Electronic Transducing Chip Platforms for Biosensing Applications

Anjum Qureshi,*[1] *Ashish Pandey*,[1,2] *Yasar Gurbuz*,[2] *Javed H. Niazi*[*1]

Summary: Growing demand for a range of portable, rapid and low cost biosensing devices for the detection of disease biomarkers, toxic chemicals/drugs and engineered nanomaterials are ever demanding. Biosensors can play a critical role especially in the early diagnosis of disease such as cardiovascular disease (CVD) without having to rely on hospital visits where expensive and time-consuming laboratory tests are recommended. Other major areas where biosensors are essential include chemical and biomedical industries. In order to address these applications, our research mainly focused on developing suitable electronic transducing platforms that enable label-free, sensitive and single/multianalyte detection. One such example successfully applied was detection of a panel of disease biomarkers such as CRP, TNFα, and IL6 to potentially determine the CVD risks. This approach can be potentially extended to developing hand-held devices for point-of-care applications. Another area we employed biosensors are for detecting toxic effects of engineered nanomaterials (NMs) that induce cellular toxicity using living whole-cells-on-chip as biorecognition elements. Here, we summarized our research work carried out in our laboratory on capacitive transducing chip platforms for biosensing applications.

Keywords: bacteria; biosensors; dielectric properties; nanoparticles; nanotoxicity; protein

Introduction

Biosensors have now become an integral part of human life and safety. These have started to emerge as important tools in health and medicine, high-throughput screening of toxic chemicals and unknown drugs and environmental monitoring. Biosensors can be classified into different types depending upon the type of biologically derived capturing element, such as antibodies, hormones, receptors, enzymes or nucleic acids (DNA/RNA) or, the type of electronic transducer that is interfaced to capture the biological reaction. Most common are those driven by antigen/antibody dependent reactions. There are many antigen/antibody based bioassays that utilize intermediary chemical/physical mediators, such as chromo-/fluoro-genic substrates for measuring color or fluorescence, respectively. Biosensors based on such type of reactions are often expensive, cumbersome process and require trained personnel to operate. These types of biosensors are currently being used for the diagnosis of one of the major diseases such as CVD worldwide.

CVD is a major cause of human death in both developing and developed countries. According to the World Health Organization (WHO), an estimated 17.5 million (30%) of all global deaths in 2005 are associated with CVD and it is estimated that by 2015, CVD can be the leading cause of death in the developing countries.[1] Early and quick diagnosis of cardiovascular

[1] Sabanci University Nanotechnology Research and Application Center, Orta Mahalle 34956, Tuzla, Istanbul, Turkey
Fax: +90 216 483 9885
E-mail: anjum@sabanciuniv.edu; javed@abanciuniv.edu
[2] Faculty of Engineering and Natural Science, Sabanci University, Orhanli 34956, Tuzla, Istanbul, Turkey

disease is extremely important and crucial not only for patient survival but also saving cost and great deal of time in successful prognosis of the diseases. Existing methods of diagnosis for CVD rely heavily on classical methods which are based on tests conducted in central laboratories that may take several hours or even days from when tests are ordered to when results are received.[2] A more sensitive and rapid technology platform is therefore needed to fulfill the rapid diagnosis requirements in CVD detection.

The elaboration of biosensors is probably one of the most promising ways to solve some of the problems concerning sensitive, fast and cost effective measurements.[3] This motivated us to work on development of electronic transducing chip platforms to detect single and multiple CVD specific biomarkers or nantoxicity induced by the nano-sized particles.

