The Effect of Nano-ZnO Surface Wettability on Modulating Protein Adsorption

Qian Hu1, Yadan Ding1, Hong Shao1, Tie Cong1, Xiaoguang Yang2,3 and Xia Hong1

1Key Laboratory of UV-Emitting Materials and Technology (Northeast Normal University), Ministry of Education, Changchun 130024, P. R. China
2College of Chemistry, Northeast Normal University, Changchun 130024, P. R. China
3National Engineering Laboratory for Druggable Gene and Protein Screening, Northeast Normal University, Changchun 130024, P. R. China

Abstract. Although surface wettability plays a major role in regulating protein adsorption and nanostructured ZnO has shown great potential in various biomedical fields, few reports have examined the influence of nano-ZnO surface wettability on protein adsorption. Herein, we explored the adsorption behavior of bovine serum albumin (BSA) on the superhydrophilic, hydrophilic, hydrophobic and superhydrophobic nano-ZnO surfaces. The adsorption amount of BSA increased with increase of hydrophilicity because of increased adsorption sites on the hydrophilic surface. The protein adsorption was proved to occur along with the desorption and conformational changes by well-fitted kinetic adsorption curves with the Spreading Particle Model and Fourier transformation infrared spectral analysis. The rates of BSA adsorption and desorption increased with hydrophobicity of the ZnO surfaces, which was considered to be related with the energy barrier created by water bound to the ZnO surfaces via hydrogen bonding. The rate of conformational change varied in a complex way, which might be influenced by the surface wettability of ZnO and some other factors. The present work may open up a new avenue to design nano-bio interfacial materials for advanced biological study and clinical applications.

1. Introduction
As an attractive semiconductor nanomaterial with good biocompatibility and excellent optoelectronic properties, nanostructured ZnO has shown great promise in various biomedical applications, including fluorescent biological imaging1, resonant Raman scattering-based biological detection2, field effect transistor-based biosensing3, and so on4,5. It is imperative to study the interfacial properties between ZnO and biological components (e.g., proteins, genes, and cells) to provide guidance for the relevant applications. As one of the most important surface properties of solid that affect the interaction between the solid surface and biological components, wettability of various solid surfaces has been extensively investigated6. Although the wettability of ZnO has also been studied7, few reports have examined its influence on protein adsorption, whose regulation is crucial for biomedicine and tissue engineering. A systematic investigation into the effects of the wettability of ZnO on protein adsorption and the establishment of the adsorption model will shed light on the understanding of the relevant interaction mechanisms, and provide theoretical and technical basis for the development of efficient nano-biomedical platform.

In this work, the macroscopic ZnO surfaces, constructed by the ZnO crystal seed layer (ZnO SL) or the ZnO nanowire array (ZnO NA), were used as the model surfaces to study the biological adsorption
process. Their wettability was adjusted between superhydrophilicity and superhydrophobicity via ultraviolet light (UV) irradiation and dark storage. The adsorption kinetics of bovine serum albumin (BSA), an abundant protein in blood plasma and traditional blocking agent\cite{8}, on these surfaces was studied in detail with the Spreading Particle Model (SPM). And the involved rate constants were calculated and discussed.

2. Experimental section

2.1. Materials

Zinc acetate dehydrate (Zn(CH$_3$COO)$_2$·2H$_2$O, ≥ 99.0%) and hexamethylene tetramine ((CH$_2$)$_6$N$_4$, ≥ 99.0%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Monoethanolamine (HOCH$_2$CH$_2$NH$_2$, ≥ 99.0%) was bought from Tianjin Guangfu Fine Chemical Research Institute. Ethylene glycol methyl ether (CH$_3$OCH$_2$CH$_2$OH, ≥ 99.0%) was purchased from Tianjin Tiantai Fine Chemicals Co., Ltd. Fluorescein isothiocyanate-labeled bovine serum albumin (BSA, ≥ 95.0%) was bought from Beijing Zhongke Universal Technology Co., Ltd. Phosphate saline buffer (PBS, powder) was bought from Beijing Dingguo Biotechnology Co., Ltd. All the reagents were of analytical grade and used directly as received without further purification.

2.2. Preparation of the ZnO SL and ZnO NA

The ZnO SL was prepared using sol-gel dip coating method. Firstly, Zn(CH$_3$COO)$_2$·2H$_2$O (36.2 g) was dissolved in CH$_3$OCH$_2$CH$_2$OH (220 mL) and HOCH$_2$CH$_2$NH$_2$ (10 mL). Then, the cleaned glasses were immersed into the above solution for 3 s, and lifted at a constant speed. The films were heated in air to 100 °C and kept for 30 min, and then heated to 450 °C to anneal for another 30 min. The heating rate was 2 °C·min$^{-1}$. Subsequently, the films were cooled down to room temperature naturally.

