

RADIOLABELLING OF GOLD NANOPARTICLES FOR ASSESSING THE ABILITY TO MULTIMODAL IMAGING: INVESTIGATION OF THEIR POTENTIALS TO TRACK STEM CELLS IN MUSCLE REGENERATION MODELS AND THEIR PRELIMINARY *IN VIVO* EVALUATION WITH SPECT/CT 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.

To overcome the current barriers of cell therapeutics on tracking non-invasively the transplanted cells and monitor their viability, the EU-funded project nTRACK aims to develop biocompatible and functional nano-based agents highly sensitive multimodal imaging modalities. 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.

More specifically, modified and labelled with the long-lived radio-isotope Indium-111 ($[^{111}\text{In}]\text{In}(\text{III})$), gold coated-magnetic core NPs (Au@IONPs) were developed aiming at real-time non-invasive whole-body monitoring of living stem cells in small animal models through the simultaneous use of different imaging techniques.

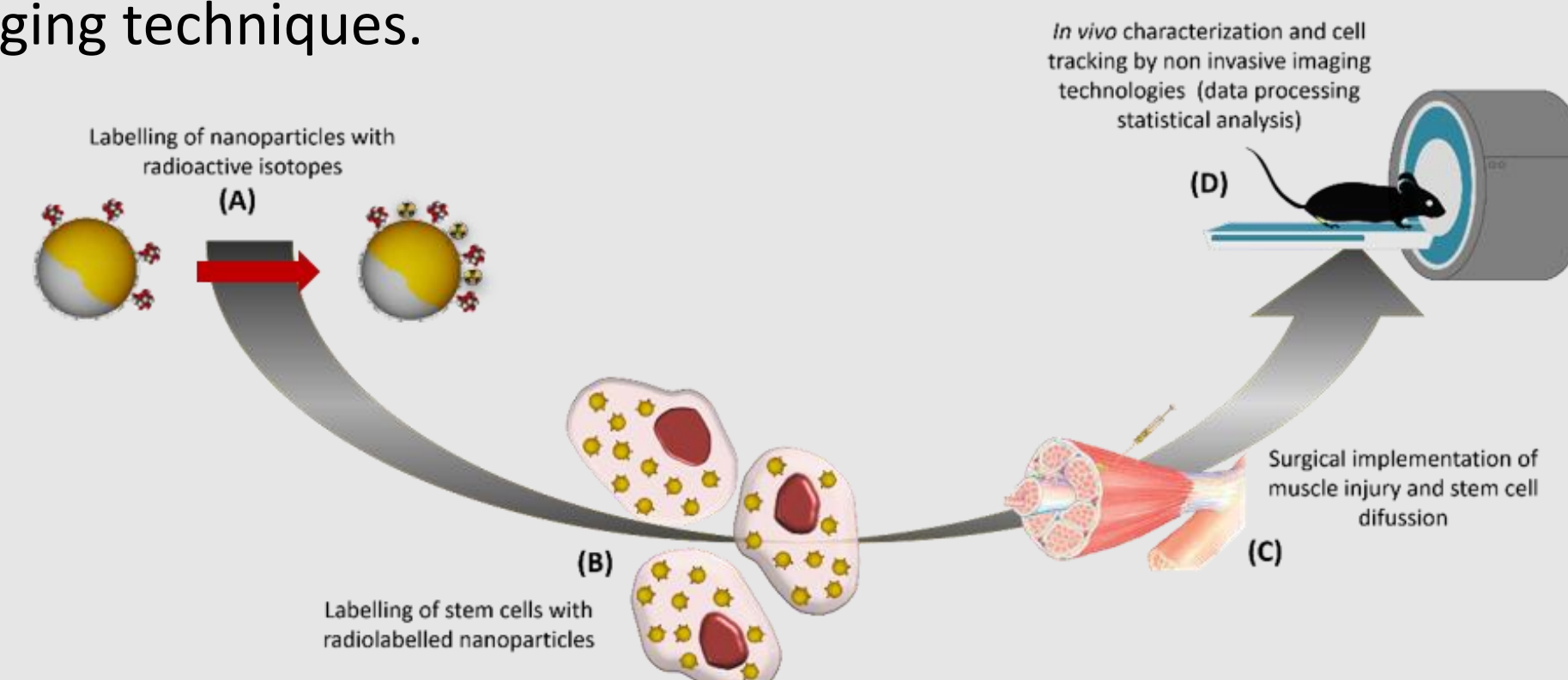


Figure 1: Schematic illustration of our approach.

