Modelling and fabrication of Surface Plasmon Resonance (SPR) based sensor for the investigation of protein-protein interaction

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5.1 Conclusions

The design and fabrication of SPR biosensors for the investigation of protein-protein interaction is presented in this thesis. The SPR sensors were designed based on multi-layer mathematical models using an optimal combination of novel materials with appropriate design parameters such as Refractive Index (RI), layer thickness etc to achieve plasmonic performance enhancement. The performance parameters of the SPR sensors viz, Sensitivity, Full Width at Half Maximum (FWHM) and Quality Factor (QF) are evaluated at an operating wavelength of 633 nm for RI range of 1.33-1.335 RIU of the sensing medium. The effect of the design parameters on the performance parameters of the SPR sensors was studied in detail. Three SPR sensors were designed based on the three-layer (Design I), four-layer (Design II) and five-layer (Design III) mathematical models. Design I consists of a prism, gold (Au) and sensing medium, with an optimized thickness of 55 nm Au demonstrating a sensitivity of 172˚/RIU. Design II consists of a heterostructure of Blue Phosphorus (BlueP) and Molybdenum Disulfide (MoS_2) over the Au layer and sensing medium, producing a sensitivity of 250˚/RIU at an optimized thickness of 54 nm Au and monolayer of BlueP-MoS_2 (3 x 0.75 nm). Design III consists of an adhesive layer of Zinc Oxide (ZnO) sandwiched between BK7 prism and Au, BlueP-MoS_2 heterostructure and sensing medium, exhibiting a sensitivity of 260˚/RIU at an optimized thickness of 48 nm Au, 6 nm ZnO and a monolayer of BlueP-MoS_2 (0.75 nm). A comparative study of the performances of the three designs of the SPR sensors demonstrates that Design III exhibited a sensitivity enhancement of 51.16% and 4% over Design I and Design II respectively. Further, Design III also showed a QF enhancement of 19.3% and 3.97% over Design I and Design II respectively. The enhanced plasmonic performance of the SPR sensors designed using a combination of novel materials also has a scope for experimental realization as presented in the future direction of the conducted research.

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 for the patterning of multiple proteins. Polydimethylsiloxane (PDMS) is a widely used silicon-based organic polymer for the fabrication of microfluidic devices owing to its biocompatibility, non-toxicity, optical transparency, chemical inertness etc. The bonding of the PDMS microfluidic flow cell

with the plasmonic metal (Au) film is a critical step in the fabrication of the SPR biosensors due to the poor adhesion of PDMS directly with Au. A novel bonding protocol was used to ensure the irreversible and leakage-free bonding of PDMS microchannels (length=20 mm, diameter=1.5 mm) with Au substrates at room temperature for the construction of SPR biosensors. Polyclonal antibodies viz; Mouse anti Human Immunoglobulin-G (M-aHIgG), Goat anti Human Immunoglobulin-G (G-aHIgG) and Rabbit anti Human Immunoglobulin-G (R-aHIgG) were immobilized through the PDMS microchannels over the surface activated Au substrates. During testing, the target analyte, Human Immunoglobulin-G (H-IgG) protein was injected through the microchannels reacting with the immobilized ligands on the sensor surface. Three SPR biosensors viz; SPR Biosensor I, SPR Biosensor II and SPR Biosensor III were fabricated with different concentrations of antibodies followed by exposure to different doses of H-IgG antigen. The SPR biosensors were structurally characterized using Ultraviolet-Visible (UV-Vis) spectroscopy and the sensing performance was determined in terms of sensitivity, selectivity and Limit of Detection (LoD). A custom-made Kretschmann configured SPR measurement prototype measuring 300 mm x 250 mm x 250 mm and weighing ~3.5 kg was designed and fabricated in order to perform the sensing study. The results demonstrated that H-IgG antigen was selective to all the three varieties of antibodies, viz; M-aHIgG, G-aHIgG and R-aHIgG, however, the affinity and the degree of the interaction capacity of the antigen varied with the type of antibody. The SPR Biosensor II exhibited the highest average sensitivity (0.06663(˚/µg/ml), lowest LoD (15 µg/ml), wide linear response (15 µg/ml- 225 µg/ml) with a response time of ~12 min exhibiting selective detection of H-IgG with negligible response towards other interfering proteins like BSA and Polyvalent Antivenom.

