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IRON OXIDE NANOPARTICLES : QUESTION OF NANOSAFETY FOR NANOMEDICINE APPLICATIONS

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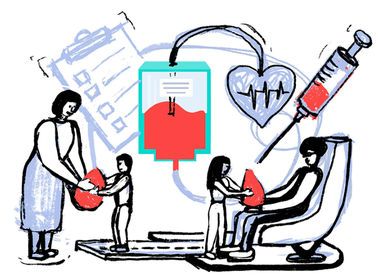
BACKGROUND AND OBJECTIVE

Iron oxide nanoparticles (Fe_3O_4 NPs) are probably the NPs that have received the most increasing attention in nanomedicine. These NPs have been found to possess some properties related with their superparamagnetic behaviour. In fact, this characteristic offers them a great potential to develop a variety of applications in medicine, including the treatment of iron deficiency, thermotherapy, drug delivery and so on. However, many safety concerns are rising, mostly regarding their interactions with innate immune cells. For example, their capacity to induce inflammation, which is one of the most undesired side effects associated with NP exposures, needs to be studied more in depth. The aim of this project is to understand the effects of Fe_3O_4 NPs on the biology of human neutrophils, key player cells in inflammation and the most important leukocyte population present in the circulation.

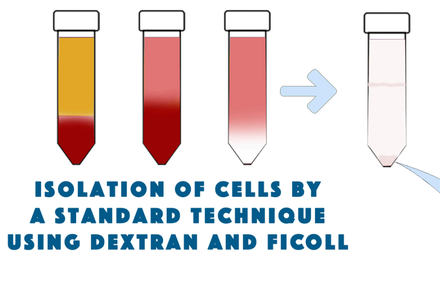
Fe_3O_4 NPs

The iron oxide nanoparticles (Fe_3O_4 NPs) were purchased from Sigma. According to the manufacturer, the particle size is 9-11 nm as assessed by transmission electronic microscopy (TEM). The solution is at 5 mg/ml in de-ionized water and a fraction was further diluted to obtain a stock solution at 1000 X to work with and was used as is. The endotoxin level of the NPs suspension was determined by the classical Limulus amebocyte lysate (LAL) assay using the ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit and were under the detection limit of 0.01 EU/ml.
Before performing any experiments, we previously determined that Fe_3O_4 NPs do not induce cell necrosis in our experimental conditions used as assessed by the trypan blue exclusion assay.

MATERIALS AND METHODS



BLOOD SAMPLES ARE OBTAINED FROM VOLUNTARY DONORS



ISOLATION OF CELLS BY A STANDARD TECHNIQUE USING DEXTRAN AND FICOLL

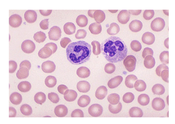
% OF NEUTROPHILS IS ≥ 95%

FUNCTIONS STUDIED: APOPTOSIS; INFLAMMATORY CYTOKINES; DE NOVO SYNTHESIS; REACTIVE OXYGEN SPECIES; PHAGOCYTOSIS

LONG RESPONSE 24H INCUBATION

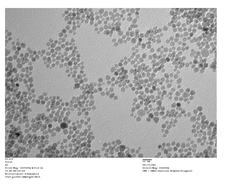
COURT RESPONSE 30' INCUBATION

TECHNIQUES USED: CYTOLOGY; ELISA; SPECTROMETRY; CYTOLOGY



H2DCFDA DYE

RED BLOOD SHEEP CELLS + ANTIBODY



ENDOTOXIN CHECKED BY LAL ASSAY

NANOPARTICLES

IRON OXIDE NANOARTICLES ARE SPHERIC WITH SIZE OF 9-11 NM, ANALYSED BY TEM. 10 UG/ML AND 100 UG/ML ARE TESTED.

RESULTS

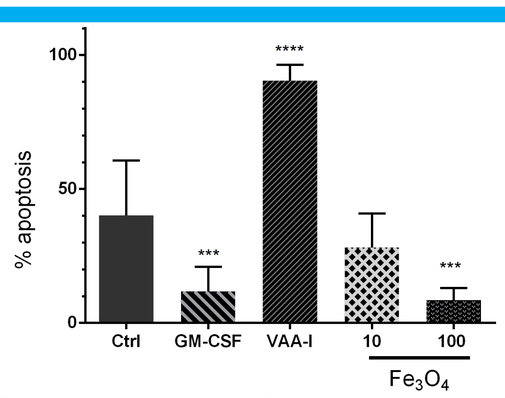


Figure 1: *Iron oxide nanoparticles (Fe_3O_4 NPs) can delay human neutrophil spontaneous apoptosis.* Freshly isolated human neutrophils were incubated for 24h in the presence of buffer (Ctrl), the antiapoptotic GM-CSF groth factor, the proapoptotic plant lectin VAA-I or the indicated concentration of Fe_3O_4 NPs. Results are expressed as means ± SEM, n=4). *, p<0.05 vs Ctrl.

