Internalization of core-shell superparamagnetic nanoparticles into granulocytes

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Nanoparticles are for definition those materials that have at least one dimension smaller than 100 nm and that can find employment in biomedical field because of their peculiar features different from the corresponding bulk materials. The main purpose of nanobiotechnology and nanomedicine is the synthesis and application of nanomaterials in the treatment of different diseases, including tumors. The peculiar physico-chemical features of materials at the nanoscale make them suitable tools for diagnosis and therapy and so for their employment as theranostic agents. Superparamagnetic nanoparticles of Fe_3O_4, for example, can be used as MRI contrast agents and once administrated can be confined in a particular region of the body by the application of an external magnetic field[1], while Au nanoparticles can be exploited not only as contrast probes in optical imaging, but also as therapeutic agents if their surface is engineered with receptor proteins, antibodies and drugs[2]. Nanoparticles (NPs) can find application both in vitro and in vivo experiments but one of the major problems in living organism are the rapid clearance performed by the immune system, the accumulation in the reservoir organs such as liver, lung and spleen and consequently the low NPs concentration that reaches the tissue of interest. It is known that the NPs properties such as their size, shape, surface charges and functionalization can affect their distribution within the body[3] and one of the most studied problems is the formation of the protein corona on their surface that may influence not only their properties but also the role of the linked molecules[4]. Many stratagems have been employed to avoid the lost of active nanoparticles concentration through the functionalization with polymers (PEG), peptides and proteins, but not too many successes have been achieved. To overcome this drawback, a new approach to the use of nanoparticles as theranostic agents has been developed by engulfing nanoparticles into the same cells (leukocytes) that are appointed to remove from the blood flow foreign bodies, such as pathogens, cellular debris, and in this case circulating nanoparticles. More in details this new delivery strategy is refereed as the Trojan Horse method[5], and consists in incubating engineered nanoparticles with ex vivo leukocytes cells and in their following readministration to patient. In this way it could be possible to avoid many of NPs application side effects and it might allow to reach regions of tumors that are inaccessible through the EPR effect (enhanced permeability and retention effect) commonly exploited to deliver nanomaterials into tumoral tissues.

In this context we synthesized, characterized and studied the engulfment of multitasking superparamagnetic nanoparticles (MNPs) of Fe_3O_4@Cu@Au into human granulocytes. NPs had been previously functionalized with methotrexate (MTX) and folic acid (FA)[6] (fig.1), respectively a chemotherapeutic drug and a vitamin which is a MTX structural isomer. We chose to synthesize this type of core-shell nanoparticles because they allow to combine on the same nanodevice not only therapeutic agents linked to the Au shell, but also different contrast agents for PET and MRI analysis such as the ^{64}Cu isotope, dispersed into Cu shell, and the superparamagnetic core of Fe_3O_4. The MNPs obtained had been characterized by different physico-chemical techniques (XRPD, VSM, AFM and TEM) to study their properties. Subsequently MNPs had been coated with Poly-L-lysine that contributes to improve the NPs dispersion into polar solvents, the cellular uptake and allows the further functionalization with MTX and FA. Furthermore, to collect confocal fluorescence microcopy images of these nanosystems internalized into cells, we labeled the residues of Lys with the fluorescent probes (Texas Red, FITC). Afterwards we incubated engineered nanoparticles with human granulocytes in order to investigate their internalization and the possible adverse effects of MNPs on cells viability.

Experimental evidence of granulocytes engulfment had been collected by SEM and fluorescence microscopy of the cellular samples, before and after incubation with the activated nanoparticles. After incubation not evident adverse effect on cells were revealed. Both SEM and fluorescence microscopy images confirmed the phagocytosis of MNPs, the former by the EDX analysis and the later by collecting by the Z-stack modality images.
Taking advantage of the physiological function of granulocytes, their great mobility in blood flow that leads to a detectable presence also in tumoral tissues, we hypothesize to exploit the same cells as

carrier of our functionalized nanoparticles, avoiding the possible aggregation of MNPs in the physiological medium, the formation of the protein corona and the capture by the reservoir organs.

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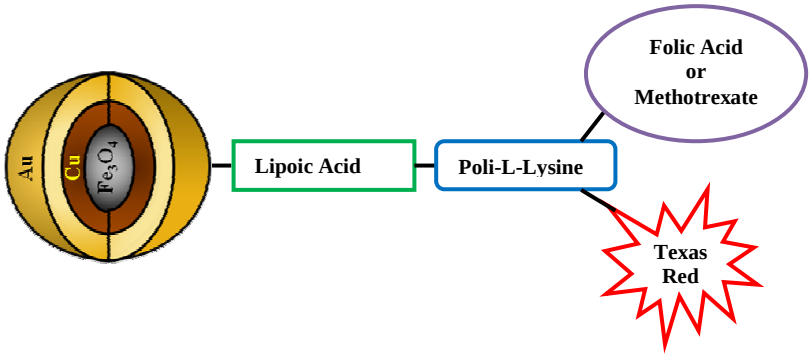

Figure 1: schematic representation of core shell MNPs engineered with folic acid or methotrexate