All the obtained results suggest that when the substances are administered on their own, they follow a gradual clearance from the body. Whilst when they will be administered within the cells are expected to stay at the region of the cells, which will allow us to test the stem cell fate *in vivo*.

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 Au@IONPS samples (gold coated - magnetic core NPs manufactured by Bar-Ilan University (1)).
- The SPECT and CT imaging studies were performed on the systems by Molecubes (Figures 4&6) and Mediso (Figure 5).

Provider	System	Modality	Resolution (in mm)
Molecubes	γ -CUBE	SPECT	0.6
	x-CUBE	CT	0.05
Mediso	nanoSPECTPlus	SPECT	0.3
	nanoScanPETCT	CT	0.75

Table 1: Spatial Resolution of the two different systems used.

OVERVIEW OF RESULTS:

- Successful labelling >95%
- Good cell uptake of NPs in PLX-PAD cells
- Simultaneous SPECT and CT imaging of $[^{111}\text{In}]\text{-3}$ NPs
- Simultaneous SPECT and CT imaging of $[^{111}\text{In}]\text{InDTPA}$ in muscle injury model
- Very fast clearance of NPs, shown *via* CT monitoring test
- Very good CT control, as if signal is detected when cells are injected, it will definitely come from the actual labeled cells
- The radioisotope complexed via the ligand indeed follows the "carrier" NPs and is not detached *in vivo* (further validation of labelling stability results)
- Significantly higher sensitivity with SPECT imaging compared to CT – small amounts of NPs can be easily detected.

CONCLUSION & FUTURE WORK:

The first results on radiolabelling Au@IONPs, examining their *in vivo* biodistribution and their potentials as multimodal imaging agents on living stem cells, presented herein. The CT enhanced of our NPs has been proven and Gold core magnetic NPs have successfully been imaged through SPECT/CT for the first time resulting in encouraging outcomes.

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

Further control *in vivo* imaging experiments will soon be completed which will be followed by the final experimental group where the fate of labelled stem cells will be monitored.

