Drug delivery nanosystems for intracellular release of doxorubicin improve the clonogenic inactivation of X-Rays in human cervical adenocarcinoma cells

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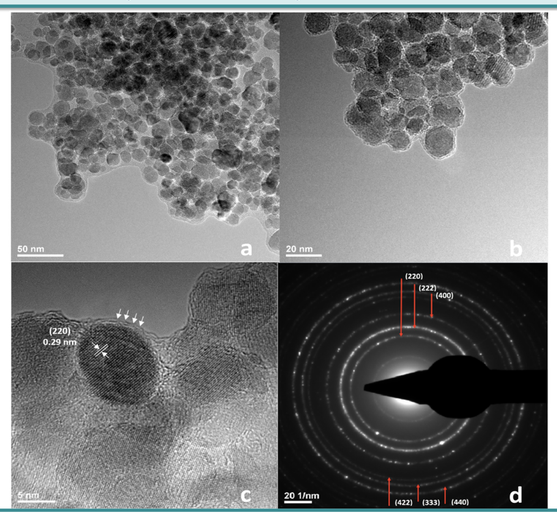
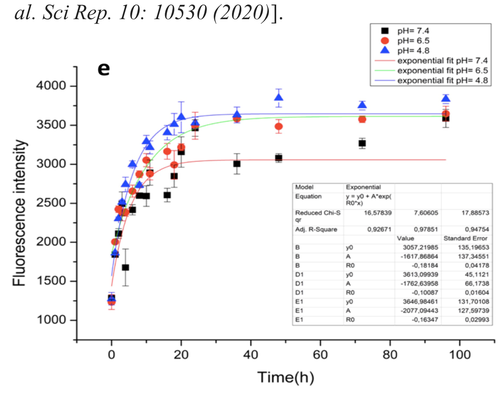
Objectives: Radiosensitization using nanoparticles is a promising approach for the improvement of radiotherapy cytotoxic effects against chemo-/radio-resistant tumor cells and simultaneous reduction of adverse effects in surrounding healthy tissues. Here, we propose a method based on iron oxide nanoparticles (IONP) for the intracellular delivery of the anthracycline doxorubicin to enhance the cytotoxic effects of X-Rays.

Materials and methods: We synthesized and characterized iron oxide nanoparticles functionalized with polyethylene glycol ($IONP_{CO}$) in order to be used as drug delivery systems for doxorubicin ($IONP_{DOX}$). The biological effects were assessed in 2D and 3D cell cultures of human cervical adenocarcinoma cells (HeLa). Uptake and retention of IONP were evaluated using optical, fluorescence and transmission electron microscopy. Clonogenic survival was used to measure the radiosensitization effect of IONP at different doses of low (50 kV), medium (150 kV) and high (6 MV) X-Rays in both 2D and 3D cell models. Data are presented as mean ± SEM (n=3).

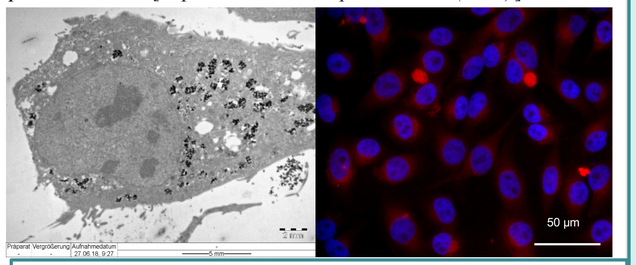


Structural and compositional characterization of IONP [*Popescu et al. Sci Rep. 10: 10530 (2020)*].

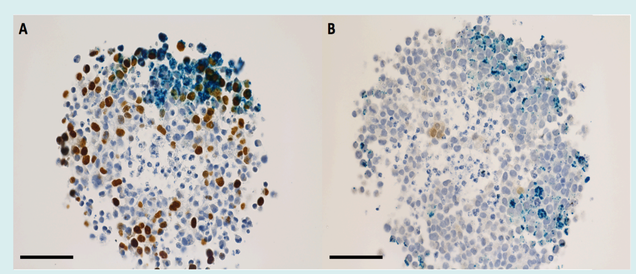


Delivery of doxorubicin from $IONP_{DOX}$ [*Popescu et al. Sci Rep. 10: 10530 (2020)*].

