Surface modification of polyurethane via creating a biocompatible superhydrophilic nanostructured layer: role of surface chemistry and structure

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ABSTRACT
Advanced surface modification approaches of biomaterials alongside the advent of sophisticated analytical techniques have provided a great opportunity to understand how the physicochemical characteristics of materials determine cell—surface dynamics at molecular and atomic scale. However, there are still many contradictory reports, which are mainly due to inadequate information about the role of the two parameters of surface chemistry and structure and their synergistic effect as an adequate predictor of biological performance. Here, surface parameters were altered by grafting of poly ethylene glycol (PEG) on polyurethane (PU) surfaces through a superhydrophilic modification method. In this study, surface modification of PU films by PEG thin layer via grafting technique and TiO2 nanoparticle entrapment in the brush polymers was investigated. The surface modification led to a reduction in protein adsorption and bacterial attachment by 8.7 times and 71% respectively with no cytotoxicity effect on HeLa cells. It was also observed that when PU surface became superhydrophilic the bacterial adhesion becomes independent of bacterium type. In general, it was observed that the impact of topographical changes on the biocompatibility and biofilm formation becomes significantly more profound than that of the surface chemistry alteration.

KEYWORDS
bacterial attachment; protein adsorption; TiO2/PEG; superhydrophilicity; nanostructured surfaces

1. Introduction
Polymeric biomaterials play an important role in health care and are used broadly in medical devices. However, creating biomaterials with biocompatibility is a challengeable task.[1] As the surface of biomaterials is the first point of contact with living cells and the initial response of immune system is related to the physicochemical properties of the surfaces such as polarity, hydrophobic interactions and van der Waals forces investigation of the surface chemistry and structure seems very important for biomaterial designing.
The interaction of the protein molecules such as immunoglobulins, proteoglycans, collagen, fibronectin, fibrin and laminin with biomaterials under the contact of living tissues may result in the attachment of bacterial cells which finally trigger the colonisation of the cells to the surface of biomaterials and may lead to the biofilm formation and infection.[2] The attachment of proteins and bacterial cells on the surfaces may also result in a series of responses such as inflammatory, foreign body reactions,[3] ultimately fibrous encapsulation, protein adsorption, complement activation and thrombus formation.[4,5]

From biological point of view, generally cells adhesions are strongly affected by the physiological activities of cells. These activates are metabolic state, cell surface hydrophobicity and charge, and the contact time of the cells and the biomaterials surfaces.[6] Bacterial cells adhere to the surface of the biomaterials via physical, molecular and cellular interaction.[1,7] From material standpoint, the cell adhesion and protein adsorption onto a substrate are highly affected by chemical and physical properties of surfaces such as surface energy, chemical composition, charge, roughness, morphology, topography, etc.[6] These physiochemical properties are integrated embodiments of surface wettability. So far, most of the researchers have focused on the smooth hydrophilic or hydrophobic surfaces rather than rough super hydrophilic/hydrophobic ones.[8–10] The outcome of these researches are not also consistence; some of them observed an increase in protein adsorption onto hydrophilic surfaces[11] whereas other works showed that proteins tended to absorb onto hydrophobic substrates.[12] Researchers have tried to increase their understanding about the interaction between cells or proteins and surfaces through extending their studies towards extreme wettability of superhydrophilic/phobic surfaces.[13–17] These extension of studies not only did not provide universal rules and better understanding on the influence of such extreme environments on the physiological response of cells but also resulted into more contradictory results and raised many new questions. For instance, some works showed that superhydrophilic modification led to almost lower or even no protein adsorption or cell adhesion;[18–22] others showed a significantly higher protein adsorption or cell adhesion.[11,23–25] This incoherent picture arises from the fact that the cell adhesion and protein adsorption onto a surface have never been fully quantitatively characterised and comprehensively studied. This is due to a remarkably large number of parameters which can affect the protein adsorption and adhesion process. The natural dynamisms of cells, together with the dynamic wetting factors form a very difficult system to characterise. Even a single cell can behave differently from a surface to another one depends on each surface physiochemical properties. Having a superhydrophilic surface through creating a hierarchical morphology with micron-nano roughness is inevitable and, therefore, there is still a continuing quest for studies which elucidate the role of nanostructured morphology on the protein adsorption and cell adhesion.

