CHAPTER 1

Introduction

Contents

1.1 Introduction

Protein-protein interactions are fundamental in the regulation of several biological cellular processes. The interactions between proteins are linked to several diseases such as neurodegenerative diseases, cancer etc. thereby attracting the attention of researchers in drug development [1]. The development of therapeutic drugs requires the characterization of protein-protein interactions and an understanding of their functioning in various bodily processes [2, 3]. Thus, the detection of protein and the analysis of affinity, binding kinetics etc. of protein-protein interaction is of critical importance. In this regard, immunoassays like Radioimmunoassay (RIA), Enzyme-linked Immunosorbent Assay (ELISA), Western Blot and amplification techniques such as Polymerase Chain Reaction (PCR) are the conventionally used detection methods having high sensitivity and specificity. However, not only do they involve the use of sophisticated and expensive instruments but also require long times and labour-intensive steps for processing technically complex assays in addition to the use of labels [4, 5].

Surface Plasmon Resonance (SPR) is a label-free, sensitive optical detection technique for the monitoring of biomolecular interactions involving protein, nucleic acid etc. which provides rapid, sensitive and real-time information, without the need for washing and labelling steps, requiring minute amounts of analytes advantageous for clinical applications. It is utilized across a variety of fields ranging from medical diagnosis to food technology, environmental monitoring etc. [6-8]. It relies on the measurement of change in the Refractive Index (RI) at the sensor surface which occurs due to the molecular interactions between a mobile analyte and a ligand molecule immobilized on the sensing layer. SPR can offer information not only on the specificity, affinity and concentration levels of the bio-molecular interactions but also provides an understanding of the kinetics of the reactions taking place [9, 10]. A plasmon is a collective oscillation of free electrons at the surface of a metal with respect to fixed positive ions. The Surface Plasmons (SPs) travel along the metal-dielectric interface accompanied by a longitudinal (TM or p-polarized) electric field decaying exponentially in both mediums. The Surface Plasmon Wave (SPW) is the collective electron density wave that propagates along the interface of metal and dielectric [11]. Figure 1.1 shows the schematic representation of the SPs propagating along the interface between metal and dielectric.

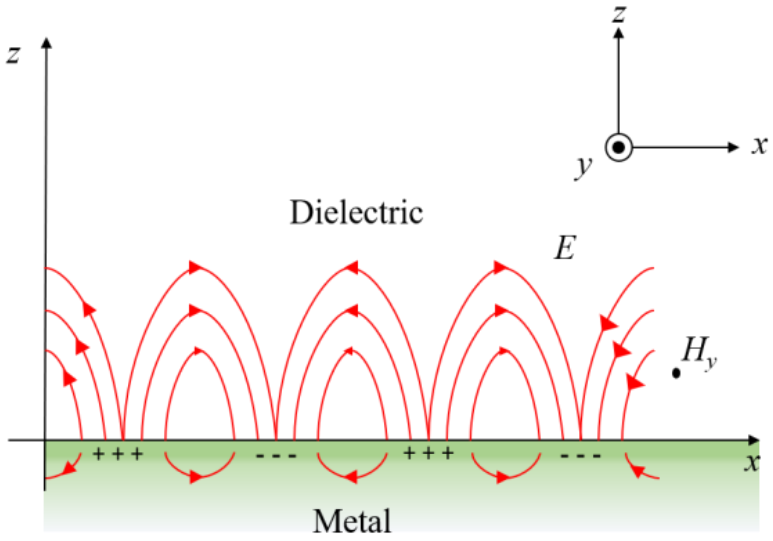

Figure 1.1: Schematic illustration of electromagnetic field of SPs propagating along the interface between metal and dielectric