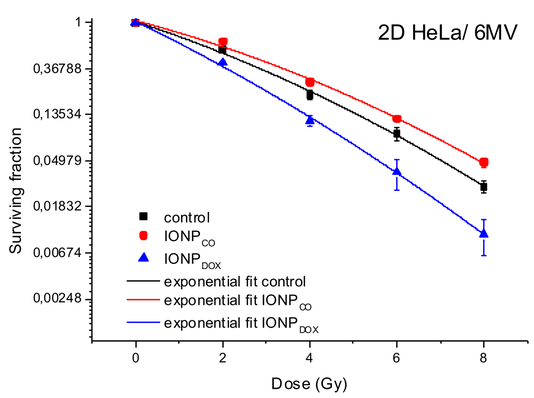
Efficient internalization of $IONP_{CO}$ and $IONP_{DOX}$ in HeLa cells occurred through pino- and endocytosis, with both IONP accumulating in the perinuclear area [*Popescu et al. Sci Rep. 10: 10530 (2020)*].

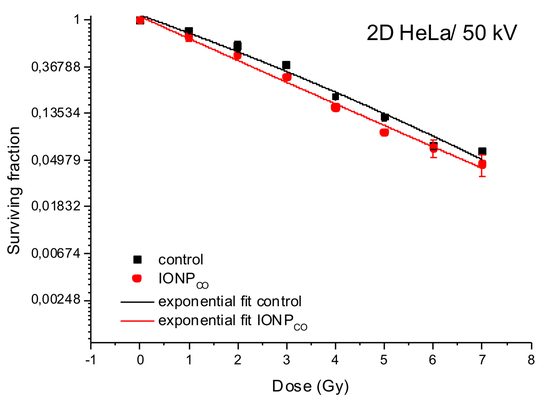


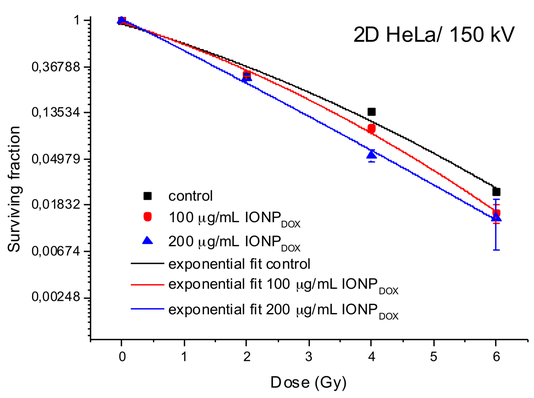
In 2D cell cultures, $IONP_{CO}$ enhanced the radiosensitivity (dose-modifying factor, DMF) of 50 kV X-Rays with a $DMF_{SF0.1}$= 1.13 ± 0.06 (P<0.05), but did not determine any radiosensiziting effect after 6MV irradiation. $IONP_{DOX}$ enhanced the clonogenic inactivation of 6 MV X-rays in 2D HeLa cell cultures with a $DMF_{SF0.1}$=1.3 ± 0.1 (P<0.05) and $DMF_{SF0.1}$= 1.29 ± 0.02 in case of 150 kV (P<0.05).

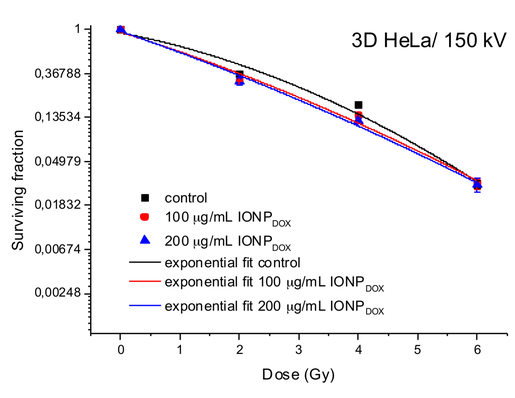


Efficient penetration of the $IONP_{DOX}$ was obtained after 48h of exposure in 3D spheroids and exposure to 150 kV led to a $DMF_{SF0.1}$=1.07 ± 0.07 (NS).









Conclusions: The radiosensitization effect was dependent on the radiation energy for drug-free nanoparticles, $IONP_{CO}$ showing a significant clonogenic inactivation compared to irradiation alone, in case of low energy X-Rays exposure, while $IONP_{DOX}$ determined radiomodulatory effects for both medium and high energy X-Rays. The IONP are good candidates for the controlled delivery of DOX to enhance the cytotoxic effects of ionizing radiation.

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