

'NTRACK: NON-INVASIVE IMAGING MODEL WITH GOLD NANOPARTICLES FOR STEM CELL TRACKING ON A MUSCLE REGENERATION'

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PURPOSE

Cell therapy provides a promising approach for diseases and injuries that conventional therapies cannot treat effectively. However, the prediction of success or failure of cell therapy is challenged by the current lack of methods to track the biodistribution and fate of the transplanted cells and to detect their viability in real time. Current clinical practice evaluates the success of cell-based treatments by assessing the disease symptoms, which can only be examined weeks or even months after the treatment was initiated. These significant drawbacks that delay the consideration of alternative treatments represent major barriers for progress and clinical acceptance of cell therapies.

OBJECTIVE(S)

nTRACK project aims developing a safe, scalable and highly sensitive nano-imaging agent. The nTRACK nanoparticles (NPs) allow non-invasive visualization and tracking of the administered labelled stem cells to be used during development of new cell therapies. Here we present the gold NPs coated with glucose to improve their cellular uptake for computed tomography (CT) imaging.

METHOD(S)

Production of the NPs are realized by using GMP compliant manufacturing methods, where sodium citrate was used as a reducing agent. NPs were stabilized by using PEG7 and to enhance cellular uptake NP surface was coated with glucose by using EDC-NHS chemistry. NPs were concentrated to 3 w % by using cross flow filtration. NPs were injected intramuscularly to healthy Swiss Albino mice and monitored through CT imaging which was performed with X-CUBE (Molecubes, Belgium) at 1hr, 3hrs and 24hr post injection. Inductively coupled plasma optical emission spectrometry (ICP OES) method was used to determine the NP concentration in mesenchymal-like adherent stromal cells (PLX PAD) and murine macrophages (RAW264.7) in vitro. Visualization of the intracellular fate of nanoparticles in PLX PAD was performed by TEM.

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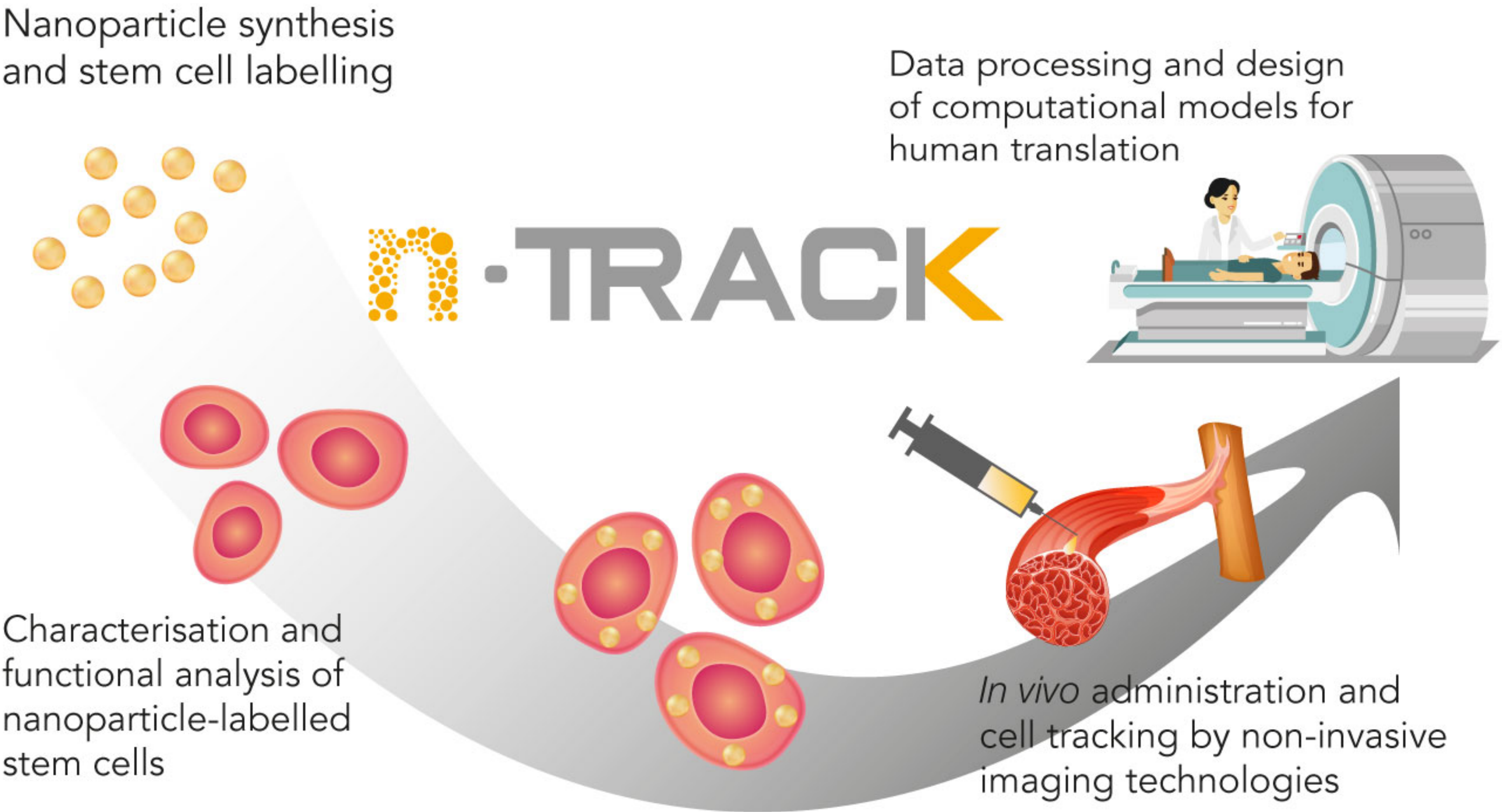


