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Titanium Dioxide Nanoparticles Binding to Human Blood Plasma Proteins

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Abstract

Titanium Dioxide (TiO_2, titania) nanoparticles are widely studied in cosmetology, food administration, and medical fields. This is due to the nanoparticles' suitable properties, specific combination of strength and high corrosion resistance. With the increasing attention to these particles, there is concern with potential health complications. Some of the complications involved with titania nanoparticles include blood clots and immune reactions (Sit et al., 2019). In this study, we continue the study conducted in the Sahai research group (J. Chen. MS thesis, 2020), on the binding of titania nanoparticles with blood plasma proteins, focusing on potential effects of shape only. Hydrophobicity of the titania nanoparticles of three different shapes (nanotubes, nanorods, and rutile hexagonal nanotubes) was established by heating the nanotube and nanorod samples. This was done to ensure that the samples had similar hydrophobic properties. The protein adsorption isotherms showed little adsorption to these samples due to their high hydrophobic properties. Subsequently, nanotubes and anatase hexagonal nanoparticles were tested because the two samples have similar hydrophilic properties but different shapes. The protein adsorption isotherms for these two samples showed similar binding affinities for the fibrinogen protein, however, the human serum albumin protein adsorbed poorly and did not yield accurate results. This study can give insight on the best design of nanoparticles that can be used in the biomedical field and cosmetology with the lowest risk of harmful effects.

Introduction

Titanium Dioxide nanoparticles (TiO_2) are used in the biomedical field for cosmetics, food biosensors, and for drug delivery. TiO_2 nanoparticles are utilized because of their suitable properties, high corrosion resistance, and the specific combination of strength with biocompatibility (Kulkarni et al., 2015). Once the nanoparticles are taken into the body, binding can occur onto proteins such as blood plasma proteins. The binding of the nanoparticles to the blood plasma proteins can change the function of the nanoparticles, which also causes concerns for health complications, such as undesired immune reactions and blood clotting (Sit et al., 2019). Plasma protein- TiO_2 interactions may also change the structure of the proteins and their function, such as regulation of blood clotting. The study of TiO_2 nanoparticles binding to blood plasma proteins has become a topic of interest because of the potential benefits of using the particles, however, there is concern for the health risk that is associated with these practices.

Deng et al., (2009) studied different metal nanoparticles, including titanium dioxide, and how their properties affect the binding abilities to blood plasma proteins. Their work suggests titania nanoparticle shape affects protein adsorption from plasma, but their work was conducted using whole plasma, and the details of how shape affects protein folding was not explored. The present study is a continuation of Jiadong Chen's work (M.S. thesis, 2020) in the Sahai group on the effects of titania nanoparticle shape on blood plasma protein adsorption and folding changes. The model proteins that were chosen for that study were human serum albumin (HSA) and Fibrinogen (FIB). These proteins were selected because HSA is the most abundant protein in the human blood plasma and FIB plays a key role in thrombus (blood clot) formation.

Chen's study focused on nanorods (NRs), nanotubes (NTs), and rutile hexagonal nanoparticles (HNPs). The rutile HNPs showed the strongest adsorption of the proteins, then the

NTs, then the NRs. (true for both proteins involved). The binding affinity for the FIB protein was greater than the binding affinity for HSA, however these various NPs were found also to have varying hydrophobicity/hydrophilicity with rutile HNPs being the most hydrophobic and the NTs and NRs being more hydrophilic. Thus, the effects of shape and hydrophobicity/hydrophilicity were confounded in that study. The goal of the present work was to prepare titania nanoparticles that have similar hydrophobicity/hydrophilicity but different shapes and to study plasma protein adsorption on these nanoparticles.

The model nanoparticles utilized for the present study are titania NTs, NRs, rutile HNPs, and hexagonal anatase hexagonal nanoparticles (AHNPs). NTs and NRs will be heated at varying temperatures to ensure that the samples are hydrophobic similar to the RHNPs, so that the shape of the nanoparticles is the sole variable parameter. Also, the NTs and AHNPs will be compared because they have different shapes, but both should be hydrophilic. The AHNPs are hypothesized to be more hydrophilic because they are produced by sol-gel synthesis method.

The results of the present study showed that the adsorption affinity of FIB on NTs was comparable to that of HNPs. The HSA protein adsorption isotherm showed little or no binding to NTs and AHNPs.

Experimental Section

1.1 Materials

All water used was ultrapure water (18.2 MΩ·cm) (Barnstead GenPure xCAD Plus Thermo Scientific, Rockford, IN, USA).

