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Raman Tweezers Spectroscopy Study of Size Dependent Silver Nanoparticles Toxicity on Erythrocytes

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Abstract : Raman Tweezers technique has become prevalent in single cell studies. This technique combines Raman spectroscopy which gives information about molecular vibrations, with optical tweezers which use a tightly focused laser beam for trapping the single cells. Thus Raman Tweezers enabled researchers analyze single cells and explore different applications. The applications of Raman Tweezers include studying blood cells, monitoring blood-related disorders, silver nanoparticle-induced stress, etc. There is increased interest in the toxic effect of nanoparticles with an increase in the various applications of nanoparticles. The interaction of these nanoparticles with the cells may vary with their size. We have studied the effect of silver nanoparticles of sizes 10nm, 40nm, and 100nm on erythrocytes using Raman Tweezers technique. Our aim was to investigate the size dependence of the nanoparticle effect on RBCs. We used 785nm laser (Starbright Diode Laser, Torsana Laser Tech, Denmark) for both trapping and Raman spectroscopic studies. 100 x oil immersion objectives with high numerical aperture (NA 1.3) is used to focus the laser beam into a sample cell. The back-scattered light is collected using the same microscope objective and focused into the spectrometer (Horiba Jobin Vyon iHR320 with 1200grooves/mm grating blazed at 750nm). Liquid nitrogen cooled CCD (Symphony CCD-1024x256-OPEN-1LS) was used for signal detection. Blood was drawn from healthy volunteers in vacutainer tubes and centrifuged to separate the blood components. 1.5 ml of silver nanoparticles was washed twice with distilled water leaving 0.1 ml silver nanoparticles in the bottom of the vial. The concentration of silver nanoparticles is 0.02mg/ml so the 0.03mg of nanoparticles will be present in the 0.1 ml nanoparticles obtained. The 25 ul of RBCs were diluted in 2 ml of PBS solution and then treated with 50 ul (0.015mg) of nanoparticles and incubated in CO2 incubator. Raman spectroscopic measurements were done after 24 hours and 48 hours of incubation. All the spectra were recorded with 10mW laser power (785nm diode laser), 60s of accumulation time and 2 accumulations. Major changes were observed in the peaks 565 cm-1, 1211 cm-1, 1224 cm-1, 1371 cm-1, 1638 cm-1. A decrease in intensity of 565 cm-1, increase in 1211 cm-1 with a reduction in 1224 cm-1, increase in intensity of 1371 cm-1 also peak disappearing at 1635 cm-1 indicates deoxygenation of hemoglobin. Nanoparticles with higher size were showing maximum spectral changes. Lesser changes observed in case of 10nm nanoparticle-treated erythrocyte spectra.

Keywords : erythrocytes, nanoparticle-induced toxicity, Raman tweezers, silver nanoparticles
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