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Gold Nano-Urchins Enhanced Surface Plasmon Resonance (SPR) BIOSENSORS for the Detection of Estrogen Receptor Alpha ($ER\alpha$)

Jian Yi Pae , Radhika V. Nair, Parasuraman Padmanabhan , Gayathri Radhakrishnan, Balázs Gulyás, and Murukeshan Vadakke Matham

Abstract—Breast cancer is the deadliest form of cancer in women, but it also has very high survival rates when treated early. Existing breast cancer detection methods such as mammography are ineffective in the early detection of breast cancer due to the low uptake among women and the need for regular screening. Therefore, biosensors have been proposed as an alternative method for cost-effective breast cancer screening which may encourage more women to go for screening more regularly. In this paper, a gold nano-urchins enhanced plasmonic sensor is demonstrated for the detection of a typical breast cancer marker, estrogen receptor alpha ($ER\alpha$). The localized field confinement effect due to the sharp features of the nano-urchins amplifies the biosensing signal and improves its sensitivity as compared to a conventional surface plasmon resonance (SPR) biosensor.

Index Terms—Bionanotechnology, biomedical applications, nanoparticles, plasmons.

I. INTRODUCTION

STATISTICS from the Global Cancer Observatory, International Agency for Research on Cancer, World Health

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Organization (WHO), has shown that breast cancer is the most common form of cancer in women worldwide and the top cause of cancer-related death among women [1], [2]. However, despite having such high mortality, the 5-year relative survival rates for patients diagnosed with Stage 2 or earlier stages of breast cancer are more than 90% [3]. This implies that patients who are diagnosed with earlier stages of breast cancer and underwent the appropriate treatment are very likely to survive for at least 5 years. Hence, it is important to detect the early onset of breast cancer to reduce the associated risks involved and for better chances of survival [4]. The existing breast cancer detection techniques such as mammography, sonography, magnetic resonance imaging (MRI), etc., are very effective for the detection of breast cancer [5]–[8]. However, for breast cancer to be detected early, consistent screening and monitoring are required which inevitably leads to greater financial burden [9], [10]. Additionally, the lack of awareness and understanding of the risk of breast cancer also resulted in low uptake of breast cancer screening due to the perceived hassle of undergoing screening at periodic regular intervals [11], [12]. This is particularly prevalent in countries where there are limited facilities and lack of accessibility to clinics dedicated to breast cancer screening [13]. All these factors may explain why breast cancer remains so deadly even with such promising survival rates.

In recent years, there is an increasing interest in the research and development of biomedical sensors as point of care (POC) devices for breast cancer diagnostic applications [14]. These biosensors can provide in situ screening of patients' physiological fluids such as blood, serum, saliva or milk for detection of different breast cancer biomarkers with relatively high sensitivity and accuracy [15]–[17]. Coupled with the development of cost-effective fabrication methods, the development of biosensors could, in the future, allow women to screen for breast cancer biomarkers at their own convenience with little discomfort [18], [19]. This would allow women to monitor for the early onset of breast cancer and hopefully prompt them to seek medical advice early.

With the advancement in optics research, optical biosensors, especially those based on the plasmonic effect, are becoming increasingly popular for breast cancer diagnostic applications [20]–[22]. Using the phenomenon of surface plasmon resonance (SPR), optical biosensors can achieve high sensitivity and low detection limits by exploiting the highly concentrated surface plasmon (SP) waves that are located at the metal-dielectric

Fig. 1. Model of a (a) Au nano-sphere and, (b) Au nano-urchin (both have a particle size of 100 nm). (c) Simulated extinction spectrum of the Au nano-sphere and Au nano-urchin.

interface of the sensor surface [23]. Additionally, signal amplification can be introduced to SPR biosensors to improve its sensitivity for detection of smaller biomolecules, such as DNA for breast cancer gene screening. This could be achieved using nanomaterials including nanoparticles and 2D materials such as graphene [24]–[26]. These nanomaterials can be used as amplification tags where they are attached to the analytes to induce a larger SPR signal or the nanomaterials could be layered over sensing surface of the SPR biosensor to couple the SP wave for secondary excitation of localised SPR (LSPR) in the nanomaterials [27]–[29]. In this recently demonstrated sensor, the surface of a conventional SPR biosensor was covered with a gold (Au) nano-dot array [30]. They showed a significant enhancement in the detection limit for the detection of streptavidin.