The ZnO NA was prepared by hydrothermal method. The as-prepared ZnO SL was suspended in an aqueous solution of Zn(CH$_3$COO)$_2$ (0.02 M) and hexamethylene tetramine (0.02 M) in a Teflon-lined autoclave, and then reacted at 90 °C for 2 h. After cooling down to room temperature, it was removed from the solution, rinsed with deionized water and dried. Subsequently, it was suspended in an aqueous solution of Zn(CH$_3$COO)$_2$ (0.01 M) and hexamethylene tetramine (0.01 M) at 90 °C for another 4 h to perform secondary growth.

In order to obtain the surfaces with different wettability for protein adsorption, part of the obtained surfaces were exposed to UV for more than 4 h, and the rest of the surfaces were stored in dark.

2.3. Protein adsorption on the ZnO surfaces

BSA solution was prepared in PBS (pH = 7.4). The adsorption experiments were carried out in weighing bottles of 5 mL at room temperature. First, 2 mL of BSA solution was added into each weighing bottle. Then, the ZnO surfaces with similar size were carefully placed into the solution and incubated at room temperature. The adsorption kinetic curves of BSA on the ZnO surfaces were obtained with the solution concentration of 800 μg/mL, and the incubation time from 5 to 1800 min. The solution concentrations before and after the adsorption experiment were determined by fluorescence intensity of the solution. And the adsorption quantities on the ZnO surfaces were calculated by equation 1.

$$q = (C_0 - C_1) \times V/S$$  \hspace{1cm} (1)

where $C_0$ is the initial concentration of BSA solution, $C_1$ is the concentration of the BSA solution at a certain time, $V$ is the solution volume, and $S$ is the area of the surface.

2.4. Characterization

X-ray diffraction (XRD) patterns were obtained using a Rigaku D/MAX-2400 X-ray diffractometer with Cu-Kα radiation (λ = 0.1541 nm). Morphologies of the ZnO SL and NA were characterized by XL-30 Field Emission Scanning Electron Microscope (SEM). The contact angle (CA) of each surface was measured by Krüss Drop Shape Analyzer-DSA100. The fluorescence intensities of BSA-FITC
solution were measured by LS-55 Perkin Elmer luminescence spectrometer. The excitation wavelength for BSA-FITC was 497 nm. Fourier transformation infrared (FT-IR) absorption spectra of adsorbed BSA on the ZnO surfaces were obtained with reflection mode using Nicolet IS10 spectrometer.

3. Results and discussion
The crystal structures of as-prepared ZnO SL and NA were investigated using XRD technique. As shown in Figure 1, all of the diffraction peaks can be indexed to hexagonal wurtzite structured ZnO (JCPDS card no. 36-1451). It is worth noting that the intensity of (002) diffraction peak of the ZnO NA surpasses others, indicating that the ZnO NA was growing preferentially along the [0001] direction, and the outer top surface is the (001) plane of the ZnO NA.

![Figure 1. The XRD patterns of the ZnO SL and the ZnO NA. And the standard diffraction pattern of hexagonal wurtzite structured ZnO.](image1)

In order to get more detailed information about the surface structure and morphology of the ZnO SL and NA, SEM observation was carried out. Figure 2a is the top view of the ZnO SL. It can be seen that the ZnO SL consists of numerous small crystals with a diameter of 22.3 ± 4.0 nm. The thickness of the film is about 48.9 ± 1.9 nm, as shown in the cross-sectional view (Figure 2b). Figure 2c and 2d are the top view and cross-sectional view of the ZnO NA, respectively. The diameters of the nanowires are about 30.9 ± 3.2 nm, and the lengths are about 1.1 ± 0.2 µm. The anisotropic structure of the ZnO NA was consistent with the XRD results.