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NTRACK

Multimodal nanoparticles for structural and functional tracking of stem cell therapy on muscle regeneration

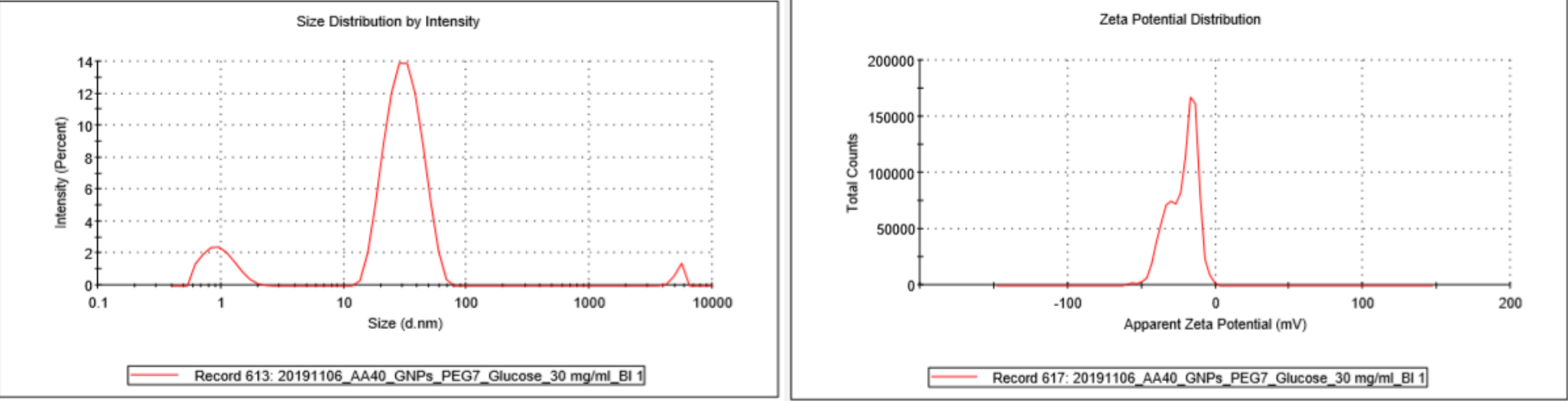


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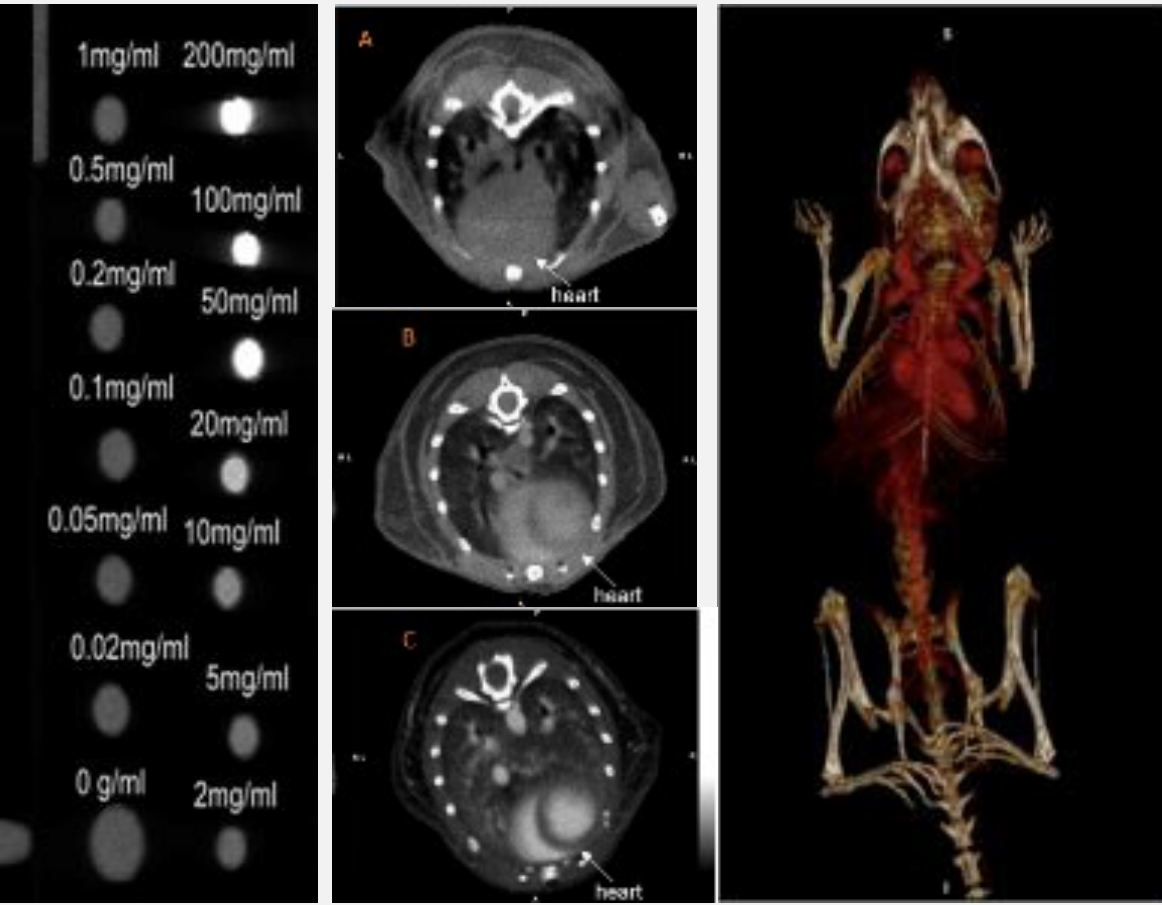


