Doctoral Dissertation
Doctoral Program in Chemical Engineering (35th Cycle)

Doped ZnO nanoparticles in biomedicine: their role as stimuli-responsive anticancer agents

By
Marco Carofiglio

Summary

The research in nanomedicine is focusing considerable attention on smart nanoparticles thanks to their multifunctional properties which gather their use in several fields like imaging, therapy or a combination of the two, i.e. theranostics. Among the various nanomaterials, zinc oxide nanoparticles (ZnO NPs) present physical and chemical properties which could fulfill the requirements for an efficient nanotheranostic system, like antimicrobial, photocatalytic, semiconducting and piezoelectric properties. However, pure ZnO NPs also suffer from poor stability in biological media and consequent possible cytotoxic effects. This important limitation to their use could be overcome by doping, i.e. the inclusion of a new element inside the ZnO crystalline structure. Depending on the doping element, the already interesting properties of ZnO NPs could be further enhanced or new properties introduced. For example, the safety of the NPs could be improved, as well as its piezoelectric properties, while novel magnetic responsiveness can be induced.

The aim of the PhD thesis here presented is to explore the potentialities of newly synthetized iron doped ZnO NPs (Fe:ZnO NPs) in the field of nanomedicine to treat pancreatic cancer. Iron doping, in particular, was chosen as it can lead to magnetic responsiveness and enhanced safety to the ZnO NPs. Fe:ZnO NPs were synthetized by means of a simple, fast and cheap wet chemical method that lead to nano-sized spherical particles. The NPs surface was functionalized during the synthesis with a capping agent, oleic acid, aimed at increasing the colloidal stability. A further amino-propyl functionalization was performed after NPs formation to provide functional groups exploited for dye labelling, useful in cell cultures. Then, a complete physical and chemical characterization of the Fe:ZnO NPs was fulfilled, and the Fe:ZnO NPs were compared to pure ZnO NPs. The doping was confirmed through X-ray diffraction, electron microscopy, energy dispersive spectroscopy and X-ray photoemission spectroscopy, while the success of the two functionalizations was verified by Fourier-transform infrared spectroscopy. Finally, the colloidal stability and surface charge were established through Dynamic Light Scattering and Z-potential measurements, respectively. The novel features

gathered by doping were also investigated. The optical behavior of the NPs was analyzed, searching for new energy states that could suggest interesting applications for medical imaging. From the therapeutic standpoint, the electromechanical response of Fe:ZnO NPs was studied and compared to ZnO NPs, finding an enhanced response for the doped NPs. However, the most interesting result was found in terms of magnetic responsiveness. In this sense, Fe:ZnO NPs acquire, thanks to doping, a considerably enhanced magnetic responsiveness that could be efficiently used in magnetic resonance imaging.

To have a first proof-of-concept application of the fabricated nanoparticles, Fe:ZnO NPs were tested on a pancreatic adenocarcinoma cell line as a sonosensitizing agent. The idea leading the research was to couple Fe:ZnO NPs with shock waves, i.e. high pressure waves, that can be remotely provided to cancer cells to kill them once they have internalized Fe:ZnO NPs. In this view, a safe dose of Fe:ZnO NPs is administered to cells and a harmless dose of shock waves is provided to the cancerous tissue. The damages to the diseased tissues are obtained only when the two treatments are combined, providing a strong control on the cell death and reducing, in turn, the risks of collateral effects. To perform this task, the cytotoxicity of the NPs on cancer cells was evaluated and compared to the toxicity of the same particles on non-cancerous cells. Then, the Fe:ZnO NPs internalization was measured and analyzed with fluorescence microscopy and cytofluorimetry to determine the best treatment conditions. With these optimized parameters, Fe:ZnO NPs and shock waves were administered together to the cells, finding an enhanced toxicity only when the two treatments are combined and proving the efficacy of the nanotheranostic agent. Finally, the cell death mechanism was analyzed through fluorescence microscopy analyses and kinetics cell death analyses to provide further insight on the whole system, in view of further tests in vivo.

The results presented in this PhD thesis consider the fabrication of a multifunctional Fe:ZnO NPs based system from its design up to its application in vitro. The main goal is to prove the effectiveness of this nanoplatform in a proof-of-concept application and to open to the exploitation of the system as a nanotheranostic device in several fields of the fight against cancer. The final results suggest the use of the nanoconstruct in further tests in vivo, before the hopeful application of them in clinical tests.