

STRUCTURAL AND FUNCTIONAL TRACKING OF STEM CELLS ON MUSCLE REGENERATION MODEL THROUGH NON-INVASIVE MULTIMODAL IMAGING

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BACKGROUND & PROJECT PLAN

Stem cell therapy keeps gaining ground as a promising approach for a broad range of diseases, with currently no alternative effective therapies. However, tools providing real-time tracking of transplanted cells on their early biodistribution and viability, are missing from the current therapeutic approaches. At the same time gold nanoparticles (GNPs) have shown that except of excellent blood pool and heart contrast agents, can also serve as can serve as imaging agents to perform cell tracking, in cell therapy schemes. Moreover their ability to be easily radiolabelled, renders them a valuable tool which could evaluate and predict the safety and success of cell-based treatments. are already used as contrast agents in Computed Tomography (CT) and as drug carriers for targeted therapy

The EU-funded project nTRACK, started in October 2017 aims to develop a safe, scalable and highly sensitive multimodal cell nano-imaging agent ready for first in humans. The nTRACK approach will enable non-invasive whole-body monitoring, longitudinal and quantitative discrimination of living stem cells in humans using CT, MRI and PET, simultaneously.

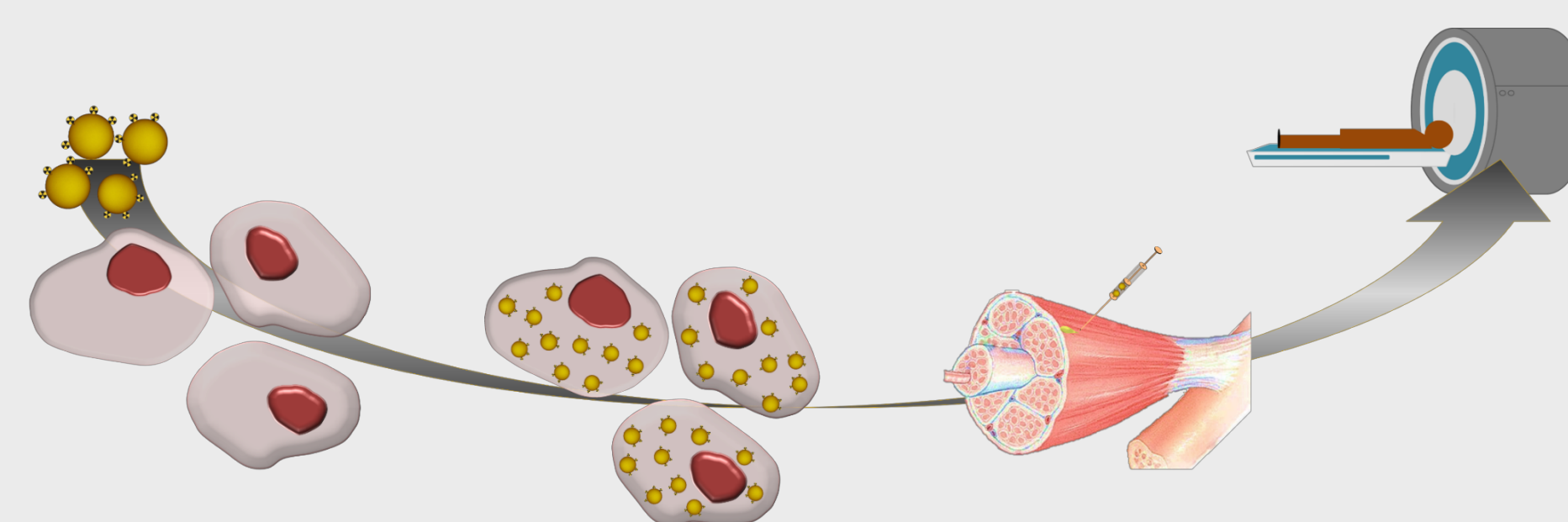


Figure 1: Schematic illustration of the nTRACK's project plan.