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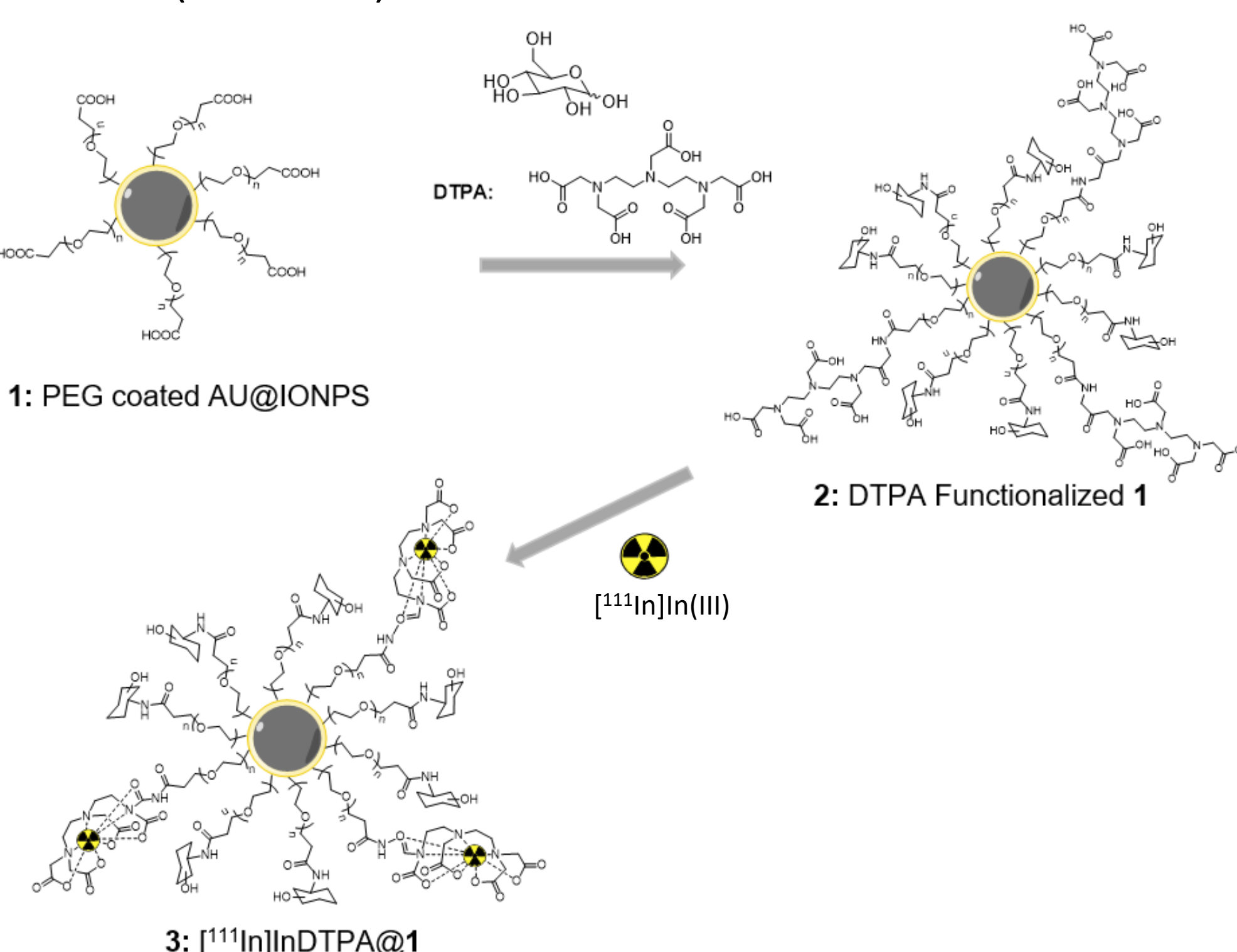
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RADIOCHEMISTRY:

A candidate batch of PEG coated Au@IONPS (**1**) was studied for its ability to be labelled with Indium-111. For this approach, diethylenetriaminepentaacetic acid [DTPA] was used to chelate the radioisotope on the NPs surface. More specifically, DTPA was first conjugated to (**1**) and then labelling with Indium-111 was followed (**Scheme 1**).



Scheme 1: Synthetic pathway towards the formation of $[^{111}\text{In}]\text{InDTPA@Cluc-PEG coated Au@IONPS } ([^{111}\text{In}]\text{-(3)})$.

The labelling protocol for the chelation of $[^{111}\text{In}]\text{InCl}_3$ to DTPA functionalised NPs (**2**) was optimised in order to obtain the highest possible radiochemical incorporation in combination with a good kinetic stability. The results showed that a successful labelling procedure was established, providing a satisfactory stability for at least up to 24 hours at 37°C (**Figure 2**).

