

Development of green approaches based on solvent-free nanoparticles and *in vitro* models for the management of metastatic melanoma

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Nanoparticles (NPs) brought many advantages in cancer therapy, but their synthesis process results still environmentally unsustainable, due to the amount of organic solvent involved.

The aim of this project is the development of green NPs synthesis technique to deliver hydrophobic drugs. Two green NPs platforms were prepared i) antibody-loaded Chitosan (CS) NPs obtained by ionic gelation and ii) siRNA-loaded phosphate-Poly(Allylamine-Hydrochloride) (PAH) NPs, obtained through electrostatic self-assembly.

Small size NPs with low polydispersity index, and positive Z potential were obtained. No sign of cytotoxicity caused by NPs was observed against melanoma and fibroblasts cell lines. NPs-induced platelet

activation was tested to investigate NPs safety after systemic injection. Platelet activation was evaluated through SEM microscopy and by FACS analysis. PAH NPs did not trigger platelet activation, at any of the tested concentrations, while CS NPs did not induce activation at low concentrations.

NPs showed capacity to load model payloads and to release it in a controlled fashion. FACS analysis and confocal microscopy showed that PAH NPs were able to significantly enhance siRNA delivery to cells, as compared to free siRNA administration.

According to 3R principles, a 3D-printed metastatic melanoma model is under development as a NPs testing device, representing an alternative to animal tests.

Skin fibroblasts (Hff-1) were embedded in a collagen/hyaluronic acid-based hydrogel, and allowed to grow up to four weeks. To recreate the vasculature, a channel was obtained within the hydrogel and seeded with Endothelial Cells (hUVECs). The model will be inoculated with melanoma cells and used to investigate NPs extravasation towards the primary tumor and their ability to target metastatic melanoma cells present in the channel.

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