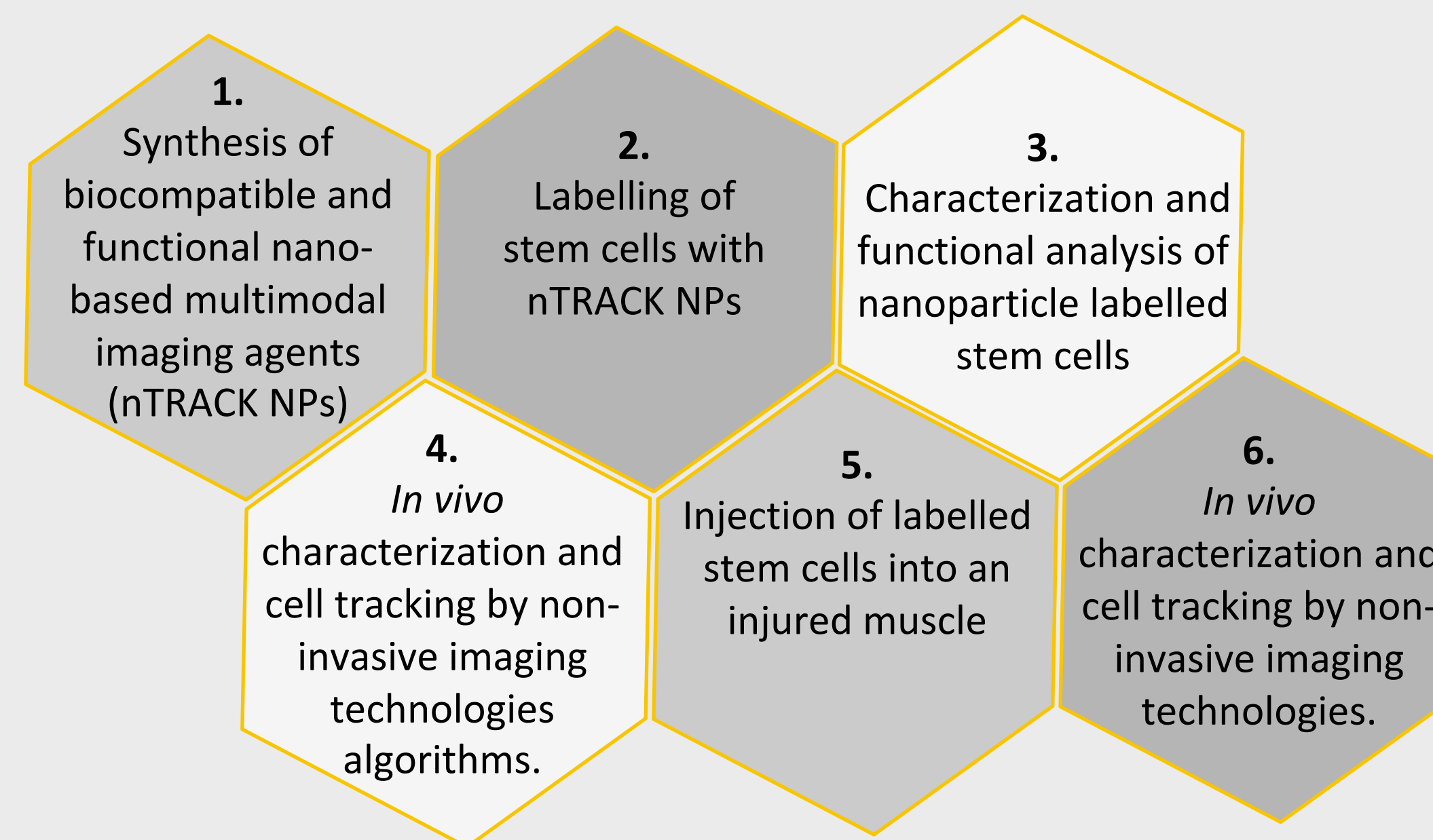


Figure 2: Images of γ-CUBE and x-CUBE from Molecubes, tomographic systems that for these studies.