The propagation constant (K_{SP}) of the SPW is given by [11]:

$$K_{SP} = \frac{\omega}{c}\left[\sqrt{\frac{\epsilon_M \epsilon_S}{\epsilon_M + \epsilon_S}}\right] \quad (1.1)$$

where ω is the frequency of the incident light, c is the velocity of light, ϵ_M and ϵ_S represent the dielectric constants of the metal layer and dielectric medium, respectively. The propagation constant of SPW is greater than that of the p-polarized light wave in a dielectric medium resulting in a momentum mismatch between the two. Thus, direct light cannot be used to excite SPs at a metal-dielectric interface. Therefore, the momentum and hence the wave vector of the light in a dielectric medium is increased using various coupling mechanisms such as prism, optical fiber, grating-coupled etc. Among them, the Kretschmann angular interrogation technique exhibits enhanced sensitivity, stability and high signal-to-noise ratio due to the use of light of a single wavelength of constant power and is the most widely used scheme for excitation of SPs [12-15]. In this technique, p-polarized light is passed through a prism, the base of which is coated with a metal (usually silver or gold) of suitable thickness to excite SPW. The incident light generates an evanescent wave at the prism-metal layer interface at an angle equal to or greater than the angle required for Attenuated Total Reflection (ATR).

The propagation constant (K_{ev}) of the evanescent wave is given by [11]:

$$K_{ev} = \frac{\omega}{c} n_p sin\theta \quad (1.2)$$

where θ, ω, c and n_p are the angle of incidence of light, frequency of light, speed of light and the RI of the glass prism respectively.

For the excitation of SPs, the wavevector of the propagation constant of the evanescent wave must match with that of the SPW of similar frequency and state of polarization. This occurs at a particular angle of incidence, Θ_{SPR}, called the resonance angle and the corresponding resonance condition is given by the following equation:

$$\frac{\omega}{c} n_p sin\theta_{SPR} = \frac{\omega}{c}\left[\sqrt{\frac{\epsilon_M \epsilon_s}{\epsilon_M + \epsilon_s}}\right] \quad (1.3)$$

The coupling of the evanescent wave with the SPW produces a dip in the intensity of the reflected light. Any changes in the RI of the sensor surface cause corresponding shifts in the resonance dip. When the change is due to the biomolecular interaction at the vicinity of the metal surface the shift in the resonance dip can be monitored to estimate the level of molecular interaction. Figure 1.2 provides a schematic illustration of SPR in the Kretschmann angular interrogation configuration.

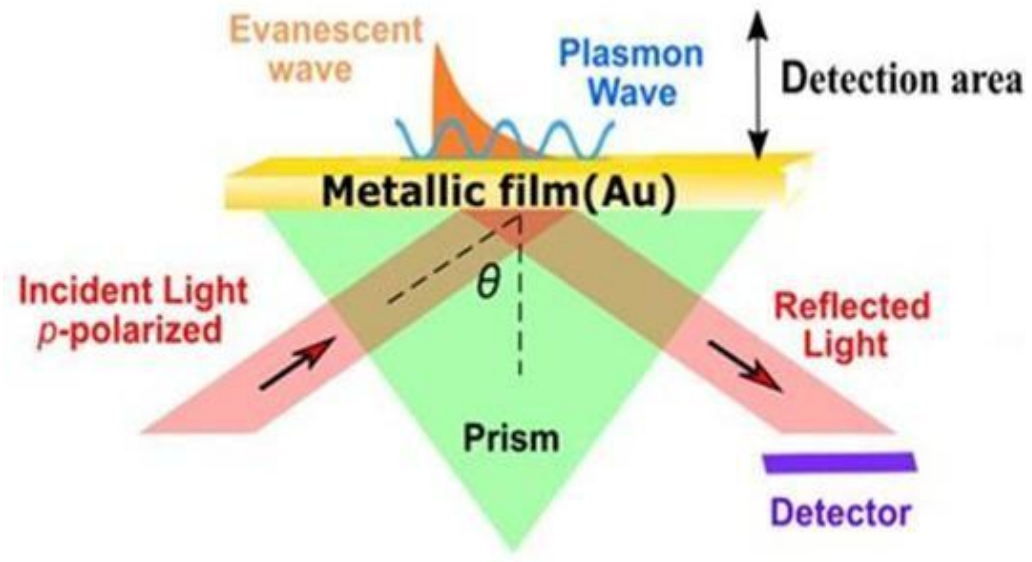

Figure 1.2: Schematic representation of SPR in the Kretschmann angular interrogation configuration [16]