Adsorption of proteins onto a surface is considered the key factor which can determine the fate of adherent bacterial cells and initiation of biofilm formation and infections. As the treatment becomes too difficult after the biofilm formation due to the antimicrobial resistance feature of the biofilms,[26] altering the surface architecture and chemistry seems to be beneficial in order to prevent or postpone the biofilm formation. For such surface engineering two different strategies can be adopted: (1) coating the biomedical surfaces by carefully selecting a suitable biomacromolecules to prevent protein adsorption, and (2) increasing the surface hydrophilicity towards superhydrophilicity.
through manipulating the surface properties to provide a permanent hydration layer.\cite{27} So far, the researchers' focuses have been mostly on the primary strategy, while the later has recently gained a significant attention as an efficient facile approach to render biocompatibility and biofilm formation resistance. Surface properties such as surface free energy (SFE), charge, roughness, micron–nano architecture morphology, the degree of hydrophilicity, porosity and composition are important factors that have effects on blood compatibility and protein/bacterial adhesion and mammalian cells adhesion to surfaces.\cite{1,6,28,29}

To create a superhydrophilic surface, the two parameters of surface chemistry with changing chemical composition of the surface of biomaterials and surface structure with changing the roughness of the surface can be manipulated.\cite{28} In general, increasing the surface energy by chemical modification could result in an increase in the hydrophilicity while reducing the surface energy could lead to an increase in the hydrophobicity.\cite{30} Since cell-biomaterial interaction occurs in nano-scale,\cite{31,32} the investigation of functionalised surface in nano-scale seems also necessary.

It was reported that grafting of hydrophilic materials onto the surface of hydrophobic polymeric films resulted in the reduction of its bacterial adhesion\cite{33} and protein adsorption.\cite{29,34,35} This is mostly due to the formation of $\text{–OH}$ functional groups on the surface of the films, which increases the amount of hydrogen bounding interactions and hydrophilicity which causes a repulsion between the thin layer of water and proteins.\cite{6,36} As the protein adsorption is the first stage of platelet deposition on the surface of blood contact materials,\cite{37,38} the hydrophilic/superhydrophilic modification of surfaces in order to decrease denaturation of protein molecules in hydrophobic surface could be useful to improve the efficiency of related biomaterials.\cite{39} In this study, polyethylene glycol (PEG) was selected as the hydrophilic moiety during grafting process because it had been known as one of the best hydrophilic polymers to reduce nonspecific attachment of the proteins on the surfaces.\cite{1,40} PEG layers have been commonly used for surface modification in order to minimise undesired cellular responses.\cite{41} Furthermore, PEG has excellent steric stabilisation effects and coordination with water molecules in aqueous medium.\cite{42} These reasons indicate the significant potential of PEG polymers for surface engineering and biomaterial designing. Polyurethane (PU) was also selected as substrate in this work as it is widely used in several industrial applications such as biomedical devices,\cite{43} engineering, adhesive and coating materials.\cite{44,45}

Many experiments on the surface modification of different substrates with PEG have been carried out. For example, Alves et al. reached the contact angle of $72.1^\circ$ from $99.6^\circ$ and surface tension of 20.53 from 32.46 mN/m by grafting of PEG6000 to the surface of thermoplastic polyurethanes.\cite{46} Tan and Obendorf modified the surface of microporous PU membrane by grafting of different concentration of PEG in order to produce moisture responsive membrane.\cite{47} Lee et al. modified segmented polyurethane with pluronics with different polyethylene oxide chain length to resist the surface of blood contacting devices against platelet adhesion.\cite{38} Feng et al. improved hemocompatibility of polycarbonateurethane surface by UV-initiated polymerisation of PEG onto the surface and suppressed the platelets adhesion onto the film by reducing the surface hydrophilicity from the contact angle of $86.0^\circ−58.2^\circ$.\cite{48} Cheo et al. grafted PEG chains into natural rubber via UV graft polymerisation and managed to decrease the contact angle by $26^\circ$ and reduce the adsorption of protein and platelet adhesion.\cite{49} Dong et al. grafted PEG into
polyamide and polyester surfaces and reduced the biofilm formation of Listeria monocytogenes about 96% and reached to the contact angle of 24° and 31° from 62° and 76°.[50] Caro et al. grafted PEG on stainless steel surface and reduced the adhesion of bovine serum albumin (BSA) and Listeria ivanovii around 97%.[51]

To the best of our knowledge, none of the previous works have managed to reach superhydrophilicity by incorporating PEG on PU surfaces. Also, there is no or limited studies which elucidate the effect of key surface parameters such as surface chemistry and structure on the biofilm formation and biocompatibility enhancement.