RESULTS:

Exposure of PLX-PAD cells to nTrack NPs in saline solution was explored and resulted in the successful staining of cells with nTRACK NPs (**Fig.3**).

The labelling protocol for the chelation of $[^{111}\text{In}]\text{InCl}_3$ to DTPA functionalised nTRACK NPs was optimised in order to obtain the highest possible radiochemical incorporation in combination with a good kinetic stability. (**Fig.4**)

The first in vivo control tests have been performed, to establish the biodistribution of the (i) ligand, of the (ii) NPs and of the (iii) labeled NPs. These biodistributions are shown in the following Figures (**Fig. 5, 6, and 7**).

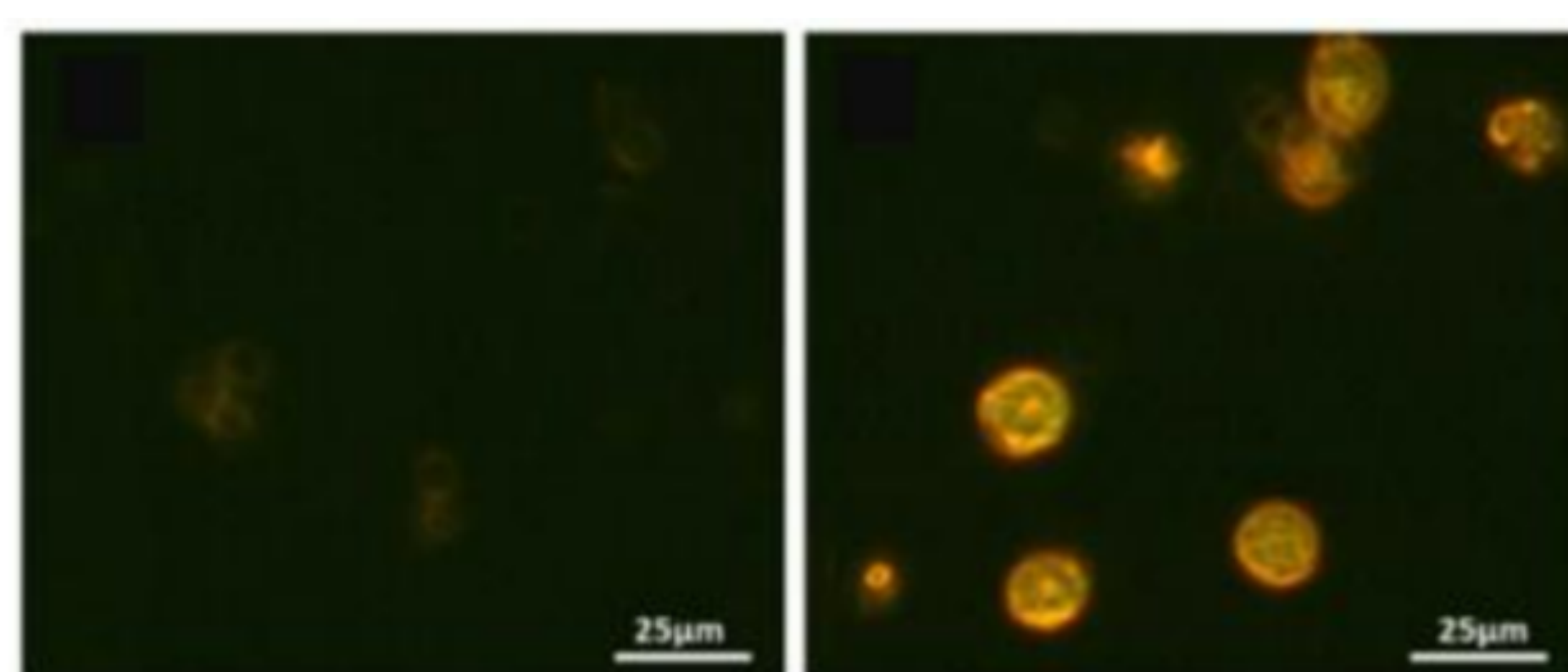
These results show that when the substances are administered on their own, they follow a gradual clearance from the body. When administered within the cells, this will allow to test that the cells indeed stay in the desired region.

