Chapter 4

Interaction of DNA with Nano-Patterned TiO$_2$(110) Surfaces

4.1 Introduction

In chapter 3, we discussed the creation of nanostructures on rutile TiO$_2$ surfaces through ion irradiation with low energy Ar ions. During the fabrication of these nanostructures, preferential sputtering of oxygen occurs which results in the creation of oxygen vacancies on the surface. In the process, surface also becomes Ti rich. This formation of nanodots as well as the creation of oxygen vacancies and development of Ti rich zones on TiO$_2$ surface after irradiation, become responsible, as discussed in the earlier chapter, for the observation of enhanced UV-Vis absorbance, increased PL and a slightly reduced bandgap. These properties display potential for photocatalytic applications. In the present chapter, we discuss the interactions and adsorption properties of DNA on these ion beam modified nanopatterned TiO$_2$ surfaces.

For DNA based biomolecular surfaces, considerable difference exists in the adsorption of the nucleobases and their derivatives on material surfaces, since adsorption properties crucially depend on the molecular architecture of the adsorbing species. Surface adsorption for even relatively simple species such as oxyanions or metal cations [1,2], or amino acids
can exhibit complex behavior. Being relatively more complex species, the nucleic acid components, which have complex geometries and multiple functional groups, should have considerably more complicated adsorption properties. Many organic compounds adsorb rapidly on the material surfaces in aqueous solution. The extent of adsorption is dependent on variables like material type, surface roughness, and the type of organic molecule getting adsorbed [4]. The basic adsorption is likely to be mediated at the molecular level by a combination of specific molecule-surface interactions. The interactions of nucleic acids and their components with material surfaces are especially interesting and of fundamental importance.

Recent research has focused on the development of nanostructured materials for the use in biomedical devices, including medical prostheses, implantable biosensors, and drug delivery devices [5]. Due to the small size and high surface area, nanomaterials can have unpredictable adsorption properties [6]. They may lead to excellent adsorption with increased biocompatibility or can cause damage to biomolecules. Thus, it has been of great concern to understand the influence of the nanostructured surfaces on the DNA morphology and its functionality.

Titanium dioxide (TiO$_2$) is considered to be an excellent and promising material for biomedical implants [7,8] due to its nontoxic nature, corrosion resistance properties and compatibility with many biomolecules. Interaction of TiO$_2$, for example, with phosphate solutions [9] influences the bioactivity of the oxide surface remarkably, through the formation of the biocompatible hydroxyapatite [10,11], after phosphate ions get incorporated in the oxide layer on titanium. The focus of research, presently, is not only on the bio-effect and interaction of TiO$_2$ with biomolecules, like DNA, but also on the modification of these interactions. Parameter like nature of the material, its surface, presence of nanostructures, their sizes etc. are essential components necessary for defining and estimating these interactions.

This chapter presents the studies of adsorption and interaction of plasmid DNA with the patterned TiO$_2$ surfaces. The TiO$_2$ surfaces have been patterned with the nanostructures,
as discussed in chapter 1 and 3, by utilizing low energy Ar ions. The nanodot patterns are induced by sputtering and are generated through self assembly. These patterns get fabricated due to the competition between the roughening mechanisms, produced by curvature dependent radiation sputtering, and various smoothening phenomenon [12, 13]. In order to understand the influence of the nanopatterned surface on the DNA morphology, and its functionality, it is essential to understand the morphological parameters of DNA. Obtaining these parameters is especially important in the systems where DNA displays high packing or entangled networks like on a 2D cell surface where several complex geometries for DNA are observed [14, 15]. In this chapter, the interaction of nanopatterned TiO$_2$ surfaces with DNA have been investigated by estimating the parameters like $\text{Persistence length}$ of DNA ($P$) and its $\text{Correlation length}$ ($\xi$). The rigidity or the stiffness of any polymer, like DNA, is defined by $P$ and an increase in this length suggests an enhancement of interactions. An increase in $\xi$, or the intermolecular separations, of DNA also indicates the promotion of interactions through an increased wetting at the surface.

