Design of a Nucleic Acid Biosensor Using COMSOL Multiphysics®

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Abstract: The detection of target biomarkers (proteins, DNA or antigens) from biological samples such as serum, urine or blood in high-throughput manner with high specificity is the starting point of developing effective therapy for many diseases including cancers. Currently available bio sensing techniques that are involving in the detection of biomarker molecules in patients' samples do not have the adequate sensitivity and limit of detection to be effectively detect early stage of cancer. Especially, the limit of detection of the current biosensing techniques varies from molar (M) to few nano/pico (10^{-12}) molar and this limit is insufficient for early detection of cancer. To address these issues, we proposed a low-cost and high-throughput technique based on dielectrophoresis (DEP). In this work, we have used the COMSOL software to design electrodes which were used in DEP based biomarker detection.
Keywords: Dielectrophoresis, Electric field gradient, miRNA

Introduction

Cancer is one of the major causes of death around the world [1]. According to the American Cancer Society, 1,688,780 new cancer cases are expected to be diagnosed in the US and about 600,920 Americans are expected to die due to cancer in 2017. For example, pancreatic cancer (PC) is the fourth leading reason of cancer death. In 2015, there were approximately 50,000 deaths occurred due to PC in the US [3]. The prevalence of PC has steadily increased in the last decade, making PC a major health trouble in the US [3]. Current imaging based detection methods available in medical industries do not have the capability of detecting PC at a treatable stage; therefore, many patients were not treated successfully. Deep location of the PC in the body made the imaging based detection into failure. Conventional imaging methods do not have the capability to image the entire pancreas with sufficient resolution for diagnosis; therefore, alternative biomarker detection methods are needed. Previous studies have shown that various cancers, including PC, can be detected earlier using clinical blood biomarkers [4, 5].

The term biomarker generally refers to a broad subcategory of measurable pointers of some biological condition, pharmacological responses or pathogenic process [6, 7]. World Health Organization (WHO) and the United Nations and the International Labor organization, defined the term biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [8]. Among biomarker categories, proteins, DNAs, miRNAs are included in one of the most important group of biomarkers, which usually containing in blood, saliva, serum or tissue, and can be used as an effective pointer of the disease states [7].

Currently, the ELISA (enzyme-linked immunosorbent assay) is the gold standard for protein detection in biological samples [16]. The detection limit of ELISA is about 250 pg/mL [17]. However, to detect/quantify analytes (proteins) that are related to various stages of tumors including early detection require to detect well below the current limit of the ELISA. For example, Interleukin 6 (IL-6) levels of early oral cancer patients have <100 pg/ml and prostate specific antigen (PSA) level of the early stage of prostate cancer patients is about 1ng/mL [18]. Further, it has been reported that there are << pg/mL analytes in the early stage of tumors [18].

Real-time polymerase chain reaction (RT-PCR) and next-generation sequencing are the gold standard for DNA, miRNA detection in biological samples [6]. Next-generation sequencing is very costly (>$1000) and low throughput; therefore, it is not suitable for routine clinical testing. RT-PCR requires stable genes to serve as a reference. In the context of cancers, finding stable genes to use as references is technically difficult. Further, RT-PCR reactions are expensive and time consuming (>4 hrs); thus, it is also not suitable for routine testing [7]. There are other techniques, including microarrays and electrochemical and hybridization-based sensors, but they are fundamentally incapable of detecting rare miRNAs (<pM) from a complex mixture of RNAs [6]. These techniques use diffusion or a combination of diffusion and sample flow to apply target miRNAs to detection electrodes or complementary capture molecules [6,7].