Here, an efficient approach of superhydrophilic surface modification of polyurethane with systematic characterisation was performed to investigate the influence of different parameters, i.e. grafting density, SFE, protein adsorption and surface topography on cell viability, protein adsorption and bacterial attachment. Moreover, the independent of bacterial outer layer properties like surface hydrophobicity and surface charge of micro-organisms on the adhesion to the surface with extreme wettability was investigated. Based on our previously published works,[52–54] we found that addition of TiO2 nanoparticles on polymeric surfaces can significantly alter surface characteristics and BSA protein adsorption. In this work, TiO2 nanoparticles alongside PEG molecules were also incorporated to see how an organic—inorganic pattern on a surface can change surface morphology, chemistry, protein adsorption and bacterial attachment.

In addition, an attempt has been done to introduce a mechanism which explains how the creation of micron- and nano-scale hierarchical structure can change the surface properties and its biological activity. The biocompatibility of the modified sample was evaluated by MTT assay and protein adsorption test and the hydrophilicity of the samples were measured by contact angle measurement (sessile drop) method. The hierarchical structure and surface functional groups were analysed with the help of scanning electron microscopy (SEM) and Fourier transfer infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. Materials

Toluene, hexamethylene diisocyanate > 99% (HDI) and dibutyltin dilaurate > 97% were obtained from Merck. Poly (ethylene glycol) (PEG; 2000 Da) and absolute ethanol from Merck were used without further purification. RPMI-1640 medium, fetal calf serum (FCS), dimethyl sulfoxide (DMSO), nutrient broth bacterial culture media, titanium (IV) iso-propoxide (TTIP), acetylacetone, perchloric acid and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma.

2.2. Polyurethane film preparation

Polyurethane films with the dimension of 2 × 3 cm² were made by the injection moulding of thermoplastic granules of commercial polyurethane and were washed and sonicated to remove any processing agents. Then the substrates were dried for 24 h in 60 °C. The samples were sonicated again for 20 min before use.
2.3. **TiO₂ nanoparticle preparation**

Titanium nanoparticle sol–gel solutions were prepared following the route used by Razmjou et al.\cite{53} In this method, nanoparticles were prepared by mixing of titanium (IV) iso-propoxide (TTIP), anhydrous ethanol, perchloric acid, 2,4-pentanedione and H₂O in the molar ratio of 1:0.45:0.5:0.5:4.76 for an hour at room temperature.

2.4. **Grafting of PEG onto PU surfaces**

As mentioned in the introduction, to create superhydrophilic polyurethane surface, PEG was chosen due to its excellent biocompatibility,\cite{55} good water absorbance,\cite{56} strong flexibility \cite{57} and nonimmunogenicity.\cite{58} To make a stable PEG modified surface, covalent attachment of PEG is needed. To avoid steric hindrance and increase the grafting density of PEG chains, hexamethylene diisocyanate was used as coupling agents.

In brief, the surface modification was performed by grafting of PEG onto the surface in two steps under nitrogen atmosphere. First, the polyurethane surface was functionalised with 5.00%, 2.5%, 1.25%, 0.62%, 0.31% and 0.15% v/v of HDI. The PU samples were immersed in the solution of toluene as the solvent and swelling agent. Then the samples were washed with toluene for 30 min. In the second step, samples were grafted with PEG in 10% w/v solution of toluene and PEG for 24 h at 45 °C. Finally, samples were rinsed with toluene and dried at 35 °C for 24 h. Figure 1(a,b) shows the schematic diagram of PEG grafting process on the surface of PU films.

2.5. **TiO₂ nanoparticles entrapment**

The TiO₂ nanoparticles were entrapped in the surface of modified PU films by the low temperature hydrothermal process for its hydrophilic and photocatalytic nature to produce a biocompatible layer. In brief, the modified samples were dip-coated in TiO₂ solution with the coating speed of 2 mm/s for 8 s holding time. Then the samples were dried in 120 °C for 16 h and treated in the water bath in 90 °C for 24 h. The samples were rinsed three times and dried in a vacuum oven at 35 °C for a day.