The $\text{persistence length}$ of DNA is an important morphological parameter and its definition depends on the chosen model [16]. The $\text{Worm Like Chain}$ model, proposed by Kratky-Porod [17], has often been employed to describe the average conformation of long, intrinsically straight polymer molecules, including DNA [18]. In this model, DNA is considered to be an intrinsically straight polymer chain which is partly relaxed by the effect of a thermal bath [19] and the persistence length of DNA is the orientational correlation length along the length of the chain. This Persistence length, as shown by Landau and Lifshitz [20], can be expressed as $P = \frac{YI}{\kappa_B T}$ and it depends on Young’s modulus ($Y$), inertial moment ($I$) of the chain, and the absolute temperature of the medium ($T$) with $\kappa_B$ being the Boltzmann constant. Another widely used, $\text{Freely Jointed Chain Model}$ [21], considers the polymer as a chain of uncorrelated independent segments of length $2P$ (Kuhn segments). Independent of any model, $P$ is the direct measure of the polymer’s intrinsic physical property, its stiffness. The larger the value of $P$, the higher will be the stiffness or rigidity of the polymer [22, 23]. Evaluation of $P$ can, thus, be used to assess the interaction of DNA with other molecules.
Experimentally, the morphological parameters, $P$ and $\xi$, of DNA polymer can be estimated by vectorization and analysis of individual DNA molecule imaged by AFM, and then averaged over the full statistical set of discrete images. This process, however, has several limitations like choice of statistical model and dimensionality [23]. Thus, while it is very important to focus on each single DNA molecule with very high pixel of information, it is a tedious process especially if the surface is covered with a very large number of DNA molecules. Recently, studies by Calò et. al. [23] have shown that Power Spectral Density (PSD) [24] analysis, of AFM images, is a powerful technique to obtain the morphological parameters of DNA. Containing full information on the contribution of each spatial frequency to the topography, PSD can provide spatial distribution of the DNA molecules, over the surface, across a multiple length scales [25]. Owing to the isotropic nature of the surfaces adsorbed with DNA molecules, one-dimensional PSD data is obtained by Fourier transforming the AFM image line by line along the fast scan direction and averaging. The range of frequencies, $\nu$, investigated by this method fall between $1/L$ (L being the image size) and the Nyquist frequency $N/2L$ (N being the number of pixels of the image).

### 4.2 Experimental

Single crystals of Rutile TiO$_2$(110) were sputtered in UHV (see section 2.2.2 for details), with 3 keV Ar$^+$ ions, at 15$^\circ$ incident angle. The flux of Ar ions was $1 \times 10^{13}$ ions/cm$^2$·sec. The TiO$_2$ samples were irradiated for two different durations, 10min and 30 min, giving the respective fluences of $6 \times 10^{15}$ and $1.8 \times 10^{16}$ ions/cm$^2$. These ion beam modified surfaces were interacted with the plasmid DNA (pBR 322) that was isolated from DH5$\alpha$ bacterial cell. The plasmid DNA is circular in shape and consists of about 4361 base pairs. The surface morphology, both prior to and after interaction with DNA molecules (concentration of 1ng/ml), has been investigated by AFM. Similar investigations on the ion beam modified surfaces have also been carried out. The AFMs (Nanoscope IIIa and V) were operated in
the tapping mode under ambient conditions. X-Ray Photoelectron Spectroscopy (XPS) measurements were performed on a VG instrument with a Mg Kα source in UHV.

