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Microfluidic Plasmonic Bio-Sensing of Exosomes by Using a Gold Nano-Island Platform

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Abstract**—**A bio-sensing method, based on the plasmonic property of gold nano-islands, has been developed for detection of exosomes in a clinical setting. The position of the gold plasmon band in the UV-Visible spectrum depends on the size and shape of gold nanoparticles as well as on the surrounding environment. By adsorbing various chemical entities, or binding them, the gold plasmon band will shift toward longer wavelengths and the shift is proportional to the concentration. Exosomes transport cargoes of molecules and genetic materials to proximal and distal cells. Presently, the standard method for their isolation and quantification from body fluids is by ultracentrifugation, not a practical method to be implemented in a clinical setting. Thus, a versatile and cutting-edge platform is required to selectively detect and isolate exosomes for further analysis at clinical level. The new sensing protocol, instead of antibodies, makes use of a specially synthesized polypeptide (Vn96), to capture and quantify the exosomes from different media, by binding the heat shock proteins from exosomes. The protocol has been established and optimized by using a glass substrate, in order to facilitate the next stage, namely the transfer of the protocol to a microfluidic environment. After each step of the protocol, the UV-Vis spectrum was recorded and the position of gold Localized Surface Plasmon Resonance (LSPR) band was measured. The sensing process was modelled, taking into account the characteristics of the nano-island structure, prepared by thermal convection and annealing. The optimal molar ratios of the most important chemical entities, involved in the detection of exosomes were calculated as well. Indeed, it was found that the results of the sensing process depend on the two major steps: the molar ratios of streptavidin to biotin-PEG-Vn96 and, the final step, the capture of exosomes by the biotin-PEG-Vn96 complex. The microfluidic device designed for sensing of exosomes consists of a glass substrate, sealed by a PDMS layer that contains the channel and a collecting chamber. In the device, the solutions of linker, cross-linker, etc., are pumped over the gold nano-islands and an Ocean Optics spectrometer is used to measure the position of the Au plasmon band at each step of the sensing. The experiments have shown that the shift of the Au LSPR band is proportional to the concentration of exosomes and, thereby, exosomes can be accurately quantified. An important advantage of the method is the ability to discriminate between exosomes having different origins.

Keywords**—**Exosomes, gold nano-islands, microfluidics, plasmonic biosensing.

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I. Introduction

EXOSOMES are a group of nano-scale extracellular communication organelles released by all cells, which transports cargoes of molecules and genetic materials to proximal and distal cells and are enclosed by a phospholipid bilayer. They are in the size range of 50 nm to 120 nm and are homogenous in nature. Exosomes are found abundant in all biological fluids, including urine, blood, ascites, and cerebrospinal fluid fractions of body fluids such as serum and plasma, as well as in the cultured medium of cells [1]-[3]. They are the intercellular communicators, which transport cargos between cells as shown in Fig. 1 and also spread proteins, lipids, mRNA, DNA, and are involved in the progression of diseases. Thus, they have the potential to be used for minimal-invasive molecular diagnostics.

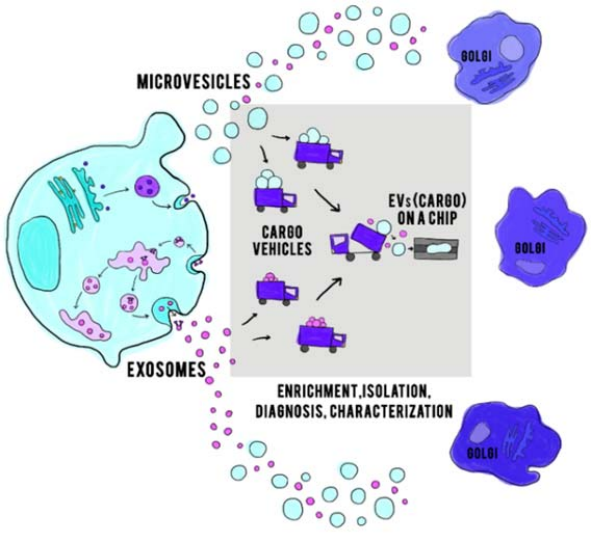


Fig. 1 Schematic of intercellular communication and transportation of cargo between cells

Presently, very few methods are available for the isolation and detection of exosomes. The golden standard method that is widely used for their isolation and quantification is ultracentrifugation. This method is time consuming, laborious, infrastructure intensive, may lack specificity so it may not be a practical method to be implemented in a clinical setting. Hence, an adaptable platform is required to selectively detect and isolate exosomes for further analysis at clinical level.