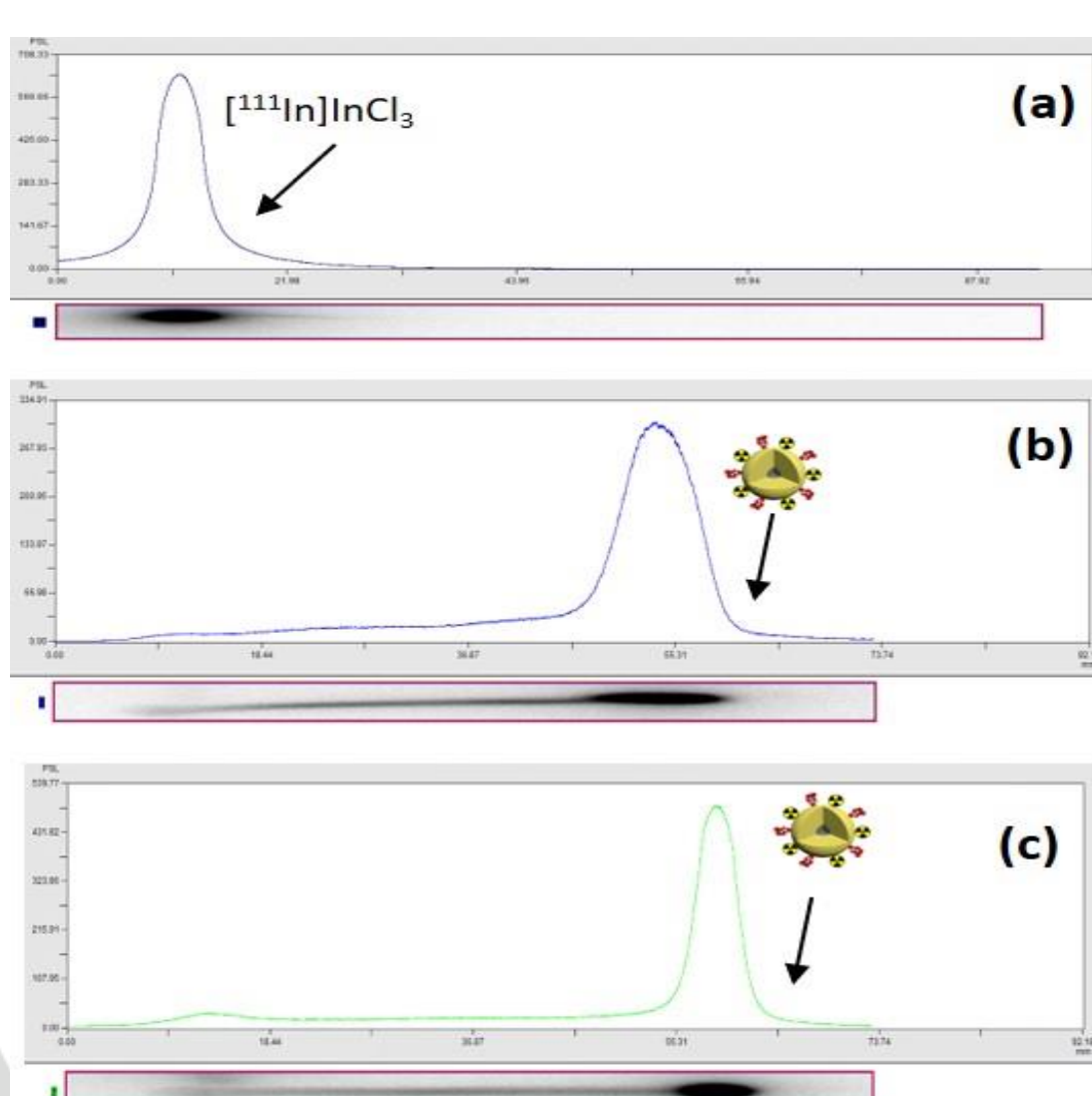


Figure 2: Radio TLC developed on C18 chromatography paper with MeOH/ NH_4OAc 5M of (a) $[^{111}\text{In}]\text{InCl}_3$, (b) 2 hrs post-preparation and (c) $[^{111}\text{In}]\text{-(3)}$ at 24 hrs post-preparation. The chromatographic papers were scanned using a phosphor imager.

IN VITRO CELL UPTAKE:

Exposure of PLX-PAD cells to PEG-Gluc coated Au@SPIONS (**1**) in saline solution was explored and resulted in the successful staining of cells with Cluc-PEG coated Au@IONPS NPs (**1**) (**Figure 3**).