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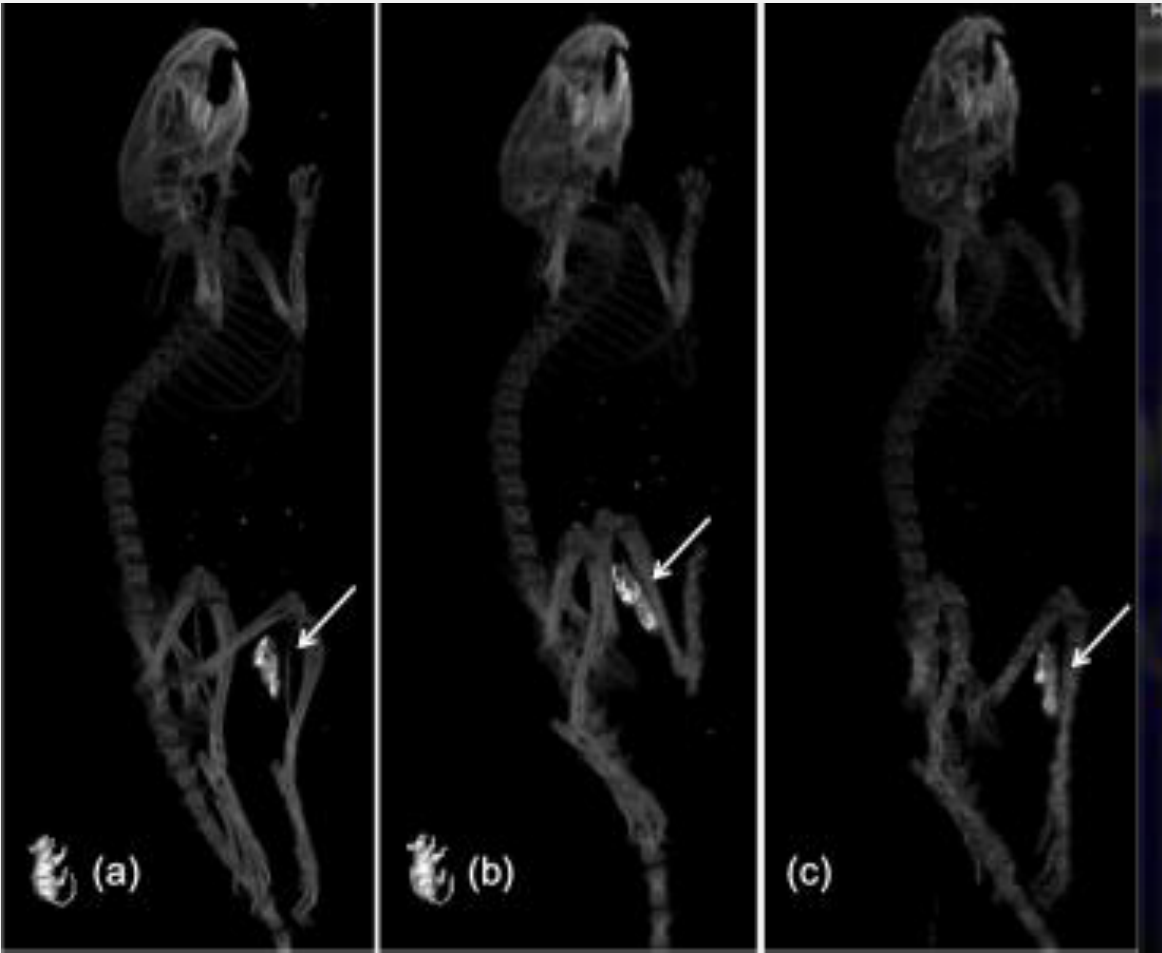
RESULT(S)



AA-40	Particle size, nm	PDI	Zeta, mV
GNPs	21.67	0.551	-29.9
GNPs_Glucose	23.15	0.433	-22.5

