribution by each bacteria could be also affected by agglomerations that could occur during their immobilization on the device surface.

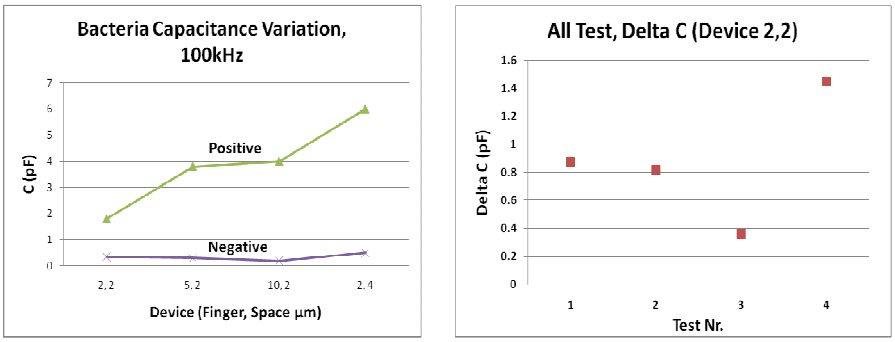

Fig. 2. (left) Capacitive variation for different ID finger dimensions (as indicated in the x-axis legend), (right) Variation of capacitance (i.e. positive minus negative) for the 4 test repetitions using the (2, 2) µm system.

Table 3. Bacteria count and sensitivity calculation.

ID	Number of bacteria (N)	Capacitance change ΔC (pF)	Sensitivity ΔC/N (pF)
Sensor “a”	70	1.18	0.017
Sensor “b”	30	0.41	0.014
Sensor “c”	90	0.83	0.009
Sensor “d”	50	3.19	0.064

4. Conclusions

A low cost device, i.e. simple-to-manufacture and test, is presented for the capacitive detection of bacteria. Results show a successful selectivity between positive and negative samples. Bacteria contribute to the measured capacitance increase by adding well-defined aluminum oxide capacitive unit elements in the electrical equivalent circuit of the sensor. A very low number of bacteria is sufficient to have a good detection (as low as 30 units). Our device is now ready for more application tests using other bacteria as well as different concentrations.

References

1. P.P. Banada, K. Huff, E. Bae, B. Rajwa, A. Aroonnual, B. Bayraktar, A. Adil, J. P. Robinson, E. D. Hirleman, A. K. Bhuni. Biosensors and Bioelectronics (2008) article in press.

2. A. Choi, J.-S. Park, H.-I. Jung. Sensors and Actuators B **137** (2009) 357–362.

3. Y.-H. Kim, J.-S. Park, H.-I. Jung. Sensors and Actuators B (2009) article in press.

4. P. D. Skottrup, M. Nicolaisen, A. F. Justesen. Biosensors and Bioelectronics **24** (2008) 339–348.

5. R. Miranda-Castro, N. de-los-Santos-Álvarez, M. J. Lobo-Castañón, A. J. Miranda-Ordieres, P. Tuñón-Blanco. Biosensors and Bioelectronics (2009) article in press.

6. H. Sun, T.S. Choy, D.R. Zhu,W.C. Yam, Y.S. Fung. Biosensors and Bioelectronics **24** (2009) 1405–1410.

7. R. A. Villamizar, A. Maroto, F. X. Rius, I. Inza, M. J. Figueras. Biosensors and Bioelectronics **24** (2008) 279–283.

8. L. Yang. Talanta **74** (2008) 1621–1629.

9. Y.-C. Lu, Y.S. Chuang, Y.Y. Chen, A.C. Shu, H.Y. Hsu, H.Y. Chang, T.R. Yew. Biosensors and Bioelectronics **23** (2008) 1856–1861.

10. L. Moreno-Hagelsieb, B. Foultier, G. Laurent, R. Pampin, J. Remacle, J.P. Raskin, D. Flandre. Biosensors and Bioelectronics **22** (2007) 2199–2207.

11. L. Moreno-Hagelsieb, B. Foultier, G. Laurent, C. Poleunis, P. Bertrand, J.P. Raskin, J. Remacle, D. Flandre. MicroTAS 2004, Sweden, Sep. 2004.

12. I..P. Van Gerwen, W. Laureyn, W., Laureys, G. Huyberechts, M. Op De Beeck, K. Baert, et al, Sensors and Actuators B **49**, (1998) 73-80.

13. .P. Olthuis, A.J. Sprenkels, J.G. Bomer, P. Bergveld, Sensors and Actuators B **43** (1997) 211-216.