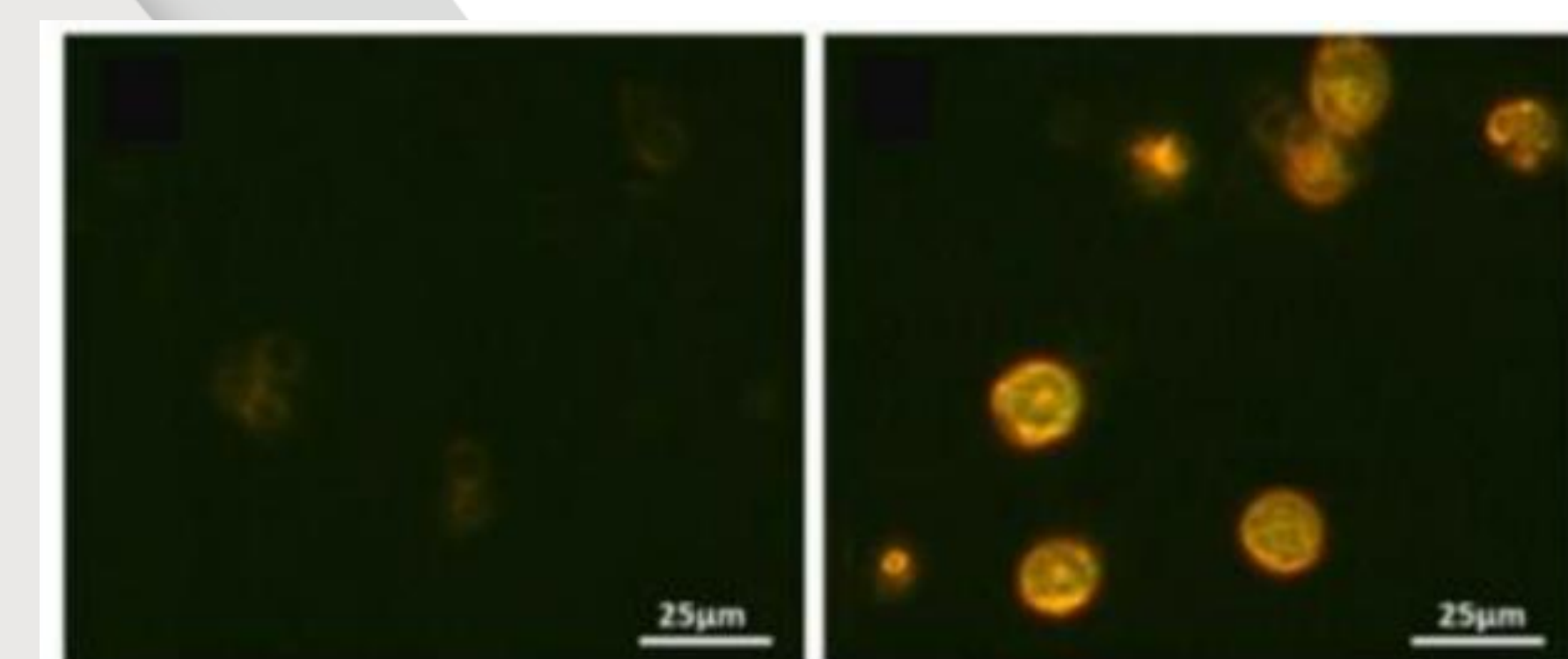


Figure 3: Microscope Images of NP stained cells.

IN VIVO IMAGING:

Preliminary experiments have been performed, in healthy mice to explore the biodistribution and the contrast induction ability of (i) (**2**) NPs and (ii) $[^{111}\text{In}]\text{-(3)}$ NPs when administered intramuscularly (**Figures 4 & 5**). Then first *in vivo* control experiments performed to rats where a muscle injury model was applied. (**Figure 6**).