The nanotechnology industry is rapidly growing with promises of substantial benefits that will have significant economic and scientific impacts, applicable to a whole host of areas ranging from aerospace engineering and nano-electronics to environmental remediation and medical healthcare. Nanotechnology has tremendous potential to change and improve many sectors of the economy, including consumer products, healthcare, transportation, energy and agriculture.[4] It is estimated that over 500 consumer goods that are already available consist of NMs. Nanoparticles (NPs) are present in some sunscreens, cosmetics, toothpastes, sanitary-ware coatings, silicon chips and even in food products. Worldwide investment on nanotechnology is on the rise[5,6] and the trend is expected to continue over the next decade. Unusual physicochemical properties of engineered nanomaterials (NMs) are attributable to their small size, chemical composition, surface structure, solubility, shape, and aggregation.[7] Yet concerns have been raised that the properties of nanostructured materials that make them so attractive could potentially lead to unforeseen health or environmental hazards. Currently, a complete understanding of the size, shape, composition and aggregation-dependent interactions of nanostructures with biological systems is lacking due to multiple choices of possible parameters that need to be considered for characterizing the toxicity, such as variability of methods, materials, and cell-types utilized.[8,9] Therefore, capacitive chip platform can be integrated with living cells to develop a whole-cell based lab-on-chip (LoC) platform for detecting toxicity of engineered nanomaterials (NMs). The whole living-cells-on-chip enables understanding the impact of any toxic chemical or engineered NM that come in contact with living cells at the interface of living cell-electronic transducers. In this paper, we highlight some of the striking features of biosensing and the related work conducted in our laboratory.

Experimental Section

Fabrication of Capacitor Arrays

Gold interdigitated electrode based capacitor sensor arrays were patterned on SiO_2 surface using image reversal technique. In this process, the metal layers were patterned using the dual tone photoresist AZ5214E. A 2 μm thick AZ5214E photoresist was patterned with the help of a mask for a lift-off process in pure acetone as a solvent. Following this step, a very thin tungsten layer of 50–60 nm size was layered to improve the adhesion of gold on the SiO_2 film by DC sputter deposition and about 200–210 nm thick gold layer was deposited. The dimension of each electrode was 800 μm in length, 40 μm in width with a distance between two electrodes of 40 μm. Each capacitor sensor contained 24-interdigitated gold electrodes within a total area of 3 mm^2.

Immobilization of Antibodies (Anti-CRP, -TNFα, and -IL6) on Electrodes

Sensor chips were first surface functionalized by self-assembled monolayer (SAM) with β-mercaptopropionic acid and the

commercial monoclonal antibodies (50-100 μg/mL) specific to target biomarkers were covalently coupled using 50 mM of 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 50 mM *N*-hydroxysuccinimide (NHS) reaction. Details of methods for protein immobilization procedure and sensor response measurement were same as reported in previous studies.[10]

E. coli Cell Culture and Immobilization of Cells on Capacitor Chip Platform

A wild-type *E. coli* (DH5α) was grown overnight in Luria-bertani (LB) broth at 37 °C. The cells were harvested by centrifugation at 5000 rpm and washed with phosphate buffered saline (pH 7.4). The washed cell-surface proteins were subjected to a rapid activation by EDC/NHS and the entire mixture was incubated on sensor surfaces. The sensor surfaces had previously layered with SAM of β-mercaptopropionic acid that provided free COOH groups on to which –NH2 groups of the cell-surface proteins were covalently coupled. The sensors were finally washed with PBS and all the remaining details for immobilization, confirmation and toxicity response measurements were same as reported in our previous work.[11]

Results and Discussion

Capacitive immunoassays are promising alternatives to existing immunochemical tests for the development of hand-held devices which can be used for point-of-care applications. The attraction of affinity-based capacitive sensors is that they are able to determine the analyte directly in a sample with no or very little sample preparation. The sensing principle of these sensors is based on changes in dielectric properties, charge distribution, and/or conductivity change that occur upon antibody-antigen complex formation on the surface of the electrodes. Capacitive affinity biosensors can be constructed by immobilizing recognition elements, such as antibodies on the electrodes, and measuring changes in the dielectric/surface properties when an analyte binds. For providing larger sensor surface, conductors can be made into a pattern of interdigitated fingers. The capacitance between the interdigitated electrodes can then be described by the basic capacitance equation:

$$C = 2n\varepsilon\varepsilon_0 A/d \quad (1)$$

where ε is the dielectric constant of the medium between the plates, ε_0 is the permittivity of free space, A is the area of the electrodes and d is the distance between the two electrodes, n being the number of electrodes and finally the factor 2 in equation represent each electrode forming two capacitors. Thus, when there is a change in the dielectric properties of the material between the electrodes, a change in the capacitance will occur and it is correlated to the bound antigen molecules and amount captured by antibodies on the surface, as well as between the electrodes.

