Development of multifunctional iron-doped ZnO nanoparticles addressing pancreatic cancer cells

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Marco Carofiglio, Marco Laurenti, Luisa Racca, Sugata Barui, Nadia Garino, Valentina Cauda*

Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi, Turin, Italy

**marco.carofiglio@polito.it, Phone number: +39 334 7606 030*

OBJECTIVES

Pancreatic ductal adenocarcinoma (PDAC) is currently one of the most devastating diseases for which current therapeutics are of limited benefit. PDAC has an incidence of 5 per 100'000 women worldwide in developed country and an enormous mortality rate of about 90 % [1]. The overall patient survival is improved by "only" 4 months in the last decade. Therefore, novel therapeutic and early diagnostic techniques are required to address this tumor.

Zinc oxide nanoparticles (ZnO NPs) are gaining attention in nanomedicine, due to their high versatility and properties, which can be easily tailored by means of various strategies. One of the most promising ones is doping: introducing ions of different elements may induce new functionalities to ZnO NPs [2] which can be exploited to design a theranostic nanoparticle. Here iron-doped ZnO NPs (Fe:ZnO NPs) functionalized with oleic acid and amino groups are developed to achieve a multipurpose theranostic nanomaterial: on the one hand, Fe doping elicits magnetic responsiveness with potential uses as contrast agent in magnetic resonance imaging (MRI); on the other hand the amino-functionalized ZnO NP is able to develop reactive oxygen species (ROS) generation under ultrasound (US) activation, aiming to kill pancreatic cancer cells.

METHODS

Fe:ZnO NPs were synthesized with different iron concentrations (0, 6, 12 at.%) by a wet chemical method with an oleic acid shielding, while the

amino group functionalization was performed with a post-synthetic grafting approach [3] and exploited for dye labelling. The resulting nanoparticles were characterized from the crystallographic and morphological standpoints by X-Ray diffractometry (XRD) and scanning electron microscopy respectively. Energy Dispersive X-ray Spectrometry (EDS), Fourier transform infrared spectroscopy (FTIR) and X-ray photoemission spectroscopy were used to establish the composition of the resulting particle. Furthermore, their magnetic response was evaluated through a DC magnetometer [3], while the ROS generation under US stimulation was established through electron paramagnetic resonance spectroscopy (EPR) coupled with the spin-trapping technique [4].

To assess the biological behavior of the Fe:ZnO NPs, viability and nanoparticles uptake tests were carried out on pancreatic cancers cells (e.g. BxPC-3) by means of the WST-1 assay and cytofluorimetry analysis.

RESULTS

Fe:ZnO NPs developed in this work revealed to be spherical 8 nm ZnO particles presenting a wurtzitic crystalline structure (Figure 1a) and an actual doping level of 0, 4.8 and 7.8 at. %. EDS and FTIR analyses confirmed the functionalization with oleic acid and amino groups. Iron doping was proven to be effective, with concentration dependence, in increasing the magnitude of the paramagnetic signals obtained in DC magnetization measurements, as well as the reactive oxygen species generation under US stimulation (Figure 1b and c).In addition, Fe:ZnO NPs showed to be safe up to 20 μg/mL concentration and no significant cell viability reduction was found at this concentration, considering the different levels of doping. Nanoparticles uptake experiments were used to assess the number of NPs that could contribute to intracellular reactive oxygen species generation upon US stimulation, establishing that a high percentage of cells have internalized the NPs with a fast internalization rate.

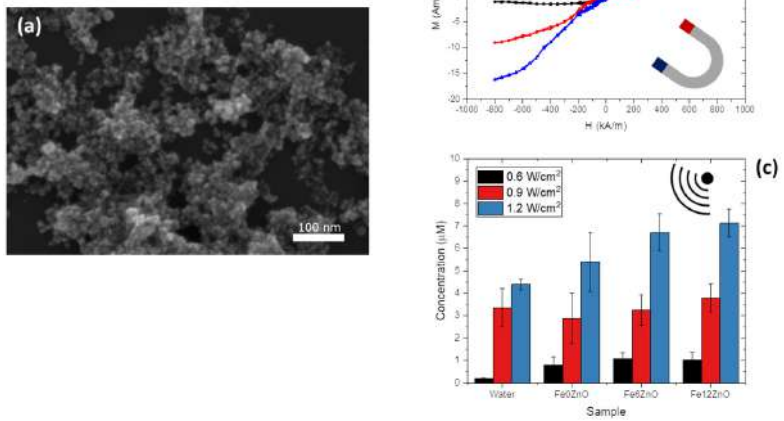

FIGURE 1: (**a**) FESEM, (**b**) magnetic properties and (**c**) ROS generation under US stimulation characterization of iron-doped ZnO nanoparticles.

CONCLUSIONS

Iron-doped ZnO NPs were successfully synthetized and characterized. The physical and chemical characterizations reveal the potential use of these nanoparticles as a powerful theranostic system for cancer cells, where the imaging is accomplished by MRI and the therapy is performed through NP-assisted US stimulation. Viability tests suggest the safety of the developed device up to 20 μg/mL, allowing to go further with biological tests in which cells are physically stimulated with NP-assisted US.

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