2.6. **Surface characterisation**

2.6.1. **Fourier transform infrared spectroscopy**

Fourier transform infrared spectra for the samples were obtained by Fourier-transform infrared spectrometer (PerkinElmer Spectrum 100, Waltham, MA, USA) to analyse the changes in the functional groups of the surfaces.

2.6.2. **Contact angle goniometry**

The measurement of water contact angle can reflect microscopic chemical and structural heterogeneities.\cite{53} The hydrophilicity of the polyurethane surfaces coated with different concentration of HDI was characterised by using sessile drop techniques using contact angle goniometer (KSV Cam 200 instrument, Helsinki, Finland). The mean contact angles of at least eight water droplets on the random selected regions of each sample were reported.
Figure 1. (a) Schematic picture of chemical reaction of pegylation and (b) schematic diagram of PEG grafting reaction on the surface of poly urethane films; (a) HDI with two isocyanate (NCO) ends of which one (b) is converted to NCOH. The grafting of HDI occurs in 70 °C and NCO anchor group appears at the surface (c), then PEG with OH ends (d) converts to O end (e) and attaches to the HDI with covalent band (f).
2.6.3. **Thermal, chemical and mechanical stability**
To analyse the stability of the coating layers, the samples were immersed in deionised water at 30 to 100 °C for 20 min, were undergone sonication from 5 to 30 min and were maintained in a pH range of 0.2–13 for 24 and 48 h. Then their contact angles were measured to assess whether the coating can still maintain its superhydrophilicity.

2.6.4. **Grafting yield**
The grafting yield (GY) of the samples before and after HDI cross-linking and pegylation was calculated based on the percentage of increase in the weight of sample using the below equation:

\[
GY(\%) = \frac{W_g - W_o}{W_o} \times 100
\]

where \(W_0\) is the initial weight of each sample after rinsing with DI water and 15 min sonication. \(W_g\) is the weight of the samples after cross-linking and PEG grafting. In order to calculate grafting density, the weight of samples of PU films were measured by 6 decimal digit precision analytical balance.

2.6.5. **Surface free energy measurement**
In order to explore the effect of pegylated layer on the surface properties, the surface free energies of samples were measured according to acid–base Van Oss method using three different liquids of water, glycerol and diiodomethane with known parameters which are represented in Table 1.[59]

2.6.6. **Scanning electron microscopy analysis**
To analyse surface structural changes of the grafted samples with different concentration of HDI and TiO\(_2\) nanoparticles, a field emission scanning electron microscope (JEOL 7001F FEG FE-SEM, Peabody, MA, USA) at 5 keV acceleration voltages was used. A 1 nm layer of gold–palladium coating was sputtered on the sample surfaces to render them conductive.

2.6.7. **Protein adsorption capacity**
The ability of the coating to resist adsorption of proteins was investigated by Bradford protein assay. 6 cm\(^2\) PEG-grafted PU segments and TiO\(_2\) coated segments were exposed to 2 mg/mL solution of BSA solution for 24 h. Then, the absorbance of the solution was measured by Bradford assay at 595 nm by UV-visible spectrometry (Biowave II spectrophotometer, United Kingdom) before and after the removal of the segments. The amount

### Table 1. The parameters of acid–base (Van Oss) approach.

|          | \(\gamma^{\text{tot}}\) | \(\gamma^d\) | \(\gamma^+\) | \(\gamma^-\) |
|----------|---------------------|----------|-----------|---------|
| MQ water | 72.8                | 21.8     | 25.5      | 25.5    |
| Glycerol | 64.00               | 34.00    | 03.92     | 57.40   |
| Diiodomethane | 50.80               | 50.80   | 00.00     | 00.00   |

Note: \(\gamma\) refers to surface tension and superscripts d, + and – refer to dispersive, acid and base component, respectively.
of adsorbed protein was reported in $\mu$g/mm$^2$ according to the literature.[53] Results were reported based on the average of five independent measurements.

