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The increasing global production of plastics over many years, combined with improper waste management and low recycling rates, has led to widespread plastic pollution. Under the influence of various physico-chemical factors (e.g., UV radiation), as well as microbiological biodegradation and chemical degradation, plastics break down into smaller microplastics (<5,000 μm) and, subsequently, into nanoparticles with diameters below 0.1 μm. The extent of environmental contamination with micro- and nanoplastics results in exposure of living organisms to these particles, as evidenced by their detection in various human tissues, including blood. One of the most commonly produced plastics is polystyrene, used, among other applications, as an insulating material known as Styrofoam. This material has also been detected in human-derived samples. Chronic human exposure to plastic particles, together with their presence in human tissues, particularly in blood, highlights the need to examine the effects of polystyrene nanoparticles on individual morphotic elements, including erythrocytes, which are the most abundant cells not only in the blood but also in the entire human body. Accordingly, this doctoral thesis aimed to assess the impact of non-functionalized polystyrene nanoparticles (PS-NPs) of different diameters (~30 nm, ~45 nm, and ~70 nm) on the structure and function of human erythrocytes. Moreover, given the presence in blood of numerous proteins capable of interacting with PS-NPs, the study also included an evaluation of the effects of these nanoparticles on the most abundant human blood protein, human serum albumin (HSA). Specifically, the haemolytic potential of PS-NPs was assessed, together with their effects on erythrocyte membrane fluidity and cell morphology. The interactions of PS-NPs with the model protein albumin and with erythrocyte proteins were also examined. In addition, the research evaluated the effects of PS-NPs on reactive oxygen species (ROS) generation and lipid oxidation, as well as parameters associated with the occurrence of eryptosis in red blood cells. The following techniques were employed: electron paramagnetic resonance, flow cytometry, spectrofluorometry, spectrophotometry, circular dichroism (CD), dynamic light scattering (DLS), and optical microscopy. Initial analyses demonstrated that PS-NPs at high concentrations induce diameter-dependent haemolysis of erythrocytes. Subsequent experiments showed that PS-NPs stiffen the hydrophobic regions of the erythrocyte membrane and induce rheological changes in red blood cells. Further studies revealed that PS-NPs interact with both HSA and erythrocyte proteins.

With respect to HSA, PS-NPs were shown to induce the formation of a stable protein corona, which may influence in vivo interactions of PS-NPs with blood cells. In erythrocyte proteins, exposure was associated with altered intracellular microviscosity and oxidation of membrane proteins. The observed increase in carbonyl group levels may result from mechanical interactions between PS-NPs and the membrane, as well as from the rheological alterations induced by these nanoparticles. No oxidation of haemoglobin or changes in acetylcholinesterase activity were detected. Experiments carried out in the final stage of the study demonstrated that PS-NPs do not induce ROS generation, although at high concentrations they do cause lipid oxidation. No changes in caspase-3 activity were observed. Phosphatidylserine externalization and increased intracellular calcium levels were detected only at the highest concentration tested, which was haemolytic. In contrast, calpain activation was already observed at pre-haemolytic concentrations (1–50 μg/ml), which may be associated with mechanically induced alterations caused by PS-NPs. Overall, analysis of all proeryptotic erythrocyte parameters examined indicates that PS-NPs do not induce programmed red blood cell death. In summary, non-functionalized PS-NPs exert a relatively mild, size-dependent detrimental effect on human erythrocytes. Exposure led to alterations in erythrocyte membrane mechanical properties, such as membrane stiffening and rheological disturbances, which ultimately resulted in haemolysis at high concentrations (100–250 μg/ml). PS-NPs did not exhibit direct pro-oxidative effects on haemoglobin and did not increase ROS levels; however, at high concentrations (100 μg/ml) they induced lipid peroxidation and increased carbonyl groups in membrane proteins. PS-NPs, regardless of size, did not initiate eryptosis at pre-haemolytic concentrations but caused subtle structural membrane changes, likely mechanical in nature, as supported by calpain activation already at low concentrations. Furthermore, the study demonstrated that PS-NPs form a stable protein corona with albumin, which undergoes structural modifications upon interaction with the nanoparticles; such modifications may potentially affect proper erythrocyte function *in vivo*.