Green approaches based on solvent-free methods to prepare nanoparticles and on alternative *in vitro* models for their validation: application in the treatment of metastatic melanoma.

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Nanoparticles (NPs) are advantageous drug-delivery systems due to their ability to maximize drug efficacy and minimize side effects. However, NPs preparation techniques pose environmental issue, as they require large quantities of organic solvents.

This contribution proposes the use of green methods for the preparation of NPs to deliver protein drugs or therapeutic RNAs. 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.

NPs of small size (~200 nm), low polydispersity index, and positive Z potential were obtained. NPs toxicity was investigated against melanoma and fibroblasts cell lines, observing no significant reduction in viability, up to 1 mg/mL. Platelet activation after exposure to NPs was tested as a preliminary assessment of their safety after intravenous injection. NPs were incubated with platelets for 30 min, followed by count of platelet adhesion and SEM analysis on adherent platelets, for a qualitative assessment, and by FACS, for a quantitative measure. PAH NPs did not trigger platelet activation, at any of the tested concentrations, while CS NPs did not induce activation at concentrations below 200 µg/mL.

NPs showed capacity to load model payloads (secondary antibody for CS NPs and mock siRNA or BRAF-silencing siRNA for PAH NPs) 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 (Figure 1).

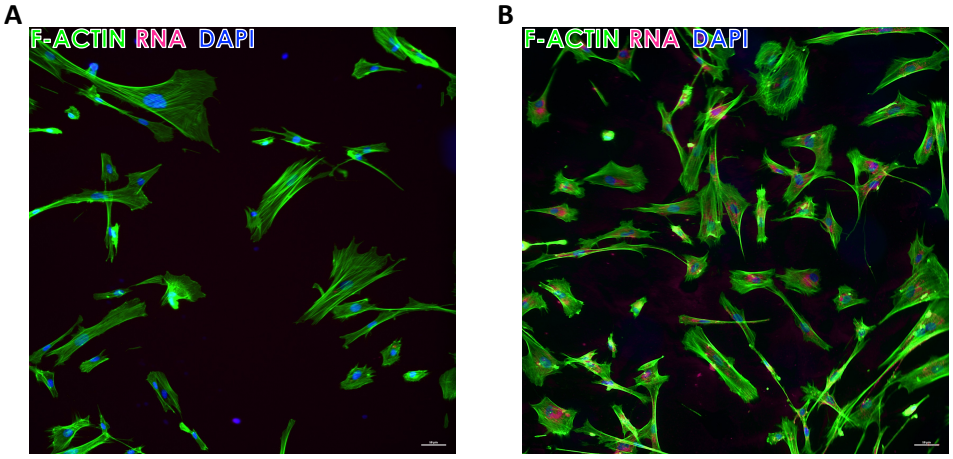

Figure 1: Human fibroblast (Hff-1) after free siRNA administration (a) and siRNA-loaded PAH NPs (b) at the same siRNA concentration

Cisplatin-loaded CS and BRAF-siRNA loaded PAH NPs were tested against human melanoma spheroids. Since the tumor response to treatment strongly depends on its interactions with the tumor microenvironment (TME), a 3D-printed model of metastatic melanoma is under development as a further NPs testing device, representing an alternative to animal tests. To obtain the model, skin fibroblasts (Hff-1) were embedded in a collagen/hyaluronic acid-based hydrogel, and allowed to grow for one week. 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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