The morphology would profoundly affect the wettability of the ZnO surfaces. For the relatively flat ZnO SL, a photoinduced switching between hydrophobicity and hydrophilicity is shown in Figure 3a. The water CA decreases from 105.6 ± 1.3° to 28.1 ± 1.1° after UV irradiation. It results from the photogenerated oxygen vacancies on the ZnO surfaces under UV irradiation, which could be occupied quickly by the hydroxyl groups in air, and thus improve the surface hydrophilicity. For the rough ZnO NA, the photoinduced wettability switching was enhanced greatly. The ZnO NA was superhydrophobic with the CA of 157.4 ± 2.2°, while it turned to superhydrophilicity with the CA of about 0° after UV irradiation (Figure 3b). The distinction between the wettability of the ZnO SL and the ZnO NA is ascribed to the difference in the surface structures of ZnO. On the one hand, the anisotropic ZnO NA oriented with the well-faceted (001) end protruding out has the lower surface free energy than the randomly oriented ZnO SL. On the other hand, air could enter the gaps between the ZnO NAs, and increase the hydrophobicity of the surface due to the shrink of the water droplet at the gas-liquid interface. After UV irradiation, more hydroxyl groups were adsorbed on the ZnO surface, and water would enter and fill the gaps between the ZnO nanowires due to the three-dimensional

![Figure 2. SEM images of (a, b) the ZnO SL and (c, d) the ZnO NA. (a, c) Top view and (b, d) cross-sectional view.](image2)

![Figure 3. Photographs of water droplet on the surface of (a) the ZnO SL and (b) the ZnO NA before (left) and after (right) UV irradiation.](image3)
capillary effect of the rough surface\textsuperscript{[11]}. Therefore, after UV irradiation, superhydrophilicity was observed with the ZnO NA, while hydrophilicity was observed with the ZnO SL.

The adsorption kinetics of BSA on the superhydrophilic, hydrophilic, hydrophobic and superhydrophobic ZnO surfaces were measured in order to study the protein adsorption process on the ZnO surfaces. It can be clearly seen from Figure 4 that the adsorption amount increases with the increase of hydrophilicity. It is because that the solution on the hydrophilic, especially superhydrophilic surfaces of ZnO tends to spread and infiltrate into the gaps between the ZnO crystal seeds or nanowires due to the three-dimensional capillary effect. Hence the contact area between the protein solution and the ZnO surfaces would be extended, and more adsorption sites would be provided for BSA. On the hydrophobic surface, the spread and infiltration of the solution would be reduced, and less adsorption sites would be available for BSA. And this phenomenon is amplified on the superhydrophobic surface because the air trapped within the gaps of the nanowires greatly prevents intimate contact of the surface with the protein solution and thus strongly inhibits BSA adsorption.

![Figure 4. Kinetic curves of BSA adsorbed onto the ZnO surfaces with different wettability. Solid lines embedded are the theoretical values fitted according to the SPM.](image)

![Figure 5. FT-IR spectra of (a) BSA in PBS solution, BSA adsorbed onto the (b) superhydrophilic, (c) hydrophilic, (d) hydrophobic and (e) superhydrophobic ZnO surfaces accompanied with curve-fitting.](image)

![Figure 6. Desorption kinetics for the (a) superhydrophilic, (b) hydrophilic, (c) hydrophobic and (d) superhydrophobic ZnO surfaces. The corresponding fitting formulas are shown above the curves.](image)

It can also be seen from Figure 4 that the adsorption amount (q) of BSA on the superhydrophilic surface reached a maximum within the first 180 min, and then decreased slightly to attain equilibrium. The similar variation tendency was observed for the other ZnO surfaces. It indicates that the adsorption process was predominant at first, and then replaced by the desorption process after a certain time. The adsorption-desorption equilibrium was attained finally. The distinction in the time to reach the maximum adsorption amount and the adsorption-desorption equilibrium on the ZnO surfaces with different wettability might be related to the difference in the rate of adsorption and desorption of BSA.
In addition, the conformational change of BSA which could cause irreversible adsorption was also a factor that might be considered.

FT-IR spectra were recorded to study the conformational changes of BSA adsorbed on these ZnO surfaces. The amide I region (~1700-1600 cm\(^{-1}\)), which was largely ascribed to C=O stretching vibration\(^{12,13}\), was employed for the secondary structure analysis of BSA. Four peak positions centered at 1681 ± 1 cm\(^{-1}\) (turns), 1656 ± 1 cm\(^{-1}\) (α-helixs), 1636 ± 1 cm\(^{-1}\) (β-sheets) and 1617 ± 1 cm\(^{-1}\) (side chains) were used in the curve fitting (Figure 5). As the absorption band at 1617 ± 1 cm\(^{-1}\) was assigned to the side chains of BSA\(^{14}\), not the amide carbonyl group in the main chain, the peak areas of the other bands were computed to obtain the contents of the secondary structures. As shown in Table 1, BSA adsorption on the ZnO surfaces caused obvious changes in the contents of α-helixs and β-sheets, and slight changes in the contents of turns compared with those of BSA in PBS solution. It indicates the occurrence of conformational changes of BSA upon adsorption on the ZnO surfaces.