METHODS & MATERIALS:

- Biocompatible and functional nano-based multimodal imaging agents were developed in order to enable non-invasive monitoring of living stem cells in small animal models through SPECT and CT imaging.
- The *in vivo* platform and methodology to track stem cells fate was established based on GNPs samples (gold coated - magnetic core NPs manufactured by Bar-Ilan University).
- The *imaging studies* were performed on a SPECT and on a CT imaging system either by Molecubes (γ-CUBE and x-CUBE), providing spatial resolution of 0.6 mm and 0.05 mm, respectively or by Mediso (nanoSPECTPlus and nanoScanPETCT), providing spatial resolution of 0.3 mm and 0.01 mm, respectively



FIGURES:



Control Sample (No nTRACK NPs) Cell stained with nTRACK NPs

Figure 3: Microscope Images of NP stained cells.

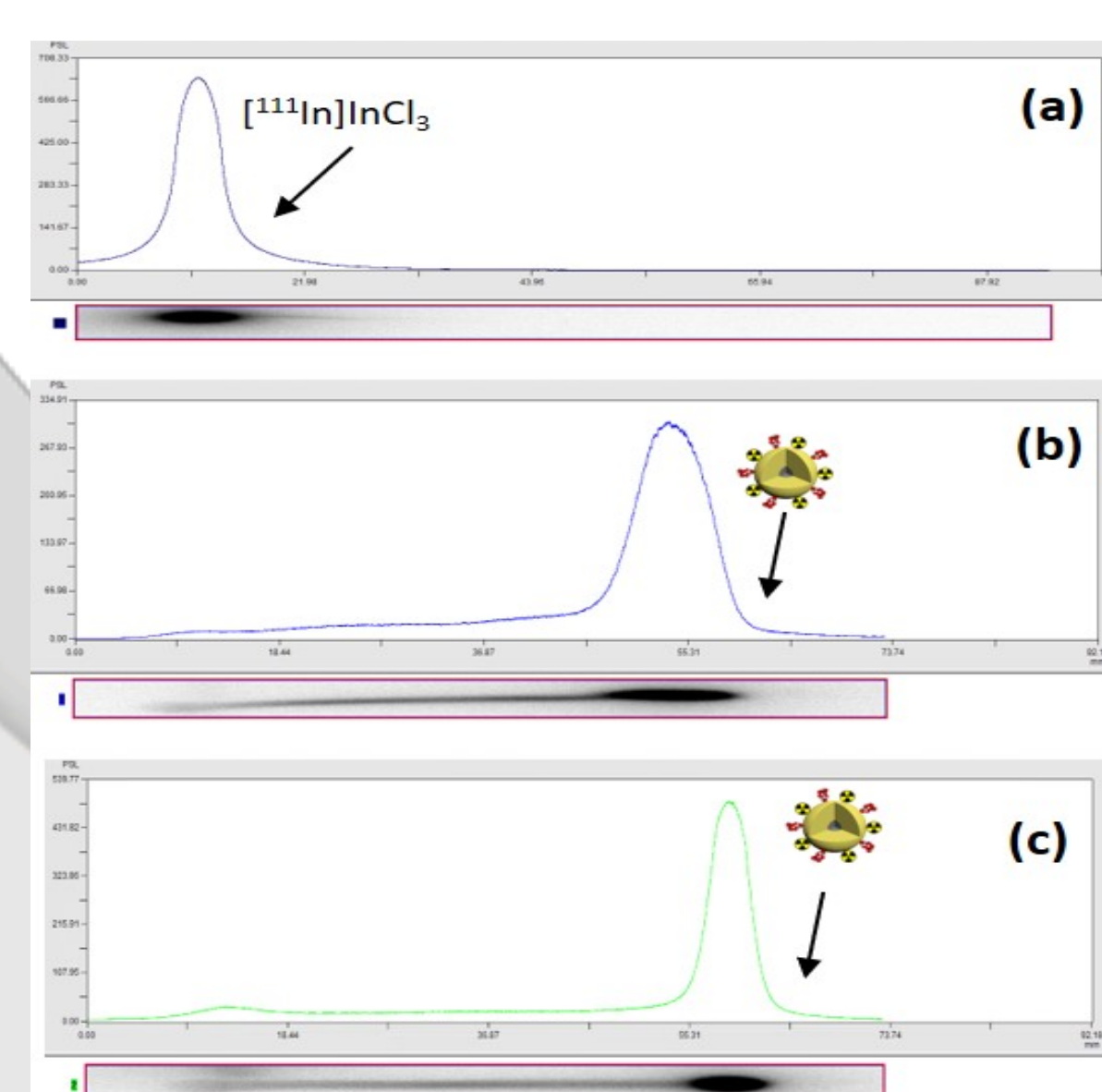


Figure 4: Radio TLC of (a) $[^{111}\text{In}]\text{InCl}_3$ and (b,c) $[^{111}\text{In}]\text{In-DTPA-nTRACK}$ NPs developed on C18 chromatography paper with MeOH/ NH_4OAc 5M at 2 h post preparation (b) and 24 h post preparation (c) The chromatographic papers were read using a phosphor imager.

