CHAPTER – 7
PDMS SURFACE FUNCTIONALIZATION AND ITS CHARACTERIZATION

7.1 Overview

Polydimethylsiloxane or dimethicone (PDMS) is one of the most commonly used polymeric materials for fabrication of fluidic devices and chips [1]. Chemically, it is an organo-silicon compound consisting of backbone of oxygen and silicon atoms with empirical formula \((\text{C}_2\text{H}_6\text{OSi})_n\), where \(n\) is number of repetitive units. Due to its low cost, ease of fabrication and transparency PDMS has replaced its conventional counterparts i.e. glass and silicon in numerous applications[2].

In biological applications, low toxicity, easy molding and low temperature polymerization give PDMS an edge over other polymeric materials [3]. However one of the major drawbacks of PDMS is its extreme inertness due to closely packed methyl groups [4]. As a number of biological processes like bioassays and micro-bioreactors require biofunctionalized surfaces therefore an easy way to functionalize PDMS surface is crucial [5], [6]. The preliminary step to functionalize PDMS surface is plasma oxidation treatment to oxidize Si-CH\(_3\) groups to Si-OH [7]. The most commonly utilized is oxygen plasma. High energy photons, ions and electrons in plasma breaks polymer backbone thus forcing carbon containing groups to form volatile organic species and combining silicon and oxygen through Si-O bonds forming a silica like surface [8]. The major drawback of this technique is the hydrophilic surface is short lived and hydrophobicity is regained within several minutes [9]. UV and ozone treatment are also used as an alternative to oxygen plasma but are equipped with similar drawbacks. Ionic polyelectrolyte modifications using ionic polymers and surfactants are also widely studied [10], [11]. These techniques are less preferred due to non specific and weak coating and poor control over surface functionalization. Covalent modification of PDMS using chemical derivatization by acidic solutions is another easy and cost effective technique to generate hydroxyl functionalities. Harsh acidic solutions like aqua regia and piranha are used for generation of hydroxyl groups over PDMS surface [12]. The hydroxyl groups generated by these treatments are then easily converted into amines by treatment with triethoxysilyl derivatives of amines like aminopropyltriethoxysilane (APTES) [13]–[15].
However the major complications start after amine generation. A number of reactive chemical reagents are reported in literature for conjugating biomolecules on PDMS surface through a wide array of chemical reaction. However, all these mechanisms employ either amine or carboxyl groups of biomolecules. A few noteworthy mentions are amine-glutaraldehyde-amine \[16\], amine-NHS(N-hydroxysuccinimide)[17], amine-EDC (carboxylate-1-ethyl-3-(3-dimethylamonipropyl) carbodiimide)[7], amine-NHS-carboxylate-EDC and sulfydryl-melamide [4]. As can be easily noted most of the chemistries employ toxic and costly reagents, generation of byproducts producing artifacts which restricts their broad applicability. Also it is worthy to mention a strict control over pH is required in these chemical conjugations to enhance surface functionality. Clearly, a soft and economical chemical modification strategy is need of hour.

Herein, this chapter elucidates a novel soft diazonium route to functionalize PDMS surface. Indeed, although diazonium chemistry is widely used in antibody conjugation yet their true potential on PDMS based devices have not been evaluated so far. Amine terminated PDMS surface was activated with cost effective reagent sodium nitrite to form a diazonium film on surface. This diazonium film is highly stable and reacts with amino acids present on the outer surface of protein to form a stable conjugated PDMS surface. This soft method does not require a strict control over pH and neither generates any byproduct and artifact.

**7.2 Material and Methods**

**7.2.1 Reagents**

PDMS elastomer and curing agent was procured from Dow corning inc. USA. Amino propyl triethoxysilane (APTES) and hydrogen peroxide was purchased from Sigma Aldrich USA. Sodium nitrite and sulphuric acid were procured from central drug house, Mumbai. Alexa flour labeled goat anti-rabbit antibody was procured from invitrogen. Anti-Salmonella antibody used in this work was raised inhouse at Imtech, Chandigarh as reported in earlier chapters.

**7.2.2 Biofunctionalization of PDMS surface**

A uniform PDMS film of 1 mm thickness was generated using Bio-rad gel electrophoresis assembly. Piranha solution was prepared from hydrogen peroxide and
sulphuric acid in 1:3. PDMS film was initially sonicated in water bath sonicator for 2 minutes to remove dust and other microparticles. After surface cleansing the PDMS surface was then treated with piranha solution for 30 seconds. The surface was then washed with ethanol and treated with 3% APTES solution in ethanol for 20 minutes at room temperature. This step was followed by incubation at 80°C for 2 hours to evaporate extra APTES. With amine coated on PDMS surface, to create diazonium groups sodium nitrite (100 mM) was used. The PDMS films were immersed in sodium nitrite solution for 30 minutes, followed by washing with deionized water. Thus created diazonium groups are reactive to proteins through histidine and tyrosine amino acids.