Biosensors for Detection of Disease Biomarkers

Capacitive biosensors for single biomarker (CRP) detection have been previously well developed in our laboratory, where synthetic RNA aptamers and antibodies were employed as affinity ligands on gold interdigitated (GID) capacitor arrays.[12,13] Although, most protein biomarkers are not highly specific to a particular disease, for example, CVD risk is associated with more than one biomarker for its incidence. Thus, detection of a panel of biomarkers such as C-reactive protein (CRP), TNFα, and IL-6 allows accurate prediction of the CVD at the early stages. These multiple biomarkers are shown to have strong and consistent relationships with each other that may aid detecting the inflammation and future CVR events.[14] Thus, development of a multianalyte immunoassay for panels of biomarkers holds significant importance for diagnosis of disease.[15] Some studies have shown that the measurement of a biomarker panel can prevent false-positive or false-negative results, thus improving

the diagnostic value of the biomarkers.[16] Therefore, one of our aims was to develop a new label-free multianalyte capacitive immunosensor based on gold interdigitated electrodes (GID) fabricated on SiO_2 surface (capacitors) to detect a panel of disease biomarkers that include CRP, TNFα, and IL-6. This study prompted us to determining the CVD risk in a synthetic sample with a unique approach distinct from conventional biosensing with several important considerations. These include, (a) efficient covalent immobilization of pure/mixture of antibodies directly on an optimized GID electrode geometry compared to epoxy-silanization,[13] which is prone to less sensitivity, and (b) use of a less-expensive SiO_2 background of the capacitors with high sensitivity compared to that reported with nanocrystalline diamond (NCD) background.[12]

Label-free detection of multiple biomarkers (CRP, IL6 and TNFα) for the diagnosis of CVD risk was mainly carried out using capacitor arrays made of GID electrodes immobilized with multiple antibody types in mixture for the detection of multiple biomarkers through capacitance/dielectric measurements (Figure 1). In the first step, the chips were incubated with different concentration of CRP for 1 h and the chips were washed, dried and measured the capacitance and dielectric properties. This process was repeated in the second and third steps with the same chips by incubating with TNFα and IL6 antigens, respectively and the change in capacitance/dielectric properties after each step was recorded (Figure 2). When the immobilized antibodies interacted with antigens, the interactions induced the change in the dielectric layer on capacitor surface and thus change in capacitance/dielectric properties, which were corresponding to the specific antigen. The detection limit was in the range 25 pg/ml to 25 ng/ml under standard assay conditions (Figure 2).[10] The high sensitive method demonstrated in this study can potentially be applied to detecting multiple biomarkers in real serum for potential diagnostic application.

Chips immobilized with multiple antibodies can therefore determine the levels of one or more biomarkers in an unknown sample. However, the unknown sample if contained normal level of one biomarker and elevated level of the other, in such cases, the chips once tested with unknown sample can be tested again, but this time using known standard biomarker proteins. This approach eventually allows determining as to what type of biomarker's level was elevated in the sample. Currently, our research group is working toward the

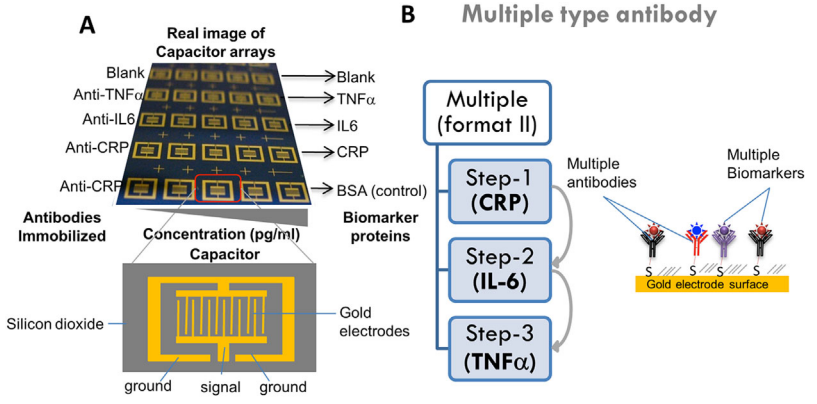

Figure 1.
Schematic representation of (A) GID capacitor array chip and (B) multiple antibodies and capturing of protein biomarker target/s.[10]