4.3 Results and Discussion

Figure 4.1 shows the AFM images of the morphological evolution of the TiO$_2$ surfaces, both prior to and after ion irradiation. The figure also displays the modification in morphology of surfaces after their interaction with DNA. The left panel (of fig 4.1), shows the AFM images for virgin TiO$_2$ as well as for sputtered surfaces. Although the virgin sample displays a smooth surface, after sputtering with a fluence of $6 \times 10^{15}$ ions/cm$^2$ an increase in roughness is observed. The sputtered surface also exhibits the formation of some nanostructures but these, however, are not prominent at this stage and appear as small patches. After irradiating with higher fluence, $1.8 \times 10^{16}$ ions/cm$^2$, the surface shows the presence of well defined nanostructures. These nanostructures are about 30 nm in size and, as discussed earlier, are self assembled. The rms surface roughness for the surfaces irradiated with the fluences of $6 \times 10^{15}$ and $1.8 \times 10^{16}$ ions/cm$^2$ are found to be 0.127 nm and 2.153 nm, respectively. Right panel in fig. 4.1 displays the AFM images of the surfaces after their interaction with DNA.
Figure 4.1: $1 \times 1 \mu m^2$ AFM images from un-reacted surfaces are shown in the left panel for (a) virgin TiO$_2$ and surfaces irradiated with fluences of (b) $6 \times 10^{15}$ ions/cm$^2$, and (c) $1.8 \times 10^{16}$ ions/cm$^2$. Right panel shows the AFM images of the corresponding surfaces after they are interacted with DNA.
The size (diameter) distributions of the plasmid DNA on the virgin as well as the sputtered surfaces are shown in fig.4.2. The average diameter of the DNA on the virgin surface is about 60 nm which increases to ∼220 nm and 350 nm on the surfaces irradiated with the fluences of $6 \times 10^{15}$ and $1.8 \times 10^{16}$ ions/cm$^2$, respectively. Figure 4.2 clearly demonstrates an increase in DNA-diameter on the sputtered surfaces compared to the virgin TiO$_2$ surface. Moreover the surface, sputtered with higher ion fluence, consists of DNA with larger average diameter. These results indicate the crucial effect of surface morphology on the DNA conformations. Also, the enhancement in DNA-diameter, after sputtering, suggests a decrease in contact angle between DNA and the surface. The surfaces, thus, become more hydrophilic, and so more biocompatible, as the irradiation fluence is increased. Better biocompatible and hydrophilic nature, which increases with ion fluence, is related to the higher rms roughness and the formation of nanostructures on the ion irradiated surfaces.
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Figure 4.2: Size (diameter) distribution of plasmid DNA after it is interacted with (a) Virgin TiO$_2$ and surfaces irradiated with fluences of (b) $6 \times 10^{15}$ ions/cm$^2$, and (c) $1.8 \times 10^{16}$ ions/cm$^2$.

The AFM images similar to those shown in fig.4.1 were utilized to obtain the PSD plots for the virgin surface as well as from the ion sputtered surfaces after their interaction with DNA. Figure 4.3 displays these PSD plots in log-log representation as a function of spatial...
wave vector ($\kappa$) with $\kappa = 2\pi v$. The PSDs display a low frequency plateau which is related to the white spectrum. This flat region is followed by frequency -decaying region, that can be approximated by two power law decays, each spanning about 1 order of magnitude of $\kappa$, the spatial frequencies. The spatial correlations, or multi-affine behavior, in any system lead to power law decaying regions. The specific frequencies where the different regions intersect correspond to the inverse of correlation lengths. The larger correlation length, related to the smaller frequency, corresponds to the intermolecular separations ($\xi$) whereas the smaller correlation length is associated with the Persistence length ($P$) of the DNA.

The PSD from the virgin surface (see in fig.4.3), after it was interacted with DNA, provides two correlation lengths, the persistence length ($P$) of 41.5 nm (at $\kappa = 151 \mu m^{-1}$) and the intermolecular separations between plasmid DNA ($\xi$) of 174.4 nm (at $\kappa = 36 \mu m^{-1}$). The value of persistence length for DNA, obtained here, is consistent with the values reported in literature [26]. After the interaction of DNA with the surface, sputtered at lower fluence ($6 \times 10^{15}$ ions/cm$^2$), the correlation lengths were found to be $P = 67.5$ nm (at $\kappa = 93 \mu m^{-1}$) and $\xi = 261.6$ nm (at $\kappa = 24 \mu m^{-1}$). Interaction of DNA with surfaces sputtered at higher fluence ($1.8 \times 10^{16}$ ions/cm$^2$) also leads to two correlation lengths $P = 89.7$ nm (at $\kappa = 70 \mu m^{-1}$) and $\xi = 483.0$ nm (at $\kappa = 13 \mu m^{-1}$). The results indicate that both the correlation lengths, $P$ and $\xi$, become larger, increasing with fluence, on the ion sputtered surfaces. The increased persistence length ($P$) indicates an enhanced stiffness and so higher interaction of the DNA molecule on the sputtered surfaces. The larger intermolecular separations ($\xi$) also suggest more interaction as well as an increased biocompatibility and hydrophilicity of the sputtered surfaces.
Figure 4.3: 1D-iso PSD plot from DNA interacted with (a) Virgin TiO$_2$ and surfaces irradiated with fluences of (b) $6 \times 10^{15}$ ions/cm$^2$, and (c) $1.8 \times 10^{16}$ ions/cm$^2$. 
Figure 4.4: XPS spectra of Ti(2p) region for (a) virgin TiO$_2$ and surfaces irradiated with fluences of (b) $6 \times 10^{15}$ ions/cm$^2$, and (c) $1.8 \times 10^{16}$ ions/cm$^2$.