2.6.8. Cell viability assay

Since the surface properties like wettability and topography are critical factors that affect the cell-biomaterial interactions, biocompatibility assay is crucial.[14] To investigate the biocompatibility of the coating layers, 1 cm$^2$ of each sample was incubated in 1 ml of Milli-Q water for 24 h and the extracts were filtered (0.45 $\mu$m) and used for MTT assay. MTT assay was performed according to the procedure reported previously.[60] Briefly, human cervical cancer cells (HeLa) were grown in RPMI-1640 medium supplemented with 10% FCS. The cells were seeded on 96-well plates containing 200 $\mu$L medium (0.5 mg mL$^{-1}$ in media) at a density of $10^4$ cells ml$^{-1}$ and cultured in a humidified incubator at 37 °C in 5% CO$_2$. The cultured cells were then treated in separate wells with 10 and 20 $\mu$g ml$^{-1}$ of the treated waters and incubated for 48 h in the same conditions. Then the medium was discarded and 100 $\mu$L MTT was added into each well and incubated again at 37 °C for 4 h. Subsequently, 150 $\mu$L DMSO was added to each well and the absorbance was measured at 570 nm by using microplate reader. The cell viability was determined as ratio of absorbance values from each treatment and the control. All the experiments were repeated three times.

2.6.9. Microtiter plate assays

The adhesion behaviour of bacteria on the surface of biomaterials is an important property which can strongly affect the incidence of infection. To investigate the effect of coating on the bacterial attachment and formation of biofilm in the surface, three strains of bacteria (Streptococcus pneumonia, Staphylococcus epidermidis and Staphylococcus aureus) with strong ability to form biofilm and known as nosocomial infection strains were cultured in nutrient broth (NB) medium at 37 °C for 24 h. Then the optical density adjusted to 0.1 at 600 nm to reach $10^8$ cfu/ml. Three replicates of each sample were placed in 24-well polystyrene plates and 2 ml of bacterial suspension was dispensed in each well. The plates were incubated at 37 °C for 24 h. After incubation, the samples were washed three times with Milli-Q water and transferred into a new well plate. Two millilitre of crystal violet 0.3% was then added to each well. After 15 min, samples were gently rinsed again three times to remove non-bound bacterial cells and extra stain. The washed samples were immersed in a new well plate containing 2 ml of ethanol 96% for 20 min to release crystal violet from the attached cell walls of bacteria. The optical density of the solution of each well was measured at 540 nm by UV-visible spectrometry. Three replicates of each sample, incubated with fresh sterile NB without bacteria, in the same situation, were used as the negative control. The optical density of crystal violet from bacteria was corrected by subtracting its mean OD from the negative control prior to statistical analysis. The relative percentage of bacterial attachment inhibition was calculated by the below equation:

$$\text{Percentage of inhibition (\%)} = \frac{OD_u - OD_m}{OD_u} \times 100$$  \hspace{1cm} (2)

where $OD_u$ is the optical density of the unmodified sample, while $OD_m$ is the optical density of the modified sample.
3. Result and discussion

3.1. Fourier transforms infrared spectra

The appearance of functional groups in the surfaces can be observed by FTIR spectra. Figure 2 shows the effect of different concentration of HDI on the presence of functional groups at the surface of polyurethane films. The isocyanate (NCO) absorbance at 2348 and 2216 cm$^{-1}$ shows a successful covalent attachment of HDI on the surface of PU films. Without HDI treatment the adsorption of PEG is weak and can be easily washed away by rinsing. As can be seen in the figure, the NCO peak becomes very weak after pegylation (samples (d–f)) which could be related to the appearance of covalent bond between isocyanate groups and PEG branches. Apparently, by increasing the concentration of HDI the OH band groups at 3351 cm$^{-1}$ become stronger, which might be an indication of higher PEG graft density on the PU surfaces.

3.2. Superhydrophlicity

The hydrophilic or hydrophobic quality of a smooth and clean surface arises from its chemical and geometrical structure. Surface wettability can be modulated by

![Figure 2. IR spectra of the samples (a) unmodified PU, (b) pegylation without HDI cross linker, (c) 5.00% HDI concentration without further pegylation, (d) 1.25% HDI with pegylation, (e) 2.50% HDI with pegylation and (f) 5.00% HDI with pegylation.](image-url)
modifying surface chemistry and structure which direct the surface tensions at microscopic scale.

One of the definitions of superhydrophilicity is having the surface with water contact angle less than 5°. Others define it as a surface with complete water spreading and roughness factor larger than 1.[61] Another definition is a surface in which the contact angle is enhanced by small roughness and is less than 5°.[9] Here, we considered a surface superhydrophilicity based on the definition introduced by Wang et al. [53] which is more widely accepted and is achieving zero contact angle within the first 5 s.