In this paper, an Au nano-urchins enhanced SPR biosensor was developed to increase its sensitivity as compared to a conventional SPR biosensor. Due to the sharp urchin-like features, the plasmonic waves can be further confined to an even smaller region as compared to traditional nanoparticles which are spherical [31]–[33]. To support this finding, theoretical simulations were used to compare the field enhancement of nanoparticles with different geometrical shapes and sizes. Based on the obtained result, it was discovered that the Au nano-urchins would have a significantly larger field enhancement factor as compared to Au nano-spheres. For demonstration, an Au nano-urchins enhanced SPR biosensor was experimentally validated for the detection of estrogen receptor alpha ($ER\alpha$). $ER\alpha$ is an important breast cancer marker and the overexpression of $ER\alpha$ is used as a diagnostic indicator of the onset of hormone-dependent breast cancer [34]–[36]. For comparison, the experiment was repeated using a conventional SPR biosensor and an Au nano-spheres enhanced SPR biosensor.

II. MATERIALS AND METHODS

A. Field Enhancement in Au Nano-Urchins

The geometrical shape and sizes of the nanoparticles have a strong influence on their ability to support the localized surface plasmons wave. Therefore, finite-difference time-domain (FDTD) simulation was first used to compare the field enhancement factor between two different nanoparticle geometry – namely, an Au nano-spheres (Fig. 1a) and an Au nano-urchins (Fig. 1b) design. Au is chosen as the material due to its biocompatibility and high affinity with the biomolecules. To start, the

Fig. 2. Simulated electric field intensity and the field enhancement factor of the (a) Au nano-sphere, and (b) Au nano-urchin.

size of each design was modelled at 100 nm and their respective extinction spectrum was simulated as shown in Fig. 1c. From the simulation, the peak extinction is identified to be 608 nm and 637 nm for the Au nano-spheres and Au nano-urchins, respectively. This value would correspond to the resonance wavelength of the nanoparticles where the electric field enhancement is the greatest.

Using a frequency-domain power monitor, it is possible to determine the electric field intensity distribution of the localised surface plasmon wave around the nanoparticle (refer to the supplementary data for the FDTD simulation window). The excitation source is set to be a plane wave which corresponds to the respective resonance wavelength values obtained from the previous simulation. The field enhancement factor of Au nano-spheres is 135 (Fig. 2a) which is much lesser as compared to the Au nano-urchins value of 1050 (Fig. 2b) (refer to supplementary data for the unscaled results). From this result, the Au nano-urchins would be a more effective nanoparticle choice for the development of biosensors as it has a higher field enhancement factor which would lead to better sensitivity.

Different sizes of Au nano-urchins were also simulated to investigate its effect on the field enhancement factor. First, the extinction spectrum of Au nano-urchins with varying particle sizes was simulated to determine its resonance wavelength (refer to supplementary data for the extinction spectrum). Based on this, the respective electric field intensities were then simulated by fixing the excitation wavelength at the resonance wavelength as shown in Fig. 3 (refer to supplementary data for the unscaled results). Based on the results, the field enhancement factor for the Au nano-urchins with 60 nm, 80 nm, 100 nm, and 120 nm particle sizes are 243, 454, 1050, and 804, respectively. Therefore, Au nano-urchins with 100 nm particle sizes were determined to be the most optimal geometry and size for the field enhancement in the biosensor as it has the highest field enhancement factor.

The Au nano-urchins will then be coupled to a conventional SPR biosensor by layering it over the Au surface. Therefore, the simulation model was modified to validate the electric field intensity distribution across the metal-dielectric interface of the SPR biosensor. The light source for the simulation is selected to be 632.8 nm wavelength which is the emission wavelength of the He-Ne laser used in the SPR system. The first simulation is conducted in angular interrogation mode by scanning the angle of incidence of the p-polarized light source and monitoring the

Fig. 3. Simulated electric field intensity of Au nano-urchins with a particle size of (a) 60 nm, (b) 80 nm, (c) 100 nm, and (d) 120 nm.

