Raman Spectroscopic Study of TiO₂ Nanoparticles effects on the Hemoglobin State in Individual Red Blood Cell

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Titanium dioxide (TiO₂) is considered as a non-toxic material and widely used in number of everyday products, such as sunscreen. TiO₂ nanoparticles (NP) are also considered as prospective agent for photodynamic therapy and drug delivery. These applications require understanding of TiO₂ potential effects on blood system and its components upon administration. In the present work we analyze the interaction of TiO₂ NP of different crystal phases (anatase and rutile) with individual rat Red Blood Cells (RBC) and the TiO₂ influence on the oxygenation state and functionality of RBC, estimated via analysis of Raman spectra of Hemoglobin (Hb).

The size and ξ-potential of TiO₂ NP suspended in ultrapure MilliQ water were 15.5 ± 0.37 mV for anatase NP, 11.1 ± 0.36 mV for rutile. The size of rutile TiO₂(r) at that condition showed a narrow distribution with maximum near 154.4 ± 16.1 nm; anatase TiO₂(a) reveals 2 fractions, one centered near size 136 nm and the other, characterizing the aggregates, with averaged size of 448.1 ± 27.3 nm. When dissolved in PBS (pH 7.3), the measured values of ξ-potentials become negative and equal to -23.4 ± 2.0 and -25.2 ± 1.43 mV, respectively for the TiO₂(r) and TiO₂(a). From the particle size distribution, the TiO₂ in PBS aggregated or agglomerated and formed a wide distribution with the hydrodynamic size up to one micron.

Conclusion Raman mapping allows analysis of distribution of Hb in different forms along RBC and localization of the TiO₂ NP. No penetration of the NP inside RBC was observed, however both kinds of TiO₂ NP adsorbed on the RBC membrane that can affect the Hb oxygenation state. The TiO₂ effect on the RBC is observed, resulting in a decrease in the oxygenation degree of RBC. We suggest that TiO₂ affects Hb oxygenation state via altering RBC membrane properties. Possible influence on the safety at TiO₂ use in advanced medical application, especially on safety and efficiency of photothermal therapy, are considered. Facilitating by TiO₂ of the disturbing treatment of laser is probable, resulting first of all in the oxygen photodissociation from Hb. These effects should be considered at development of the method of TiO₂ NP theranostic applications which imply the NP interaction with the blood.

Figure 1. Raman spectra of Rutile TiO₂(r) and Anatase TiO₂(a) NP with 488 nm wavelength laser excitation. The peaks positions and modes are marked according to Zhang, C. et al, Sci. Rep. 2017, 7, 9943

Figure 2. Raman spectra of oxyHb and deoxyHb, measured in oxygenated and deoxygenated RBC; ex. 488 nm. (O) and (D) mark the wavenumber ranges for mapping of ν3 band: oxyHb (1365-1380 cm⁻¹) and deoxyHb (1350-1360 cm⁻¹), correspondingly. Peaks for oxyHb 1588 cm⁻¹ (ν2), and 1640 cm⁻¹ (ν4) are also shown. Peaks are assigned according to [Wood, B.R. et al, In Vibrational Spectroscopy in Protein Research; Elsevier Inc./Academic Press: London/San Diego/Cambridge/Oxford. 2020: pp. 375-414.]

Figure 3. Raman mapping of individuals RBC: (a) control RBC; (b) RBC with TiO₂ (Rutile); (I) in the range 1365-1380 cm⁻¹; distribution of oxyHb; (II) in the range 1350-1360 cm⁻¹; the distribution of deoxyHb; (III) in the range 600-650 cm⁻¹; the distribution of the Rutile TiO₂ peak in the treated RBC; the ambient conditions, ex. 488 nm; 24x24 pixels. Scale bar is 1 μm.

Figure 4. Characteristic Raman spectra of an individual RBCs extended in the 1300-1425 cm⁻¹ range. Examples for (I) the most oxygenated RBCs; (2) the most deoxygenated RBCs.

Figure 5. Spectra extended in the 1500-1700 cm⁻¹ range; oxygenation state marker bands are shown. For every RBC the spectra demonstrate the higher (I) and lower (II) oxygenation states. (a) Control RBC; (b) RBC treated with TiO₂(r); (c) RBC treated with TiO₂(a). Blue and green arrows mark the ν3 band at 1356 cm⁻¹ and 1372 cm⁻¹, respectively.

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