In order to capture EVs, a synthetic polypeptide called Vn96, specifically designed and validated to capture

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exosomes, is used [4]. The detection methods were initially carried out on a self-standing substrate level, by performing the sensing protocol in a discontinuous manner. Further, in order to enhance the sensitivity of the detection and to accomplish the molecular profiling of the captured exosomes, microfluidic devices were designed, developed and tested. The concentrations of the chemical and biological entities at each stage of biosensing were optimized in order to facilitate the transfer to the microfluidic stage.

The optical properties of noble metal nanostructures are extensively adapted for biological detection. Predominantly, the LSPR property of noble metal nanoparticles is widely used as a highly sensitive label-free technique for the detection of chemical and biomolecular binding events [5], [6]. The sensing mechanism consists of monitoring the change in the position of the LSPR band due to molecular interactions. The shift of the band towards longer wavelengths is due to the change of the refractive index of the surrounding media [7]-[12]. The aim of the present work is the detection of exosomes, based on the change in the position of Au-LSPR band corresponding to different binding events.

II. Materials and Methods

The substrates used in this experiment are microscope glass slides from Technologist Choice, Bio Nuclear diagnostics Inc. with a glass transition temperature, T_g = 820 °C. Gold(III) chloride trihydrate ($HAuCl_4.3H_2O$), sodium citrate were purchased from Sigma Aldrich. Polydimethylsiloxane (PDMS) is from Dow Corning. De-ionized (DI) water with a resistivity of 18 MΩ, used in all the experiments, was obtained from the NANO pure ultrapure water system (Barnstead). 11-mercaptoundecanoic acid in ethanol (NanoThinks Acid 11), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS), phosphate buffered saline (PBS) were obtained from Sigma Aldrich, Canada. PBS tablets were dissolved in DI water at 0.1 M concentration with a pH of 7.2. Streptavidin was purchased from IBA GmBH and biotin-PEG-Vn96 and MCF7 exosomes were supplied by the Atlantic Cancer Research Institute (ACRI) in Moncton, New Brunswick, Canada.

III. Experimental Results and Discussion

This section discusses the simulation of microfluidic structures, fabrication of microfluidic device and its testing using an Ocean Optics spectrometer for detection of exosomes. A scheme of the microfluidic devices, designed and simulated in COMSOL Multiphysics 5.2 is shown in Fig. 2. The design shows the inlet and outlet, connected to a microfluidic channel, containing a collection chamber. The designs are mainly analyzed for the streamline contours of fluid flow. The rhombic and triangular designs have some unused portions of space in the collection chamber, where the velocity of the fluid is so low that it might not cover that region. The fluid covers the entire region in the collection chamber of the circular design. Therefore, the circular design is selected for the fabrication of the device. The channel is 500 µm wide, its depth is 200 µm, and the collection chamber has a diameter of 5 mm. This diameter is considered relatively large, so that the fluid covers the entire region with considerably low velocity, enabling the binding of the compounds flowing in the channel to the gold nano-islands.