Figure 3(a) shows the changes in the water contact angle as a function of HDI concentration. As seen, pegylation successfully increased the wettability of all modified samples. An increase in the amount of HDI anchor groups enhanced the grafted PEG and TiO₂ nanoparticles density onto the surfaces, which leads to a good coordination with water.
molecules such that water contact angle decreased from 84° to 0° when the HDI concentration increased from 0% to 5.00% v/v. From Figure 3(b), superhydrophilicity was achieved when HDI concentration increased to above 1.25% v/v.

### 3.3. Thermal, chemical and mechanical stability

The stability of the coating layers in terms of thermal, chemical and mechanical resistances plays an important role in maintaining the performance of biomaterials. Figure 4 shows the effect of temperature, pH and sonication time on the contact angle of 5.00% HDI sample. As can be seen, the contact angle of modified samples after exposure to different temperature and sonication time remains in the hydrophilic range considering the standard deviations. Slight observed increases in the water contact angle in Figure 4(a,b)

![Figure 4](image_url)
imply the fact that there should have been some PEG molecules that were physically entrapped in the brush polymers, and were left the surface after the treatments.

However, increase in water contact angle in acidic and basic condition shows the susceptibility of the layer to these conditions, which is obvious as the PEG layer is a sensitive organic layer. In addition, roughness can be changed at high or low pH, which could change the surface wettability (see Section 3.6).[62]

### 3.4. Grafting yield

Figure 5(a) shows the GY of different samples before and after pegylation based on Equation (1). The difference between total grafting density and HDI grafting density is the grafting density of PEG layer on the surface. As the amount of NCO anchor groups on the surface increases, the density of PEG brush polymer escalates and leads to the reduction in water contact angle (see Figure 3). This increase in GY may have a significant effect on the surface structural changes. As presented schematically in Figure 5(b), an

![Figure 5. (a) Grafting yields of the samples with different concentration of HDI and (b) schematic picture of ordered molecular orientation in the surface of PU films.](image)
increase in the concentration of HDI resulted in an increase in the density of sites for chemical adsorption of PEG molecules. This may increase the inter-molecular interaction between polymer brushes. The inter-molecular interactions play a significant role in surface engineering to graft the functional molecules in the desired geometry and to create regularly arranged molecules in an ordered molecular orientation.[51]

### 3.5. Surface free energy

As mentioned before, both surface energy and the surface roughness are characteristic properties of a solid surface, which could strongly affect its wettability.[63] An increase in SFE can increase hydrophilicity while a reduction in SFE increases hydrophobicity. This surface wettability alteration, because of change in SFE, is limited to a certain level. A further increase or decrease in wettability beyond that limit is achievable through surface roughness changes.

Figure 6 shows the effect of pegylation on the SFE of the modified samples. As seen in the figure, PEG grafting has increased the surface free energies by two folds from 29 to 55.4 mN/m. The figure also shows that SFE enhanced initially and then levelled off, which means a further addition of HDI concentration, did not lead to an increase in SFE. However, Figure 3 shows that contact angle reduced significantly when HDI concentration increased. This suggests that surface structural changes have a greater contribution in increasing the hydrophilicity than surface chemistry alteration.

### 3.6. SEM analysis

To study the surface topography in the submicron structure, different samples were analysed by Field Emission Scanning Electron Microscope (FESEM). The effect of different concentration of HDI on the structure of PU films in micron-scale is presented in Figure 7. As can be seen, the unmodified sample (a) has the smoothest surface while roughened porous structure are formed when the surface was treated by HDI (Figure 7(b), (d–f)). A comparison between Figure 7(a) and 7(c) revealed that pegylation without HDI treatment

![Figure 6](image-url)  
**Figure 6.** Surface free energies of unmodified and modified samples with 1.25%, 2.50% and v/v 5.00% (v/v) HDI.
did not lead to a porous hierarchical structure which is necessarily for shifting the surface wettability towards the extreme conditions of superhydrophilicity or superhydrophobicity. Figure 7(c) also shows that the lack of HDI linkage results only in a physical adsorption of agglomerated polymer. Increasing the HDI concentration increases the surface
porosity and roughness (see Figure 7(d–f)). The SEM images showed that after modification hierarchical structure with multilevel roughness occurred which plays an important role in shifting the wettability towards superhydrophilicity.[64] As the roughness increased in the coated surface with 5.00% HDI, water contact angle declined from 84° to 0°.