1.2 Synthesis of Titanium Dioxide Nanoparticles

Three types of TiO_2 NPs were utilized: NTs, NRs and HNPs. The NT and NR samples were utilized from J. Chen. MS thesis, 2020, and the HNPs were ordered from Sigma (St. Louis, MO,

USA). Four samples were prepared, each using 50 mg of each sample by heating the NTs and NRs using Thermo Scientific™ Lindberg/Blue M™ 1100°C Tube Furnace (Hampton, New Hampshire, USA) for 24 hours in 2 mL United Scientific porcelain combustion boats (Kingwood, TX, USA) at temperatures of 600°C and 700°C, to create a more hydrophobic sample.

1.3 Characterization of TiO2 NPs

Spectrometry and Thermogravimetric Analysis (TGA): TGA profiles were obtained using TGA550, TA Instruments-Waters LLC (New Castle, DE, USA). 10 mg of the original and heated NT and NR samples and HNP samples were placed on the TGA plate, then heated from room temperature to 600°C or 700°C at a ramp of 20°C per minute. The weight loss percent was measured and recorded. These samples are referred to as NT 600, NT 700, NR 600, NR 700 for samples heated to 600°C and 700°C respectively.

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR): About 5 mg of each sample was placed on the FTIR instrument plate to cover the imaging area fully. Infrared spectra of the original and heated NT and NR samples and the HNP samples were measured from 600 to 4000 cm^{-1}, scanning each sample 4 times (Excalibur FTS 3000, PerkinElmer, Shelton, CT, USA).

1.4 Protein Adsorption Isotherm

A 10 mg/mL stock solution of each protein was prepared in 10 mM HEPES pH 7.4 and 150 mM NaCl buffer and shaken for 5 minutes to dissolve the protein. Stock solution of 10 mg/mL was prepared of the NT 600 and NT 700 samples in 10 mM HEPES pH 7.4 and 150 mM NaCl buffer. The samples were sonicated for 3 minutes using the Branson 450 Digital Sonifier (Brookfield, CT, USA) to disperse the NPs. Protein concentrations ranging from 0 to 3 mg/mL

were prepared in PCR tubes containing a total of 100 µL protein solution. 100 µL NT solution was added to each PCR tube while continuing to tip sonicate. Controls containing 200 µL protein solution of the determined concentrations were prepared as well to account for protein adsorbing to the PCR tube wall. The samples and controls were incubated at 37°C for 4 hours (I24 shaker, New Brunswick Scientific, Edison, NJ, USA).

The samples and controls (no NPs present) were centrifuged at 20,000 RCF for 5 minutes to determine the protein concentration in the supernatant only. After the samples were centrifuged,150 µL of the supernatant was removed from each tube and added to a plastic 96-wellplate, ensuring that only the supernatant was extracted, and no nanoparticles were entrained. UV-VIS absorbance at 280 nm (Synergy H1 Hybrid Multi-Mode Reader, BioTeck, Winooski, VT, USA) was used to determine the protein adsorption in each PCR tube. The concentration of protein adsorbed on NPs (qe) was calculated by the difference between the initial concentrations in the blank control and the protein concentration in supernatant of the sample containing NPs (Ce).

The Langmuir adsorption model as outlined in J. Chen. MS thesis, 2020 was used to analyze the adsorption isotherms of the nanoparticles on each of the proteins.

Results and Discussion

3.1 Characterization of NPs

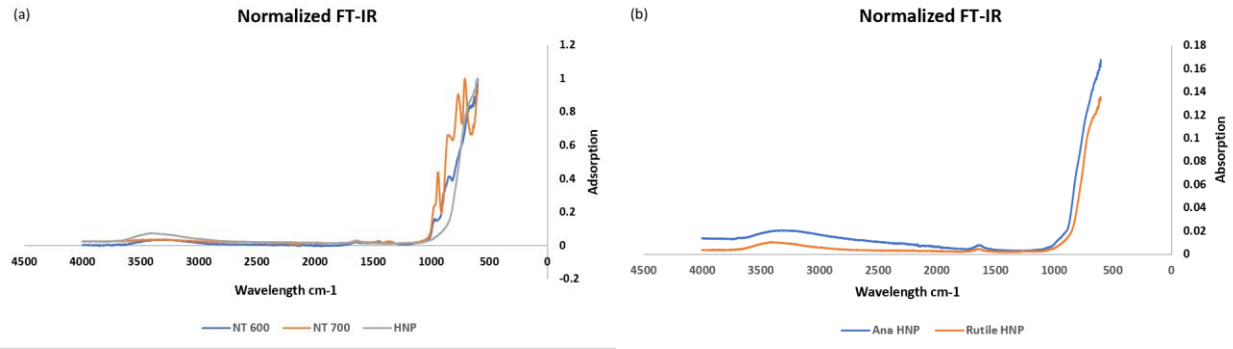

Figure 1. Comparing original NTs vs. NTs heated to 600 and 700ºC. (a) Original NT and heated NT FT-IR. (b) Anatase vs rutile HNPs