7.2.3 Characterization of surface

ELISA was performed using HRP labeled goat anti-rabbit antibody. The biofunctionalized surface was firstly treated with 5% skim milk solution to block the non-specific binding. The antibody functionalized surface was incubated with 1:20K HRP labeled antibody solution. After incubating for 15 minutes the surface was thoroughly washed and substrate solution was added.

For confocal microscopy similar protocol was adopted, but only instead of HRP labeled antibody, Alexa Flor 488 labeled anti-rabbit antibody was used and confocal microscopy was performed. For AFM micrographs analysis was performed using SPIP software.

7.3 Results and Discussions

7.3.1 Diazotization for Bio-Functionalization

Poor surface functionality is a major stumbling block in wide acceptance of PDMS based fluidic devices. To address this shortcoming of PDMS a soft diazonium based route for facile modification of PDMS surface was employed. Figure 7.1 illustrates an overview of steps required for conjugation. Firstly, hydroxyl groups were generated on PDMS surface by treatment with piranha solution. Hydroxyl groups were converted to amine groups using APTES. The amine terminated PDMS was diazotized by treating with sodium nitrite.
It is previously reported that out of 20 amino acids, histidine and tyrosine shows reactivity towards diazonium groups. However, it is also reported that not only imidazole ring of histidine and phenyl group of tyrosine are responsible but also indole group of tryptophane, aliphatic amine group and NH group of hydroxyproline and proline plays an active role [18], [19]. Till date it is well established that these reactions occurs between (i) one diazonium radical and each indolyl and sulfahdryl moiety (ii) two diazonium and each imidazole, phenolic and alpha amino group of glycine (iii) three diazonium and each guanadino group. In the present work, these reports have been utilized to generate a novel and cost effective method for bioconjugation of PDMS surface. The biofunctionalized PDMS surface was characterized by three independent techniques.

### 7.3.2 ELISA on Biofunctionalized surface

Firstly with antibody labeled surface an ELISA was performed using horseradish peroxidase enzyme labeled anti-antibody. Enzyme labeled anti-antibody binds to antibody functionalized PDMS surface and converts non-chromogenic substrate to colored solution. This colorimetric response can be easily detected using UV-Vis spectrophotometer. Figure 7.2 shows increase in absorbance of the solution corresponding to enzyme activity confirming conjugation on PDMS surface.
Figure 7.2 – Absorbance (450 nm) graph of PDMS surface functionalized with and without antibody.

7.3.3 Confocal microscopy of Bio-Functionalized surface

Next PDMS surface functionalization was characterized through confocal microscopy. Alexa fluor 488 labeled goat-anti-rabbit antibody was incubated with antibody functionalized PDMS surface to validate ELISA results. Figure 7.3 shows confocal microscopy images of biofunctionalized PDMS surface. Clearly, Figure 7.2(a) indicates green fluorescence signals elucidating the binding of anti-antibody to PDMS surface confirming the presence of antibody. Simultaneously, Z-scan images and 3-D fluorescence intensity of PDMS surface were also recovered to further clarify our findings as shown in Figure 7.3 (b) and (c).

Figure 7.3 – Confocal micrographs of functionalized PDMS surface for insights in conjugation procedure (a) 2-D micrographs (b) and (c) 3-D micrograph.
7.3.4 Atomic Force Microscopy investigations of PDMS surface

Further, to investigate conjugation mediated changes in surface morphology of PDMS Atomic Force Microscopy was performed. Figure 7.4 shows 2-D (a), 3-D (b) and surface profiles (c) images of PDMS surface before conjugation. Clearly, the surface profile shows a variation of 14 nm closely to a bare PDMS surface. Moreover, the surface is even without any island structures showing no functionalization.

Figure 7.4 – Atomic force microscopy analysis of bare PDMS surface (a) 2D micrograph (b) 3d illustration (c) size profile graph.