| Table 1. Secondary structures (%) of BSA in solution and adsorbed on the ZnO surfaces |
|--------------------------------------------------------------------------------------------------|
| BSA in PBS solution | α-Helixs | β-Sheets | Turns |
| BSA adsorbed onto the superhydrophilic ZnO surface | 65.2 | 24.7 | 10.1 |
| BSA adsorbed onto the hydrophilic ZnO surface | 41.9 | 46.2 | 11.9 |
| BSA adsorbed onto the hydrophobic ZnO surface | 49.2 | 38.3 | 12.5 |
| BSA adsorbed onto the superhydrophobic ZnO surface | 48.0 | 43.1 | 8.9 |
| | | | |

Since conformational changes existed in addition to the adsorption and desorption processes, Spreading Particle Model (SPM) was adopted to describe the adsorption kinetics of BSA on the ZnO surfaces. In SPM\(^{15}\), the kinetic equation about the adsorption of protein molecules is a function of time (t). The simplest form of the equation describes the surface coverage of protein molecules as follows: first, the protein molecules adsorb on the surface reversibly at a rate \(k_c\). Then, the protein molecules either undergo desorption or conformational change to get adsorbed on the surface irreversibly. The rates of the two events are \(k_d\) and \(k_s\), respectively. The expression for the total adsorption amount as a function of time was obtained as:\(^{[16,17]}\)

\[ q = A_1 \cdot \exp(A_2 \cdot t) + A_3 \cdot \exp(A_4 \cdot t) + A_5 \]

(2)

As can be seen from the solid lines in Figure 4, the kinetic curves of BSA adsorption were all well fitted with equation 2, suggesting that the adsorption kinetics of BSA conforms to the SPM. The values of the parameters \(A_1\), \(A_2\), \(A_3\), \(A_4\) and \(A_5\) were obtained by curve fitting. As \(A_1\cdot \exp(A_2\cdot t)\) and \(A_3\cdot \exp(A_4\cdot t)\) gradually approach zero as time increases, \(A_5\) in equation 2 is closely related with the equilibrium adsorption amount. The value of parameter \(A_5\) (plateau value) decreases with the increase of surface hydrophobicity according to Table 2. It is consistent with the experimental phenomena, confirming that the superhydrophilic ZnO surface is conducive to protein adsorption, but the superhydrophobic ZnO surface inhabits its adsorption. The parameters \(A_1\), \(A_2\), \(A_3\) and \(A_4\) are related with the initial stage of adsorption. \(A_2\) and \(A_4\) can be used to calculate the rate constants \(k_c\), \(k_d\) and \(k_s\). They are described by equation 3 and 4\(^{[16]}\),

\[ - (A_2 + A_4) = k_h \cdot C + k_d + k_s \]

(3)

\[ A_2 \cdot A_4 = k_h \cdot C \cdot k_s \]

(4)

Since the descending portion of the curve in Figure 4 indicates the dominant desorption process of BSA, the desorption rate constant \(k_d\) can be estimated by fitting this region with equation 5\(^{[16]}\).

\[ q = A \cdot \exp(-k_d \cdot t) + B \]

(5)
where A and B are constants. The fitting results and the calculated desorption rate constant $k_d$ are shown in Figure 6 and Table 2, respectively. And thus, $k_C$ and $k_s$ can be obtained with equation 3 and 4. They are also shown in Table 2.

| Table 2. Values of kinetic rate constants and plateau value |
|---------------------------------|-----------------|-----------------|
|                                | Rate constants (min$^{-1}$) | Plateau value (μg/cm$^2$) |
| Superhydrophilicity            | 0.0108           | 0.00453         | 0.0095 | 175.61 |
| Hydrophilicity                 | 0.0219           | 0.00484         | 0.0219 | 129.95 |
| Hydrophobicity                 | 0.0270           | 0.00695         | 0.0200 | 84.36  |
| Superhydrophobicity            | 0.0275           | 0.00695         | 0.0200 | 31.95  |