Figure 4.4 displays the Ti(2p) region of the XPS spectra from the virgin as well as ion irradiated surfaces. XPS spectrum from the virgin sample (in fig. 4.4(a)) shows the presence of the two spin orbit split Ti-2p features, $2p_{3/2}$ and $2p_{1/2}$. Both these features correspond to Ti$^{4+}$ coordination sites on rutile surface. Very small amount of Ti$^{3+}$ component may also be present on the surface of virgin sample. After irradiation of TiO$_2$ surface with $6 \times 10^{15}$ ions/cm$^2$, in addition to Ti$^{4+}$ sites, Ti$^{3+}$ and Ti$^{2+}$ features are also observed for
both 2p\textsubscript{3/2} and 2p\textsubscript{1/2} states. However, Ti\textsuperscript{4+} component is most prominent with the ratio of Ti\textsuperscript{3+} and Ti\textsuperscript{4+} features being only about 0.3, as seen in fig. 4.5. For the surfaces, sputtered at the higher fluence, (1.8 \times 10\textsuperscript{16} ions/cm\textsuperscript{2}) this ratio increases significantly to $\sim 4.1$ with the Ti\textsuperscript{3+} component becoming stronger than the Ti\textsuperscript{4+} (fig. 4.4(c) and fig. 4.5). The binding energy (BE) positions for various components of the 2p\textsubscript{3/2} level, for virgin and the sputtered surfaces, are mentioned in Table 4.1 for unreacted surfaces as well as after their interaction with DNA.

| Table 4.1: Binding Energy positions for various 2p\textsubscript{3/2} states of TiO\textsubscript{2} |
|-----------------|--------|--------|--------|
|                 | Ti\textsuperscript{4+} | Ti\textsuperscript{3+} | Ti\textsuperscript{2+} |
| Unreacted       |        |        |        |
| Virgin          | 458.5  | -      | -      |
| $6.0 \times 10\textsuperscript{15}$ ions/cm\textsuperscript{2} | 458.0  | 456.2  | 454.6  |
| $1.8 \times 10\textsuperscript{16}$ ions/cm\textsuperscript{2} | 458.0  | 456.5  | 455.0  |
| Reacted with DNA|        |        |        |
| Virgin          | 458.0  | -      | -      |
| $6.0 \times 10\textsuperscript{15}$ ions/cm\textsuperscript{2} | 457.3  | -      | -      |
| $1.8 \times 10\textsuperscript{16}$ ions/cm\textsuperscript{2} | 457.8  | -      | -      |

The TiO\textsubscript{2}(110) surface consists of alternating [001] direction rows of five fold coordinated Ti\textsuperscript{4+} and two fold coordinated bridging O\textsuperscript{2−}. Preferential sputtering of TiO\textsubscript{2} surface during ion beam irradiation creates oxygen vacancy with its two associated electrons getting transferred to the empty 3d orbitals of the neighboring Ti atom. This leads to the formation of two Ti\textsuperscript{3+} or one Ti\textsuperscript{2+} state on the surface [27, 28]. With preferential sputtering of oxygen, Ti rich zones also get created that become the nucleation centers for the nanodots [13, 29–33]. Thus, the Ti\textsuperscript{3+} and Ti\textsuperscript{2+} features (in fig. 4.4(b)) are related to the excess O-vacancy that form on the TiO\textsubscript{2} surface after ion sputtering. Moreover, the higher irradiation fluence, $1.8 \times 10\textsuperscript{16}$ ions/cm\textsuperscript{2}, leads to an increase in both, Ti\textsuperscript{2+} and Ti\textsuperscript{3+},
components as expected (see fig. 4.4(c)).