The application of SPR for the label-free and real-time detection of biomolecular interaction was first demonstrated by Nylander *et.al.* in 1983 [17]. They used the Kretschmann angular interrogation technique to observe the binding of anti Immunoglobulin-G (IgG) to IgG using a thin film of silver by monitoring the change in reflectivity with the angle of incident light. Since then the possible fields of application of SPR technology have expanded to several areas such as drug discovery, disease diagnosis, food safety, environmental monitoring etc. The integration of plasmonic sensing technologies such as SPR with microfluidics has the potential to deliver bio-sensing platforms offering benefits like enhanced sensing efficiency, requirement of lesser volumes of ligands and analytes, precise fluidic control and miniaturization [18, 19]. Microfluidic technology has emerged as a biochemical tool for the identification and detection of protein microarrays finding applications in a variety of fields such as food safety, drug discovery etc. [20, 21]. Polydimethylsiloxane (PDMS), a silicon-based organic polymer is widely used for the fabrication and prototyping of microfluidic devices owing to its biocompatibility, good elasticity, non-toxicity, optical transparency, chemical inertness, ease of fabrication etc. [22, 23].

1.2 Motivation

Detection of biomolecules plays a crucial role in medical diagnosis for monitoring of disease progression and treatment, as well as a wide range of other areas such as food control, environmental monitoring, drug discovery, forensics etc. Biosensors are employed in such applications for the detection of biomolecules and the measurement of biomolecular interactions by generating signals proportional to the concentration of the analytes in the interactions. Optical biosensors based on SPR have been used widely for highly sensitive, rapid and selective detection of biomolecules.

This research work aims at the design and fabrication of SPR biosensors for the detection of biomolecular interactions. SPR allows the simultaneous study of various biomolecular interactions, particularly important for the analysis of complex biomolecules such as proteins. This requires the use of microfluidic platforms such as polymer based microfluidic systems involving PDMS, for the patterning of multiple proteins so that the target analyte flows through the sensor surface and simultaneously reacts with multiple ligands immobilized on it. The bonding of PDMS microfluidic flow cell with the plasmonic metal (gold) film is a critical step in the fabrication of the SPR

biosensors as, unlike the ease of bonding of PDMS with glass, the attachment of PDMS directly with gold film is challenging [24, 25]. This research work aims to use a novel bonding protocol to ensure leakage-free bonding of PDMS microfluidic channels with gold substrates for the construction of a multiple protein-patterned SPR biosensor. The design and fabrication of a custom-made SPR measurement prototype is aimed at performing the sensing study. Following the successful proof of concept with standard proteins, the research is further targeted at the fabrication of SPR biosensors for the rapid and label-free detection of crude snake venom which is a mixture of diverse protein families.

Despite the numerous advantages of PDMS making it a suitable material for microfluidic based applications, its inherent hydrophobicity hinders its efficacy due to the poor wettability of the polymer restricting fluidic free flow through the microchannels. Its inherent hydrophobicity also results in the undesired adsorption of biomolecules such as proteins on its surface eventually affecting their detection in microfluidics based biosensing applications [26, 27]. Hence, the present research aims at the development of hydrophilic PDMS microchannels suited for microfluidic based plasmonic biosensing applications using atmospheric pressure based plasma treatment.

1.3 Research Objectives

The research work aimed at the fabrication of SPR biosensors for the investigation of biomolecular interactions. The research objectives are:

Objective I: Design of multi-layer model for Surface Plasmon Resonance (SPR) sensor.

Objective II: Fabrication of SPR biosensors with a novel bonding protocol to study Human Immunoglobulin-G (H-IgG) protein and crude snake venom protein using a custom-made SPR measurement prototype.

Objective III: Hydrophilicity improvement of Polydimethylsiloxane (PDMS) microchannels using atmospheric pressure based plasma for SPR sensing.

