CHAPTER 5
SUMMARY AND CONCLUSIONS

1. Various immobilization strategies were employed for coating IgG antibodies on different solid supports i.e. silicon, gold-coated silicon and polylysine-coated glass slides.

2. The immobilization strategies involved the use of protein A, protein G, biotin-avidin interactions, APTES, glutaraldehyde and EDC. Based on the experimental results, it was concluded that immobilization method employing protein A was more appropriate for coating antibodies on the gold-coated silicon.

3. The immobilization of biomolecules (protein A and human IgG) to gold coated silicon was assessed qualitatively by inverted fluorescence microscopy and quantitatively by (i) employing HRP labeled protein A & human IgG, and (ii) fluorescence spectroscopy using FITC labeled biomolecules. The average immobilization density was found to be 140 ng/cm² (16.9 x 10¹¹ molecules/cm²) for Protein A and 610 ng/cm² (24.3 x 10¹¹ molecules/cm²) for human IgG.

4. The uniformness of biomolecular immobilization alongwith their size and coverage was determined by atomic force microscopy (AFM). The size of the protein A molecule was found to be 2.8 nm in height and 4.9 nm in diameter. The height of the human IgG molecule was about 12 nm and its diameter was about 8.4 nm.

5. Glycine-HCl buffer (50 mM, pH 2.2) was used to dissociate the human IgG-goat anti-human IgG complex and to regenerate functional human IgG. It effectively dissociated the immune complex without affecting the association bonds between the silicon surface and the human IgG. The regenerated human IgG surface showed that the immobilized human IgG retained sufficient activity (74 - 95 % of the initial activity) to carry out four reproducible assays.

6. 3-APTES + glutaraldehyde + protein A method was employed for the immobilization of human IgG on bare silicon. The amount of protein A immobilized on the bare silicon surface was found to be about 120.0 ng/cm² (1.5 x 10⁴ molecules/cm²) for human IgG.
7. To predict the biomechanics of the cantilever based diagnostics, a MATLAB
based software was developed. The software took into account the mass, size,
structure and surface coverage of the immobilized biomolecules. It was used to
calculate the various biomechanical parameters of the microcantilever based
diagnostic kit such as force on the cantilever, stress change, strain change at the
anchor point of the microcantilever, deflection of the microcantilever, angle of
deflection, radius of curvature, relative change in resistance of the piezoresistive
substance i.e. boron integrated at the anchor point of the microcantilever. It can be
used to decide the dimensions of the microcantilever to be used for the diagnosis
of a particular disease antigen.

8. A Teflon block in the form of a cube with inlet and outlet for sample and buffers,
and having a mechanical arrangement for holding cantilever was designed and
fabricated. The flow cell set up would be used for microcantilever based
diagnosis.

9. The current-voltage measurements of biomolecules immobilized on the polylysine
coated glass slides were determined by SIGNATONE probing station. The
conductance pattern was specific and distinct for each biomolecular layer
immobilized on the polylysine-coated glass slide. The concept was successfully
tested in two clinical situations for diagnosis of Leishmania antigen and p21. The
change in conductivity after the formation of immune complex could be
employed in future as a sensor technique for protein analytes and for assessing
biomolecular interactions.