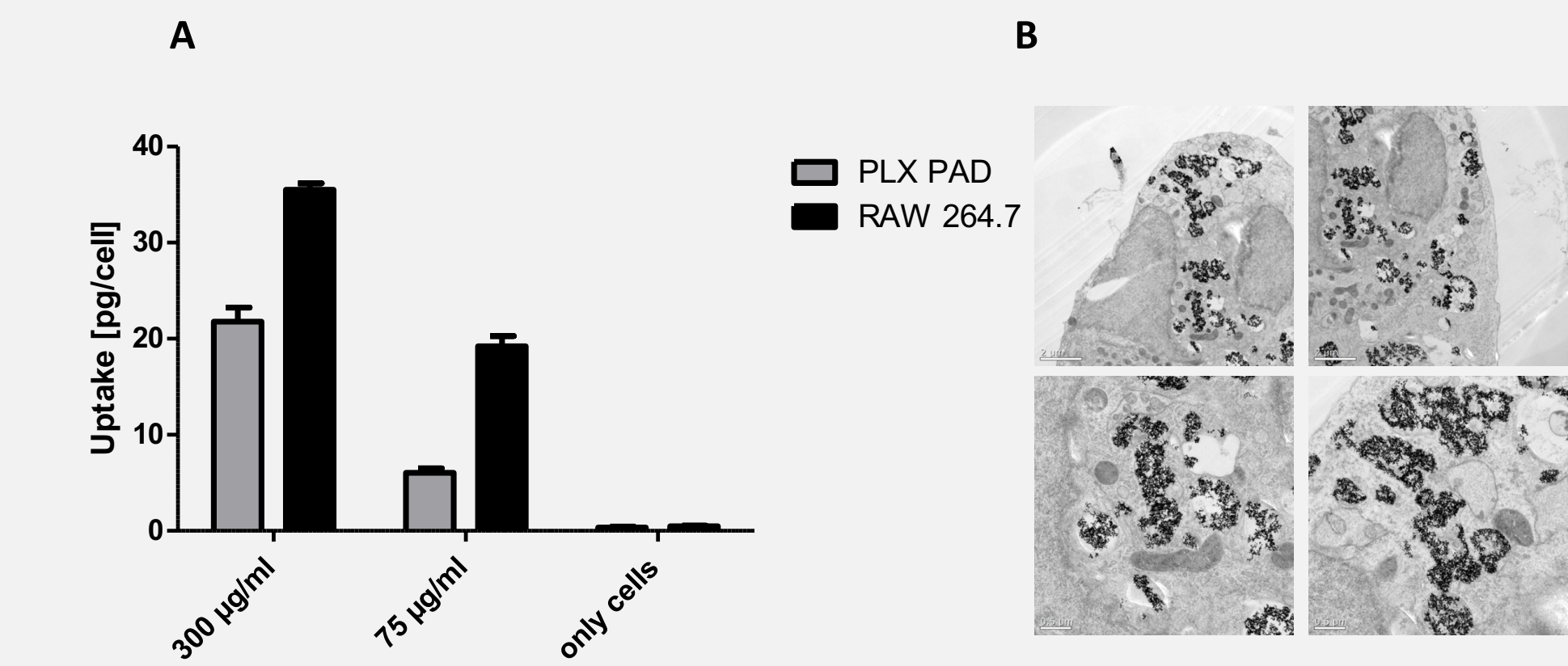


Quantification of contrast commercial GNPs (AuroVist 15nm, Nanoprobes) in different concentrations, administration to healthy muscles and imaging heart with CT, full body imaging with the highest concentration.



Intramuscular administration of GNPs & monitoring 1, 3, 24hrs with CT

CT images were acquired with a High-Resolution whole-body spiral protocol and reconstructed with the iterative algorithm and a spatial resolution of 100µm. This allowed a clear signal to be monitored on the spatial distribution of the NPs over time. CT images showed that the particles were cleared very slowly upon i.m. administration.



Cellular Uptake of iron core gold shell nanoparticles in human mesenchymal stem cells (PLX PAD) and murine macrophages (RAW264.7).
A) Determination of intracellular nanoparticle concentration with ICP OES. Cells were incubated with 75 µg/ml or 300 µg/ml nanoparticle solution, washed and afterwards dissolved in aqua regia. Gold concentration was measured with HORIBA Activa M ICP OES system, using the optical emission lines 267,595 nm and 242,795 nm. It was shown that the cells take up the particles well.
B) Visualization of the intracellular fate of nanoparticles in human mesenchymal stem cells by TEM. Hereby it could be shown that most particles are inside the cells and not on their surface. Furthermore, it can be seen that the intracellular localization seems to be the lysome or structures belonging to lysosomal pathway.

CONCLUSION(S)

nTRACK NP manufacturing method was successfully established and a GMP compliant manufacturing protocol was generated. Batch sizes were scaled-up to a few liters without any change in the critical quality attributes. NPs were shown to be well tolerated by the cells and were taken up at concentrations that would generate enough CT signal for cell tracking purposes. Strong evidence was shown that nTRACK system will allow the visualization and longitudinal monitoring of the transplanted stem cells in real time. The nTRACK NPs enhances the strength of the clinically-used imaging system by offering allowing direct clinical translation.