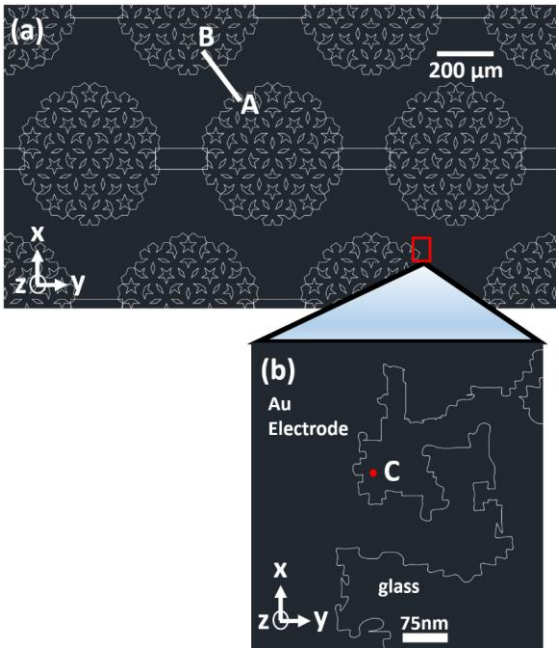

Figure 1. Electrode designs proposed for detecting biomarkers. (a) indicates the AutoCAD design of the electrode and (b) indicates the AutoCAD design of the nano-detection hotspots.

Diffusion is not a selective application method. Molecular crowding near capture-molecules or electrodes can produce steric hindrance, which greatly affects sensitivity, limit of detection, and sensing throughput, especially when trying to detect miRNAs with low molarities. Further, diffusion is not a steady-state process and produces results with a large degree of inter-sample variability, especially at sub-picomolar concentrations.

To address these issues in the current detection techniques, we designed and fabricated a new class of biosensor that use a fundamentally different sensing approach to those currently available. Rather than using a diffusion-based method, we will apply a selective attractive force named Dielectrophoretic force (DEP) on fluorescently labeled target molecules to enrich the target biomarker in the detection electrodes and eliminate the molecular crowding.

When a fluorescence particle trapped in between a metallic nano-gap and exposed to the emission wavelength, it will enhance the fluorescence emission by billion times [21,22]. Currently other research groups focusing on this MEF effect to develop a new effective method of detection. However, one of the inherent problems associated with the MEF methods is not being able to place the target molecules between nano metallic structures [22]. To overcome this problem we have used dielectrophoretic force (DEP) to selectively bring and trap the fluorescently labeled target biomarkers at the nanoscale gold detection hotspots located at the periphery of the microelectrode array.

Theory

Dielectrophoresis is a motion of suspensoid particles relative to the suspended medium resulting from polarization forces produced by an inhomogeneous electric field [9-13].

Mathematically, the time-average DEP force acting on a spherical isentropic homogeneous dielectric particle in a non-uniform electric field can be represented by

$$F_{DEP} = \frac{1}{2}\alpha \nabla|E|^2 \qquad (1)$$

where α is the polarizability of the molecule, E is the root-mean square of the electric field and $\nabla|E|^2$ is the electric field gradient [11-13]. The sign of α can be positive or negative depending on the frequency of the electric field and molecule type (proteins, miRNA, DNA, etc.) [14]. Positive indicates an attractive DEP that migrates the molecule toward the highest $|E|^2$, and negative indicates a repulsive DEP that migrates the molecule away from the highest $\nabla|E|^2$. The numerical value of α is based on the conductivity and permittivity of the buffer and the molecule type [14]. Therefore by applying a frequency that generates a large positive α on target biomarker molecules and a large negative or no α or on non-target biomarker molecules, we will be able to selectively trap target biomarker molecules at detection hotspots. Except the applied signal frequency and the voltage, we cannot physically control anything to increase the force act on the target biomarker molecules. The only way to increase the DEP force act on the target biormarker is through the electric field gradient $\nabla|E|^2$.With large DEP force, it can be easily improved the biomarker detection performance. In this paper, we have demonstrated the design of a biosensor device with detection hotspots, using COMSOL Multiphysics® software.