As shown in Table 2, both the adsorption rate constant $k_C$ and the desorption rate constant $k_d$ increase with the increase of the hydrophobicity of the ZnO surfaces. It might be related with the energy barrier induced by water bound to the ZnO surfaces via hydrogen bonding interaction. As the amount of hydroxyl groups on the ZnO surfaces decreases with the increase of hydrophobicity, the hydrogen bonding between the ZnO surfaces and water becomes weaker, and the energy barrier created by water for protein adsorption gets lower. Thus the adsorption of the protein to the ZnO surfaces becomes easier, which leads to the increased adsorption rate $k_C$ of BSA on the ZnO surfaces with increased hydrophobicity. In the meantime, lower energy barrier also makes it easier for BSA to desorb from the surface and go back to the bulk solution through the interfacial region. Therefore, the desorption rate $k_d$ of BSA also increases with the increase of the surface hydrophobicity to achieve dynamic equilibrium. As for the conformational change rate constant $k_s$, it does not change monotonically with the increase of the hydrophobicity. It indicates that the conformational change of BSA is a complex process. It might be affected by not only the surface wettability, but also some other factors, such as surface free energy, specific geometrical figures, and surface functional groups.

4. Summary and conclusions
In summary, nano-ZnO surfaces with the wettability between superhydrophobicity and superhydrophilicity were fabricated. The adsorption amount of BSA increased with the increase of hydrophilicity of the ZnO surface due to the increased adsorption sites on the hydrophilic surface. The obvious conformational changes were caused after BSA was adsorbed on the ZnO surfaces. BSA adsorption kinetics on nano-ZnO was approximated by SPM, confirming the simultaneous occurrence of the protein adsorption, desorption and conformational changes. The rates of adsorption and desorption increased with the hydrophobicity of the ZnO surfaces. It might be related with the energy barrier caused by the water bound to the ZnO surfaces via hydrogen bonding. The rate of conformational change varied in a complex way, which might be determined by the synergistic effect of the surface wettability of ZnO and some other factors. The ZnO surfaces are promising to control the protein adsorption. This work may have instructive significance for understanding biophysicochemical interactions at the nano-bio interface and optimization of the nanobiomaterial selection, design and application.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (Grant Nos. 51272040 and 11604043), the 111 project (No. B13013), and Program for New Century Excellent Talents in University of Ministry of Education of China (Grant No. NCET-12-0815).

References
[1] Ye D X, Ma Y Y, Zhao W, Cao H M, Kong J L, Xiong H M and Mohwald H 2016 ACS Nano. 10 4294.
[2] Hong X, Chu X , Zou P, Liu Y and Yang G 2010 Biosens. Bioelectron. 26 918.
[3] Arya S K, Saha S, Ramirez-Vick J E, Gupta V, Bhansali S and Singh S P 2012 Anal. Chim. Acta. 737 1.

[4] Aydin Sevinç B and Hanley L 2010 J. Biomed. Mater. Res., Part B 94 22.

[5] Talebian N, Niforoushan M R and Zargar E B 2011 Appl. Surf. Sci. 258 547.

[6] Song W and Mano J F 2013 Soft Matter 9 2985.

[7] Feng X, Feng L, Jin M, Zhai J, Jiang L and Zhu D 2004 J. Am. Chem. Soc. 126 62.

[8] Bhogale A, Patel N, Sarpotdar P, Mariam J, Dongre P M, Miotello A and Kothari D C 2013 Colloids Surf., B 102 257.

[9] Sun R D, Nakajima A, Fujishima A, Watanabe T and Hashimoto K 2001 J. Phys. Chem. B 105 1984.

[10] Cassie A B D and Baxter S 1944 Trans. Faraday. Soc. 40 546.

[11] Shalumon K T, Anulekha K H, Nair S V, Chennazhi K P and Jayakumar R 2011 Int. J. Biol. Macromol 49 247.

[12] Byler D M and Susi H 1986 Biopolymers 25 469.

[13] Roach P, Farrar D and Perry C C 2006 J. Am. Chem. Soc. 128 3939.

[14] Maruyama T, Katoh S, Nakajima M, Nabetani H, Abbott T P, Shono A and Satoh K 2001 J. Membr. Sci. 192 201.

[15] Lundström I 1985 Prog. Colloid Polym. Sci. 70 76.

[16] Goyal D K and Subramanian A 2010 Thin Solid Films 518 2186.

[17] Wang Y, Deng H, Huangfu C, Lu Z, Wang X, Zeng X, He H and Rao H 2015 Surf. Interface Anal. 47 245.

[18] Zheng J, Li L, Tsao H K, Sheng Y J, Chen S and Jiang S 2005 Biophys. J. 89 158.

[19] Kwok S C H, Wang J and Chu P K 2005 Diam. Relat. Mater. 14 78.

[20] Song W and Chen H. 2007 Chinese Sci. Bull. 52 3169.

[21] Kidoaki S and Matsuda T 1999 Langmuir 15 7639.