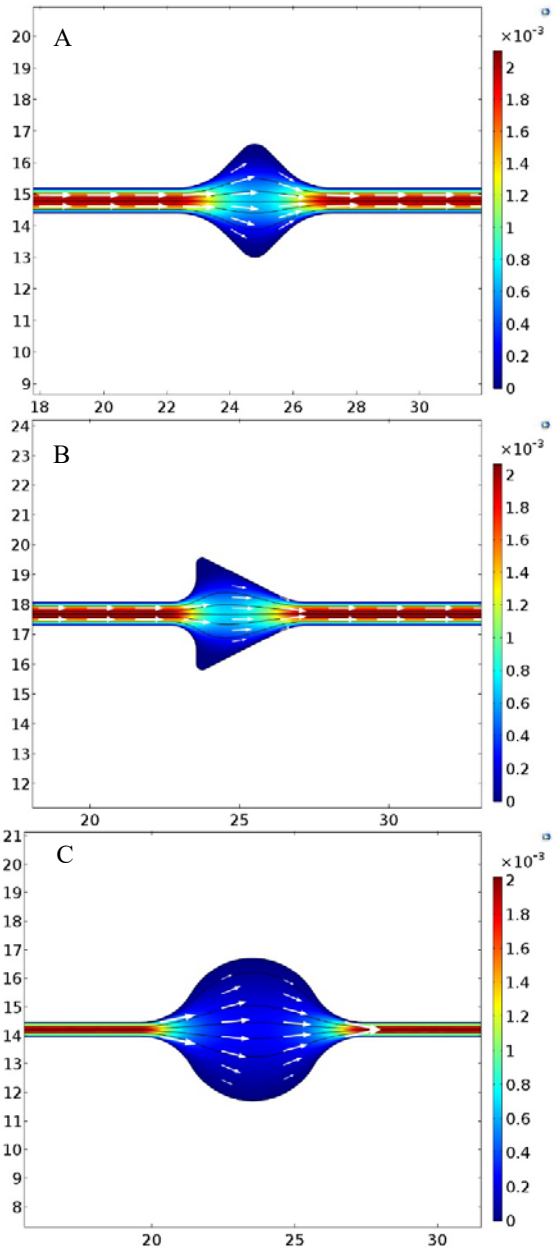


Fig. 2 Designed microchannel and predicted streamline contours (A) Rhombic design (B) Triangular design (C) Circular design

It can be seen that the device contains a wide opening around the middle of the channel which is a collection chamber where the chemical and biochemical entities will be collected during the biosensing protocol as shown in Fig. 4. The exosomes will be captured and isolated in this chamber. A mold for this design is fabricated on a silicon wafer, using a standard fabrication process [13] with a negative photoresist. The same concept can be used to create more designs for a

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better flow pattern in the channel.

The PDMS base (pre-polymer) and curing agent are mixed in the ratio of 10:1 by weight. The PDMS mixture was placed in a vacuum desiccator and degassed to remove the air bubbles. Then, the PDMS was casted onto a mold on a silicon wafer made with a standard fabrication process to make the PDMS microfluidic channel. Prior to PDMS casting, the mold was silanized by using few drops of the trichlorosilane at 60 °C for 1 hour on a hot plate, and covering it with a Petri dish. Then, the mold was placed in a Petri dish and the PDMS mixture was poured on the wafer to a thickness of ~2 mm and baked at 60 °C for 10 hours. On the wafer containing the microfluidic channel, the PDMS layer was then cut into individual samples of predefined size.

The microfluidic device is illuminated with a UV/visible light source through a 600 µm optical fiber. The transmitted light from the device is collected through another 600 µm optical fiber, which is linked to an Ocean Optics USB2000 spectrometer. This spectrometer is connected to a computer using Spectrasuite software to measure the absorption spectra. The schematic of the microfluidic device used with the Ocean Optics spectrometer setup for absorbance measurement is shown in Fig. 3.

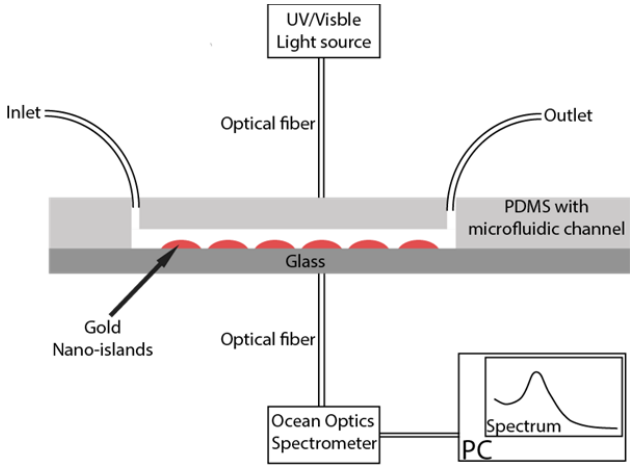


Fig. 3 Schematic of microfluidic device used in the Ocean Optics spectrometer setup for absorbance measurement

IV. Biosensing Protocol

The biosensing protocol shown in Fig. 4 used to capture the exosomes is an affinity-based approach [14] developed in our laboratory. The chemical compounds were first immobilized on a glass substrate with gold nano-islands in order to optimize the molar concentrations at each stage. They were optimized based on the stability of the LSPR shift. The optimal concentrations of streptavidin and the biotin-PEG-Vn96 complex were found to be 0.19 nM and 0.87 nM, respectively. After all the parameters were optimized, the whole protocol was transferred to a microfluidic environment.