Design of Electrodes using COMSOL

We came up a micro electrode design that can generate large electric field gradients at the detection hotspots and these electric field gradients will produce large DEP forces on target biomarkers for high throughput detection from the biological

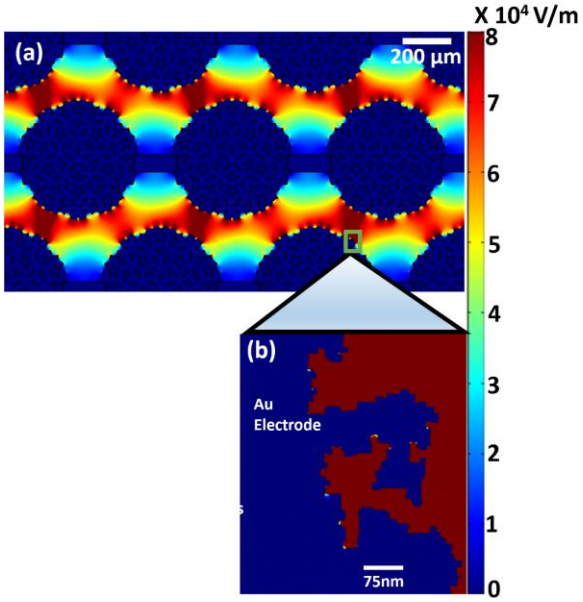

Figure 2. Calculated normalized electric field strength using COMSOL software. (a and b) indicates the normalized electric field Electric field strength of the electrode and the nano-detection hotspots respectively.

samples. Our electrode design is indicated in "Figure 1". To analyze the electric field gradient pattern and the normalized electric field pattern, first, electrode and the nano-detection hotspots were drawn to a scale using AutoCAD software. We saved the drawing files as .dxf file format, which will support COMSOL software to import and analyze the design directly. We performed the simulation separately for the electrode design and the nano-detection hotspots design to avoid the meshing problems and the memory overloading. COMSOL software was opened, and 3D space dimension was selected under the model wizard. AC/DC– electric currents (ec) was selected under the physics section and frequency domain studies was selected from the studies section for the both simulations.

For the electrode design simulation, the saved AutoCAD drawing was imported to the Geometry section and the length unit of the geometry was set as µm and the angular unit was set as degrees. After that the 2D drawing of the electrode design was extruded by 0.1 µm. Then a cube and silica glass slide were drawn on the top and the bottom of the electrode plane respectively. After the geometry section finished the gold was selected as a materiel for the electrode (conductivity: $43x10^6$ S/m, relative permittivity: 6.9) [15] and phosphate buffer saline (PBS) was selected as a material for the cube (conductivity: 1.6 S/m, relative permittivity: 80.3). Then we set the boundary conditions for the electrodes. We set 5 V to one electrode and -5 V voltage for the other electrode, we set the insulator boundary for the outer side of the cube.

Then we have solved equation 2 and calculated the electric field strength

$$E=-\nabla V \qquad (2)$$

where ∇ is the vector operator, E is the electric field strength in (V/m)[20], and V is the voltage different between the electrodes in V.

A 10 Vp-p and 120 kHz frequency was applied. Then design was meshed using swept mesh technique. First the electrode bottom plane was meshed using free triangular mesh with the maximum element size of 10 µm and the minimum element size of 0.1 µm. Secondly the free triangular mesh was swept towards the top plane of the electrode with the maximum element size of 0.005 µm and the minimum element size of 0.001 µm. Then the cube and the glass slide domain was swept with the maximum element size of 5 µm and the minimum element size of 0.1 µm. Finally, the simulation was performed and the normalized electric field strength was calculated (see "Figure 2(a) "). The normalized electric field pattern was determined by the inbuilt parameter ec.normE in the software and the electric field gradient was calculated in x, y directions by using the equation 3 given below

$$G = \sqrt{\left(\frac{d(ec.normE^2)}{dx}\right)^2 + \left(\frac{d(ec.normE^2)}{dy}\right)^2} \qquad (3)$$

where the G is the magnitude of electric field gradient and $ec.normE$ is the magnitude of the electric field (normalized electric field strength) in V/m.