Figure 4.5: Ratio of Ti$^{3+}$ and Ti$^{4+}$ XPS intensities for the virgin and ion irradiated TiO$_2$ surfaces.

Figure 4.6 shows the XPS spectra of Ti(2p) region from the virgin and ion sputtered surfaces after they have been interacted with plasmid DNA. For reference, the XPS spectra (of fig.4.4) from the un-reacted surfaces have also been shown in this figure. The interaction of DNA with the virgin TiO$_2$ surface causes a slight shift of Ti$^{4+}$ feature, compared to unreacted surface, towards the lower BE positions as seen in fig.4.6(a). This can be caused by the transfer of electrons from the negatively charged phosphate (PO$_4$$^-$) backbone of DNA to the surface. After DNA interacts with surface, irradiated with the smaller fluence ($6 \times 10^{15}$ ions/cm$^2$), the XPS results show a further shift of Ti$^{4+}$ component towards lower BE indicating transfer of more electrons from DNA moiety to surface (fig. 4.6(b)). Gaining electron can impart some mixed (Ti$^{3+}$ and Ti$^{4+}$) character to this state. Furthermore, it is noticed that there is no signature of any Ti$^{2+}$ feature, after interaction, suggesting a saturation of this state after gaining electrons from DNA backbone (see fig. 4.6(b)). Both Ti$^{3+}$ and Ti$^{2+}$ states become saturated, as seen in fig. 4.6(c), after the interaction of DNA.
with surface irradiated at high fluence \((1.8 \times 10^{16} \text{ ions/cm}^2)\). With these observations, it can be concluded that the initial transfer of electron from the DNA-phosphate backbone to the ion irradiated surfaces causes the saturation of Ti\(^{2+}\) and later of the Ti\(^{3+}\) states.

Figure 4.6: XPS spectra of Ti(2p) region for DNA interacted (red curve) surfaces of (a) virgin TiO\(_2\) as well as surfaces irradiated with fluences of (b) \(6 \times 10^{15} \text{ ions/cm}^2\), and (c) \(1.8 \times 10^{16} \text{ ions/cm}^2\). For reference, XPS spectra from unreacted (black curve) surfaces are also included (see text).

The observed XPS results can be utilized to understand the AFM and PSD results discussed earlier in this chapter. On the virgin surface, the negative charge transferred from DNA is small leading to a relatively weak interaction between them. As a result the DNA exhibits a small size (in fig. 4.1,4.2), small internuclear separation \((\xi)\) and small persistence
length \( (P) \) (in fig. 4.3). An increased charge transfer, from DNA to surface, irradiated at small fluence \( (6 \times 10^{15} \text{ ions/cm}^2) \), is observed which saturates the Ti\(^{2+} \) sites of the surface. The presence of oxygen vacancies on the ion irradiated surfaces are responsible for the enhanced interaction, resulting in an increased size of plasmid DNA along with a larger \( \xi \) and \( P \). Further increase in the diameter of plasmid DNA and larger morphological parameters, \( \xi \) and \( P \), are observed after plasmid DNA interacts with the surface irradiated at high fluence \( (1.8 \times 10^{16} \text{ ions/cm}^2) \). As the XPS results show, at this stage, charge transfer from DNA is strong enough to saturate both Ti\(^{3+} \) and Ti\(^{2+} \) states. The oxygen vacancies created on the surface, after irradiation, result in a strong conjugation of the DNA with the surface. Thus, ion irradiation leads to enhanced biocompatibility and hydrophilicity, increasing with fluence, of TiO\(_2\) surfaces.

### 4.4 Summary and Conclusion

Here, the interaction of plasmid DNA with ion beam modified TiO\(_2\) surfaces has been investigated. Results indicate that the diameter of the DNA as well as its morphological parameters, \( \xi \) and \( P \), increase on the ion irradiated TiO\(_2\) surfaces, indicating a higher biocompatibility and hydrophilicity, increasing with fluence, on these surfaces. An increased charge transfer from negatively charged phosphate backbone of DNA to the ion irradiated surfaces is observed to be crucially responsible for the observation of these properties. Oxygen vacancies created during ion irradiation become the significant centres which promote the conjugation of DNA with the surface.
References