Chung et al. showed that roughening the surface by grafting of polymer with different length chains at nano-scale can improve biocompatibility by enhancing cell growth and cell adhesion in tissue engineering.[65] It was observed that when the concentration of HDI increased to 5.00%, interesting feature was appeared. Figure 8 demonstrates that the modified PU surface with 5.00% HDI composed of many sea-urchin-like clusters in a random pattern with diameters of around 2–3 μm. Each of these sea-urchin-like cluster is composed of spear-like nanoflakes with the tip diameters of about 50–70 nm. It can also be seen that the spear-like nanoflakes grow radially and are self-assembled into micron- and nano-scale hierarchical structures. Based on the three-dimensional capillary effect, the surface roughness makes the surface more wettable and in the liquid/air interface the intermediate situation between imbibition (following the topography) and spreading will happen.[6,39,53] Here, these hierarchical structures with duel scale roughness help the water droplets spread quickly and imbibe into the grooves of the sea-urchin-like clusters and leave the top of the substrate dry in the film regime. Wenzel and Cassie–Baxter

Figure 8. SEM image of sea-urchin-like clusters formed on the PU surfaces which were modified with 5.00% v/v HDI.
models are used to understand the mechanism by which the surface wettability changes towards the extreme conditions.[66] Based on the Wenzel model, the effect of roughness is to amplify the wettability of the surface towards superhydrophobicity or superhydrophilicity. The below equation was introduced by Wenzel to describe the effect of surface chemistry and morphology on the water droplet contact angle:

\[
\cos \theta = r \cos \theta^e \tag{3}
\]

where \(\theta^e\) is the contact angle on the flat surface and \(\theta\) is apparent contact angle. The roughness factor \(r\) is the ratio of the actual solid/liquid contact area to its vertical projection. According to Equation (1), the surface chemistry represents by \(\theta^e\) whilst morphology lies within \(r\).

Producing hierarchical structure that creates multilevel roughness increases the \(r\)-value and thus renders superhydrophilicity [59] when \(\theta^e\) lies within the hydrophilic region.

### 3.7. Protein adsorption resistance

As the adsorption of proteins to the surface of biomaterials can initiate bacterial adhesion and colonisation onto the surface,[67] because of forming attractive sites for the attachment of bacteria, decrease in protein adsorption substantially reduces the biofilm formation.

The protein adsorption resistances of PEG-grafted/TiO\(_2\) entrapped surfaces were measured by Bradford assay. Figure 9 shows the amount of absorbed BSA on the surface of samples with different HDI concentration. As seen, the modified sample showed above 0.62\% more resistance in the adsorption of BSA protein. All of the modified samples show more resistance than that of neat sample. In fact, unmodified samples adsorbed the protein molecules 8.6 times more than the sample with 5.00\% v/v HDI. Molecular dynamic studies revealed that superhydrophilic surfaces produce large repulsive forces on the proteins, which lead to a lower protein adsorption.[68] The water barrier mechanism is also known as the main possible reason of reducing protein adsorption on the PEG self-assembled monolayers, which forms a physical barrier to prevent direct contact between

![Figure 9](image-url). Amount of BSA adsorption on the surface of modified samples with different concentration of HDI (% v/v).
the protein and the surface.[69–71] In another word, the tightly hydrogen bound water layer adjacent to the PEG/TiO₂ layer is mainly responsible for the large repulsive forces and the PEG layer itself has less or no direct effect on the protein adsorption reduction. It should be pointed out here that, by increasing the thickness of the modified layer in the surface, the deposition of indwelling particles like protein molecules on the surface decreases.[72] An increase in the thicknesses of inert end group of PEG/TiO₂ layer created a larger space between the surface of PU films and proteins and bacterial cells, which limit the van der Waals attraction and can delay the biofilm formation. It was also observed that the protein adsorption of the PEG layer without further addition of TiO₂ nanoparticles showed in average 8% fewer resistance than that of TiO₂ entrapped samples.

3.8. Cell viability assay

*In vitro* study of cell viability (MTT assay) revealed the small reduction of the cell viability, which depends on the composition and structure of the layers. It is known that almost all hydrophilic surfaces exhibit good cell adhesion property.[6] There was a negligible increase in the cell toxicity by increasing the volume of the treated waters. Among different treatments, minimum cell viability (83%) obtained by using 20 µl extract of bare PU while maximum cell compatibility (99.8%) achieved by adding 10 µl extract of 0.15% HDI treatments to the cells (Figure 10(a)). An appropriate cell viability (≈98.50%) also obtained by using concentrations of 5.00% and 0.15% HDI. Furthermore, cells in 5.00% concentration of HDI could live longer than others, because of higher presence of OH groups. The results obviously showed cell compatibility of the PEG-based hierarchical hydrophilic structures that indicate suitability of these structures for *in vivo* application and for surface engineering of biomedical devices.