For the nano-detection hotspots simulation the saved AutoCAD drawing was imported to the Geometry section and the length unit of the geometry was set as nm. After that the 2D drawing of the electrode design was extruded by 100 nm. Then design was meshed using the same swept mesh technique. First the electrode bottom plane was meshed using free triangular mesh with the maximum element size of 10 nm and the minimum element size of 0.1 nm. Secondly the free triangular mesh was swept towards the top plane of the electrode with the maximum element size of 5 nm and the minimum element size of 0.1 nm. Then the cube and the glass slide domain was swept with the maximum element size of 500 nm and the minimum element size of 0.1 nm. Other than this every parameter was kept similar as the electrode simulation. Finally, the simulation was performed

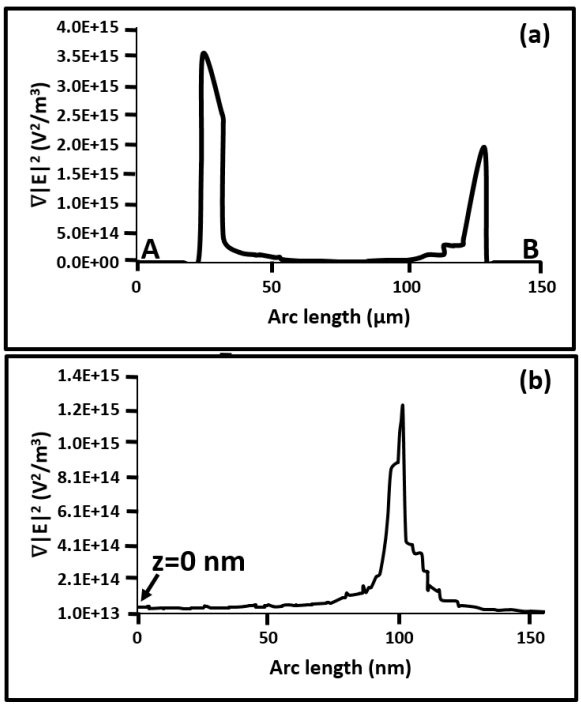

Figure 3. Electric field gradient analysis results through the cut lines mentioned in Figure 1. (a) Variation of electric field gradient along the contour mentioned as A-B in the Figure 1(a) and (b) Variation of electric field gradient along the contour C (z=0 plane to z=150nm plane) mentioned in Figure 1(b).

and the normalized electric field strength was calculated (see "Figure 2 (b)").

Finally, variations of the electric field gradients were plotted in the contours. "Figure 3" indicate the results. From the plots, it is obvious that our electrodes designs are generated extremely large electric fields and gradients necessary for high throughput biomarker detection. From the simulation results the maximum electric field gradient generated by our electrode was 3.89 x10^{15} V^2/m^3. Therefore, this electrode design can be used in high throughput biomarker detection applications. The electric field gradient pattern data was published earlier [19].

Results and Conclusions

We have designed an electrode array with nano-detection hotspots that can generate extremely high electric field gradient. We have quantitatively calculated the expected electric field gradients using COMSOL software and the calculated electric field gradient in X-Y plane is 3.89x10^{15} V^2/m^3. We found the maximum electric field gradient will be distributed on the top of the nano-detection hotspots (100nm). Therefore the fluorescently labeled target biomarkers will attracted to the nano-detection hotspots and it will be detected through the fluorescent microscope with a higher factor of enhancement. Finally, we have utilized the standard micro-fabrication techniques to develop of our electrode design. Currently, we are performing biomarker sensing experiments in human serum to find the purity, recovery and throughput of our biomarker detection device. Finally, we hope to test this device in real cancer patient biological samples to detect the cancer related target biomarkers.

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