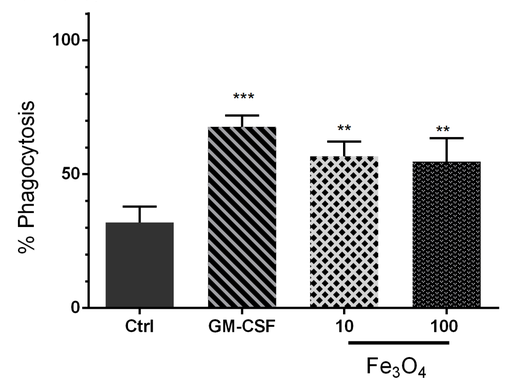


Figure 3: *Fe_3O_4 NPs slightly enhance the capacity of neutrophils to exert phagocytosis*. Briefly, neutrophils treated or not with Fe_3O_4 NPs were incubated for 30 min with opsonized sheep red blood cells (SRBCs) in a 1:5 ratio. Then, the number of neutrophil cells ingesting at least one SRBC was determined by optical microscopy. Results are expressed as means ± SEM, n=4). *, p<0.05 vs Ctrl.

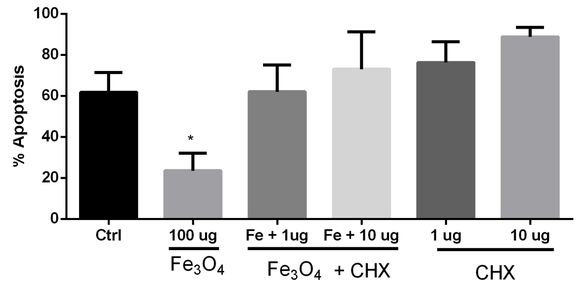


Figure 2: *Fe_3O_4 NPs delay apoptosis by a de novo protein synthesis-dependent mechanism*. Cells were incubated for 24h with cycloheximide "CHX" at a concentration of 1 µg/ml and 10 µg/ml in presence or absence of Fe_3O_4 NPs. Results are expressed as means ± SEM, n=4). *, p<0.05 vs Ctrl.

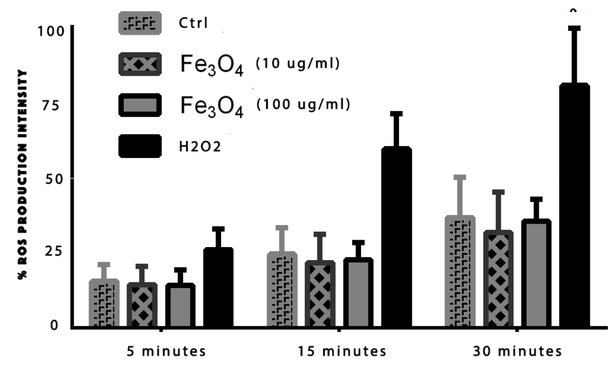
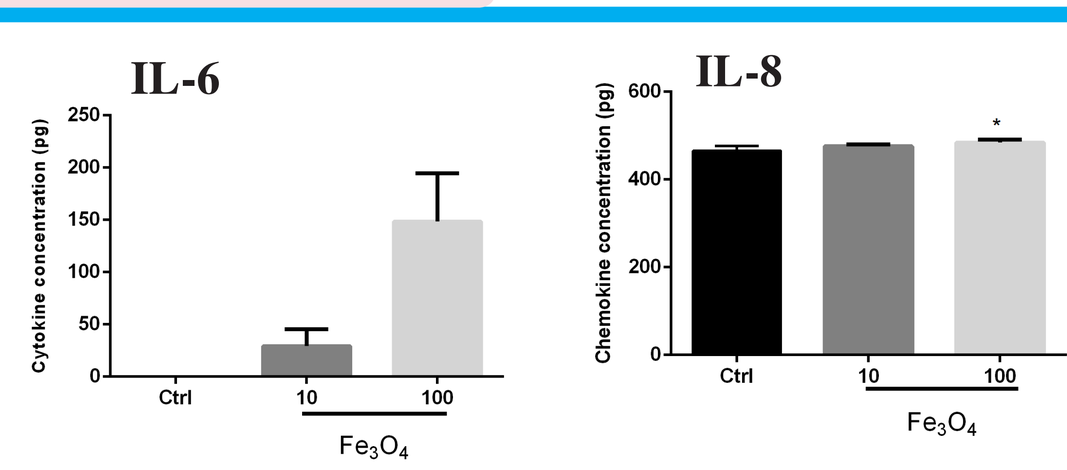