Figure 10(b,c) shows the HeLa cells after 24 and 48 h exposure to the sample with 5.00% v/v HDI, which shows that the cells could proliferate without change in their morphology. It reveals that the nanostructured modified surface exhibits excellent hydrophilic and biocompatible properties, which has no toxic effect on the cells. A comparison between samples with and without the entrapment of TiO₂ nanoparticles showed that the addition of TiO₂ nanoparticles did not induce any cytotoxic effects on the cells (data were not shown).

3.9. Microtiter plate assays

Since bacterial surface composition is a complex with a variety of hydrophobic/hydrophilic site (polysaccharide, lipids and protein), finding straight mechanism of attachment for them is too difficult. Some research study showed that the SFE of the bacteria/surface can affect bacterial attachment, while other showed no correlation.[73] To analyse the ability of bacteria to attach on the surface, the microtiter plate assay using *Pseudomonas aeruginosa*, *staphylococcus aureus* and *staphylococcus epidermidis* was conducted. The decrease in the tendency of bacteria to attach to the surface of the samples after 24 h incubation is presented in Figure 11. From the figure, a high bacterial attachment found in the unmodified samples. As can be seen, the samples with 5.00% v/v HDI show a strong potential to reduce biofilm formation according to its superhydrophilicity and protein adsorption resistance features which lead to suppress nonspecific and bio-specific selective
interactions.\textsuperscript{[6,74]} By superhydrophilic modification and by reducing the interplay of van der Waals, hydrophobic and electrostatic interaction between the bacterial cells and the surface of biomaterial, the delay in irreversible attachment of bacterial with exopolysaccharides and specific ligand occurred. While almost all bacteria are negatively charged,\textsuperscript{[75]} such inert surface can repel the initial attachment of bacteria with long and short range interaction. In particular, high PEG-grafted density surface enables steric repulsive barrier to bioadhesion of proteins and bacterial cells.\textsuperscript{[76]} Figure 11 also shows an interesting finding that when our surface became superhydrophilic (with 5\% v/v HDI and 10\% w/v PEG) the values of percentage of attachment inhibitions of different bacteria are similar considering the standard deviations, whereas they are different when the surface is not superhydrophilic. This implies the fact that adhesion of micro-organism on the surface with extreme wettability seems independent of bacterium type and its outer layer properties.

\textbf{Figure 10.} Percentage of cell viability in MTT assay with 10 and 20 $\mu$l of treated water (a), the image of HeLa cell line after 24 h (b) and 48 h (c) treatment with 5.00\% HDI sample.
4. Conclusions

A hierarchical superhydrophilic nanostructured layer was successfully created through grafting of PEG molecules and TiO₂ nanoparticles entrapment on the polyurethane (PU) surfaces using HDI coupling agent. The effect of preparing such architecture on the PU surface was studied in terms of change in surface chemistry and structure as well as biofilm formation and biocompatibility enhancement. A good thermal, mechanical and chemical stability was observed for modified samples. The results showed that increasing the concentration of HDI significantly enhanced the grafting density and consequently

Figure 11. (a) Percentage of bacterial attachment inhibition in samples with different concentration of HDI and (b) 24 well plate, in each column a, b and c are modified sample with *P. aeruginosa*, *S. aureus* and *S. epidermidis* bacteria, respectively, and d, e and f are control negative with fresh medium.
resulted in a significant reduction in water contact angle such that the surface wettability shifts towards superhydrophilicity. The surface modification leads to a lower protein adsorption capacity, higher degree of biocompatibility and biofilm formation resistance. It was found out that when PU surface became superhydrophilic the bacterial adhesion becomes independent of bacterium type. A sea-urchin-like clusters (2 to 3 μm in diameter) composed of spear-like nanoflakes with tip diameters of 50—70 nm were formed after increasing the HDI concentration to 5.00% v/v. The systematic characterisation also revealed that the contribution of surface morphological alteration on the new observed properties is more than that of surface chemistry changes.

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