FT-IR spectroscopy was used to determine the presence of water and -OH groups associated with the TiO_2 NPs to help determine relative hydrophobicity/hydrophilicity. Each chemical bond and vibrational mode gives peaks at specific wavelengths to help characterize the molecules. Water peaks were present at 3000-3600 cm^{-1} and hydroxyl groups at ~1600 cm^{-1}. The FT-IR images in Figure 1 show the change in FT-IR spectra for the original and heated NTs and anatase and rutile HNPs. The original samples show peaks at 3300 cm^{-1} and 1600 cm^{-1}, whereas the heated samples have much lower peak intensity at these wavelengths. This suggests that the water bonds and hydroxyl groups have been significantly reduced from the molecules, making the heated samples more hydrophobic compared to the original samples.

3.2 Protein Adsorption Isotherm

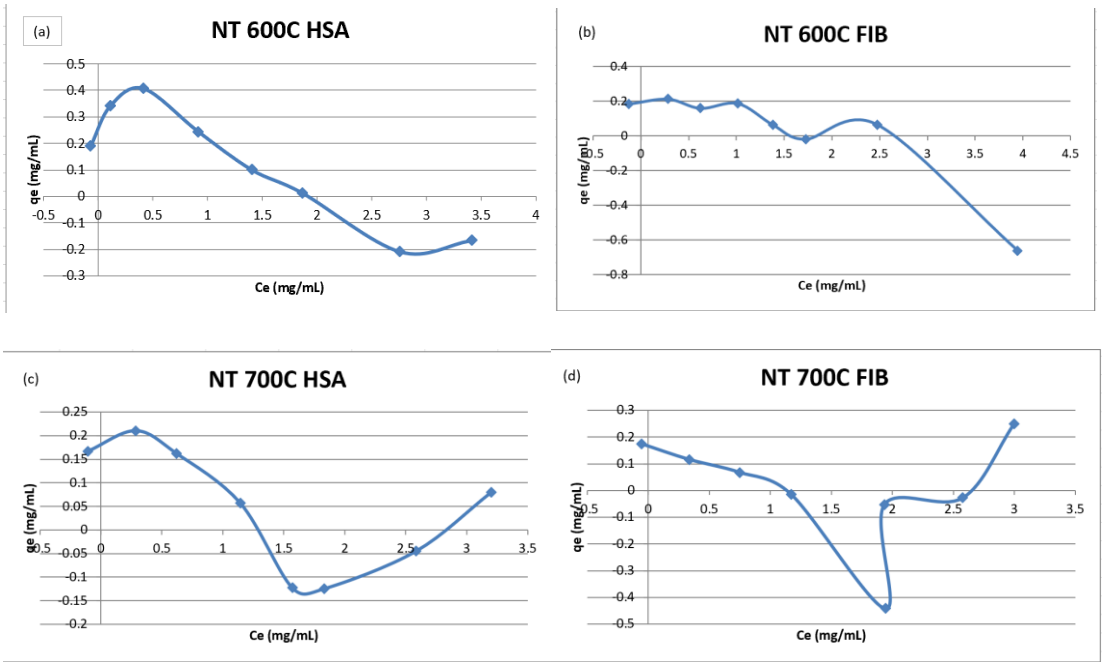

Figure 2. Protein adsorption on NTs heated to 600ºC (a, b) and 700ºC (c, d). HSA (a, c) and FIB (b, d) Ce refers to the equilibrium concentration of protein in solution and qe refers to the adsorbed concentration of the protein. These data was obtained by Ruibo Hu.

Protein adsorption isotherms are used to measure the total protein adsorption on the surface of the material. Figure 2 shows the protein adsorption isotherms for HSA and FIB on the 600ºC and 700ºC NT samples. Adsorption is limited and unusual patterns are seen, suggesting very low adsorption and therefore very large errors in the measurements. This could be caused by the low hydrophobicity of these samples. The protein adsorption isotherms were plotted as mg/mL, with the x-axis showing the Ce value, and the y-axis showing the qe values. Because of the low affinity of HSA, the subsequent adsorption isotherms were obtained only for FIB; also the NT 600 and NT 700 NPs were not used for further study.