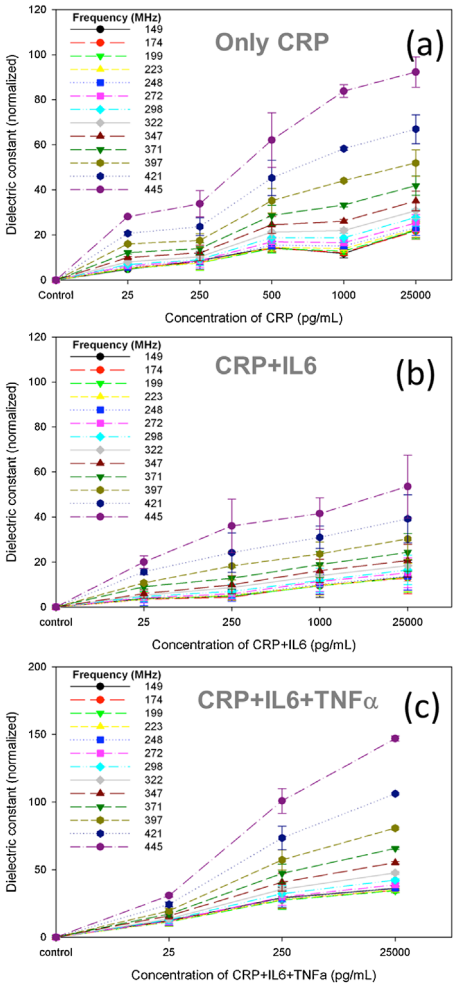

Figure 2.
Multiplexed detection of protein biomarkers (a) CRP, (b) CRP+IL6 and (c) CRP+IL6+ TNFα on chips immobilized with equimolar mixture (1:1:1) of anti-CRP, anti-IL6 and anti–TNFα antibodies and the concentration dependent increase in dielectric response within 149-445 MHz frequency are shown in the figure. BSA protein was used as a negative control.[10]

development and miniaturization biosensor to a hand-held device for point-of-care diagnosis, where we are addressing challenges with geometry of electrodes and integrating microfluidic platform that can offer the advantages with speed, simplicity in analytical procedures, minimum sampling requirement, improved test efficiency and cost effectiveness.

Biosensor for Detecting Toxicity in Living Cells Induced by Nano-Sized Materials

The capacitive biosensor developed for diagnostic application was further extended to entirely a different application. Here, we demonstrated the applicability of the capacitance based electronic transducing platform to detect the cellular stress imposed by different toxic chemicals.[17] This type of unique capacitive biosensor platform was employed for studying the size-dependent toxicity of engineered nanomaterials by integrating living bacteria at the sensor chip interface (Figure 3). As a result, a whole-cell based capacitive biosensor (WCB) was evolved to determine the biological toxicity of nanoparticles (NPs) imposed by nano-sizes using different sizes of iron oxide (Fe_3O_4) NPs as models.[11] First, biosensor chip was initially functionalized with living *E. coli* cells that served as biological reporters on sensors, while the capacitors served as electronic transducers. When the cells-on-chip allowed to interact with different sizes of Fe_3O_4 NPs (5, 20 and 100 nm), a concentration-depended cellular-responses was evident that was measured in terms of change in dielectric properties (capacitance) as a function of applied AC frequency. The WCB response showed smaller-sized Fe_3O_4 NPs (5 nm) induced maximum change in surface capacitance because of their effective cellular interaction with *E. coli* cells-on-chip indicating that the cells suffered from severe cellular deformation (Figure 4).