Following the successful proof of concept with standard proteins, the research further continued with the fabrication of SPR biosensors for the rapid and label-free detection of crude snake venom which is a mixture of diverse protein families. The biosensors were constructed by immobilizing with polyvalent antivenom and exposed to different concentrations of *Naja naja* and *Daboia russelii* snake venoms. The findings showed that the SPR biosensors offered a wide linear range (10 ng/ml-900 ng/ml, R^2=0.99), providing a sensitivity of 9.01678˚/(µg/ml) for *Naja naja* venom and 10.32268˚/(µg/ml) for *Daboia russelii* venom with LoD of 9.37 ng/ml for *Naja naja* venom and 9.89 ng/ml for *Daboia russelii* venom. Besides, the biosensors showed a

response time of ~16-20 min producing repeatable, reproducible and stable results. The SPR biosensors selectively interacted with the venom proteins showing negligible response towards other interfering proteins like BSA and H-IgG. Further, the satisfactory response of the SPR biosensors with snake venoms in real blood plasma samples establishes their feasibility for practical applications.

Despite the numerous advantages of PDMS polymer, its inherent hydrophobicity not only restricts the fluidic free flow through the microfluidic channels but also results in the undesired adsorption of biomolecules such as proteins on its surface affecting their detection in microfluidics based biosensing applications. Hence the conducted research investigated Atmospheric Pressure Dielectric Barrier Discharge oxygen (APDBD O_2) plasma for the development of hydrophilic PDMS microchannels for SPR biosensing. The PDMS microchannels were exposed to plasma treatment and the effect of plasma on the wettability, surface energy and hydrophilicity retention capacity of PDMS was determined using contact angle measurements. The plasma treated PDMS was used for the fabrication of an SPR biosensor by immobilizing H-IgG antigen through the microchannels followed by exposure to polyclonal antibodies viz; M-aHIgG, G-aHIgG and R-aHIgG of different concentrations and the results were compared with an SPR biosensor fabricated with pristine PDMS. The PDMS sample exposed to APDBD O_2 plasma for 6 min demonstrated the lowest contact angle of 35.15˚, surface energy of 62.83 mN/m and hydrophilicity retention capacity of more than 50 days in PBS. The plasma treatment induces surface hydrophilicity in PDMS due to the introduction of polar functional groups as evinced by Fourier Transform Infrared (FTIR) analysis without compromising the bulk structure and optical properties of PDMS as confirmed by X-Ray Diffraction (XRD) and UV-Vis spectroscopy measurements respectively. The complementary effect of improved hydrophilicity and higher surface energy due to plasma treatment also contributed to the reduced adsorption of proteins on the surface of PDMS as evinced by the sensitivity enhancement of 60%, 21.07% and 47.34% and LoD improvement of 61.74%, 70.07% and 91.51% of the plasma treated SPR biosensor over the pristine SPR biosensor against M-aHIgG, G-aHIgG and R- aHIgG protein respectively.

5.2 Future Direction of Research

The present research related to the fabrication of SPR biosensors for the investigation of protein based biomolecular interactions opens the door to expand the scope of the work in several areas as follows:

- Simultaneous detection of multiple proteins on a single platform.
- Experimental realization of SPR sensors designed based on multi-layer mathematical models using 2D nanomaterials, metal oxides etc. for the enhancement of plasmonic performance.
- Detection of other biomolecules like nucleic acids etc.
- Further miniaturization of the dimension of the prototype for the development of a commercially viable snakebite diagnostic kit to enable point-of-care detection.