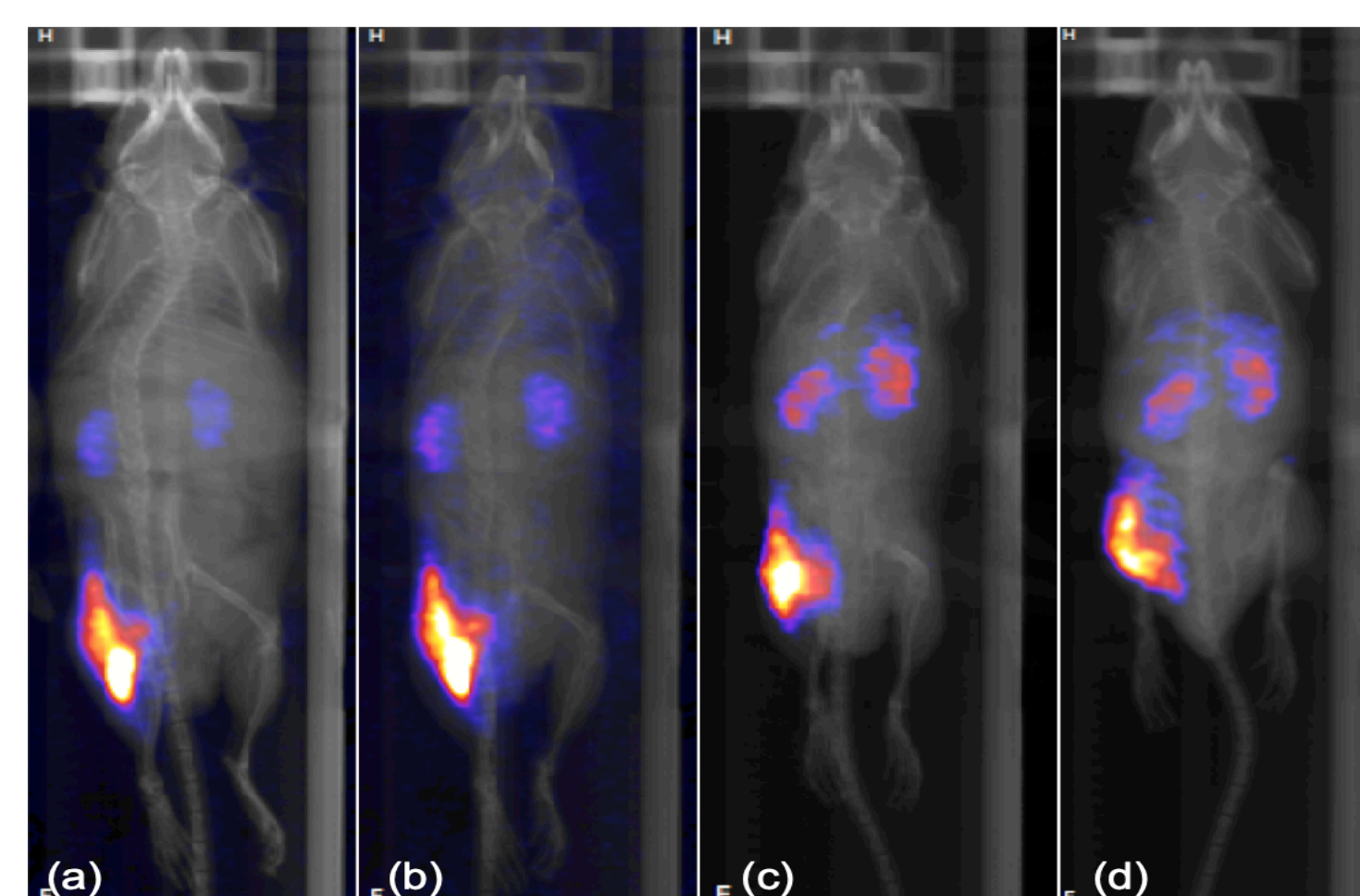


Figure 5: SPECT/CT imaging of a healthy C57Bl/6J mouse, intramuscularly injected with approximately 2.6 MBq of $[^{111}\text{In}]\text{InCl}_3$ and imaged at (a) 2 hrs p.i., (b) 4 hrs p.i., (c) 24 hrs p.i. and (d) 48 hrs p.i.

