Efficient Isolation of Exosomes and their Electric Field-Induced Lysis for Electrochemical Impedance Spectroscopy of released Biomarker: An approach towards Liquid Biopsy of Cancer

A thesis submitted

in partial fulfillment for the award of the degree of

Doctor of Philosophy

by

Pammi Guru Krishna Thej

SC16D049

Department of Avionics

Indian Institute of Space Science and Technology

Thiruvananthapuram, India

July 2023

Abstract

Early cancer detection is critical for cancer prognosis and diagnosis. Existing methods for cancer detection rely mostly on tissue biopsy or the isolation of circulating tumour cells from the individual's blood. Due to the fact that both of these methods include cell analysis, early cancer identification is not achievable in either scenario. Several researchers are aiming towards the isolation of exosomes from blood in order to circumvent this limitation. Exosomes, whose average diameter ranges from 20 to 200 nm, are cell-secreted vesicles that are common in the majority of biological fluids, including blood, urine, tears, sweat, breast milk, etc. Variations in exosomal size and composition can be attributed to several variables, including the individual's age, gender, and illness conditions. Exosomes, which are released by cells, facilitate communication between cells. Due to their diminutive size, exosomes can enter the bloodstream and travel to any distant site within the body. Exosome characteristics, such as size, protein load, and other factors, are cell-specific; therefore, their capture is essential for analysing the physical health condition of an individual, be it cancer or another disease. Although there are a number of approaches for extracting exosomes, there is a dearth of literature on their effective use in cancer prediction. The purpose of the thesis as a whole is to extract exosomes from human serum and use their protein for sensing. Under this overarching objective, this thesis has three objectives. The first objective is to investigate various nanoparticle−based exosome isolation techniques. The second goal is to lyse the exosomes in order to extract their protein content. Finally, we conduct an Electrochemical Impedance Spectroscopy(EIS) analysis of the protein content.

The primary objective was to be able to separate exosomes from serum using Colloidal Gold Nano Particles (GNP), so eliminating the requirement for an Ultracentrifuge. For this purpose, GNP were synthesised, and their concentration was determined using a UV-vis spectrophotometer. TEM was also used to examine the GNP structure. Functionalization of the prepared GNP with Polyethylene Glycol (PEG) improved the stability of the GNP in buffer solutions. The PEG-functionalized GNP were added to EDC/SNHS in order to activate the COOH of the PEG and render it appropriate for conjugation with Antibody specific for exosomal surface proteins. Antibody-conjugated GNP was treated with serum to establish GNP-Exosome complex, which can be isolated by centrifugation on a bench-top system. Several techniques, including Western blot, Transmission Electron Microscope(TEM), Nanoparticle Tracking Analyzer(NTA) and Dynamic Light Scattering(DLS),

are used to characterize the isolated exosomes. Membrane structures observed by TEM and the presence of surface proteins (CD9, CD63, CD81) and transmembrane proteins (HSP70) in the western blot indicated the extraction of exosomes from serum with minimal contaminating protein (such as albumin).

By immobilising serum exosomes on a flat surface, the second objective was to obviate the need for a bench-top centrifuge. Existing techniques for separating exosomes, such as ultracentrifugation and density gradient ultracentrifugation, are instrument-dependent, time-consuming, and lack specificity. In addition, centrifuge-based methods require resources such as centrifugation tubes, which can be completely eliminated if the exosomes are immobilised on the surface. In order to accomplish this GNP were coated on a silicon (Si) wafer and functionalized with PEG. Using EDC-NHS chemistry, anti-CD63 antibody was coupled to PEG, and serum was employed to immobilise exosomes on the Si surface. Exosome surface immobilisation has been confirmed by western blotting and quantified by NTA. It has been demonstrated that increasing the GNP density on the Si wafer increases the exosomes' size range and total amount. The approach provides various benefits, including the reusability of the Si surface for multiple isolations, the elimination of the centrifuge requirement, and the separation of exosomes in two hours. By altering the GNP size used to coat the Si wafer, we believe the suggested approach might be utilised to effectively isolate any specific subset of exosomes.

After successfully isolating exosomes on a flat surface, the isolation method is modified to be compatible with microfluidic platforms. This work comprises of two subparts, the immobilization and lysis of exosomes on Screen Printed Electrode (SPE), and the detection of the lysed protein. Separation of exosomes from human serum by immobilized on SPE was carried out in the same way as with the Si wafer. For lysis, immobilized exosomes were exposed to varying frequency and voltage sinusoidal and square wave volatges. In order to optimize the lysing-voltage and frequency, the lysate solution was incubated and analyzed for the presence of HSP70 protein on the SPE. EIS determined that, in contrast to square signals, low frequencies and specifically sinusoidal signals are ideal for lysing exosomes. Following exosome lysis by an electric field and signal optimization, EIS effectively detected HER2, a biomarker for breast cancer, in serum. The linear range of HER2 detection proven by the intended experiments renders the system suitable for detecting HER2 in the blood of cancer patients, hence making it useful to real-world applications. The results suggest that the electric field lysis of exosomes not only plays a significant role in the release of the cargo protein, but also increases the detection of surface proteins associated with exosomes.