Doctoral Dissertation
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The manifold roles of zinc oxide nanocrystals in interaction with acoustic waves

by
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Summary:

Nanoparticles (NPs) are a wide class of new materials, representing an innovative branch of scientific research, able to address various challenges in different scientific areas. The unique nano-dimension of particles provides them with specific physical and chemical properties, related to their size, shape, crystalline structure and surface-to-volume ratio. Within this frame of reference, zinc oxide has become one of the most popular metal oxide nanoparticles for biological applications. This material is characterized by an excellent biocompatibility, hemocompatibility, high surface reactivity, and it is a semiconductor with a wide band gap, which makes it excellent for optical imaging purposes.

This dissertation focuses its attention on the interaction between zinc oxide nanoparticles and ultrasound irradiation. Ultrasound exposure of ZnO NPs solution causes the occurrence of complex mechanisms, comprising stable and inertial cavitation, microstreaming and the generation of photons as well as radiation forces, having as consequences synergistic physical and chemical processes. These phenomena can be exploited for different paramount proposes, from imaging to anticancer treatment.

The use of zinc oxide combined with ultrasound (US), in accordance with numerical simulation, shows the ability to enhance and control the production of Reactive Oxygen Species (ROS), anticipating their potential as therapeutic agent. Furthermore, the combination of US and ZnO NPs reveals to increase the sonoluminescence (SL) emission, together with the ability to modify the SL spectrum when compared to the pure water behavior, making ZnO NPs very good candidates as efficient nanocontrast agents for SL imaging for biological and biomedical applications.

As first proof of concept of therapeutic application, cytotoxicity and internalization of ZnO NPs were evaluated in cervical adenocarcinoma (KB) cells, as well as the safety of the highly intense mechanical pressure waves (SW) treatment alone. The remarkably high cytotoxic combination of ZnO NP and SW was demonstrated, comparing the effect of multiple (3 times/day) SW treatments toward a single one, highlighting the killing efficiency of the combined strategy here proposed.

To overcome the problems derived from the interaction of nanoparticles with the human body, different shielding approaches for ZnO NPs were investigated. At first, re-engineered extracellular vesicles derived from healthy B-lymphocytes donor cells were studied as shell for ZnO NPs. They were decorated with monoclonal antibody (anti-CD20) to target $CD20^+$ cells, as Burkitt's lymphoma cells (Daudi). The hybrid nanoconstructs demonstrate to be characterized by high biocompatibility, targeting specificity and cytotoxic capability when remotely activated with SW. An important aspect of this nanotool is the significantly higher selectivity, and consequently the selective cytotoxicity, demonstrated towards the targeted cancerous Daudi cell line compared to the $CD20^-$ cancerous myeloid cells (HL60) and the healthy cell line.

A diverse strategy for ZnO NPs encapsulation was adopted and tested on the same hematological cell lines. Artificial, less time consuming, and self-assembled liposomes were designed and decorated with fragments of anti-CD38 antibody, as targeting agent toward cancerous Daudi cells. The biosafety of the construct itself was here proved in both healthy (B-lymphocytes) and cancerous cell lines. The remote activation of the nanoconstruct by ultrasound exposure makes it become toxic for cancerous cells, without having any significant impact on healthy cells. The mechanism of killing was examined, determining that mechanical damages were created at first, then apoptotic pathways were activated increasing the therapeutic efficiency against cancerous cells.

In conclusion, this work demonstrates the multifaced potential of nanosized zinc oxide as biomedical imaging agent and therapeutic nanotool for anticancer applications.