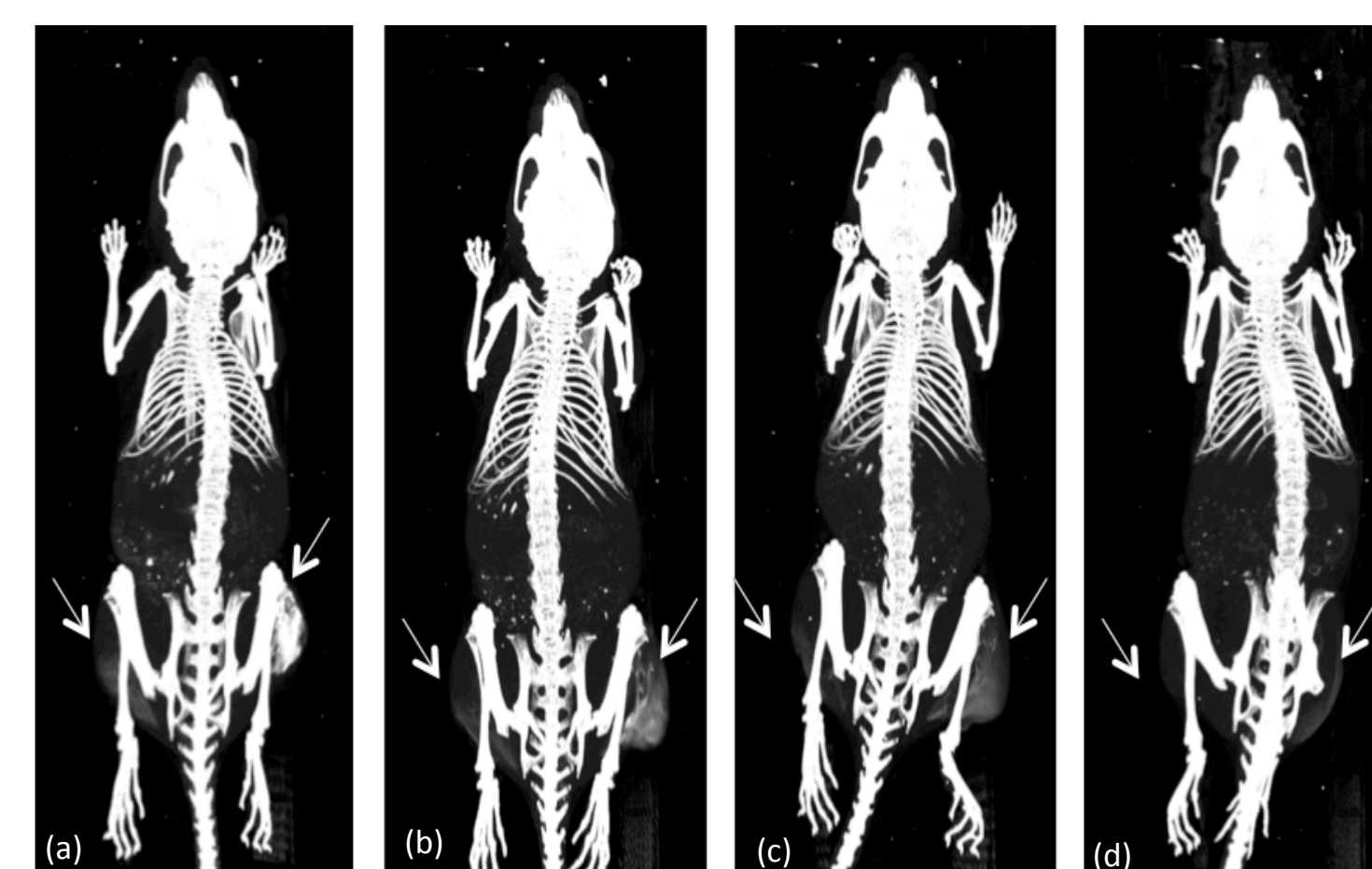


Figure 6: CT imaging of a healthy WT female mouse, intramuscularly injected with 50 μL of NPs solution BIU-DTPA-Au@IONPS the right leg is injected with 30 mgAu/mL NPs and the left leg with 7.5 mgAu/mL NPs at (a) 0 hr p.i., (b) 2 hrs p.i., (c) 4 hrs p.i. and (d) 24h p.i.