Initially, the absorption spectrum of the gold nano-islands is measured in the collection chamber. Then, a Nano Thinks 11 solution is passed through the microfluidic channel at the flow rate of 10 µl/min continuously for 30 minutes and next incubated for 3 hours for the formation of hydroxyl bonds. Then the spectrum is measured, and EDC-NHS mixture is infused at same flow rate and the device is incubated again for 4 hours for the amidation. The same procedure is repeated with the streptavidin, biotin-PEG-Vn96 and the exosomes. With the adopted procedure, a shift in the peak of the Au LSPR in each spectrum is observed at every stage, confirming the binding of each compound.

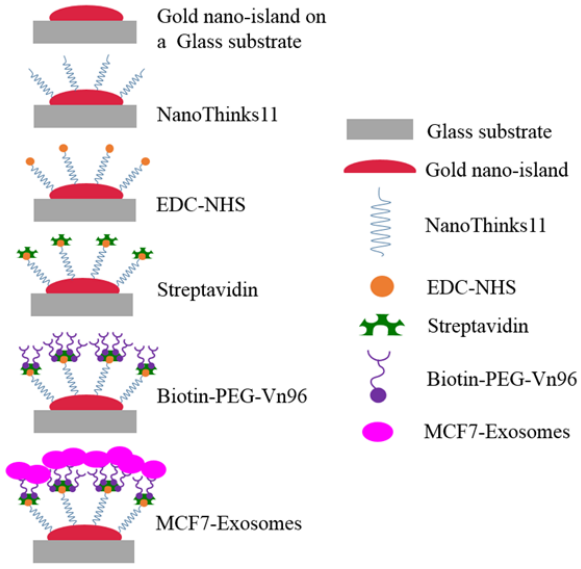


Fig. 4 Schematic of biosensing protocol used for detection of exosomes

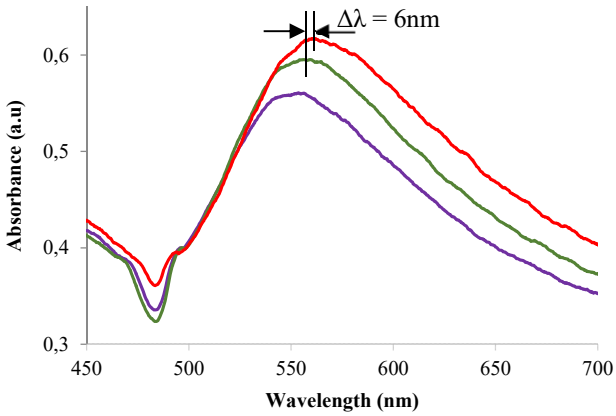


Fig. 5 Absorption spectra measured from Ocean optics spectrometer for last three stages

The Au-LSPR band is recorded at each stage of the biosensing protocol using an Ocean optics 2000 USB series spectrometer, with a fiber of 600 µm diameter. The Au-LSPR band is recorded after the functionalization of the nano-islands with the linker, followed by binding of the cross linker, the streptavidin, Vn96-linker-biotin and MCF7 exosomes. The observed spectra showed a shift towards longer wavelength of Au LSPR band. The shift upon the interaction of biotin-PEG-Vn96 and exosomes is found to be around 6 nm as shown in Fig. 5. For detection purposes, only the shift due to the final

capture step is considered.

V. Conclusion

In this work, a microfluidic method for the isolation and detection of exosomes has been developed. The detection is based on the sensitivity of the LSPR property of gold nano-islands to any change in the surrounding environment. The biosensing protocol is carried out in a microfluidic device, specially designed for the collection and detection of exosomes. The detection of exosomes in this work is possible because of the high affinity of exosomes toward the proteins located at the periphery of exosomes. This results in a red shift of the Au LSPR band and allows the quantification of exosomes.

The results indicate that label-free technique, based on the sensitivity of the Au-LSPR band to the surrounding environment is promising for the detection of MCF-7 exosomes by the immune-affinity approach using the Vn96 polypeptide. This approach seems to be better than the previous approaches. Thus this technique may lead to the miniaturization of device for point of care application.

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