Figure 4: *Fe_3O_4 NPs do not trigger oxidative stress in human neutrophils.* After cell treatment with buffer (Ctrl), H2O2 (positive control) or Fe_3O_4 NPs, the production of ROS (reactive oxygen species) production was assessed by spectrometry using the H2DCFDA probe. Results are expressed as means ± SEM, n=4). *, p<0.05 vs Ctrl.



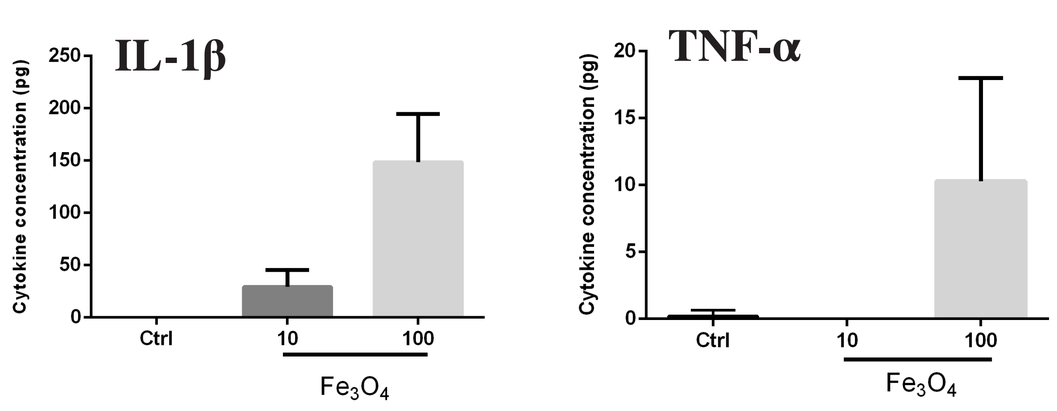


Figure 5: *Fe_3O_4 NPs can induce cytokine production.* Neutrophils were treated with buffer (Ctrl) or the indicated concentrations of Fe_3O_4 NPs for 24h. Then the supernatants were harvested and used to determine the concentration of the cytokines IL-1β, TNF-α, IL-6 and IL-8 using commercially available ELISA kits. Results are expressed as means ± SEM, n=4). *, p<0.05 vs Ctrl.

CONCLUSION AND PROGRESSION

This study indicates that Fe_3O_4 NPs can alter the biology of human neutrophils, an issue that has not been previously investigated. Indeed, these NPs were found to delay spontaneous apoptosis by a mechanism requiring de novo protein synthesis. Although Fe_3O_4 NPs were found to induce the production of IL-1β, TNF-α, IL-6, but not IL-8, they do not induce ROS production and only slightly enhance the capacity of neutrophils to exert phagocytosis.

The capacity of Fe_3O_4 NPs to delay neutrophil apoptosis and enhance phagocytosis indicate that these NPs are not cytotoxic for neutrophils corroborating our observations that Fe_3O_4 NPs do not induce cell necrosis. However, the fact that Fe_3O_4 NPs were found to induce the production of some pro-inflammatory cytokines together with their ability to delay apoptosis, indicate that they possess some pro-inflammatory activities. In future, it would be interesting to identify some proteins involved in the ability of Fe_3O_4 NPs to delay human neutrophil apoptosis as well as to study the production of other cytokines and to determine their capacity to induce by themselves an inflammatory response in vivo. This will be of great help to better design a safer use of these NPs in diverse medical applications.

REFERENCES

Goncalves et al. Activation of neutrophils by nanoparticles. ScientificWorld Journal. 2011;11:1877-1885.

James G. Cronin e al. Nanomaterials and Innate Immunity: A Perspective of the Current Status in Nanosafety. Chemical Research in Toxicology 2020 33 (5), 1061-1073.

Himly e al. When Would Immunologists Consider a Nanomaterial to be Safe? Recommendations for Planning Studies on Nanosafety. Small 2020, 16, 1907483.

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