Figure 7.4 (a) and (c) clearly indicates change in surface morphology as the antibody coated surface is rough, elucidating islands of various heights i.e a peak and ridge structure. Moreover further observations on these 3D images elucidate a clear wrinkling behavior which is more predominant after antibody conjugation. This wrinkling is further substantiated by graphical illustrations in Figure 7.4 (b). The graph illustrates that the height of peaks is about 40 nm roughly corresponding to size of protein cluster. Further evaluation through SPIP software also provided the mean square roughness values that increase from 6.1771 nm to 18.446 nm after antibody conjugation.
Figure 7.5 – Atomic force microscopy analysis of functionalized PDMS surface (a) 2D micrograph (b) size profile graph (c) 3d illustration.

7.3.5 Investigation of Antibody Functionality after Bio-conjugation

One of the major drawbacks of covalent bioconjugation routes is loss of biomolecule functionality after conjugation. To evaluate the functionality of antibody after conjugation through diazotization route, antigen binding studies were performed. As the antibodies exploited in this work are anti-Salmonella antibodies generated in rabbit, biofunctionalized PDMS surface was blocked for non-specific binding and was incubated with bacterial suspension of Salmonella Typhimurium. The incubation was followed by surface treatment with DNA binding dye DAPI. The dye stained bacterial cells and were then visualized under confocal microscope. Figure 7.6 shows DIC and fluorescence images of PDMS surface confirming the presence of bacterial cell on the surface. The experiment thus validates that the antibody does not lose its functionality after conjugation. Moreover the results also elucidates that biomolecules functionalized through this conjugation process are active and their functionality remains intact. Control experiments without Salmonella Typhimurium were also performed and show no response.
Figure 7.6 – Confocal microscopy images of PDMS surface labeled with anti-Salmonella antibody (a) DIC image (b) DAPI stain (c) Merged.

7.3.6 Microfluidic channel biofunctionalization and detection

The well characterized and novel PDMS biofunctionalization technique was then applied for detection of proteins inside microchannels. Y-shaped micro channels were fabricated from CSIR-Central Electronics Engineering Research Institute, Pilani. The microchannels were functionalized through low cost diazonium method described earlier in this chapter. Bovine serum albumin (BSA) was taken as a model antigen system and the polymerization was observed through OLYMPUS BX51 optical microscope. The aspect ratio of microchannels was 2.8 with rectangular cross section. Figure 7.7 shows optical microscope images of Y shaped cross section.

Figure 7.7 – Optical microscope image of Y shaped microchannel with an aspect ratio of 2.8. (a) COMSOL drawing (b) Optical microscope image with scale bar 200 µm (c) and (d) phase contrast optical microscope images.
The microchannels were then tested for polymerization assay for detection of BSA as discussed in Chapter 7. A brief illustration of the assay is shown in Figure 7.8. BSA conjugated through diazonium route and detected through gold nanoparticle (GNP) labeled IgG antibody. The polymer formation was then observed after the addition of optimized substrate solution (see chapter 7, section 6.2.4).

![Diagram of polymer formation](image)

**Figure 7.8** – Schematic overview of polymer formation inside the microchannel.

After an incubation of 30 minutes the polymer formation was observed under optical microscope. Figure 7.8 (a) depicts clogging of microchannels after polymer formation. This is an interesting observation as it opens new ways of doing controlled polymerization inside microchannels. Moreover the system can also be used to develop pressure based amplifications as the blockage of channels provides resistance to fluid flow. Figure 7.9 (b) depicts a magnified image of polymer formation inside microchannel. Moreover, observing closely we can easily visualize formation of siloxane microspheres inside the channels in a close agreement with FESEM observations.
Figure 7.9 – (a) optical microscope image of polymer formation inside the microchannel (b) magnified image of the microchannel showing formation of siloxane microspheres.

7.4 Conclusions

Facile functionalization of PDMS is crucial for realizing low cost microfluidic devices. There are numerous strategies reported in literature for easy modification of PDMS surfaces with biomolecules. Ironically, all the reported techniques employ either costly apparatus like plasma or use of costly and harmful chemicals like EDC, NHS, glutaraldehyde etc. These techniques also generally results in unwanted by-products. In this chapter, this problem was addressed by realizing functionalization through a soft diazonium route. Sodium nitrite, a cost effective and readily available chemical was used to generate diazo groups on PDMS surface. The activated surface was then treated with biomolecules i.e. antibody. Through effective linkage between histidine and tyrosine amino acids of biomolecule and diazo group a bioactivated PDMS surface was recieved. The surface was thoroughly characterized by ELISA, AFM and confocal studies. In conclusion an easy to use and cost effective strategy was developed to biofunctionalize PDMS for various biomedical and microfluidic applications. The developed functionalization technique was then applied for detection inside microchannels through polymer (siloxane) formation.
7.5 References