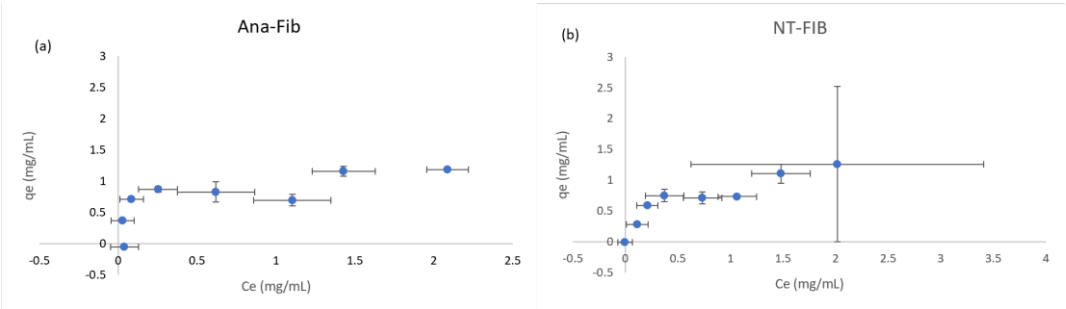

Figure 3. FIB protein adsorption on (a) anatase HNPs and (b) original NTs

All protein adsorptions were completed by Ruibo Hu in the Sahai lab. Anatase HNPs and original NTs were employed because they are both hydrophilic but have different shapes and FIB is relatively hydrophilic. Figure 3 shows the FIB protein adsorption isotherms AHNP and original NTs. For both protein adsorption isotherms in Figure 3, there first shows an increase in the amount of adsorption and eventually the adsorption concentration reaches a plateau. This means that the maximum adsorption onto the nanoparticle surfaces was achieved.

The AHNPs shows a faster initial increase in the amount of FIB adsorbed at low concentrations of protein in solution (Ce 0-0.25 mg/mL) compared to that of NTs suggesting a stronger binding affinity on AHNPs than on the original NTs. However, the two types of NPs appear to reach the maximum adsorption at the same concentration levels for the FIB protein. The adsorption isotherms were fit using the linear Langmuir model to extract the adsorption equilibrium constant (K_{ads}) and S_T. The maximum surface site concentration (S_t) of FIB on AHNPs and original NTs (Table 1). These model fits were completed by Mr. Ruibo Hy in the Sahai group. As expected from the initial adsorption behavior, the K_{ads} for Ana was greater than for the NTs by a factor of 3 or 4 times. Thus, FIB adsorbed more strongly on anatase than the original NTs, which was similar to Chen's study and the S_T values were similar (Table 1). S_T values from the Langmuir model fits were comparable for AHNPs and original NTs (Table 1) contrast to J. Chen. MS thesis, 2020 finding that S_T was lower for the original NT samples compared to rutile HNP samples.

Table 1. Binding affinity and maximum surface site concentration of anatase HNP and NT samples. The data for this table was calculated by Ruibo Hu. The units are measured in mL/mg for K_{ads} and mg/mL for ST.

FIB	**K_{ads} (mL/mg)**	**S_T (mg/mL)**
Anatase HNP	11.7013699	1.17068602
NT	3.07082833	1.30310138

Conclusions

Blood plasma protein adsorption is known to affect the performance of biomedical materials, like nanoparticles. The protein adsorption in this study showed the binding effects of hydrophobic titanium dioxide nanoparticles. It is known that the binding affinity for FIB is

greater than the binding affinity for HSA (J. Chen. MS thesis, 2020). The NT 600, NT 700, NR 600 and NR 700 NPs prepared in this study were too hydrophobic to adsorb either protein.

Because of the low binding affinity of the hydrophobic materials, there would be less change to occur to the function of the nanotubes when used in the human body. This means that hydrophobicity does play a role in the binding affinity of the nanoparticles, not just shape.

The protein adsorption performed on the anatase and the NT particles showed a comparison of shape with two hydrophilic NPs. The binding affinity of FIB on anatase HNPs was stronger than on the original NTs but the maximum amount of adsorption was comparable. This result showed that the shape of the TiO_2 did have a measurable effect on the adsorption of FIB on the two hydrophilic NPs. Future work would involve determining whether the differences in adsorption affinity between the two types of NPs influence the secondary structure of the adsorbed FIB. If they do, then shape will have been shown to affect protein structure and hence potentially its role in regulating thrombosis.

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