Fig. 4. FDTD simulation screenshot window of an (a) conventional SPR biosensor, (b) Au nano-spheres, and (c) Au nano-urchins enhanced SPR biosensor. (d) Electric field intensity simulation of the SPR sensor without Au nano-urchins, (e) with Au nano-spheres, and (f) with Au nano-urchins (the 50 nm gold film layer is located above $y = 0$).

reflectance. In this mode, the resonance angle is identified by a sharp drop in the reflectance. By running a parameter sweep, the Au layer thickness is optimised to be 50 nm which gives the best coupling efficiency between the light source and the SP wave as shown in Fig. 4a. This result is then compared against two different models where a single particle of 100 nm Au nano-sphere is added to the dielectric interface of the Au layer as shown in Fig. 4b, and a single particle of 100 nm Au nano-urchin is added to the other model as shown in Fig. 4c.

The electric field intensity (along the x - z plane) of each model, at their respective resonance angles, is then simulated to determine the maximum electric field enhancement of all the biosensors configurations. Fig. 4d shows the electric field intensity of a conventional SPR biosensor while Fig. 4e and Fig. 4f shows the electric field intensity distribution of the Au nano-sphere and Au nano-urchin enhanced SPR biosensor, respectively. There is a significant enhancement to the maximum electric field (1.78 to 160) when the Au nano-sphere is added and the electric field increased further (160 to 2×10^4) when

Fig. 5. Schematic of the proposed Au nano-urchins based SPR biosensor.

the Au nano-sphere is replaced with the Au nano-urchin. The surface plasmon wave generated by the Au film is coupled to the nanomaterials leading to localized confinement of the surface plasmon wave. This could be a possible explanation behind the mechanism for the significant electric field enhancement in the proposed SPR sensor.

However, it is important to note that in the Au nano-sphere enhanced SPR biosensor, the electric field is only confined to the region near the interface of the Au film and the Au nano-sphere. In comparison, for the Au nano-urchin enhanced SPR biosensor, the electric field is highly concentrated all around the spikes of the Au nano-urchins. This could explain why the Au nano-urchin have a better field enhancement factor as compared to the Au nano-sphere. Another possible reason is because the excitation wavelength (632.8 nm) is very close to the extinction wavelength of the Au nano-urchin (637 nm) as compared to the Au nano-sphere (607 nm) which allow for better coupling.

B. Au Nano-Urchins Enhanced SPR Biosensor Design

The schematic of the proposed Au nano-urchins based SPR biosensor is shown in Fig. 5. A high refractive index prism ($N_{\text{SF11}}, n_{632.8} = 1.77860$) is selected after a series of simulation to determine the most optimal material to allow the incident laser (632.8 nm) used in the SPR system to be coupled for excitation of surface plasmons at the interface with the sample fluid. Au is selected as the substrate for the SPR biosensor for its good affinity with the Au nano-urchins and good biocompatibility. The Au surface is functionalized by immobilizing a layer of anti-ER α antibodies which will function as the bioreceptor for label-free detection of the analyte (ER α). A microfluidic cell is added to contain the buffer fluid and allow the analyte to be introduced for the biosensing measurement.

C. Chip Fabrication and Functionalization

50 nm thick Au is sputtered directly on the hypotenuse face of the prism with a thin layer of Cr as the adhesion layer. Next, Au nano-urchins with an average particle size of 100 nm were purchased commercially (0.1 mM concentration suspended in phosphate-buffered saline (PBS) solution, Sigma-Aldrich) and characterized using transmission electron microscopy (TEM) as shown in Fig. 6.

The Au nano-urchins were immobilized on the Au surface by first functionalizing the Au surface using EDC/NHS coupling method as detailed in [37]. Fig. 7 shows the scanning electron

Fig. 6. TEM image of the Au nano-urchins.

Fig. 7. SEM image of the Au nano-urchins on the Au surface.

microscope (SEM) image of the Au surface after the Au nano-urchins have been immobilized.

The anti-ER α antibodies (1:100 dilution in PBS, ab16660, abcam) are then bound to the Au surface by dipping the prism in a solution containing the antibodies for more than 8 hours overnight. The microfluidic cell is fabricated from polydimethylsiloxane (PDMS) by adopting a soft lithography process detailed in [38].

