The interferences of nanomaterials with hemoglobin a handicap to study hemocompatibility

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Abstract

The interactions of nanomaterials with membrane cells are an important research area because such interactions are critical in many applications such as biomedical imaging, drug delivery, disease diagnostics and DNA/protein stricter probing [1]. More and more nanomaterials are designed for biological applications, and this raises new concerns about the safety of nanotechnology [2,3].
Nanotechnology-derived devices and drug carriers are emerging as alternatives to conventional small-molecule drugs, and in vitro evaluation of their biocompatibility with blood components is a necessary part of early preclinical development. Special attention should be paid to the interaction of nanomaterials (NMs) with erythrocytes and for this reason the haemolysis assay is recommended as a reliable test for material biocompatibility [4].

The method used was the hemolysis assay as described in previous papers [5] and adapted to the study of NMs. Briefly, red blood cells obtained by centrifugation from fresh blood were incubated at room temperature for 1, 3 and 24 hours with different concentrations of the different nanomaterials studied. At the end of the incubation period, tubes were centrifuged and the amount of hemoglobin on the supernatant has been determined by spectroscopy at 540 nm to determine the percentage of hemolysis induced by the chemicals, compared to red blood cells totally hemolysed. We have used red blood cells from human, rat and rabbit. One of the possible limitations of the hemolysis assay is the absorption of the NMs at 540 nm and this should be discarded [6,7]. Another the possible interference of the nanomaterials with the endpoint of the hemoglobin determination is the adsorption of the hemoglobin by the nanomaterial and/or the protein denaturation. In order to study these possible interferences we have exposed the hemoglobin obtained from erythrocytes by hypotonic haemolysis to the nanomaterials under study. The haemoglobin spectrum was recorded with an UV/visible spectrophotometer.

We have studied different nanomaterials such as nano aluminum oxide as nanopowder (13 and 50 nm) and nanowires, zinc oxide nanopowder (50 and 100nm) (Sigma-Aldrich) and a commercial preparation of hydroxyapatite (nanoXIM.CarePaste®, supplied by Fluidinova). This is a highly dispersed hydroxyapatite aqueous paste specially designed to be incorporated in high performance Oral Care products, with special highlight in toothpastes and mouthwashes aiming enamel remineralization and reduction of teeth sensitivity.

The hemolysis phenomena is usually concentration-dependent (higher test concentration induces higher hemolysis). In some cases this is not observed, a decrease in the hemolysis is observed when the concentrations of the test substances increase. This can be observed in the hemolysis induced by Al_2O_3 nanowire after 3 hours incubation (Figure 1). This effect could be done by the adsorption of the hemoglobin by the nanowire and this could be demonstrated by the spectrum of hemoglobin treated with the nanowire (Figure 2). Clearly, we can observe that the spectrum is not modified, then there is no denaturation and the decrease in absorbance could be attributed to the adsorption phenomena.
Similarly, we have observed this phenomenon with a commercial hydroxyapatite preparation (nanoXIM) (Figures 3). In figure 4 we can observe the decrease in the supernatant color and increase in pellet color with increasing concentrations of the nanomaterial.

In the case of nano zinc oxide we can observe a significant color change and the hemoglobin spectrum shows an alteration due to the protein denaturation induced by the nanoparticle at higher concentration. This effect is not observed with lower concentrations.

References

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Figures

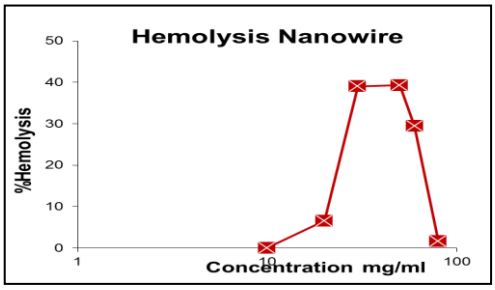

Figure 1: *Hemolysis induced by nanowire of Al_2O_3 after 3 hours incubation*

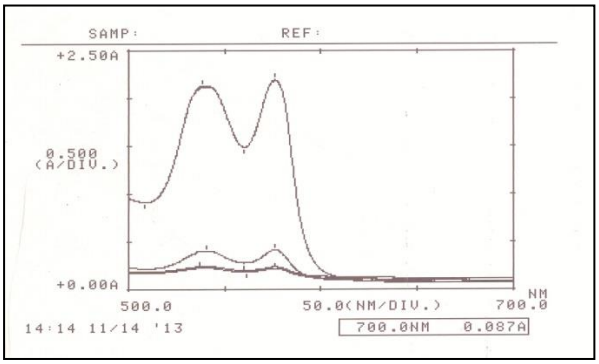

Figure 2: *Rabbit hemoglobin spectrum and effect of Al_2O_3 nanowire at 60 and 80 mg/ml after 3 hours incubation time*

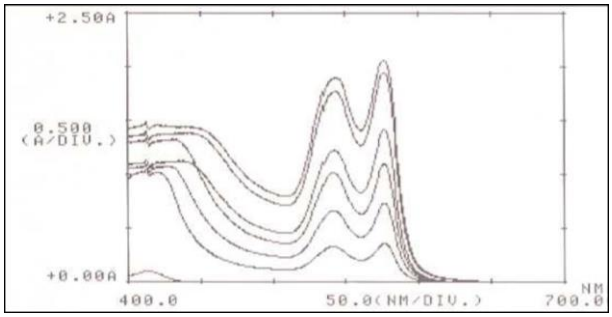

Figure 3: *Human hemoglobin spectrum and effect of nanoXIM after 24 hours incubation time (31 to 1.5 mg/mL)*

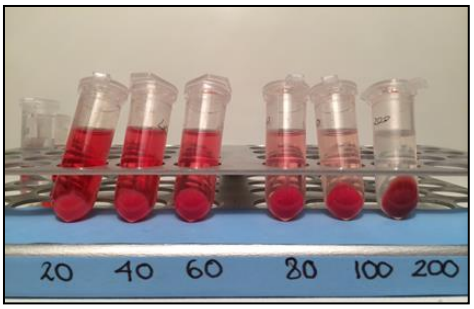

Figure 4: *Human erythrocytes treated with increasing concentration of nanoXIM. The supernatant shows decrease in color with concentration and the pellet shows the adsorption of hemoglobin*

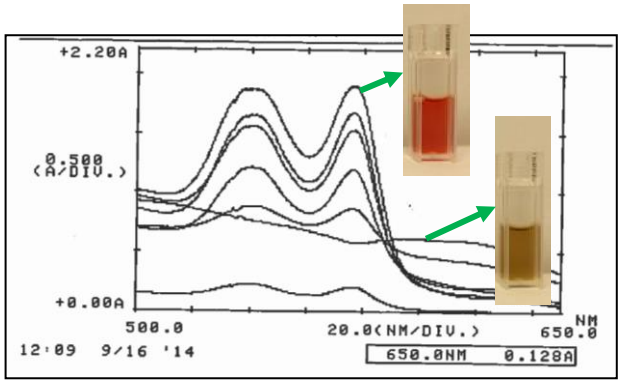

Figure 5: *Human hemoglobin spectrum and effect of nano ZnO 100 nm after 24 hours incubation time at 37ºC. Spectrum alteration after treatment with 2 mg/mL*