Further, the results of this study were validated through their cell viability and *E. coli* cellular responses at the interface of cell-membrane and NPs as a proof-of-concept. The WCB-chip was sensitive and highly specific, which was confirmed by employing heat-killed cells-on-chip in which dead cells failed to respond against NPs stresses. These results inferred that the transduced signal on WCB occurred due to the interactions between living bacteria and NPs was not only associated with the interplays at nano-bio interface, but is also strongly associated with the toxicity of NPs

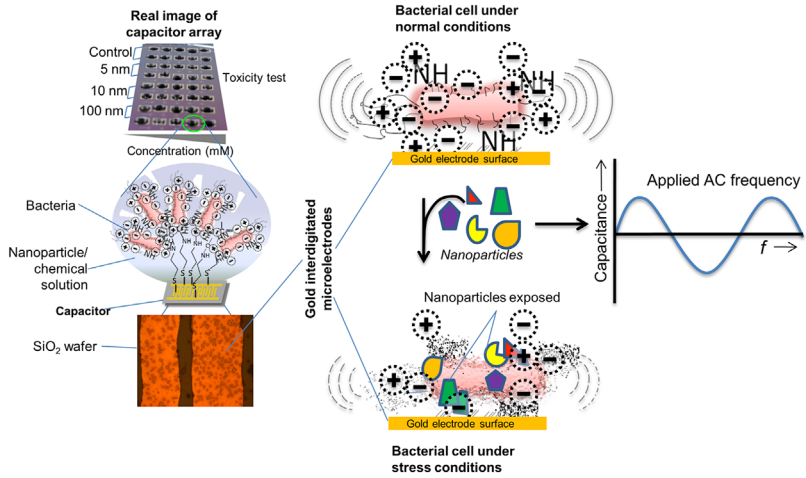

Figure 3.
Schematic illustration of transducing capacitor chip platform functionalized with living bacterial cells and exposure of different sized nanomaterials for determining nano-toxicities.

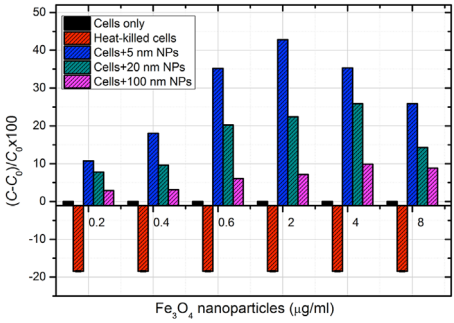

Figure 4.
Relative capacitance responses of WCB chip against difference concentration of Fe_3O_4 NPs at different sizes of sizes such as 5, 20 and 100 nm of NPs function of applied frequency at 200 MHz.[11]

on bacterial cells. This type of whole-cell based biosensor can potentially be applied for the rapid detection of chemicals that induce cytotoxicity in contaminated food samples, pharmaceutical preparations and environmental samples. Our studies also provided a means for future whole-cell based lab-on-a-chip platform for wide applications in screening drugs, toxic chemicals, gases and other contaminated chemicals that pose serious threat to living cells.

Conclusion

A novel transducing electronic transducing platform, named interdigitated capacitor has been developed for biosensing applications. We have demonstrated over its capabilities for the detection of panel of CVD risk proteins in single and multiple biomarkers detection formats. This strategy was later applied to determining size dependent toxicity of engineered nanomaterials through interfacing living cells as biological reporters as opposed to conventional antigen/antibodies. The specificity of the biosensor mainly comes from the biological element that is interfaced on electronic transducer. While the transducer itself have the same function but the level of signal it produces heavily relies on the biological component of a biosensor. In this study, the capacitive response was measured using non-Faradaic impedance spectroscopy (nFIS) method. We are further working toward the development and miniaturization of biosensors in a way that it can suit to the current requirement of point-of-care applications that is commonly referred to as a hand-held device. The basic principle of these biosensors remains the same while the Moore's law makes all of

these devices much smaller, yet more useful and more powerful. Therefore, we foresee the challenges that lead us to developing a miniaturized point-of-care device, encompassing all of the features that is seen in a conventional larger dimension (equipment) of a current biosensor.

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