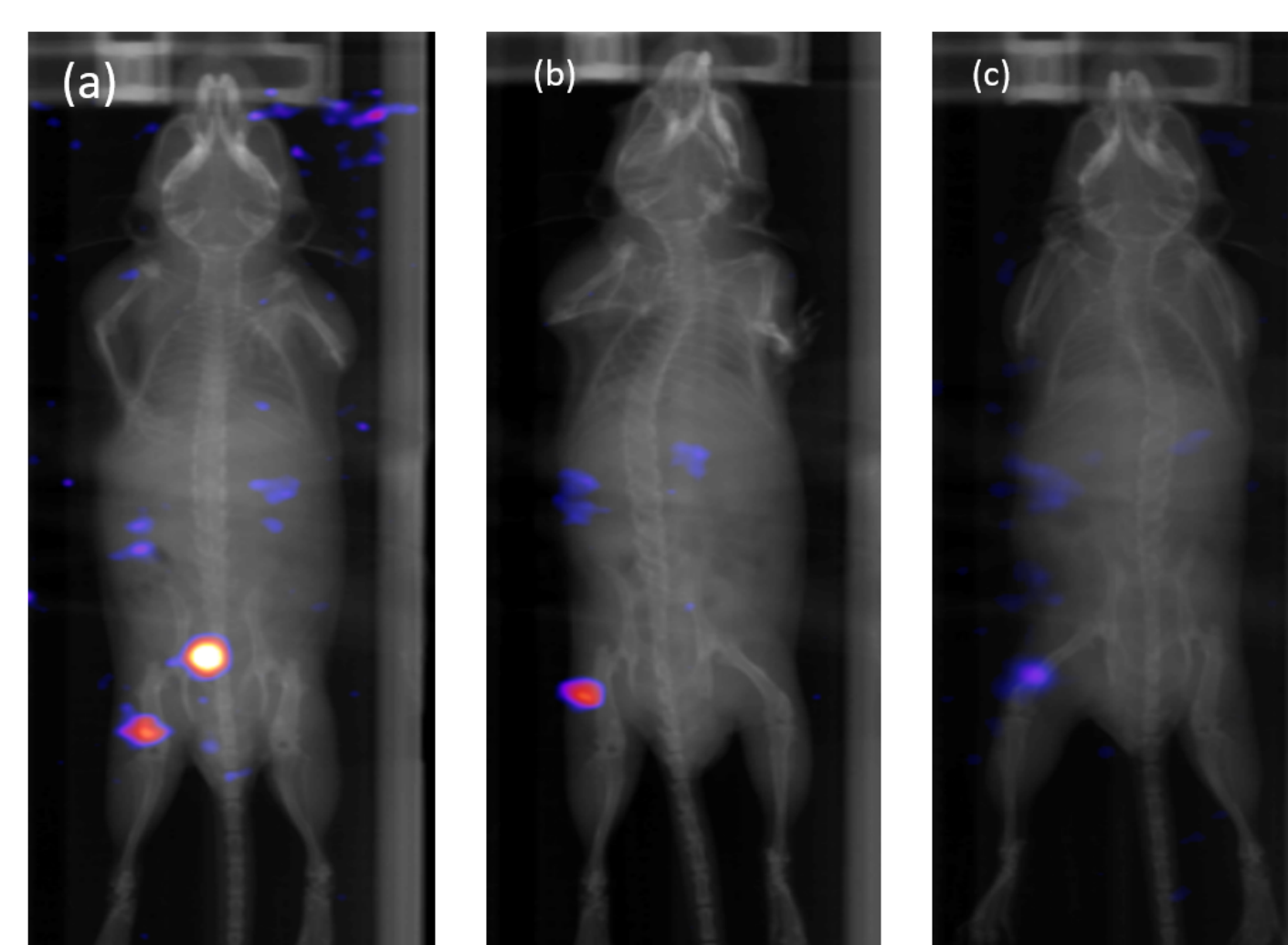


Figure 7: SPECT/CT imaging results of a healthy male C57Bl/6J mouse, intramuscularly injected with 50 μL of NPs solution BIU-DTPA-Au@IONPS_Feb2019 Feb2019 the left leg is injected with 1.5 mgAu/mL NPs at (a) 2 hr p.i. (40 min scan), and (b) 4 hrs p.i. (1 hr 10 min scan) and (c) 24 hrs (1 hr 30 min scan)

CONCLUSION & FUTURE WORK:

Radiolabelling of nTRACK NPs has successfully been performed and the first imaging results were obtained. The CT enhanced of the nTRACK NPs has been proven from CT and Gold core magnetic NPs have successfully been imaged through SPECT/CT for the first time. So the ability of nTRACK NPs to provide a multi-purpose imaging tool is already demonstrated. On the other side, the labelling protocol of the nTRACK NPs to the stem cells has been established.

The *in vivo* platform and methodology to study GNPs as an imaging tool, for cell tracking applications have been prepared and it will be further optimised in the near future.

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