1.4 Thesis Organization

The design and fabrication of SPR biosensors to study biomolecular interaction is presented in this thesis. The design of the SPR sensor is supported by multi-layer

mathematical models based on Transfer Matrix Method (TMM) and Fresnel Multilayer Reflection Theory. The effect of design parameters like the choice of materials and the layer thickness etc. is studied in detail. SPR biosensor is fabricated with a novel bonding technique and the sensor performance is investigated using a custom-made SPR measurement setup. Following the successful proof of concept with standard proteins, SPR biosensors were fabricated for the detection of crude snake venom protein. The performance of the biosensors was determined in terms of the sensitivity, selectivity, Limit of Detection (LoD) etc. Thereafter, the research work explored the use of atmospheric pressure based plasma treatment for the development of hydrophilic and protein repulsive PDMS microchannels for SPR biosensing application.

The dissertation is organized as follows:

Chapter 1 presents an introduction to the research undertaken in this thesis and the motivation behind carrying out the proposed work. The objective of the research work is defined and a chapter wise organization of the thesis is also presented.

Chapter 2 presents the design of SPR sensors using novel materials based on multilayer mathematical models. A brief review of promising materials like two-dimensional nanomaterials such as graphene, Blue Phosphorus (BlueP), Black Phosphorous (BP), Transition Metal Dichalcogenides (TMDCs) and metal oxides in the design of multilayer SPR models is presented in the beginning. The multilayer mathematical models were explored for optimal choice of materials with appropriate design parameters such as RI, Layer thickness etc. in order to achieve superior plasmonic response. The performance parameters of the sensors viz, Sensitivity, Full Width at Half Maximum (FWHM) and Quality Factor (QF) were evaluated (at an operating wavelength of 633nm) for RI change in the range 1.33-1.335 RIU. A comparative study of the performance analysis of the various SPR designs is elaborately presented.

Chapter 3 details the fabrication of a table-top SPR measurement prototype based on the Kretschmann configuration and SPR biosensors for the detection of biomolecular interaction. It presents a review of the various SPR biosensing platforms and biosensors to study biomolecular interactions. The biosensors were immobilized with three varieties of polyclonal antibodies, raised in mouse, goat and rabbit, viz; Mouse anti-Human Immunoglobulin-G (M-aHIgG), Goat anti Human Immunoglobulin-

G (G-aHIgG) and Rabbit anti Human Immunoglobulin-G (R-aHIgG) respectively. For this purpose, PDMS based microfluidic channels were fabricated and bonded to gold coated glass substrates using a novel bonding protocol. The as-fabricated PDMS microfluidic channels were used to immobilize polyclonal antibodies (M-aHIgG, G-aHIgG and R-aHIgG) for the construction of SPR biosensors patterned with multiple proteins. H-IgG was used as the target protein to evaluate the interaction capacity of the developed SPR biosensors. A custom-made SPR measurement prototype was fabricated to perform the sensing study. The prototype, driven by a single stepper-motor ensured the synchronous movement of the light source and the detector which is a critical requirement for an effective prism based SPR measuring system. Following the proof of concept using IgG protein, SPR biosensors were fabricated for the detection of crude snake venom protein of Indian cobra (*Naja naja*) and Indian Russell's viper (*Daboia russelii*).

Chapter 4 introduces the various approaches and surface modification strategies to address the issue of inherent hydrophobicity of PDMS polymer hindering its application, particularly in biological assays and biosensors. The chapter presents the use of atmospheric pressure dielectric barrier discharge oxygen (APDBD O_2) plasma in the development of hydrophilic PDMS microchannels for plasmonic sensing applications. PDMS samples were fabricated using a standard technique based on a developed master mould and then subjected to atmospheric pressure dielectric barrier discharge oxygen plasma. Plasma treated PDMS was used to fabricate an SPR biosensor to investigate biomolecular interaction. The effect of the plasma treatment on the wettability, surface energy and hydrophilicity retention capacity of PDMS was determined using contact angle measurements and Fourier Transform Infrared (FTIR) analysis whereas the bulk structural property and optical transparency were studied using X-Ray Diffraction (XRD) and Ultraviolet-visible (UV-Vis) Spectroscopy respectively.

Chapter 5 of the thesis provides a summary of the research work conducted. It also discusses the future prospects of the work for further research.

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