D. Analytes Preparation

Estrogen receptor alpha (ER α) (recombinant protein, concentration: 4 μ g at 0.15 mg/ml, ab82606, abcam) is specifically chosen to work with the anti-ER α antibodies. Upon delivery of the ER α , PBS is added, and it is aliquoted into different

Fig. 8. Schematic illustration of the SPR system.

concentrations before being stored at -20°C . Samples were then taken out of the freezer and used immediately.

III. EXPERIMENTAL SETUP

A linearly polarized helium-neon (He-Ne) gas laser with approximately 632.8 nm emission wavelength is selected as the light source and a linear polarizer is used to ensure that the light is p-polarised for excitation of the SP. To vary the incidence angle of the light, the prism is attached to a prism table which is mounted on a rotation stage. The intensity of the reflected light is then measured by a photodetector which is mounted on a separate rotation stage and the detected intensity value is displayed on a power meter. The two rotation stages (Standa Ltd.) are mounted on a special mount which allows both rotation stages to rotate about the same axis simultaneously with full 360° continuous travel range as shown in Fig. 8. They are also motorised and controlled via a LabVIEW program to achieve high repeatability.

Before the start of the measurement, PBS is first injected into the microfluidic cell which is also used as the buffer solution. The analyte is then introduced into the microfluidic cell and the reflectance is then measured in rotation steps of 0.1° . The measurement is repeated every 5 min for about 30 min. Based on the obtained SPR reflectance curve, the resonance angle can be identified by the angle where there is a sharp drop in reflectance.

IV. RESULTS AND DISCUSSION

By comparing the respective resonance angles obtained for the various ER α concentrations against the control experiment, it is possible to calculate the resultant shift in the resonance angles due to the presence of ER α . The plot of the resonance angle shift against the ER α concentration would then indicate the SPR biosensor sensitivity which is defined by the shift in the resonance angle due to the presence of the ER α . To keep the data consistent, the maximum shift in resonance angle values for each sample were used.

To further validate the results, the ER α sensing experiment was repeated with an Au nano-spheres enhanced SPR biosensor and a conventional Au film SPR biosensor without any nanoparticles. 100 nm diameter Au nano-spheres were purchased commercially (0.1 mM concentration suspended in PBS solution, Sigma-Aldrich) and functionalised using the same method described in the previous section. Fig. 9 shows the combined plot of the resultant shift in the resonance angles with respect to the various ER α concentrations for the three different biosensor configurations.

Fig. 9. Plot of the shift in resonance angle against the various ER α concentrations for the different SPR biosensor configurations.

The sensitivity of the SPR biosensors can be determined from the slope of the linear fit. For the Au nano-urchins enhanced SPR biosensor, the slope is approximately 6.7°/nM while the slope for the Au nano-spheres enhanced SPR biosensor is 5.6°/nM. The conventional SPR biosensor has a slope of approximately 2.5°/nM. Therefore, it is evident that both the nanoparticles enhanced SPR biosensor has a better sensitivity than the conventional SPR biosensor. Furthermore, the Au nano-urchins enhanced SPR biosensor has the best sensitivity out of the three biosensor configurations which support the findings from the theoretical simulations.

Based on the obtained results, it is shown that Au nano-urchins showed high field enhancement factor compared to other nanoparticles of different geometry leading to best sensitivity. The theoretical results are validated experimentally. The addition of a layer of anti-ER α antibodies which acts as the bioreceptor in the developed biosensor, created a gap between the Au nano-urchins and the ER α analytes. This reduces the effectiveness of the localized field confinement as it is only at the surrounding regions near the tips of the Au nano-urchins and leads to a lower than expected sensitivity in the developed SPR biosensor. Nonetheless, the Au nano-urchins enhanced SPR biosensor still showed the best sensitivity out of the three different biosensor configurations discussed.

V. CONCLUSION

An Au nano-urchins enhanced SPR biosensor was developed to enhance the sensitivity for the sensing of ER α . Different concentrations of ER α were sensed by the developed SPR biosensor to validate its sensitivity. A significant improvement in the SPR signal was observed when comparing the developed Au nano-urchins SPR biosensor against an Au nano-spheres SPR biosensor and a conventional SPR biosensor. This was also verified using theoretical FDTD simulations. It is envisioned that in the future, the Au nano-urchins SPR biosensor can be

developed into a highly sensitive ER α biosensor which could be potentially used for the early detection of breast cancer.

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