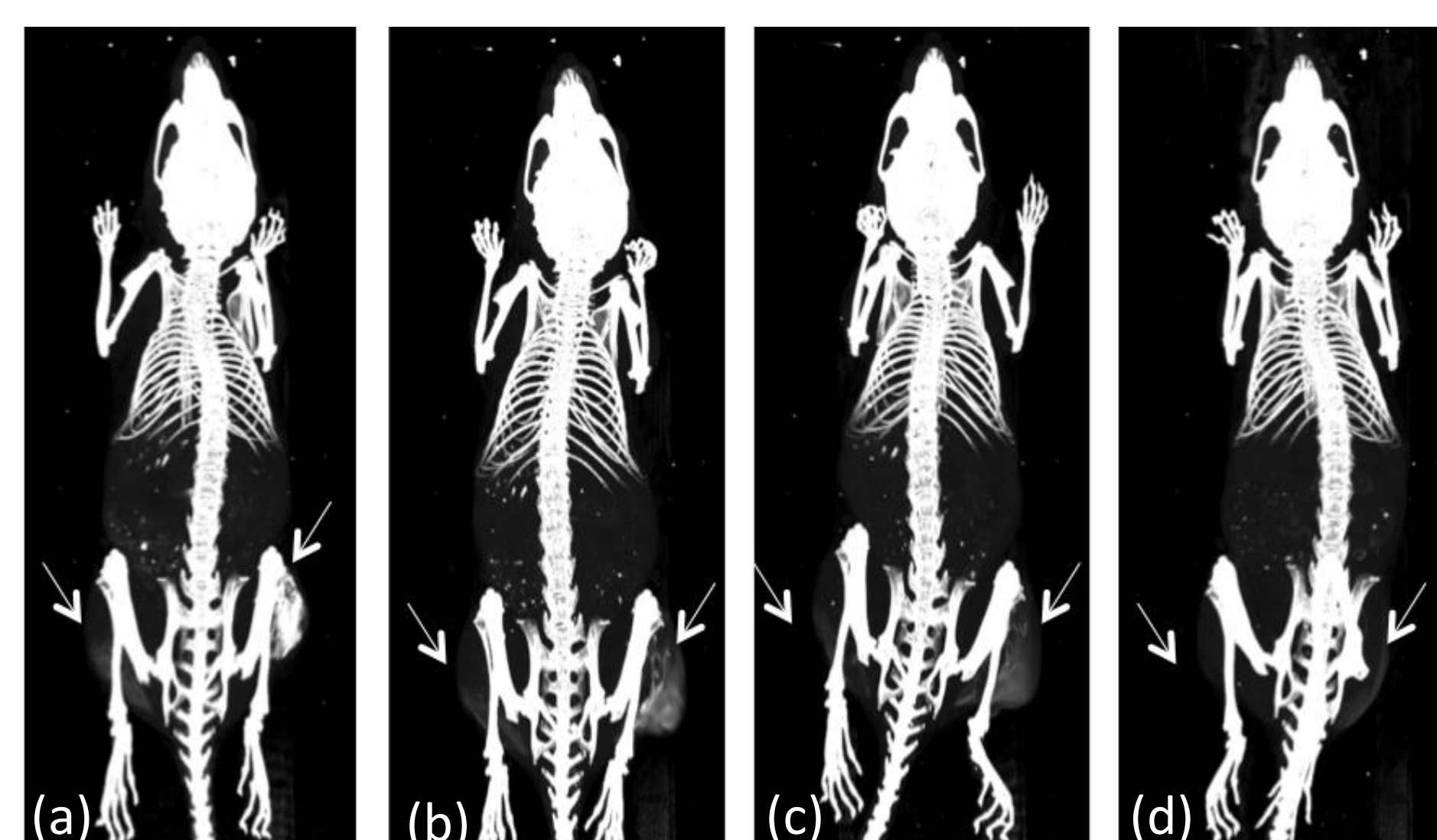


Figure 4: CT imaging of a healthy WT female mouse, intramuscularly injected with 50 uL of NPs' solution or suspension (**2**) the right leg is injected with 30 mg Au/mL NPs and the left leg with 7.5 mg Au/mL NPs at (a) 0 hr p.i., (b) 2 hrs p.i., (c) 4 hrs p.i. and (d) 24hrs p.i.

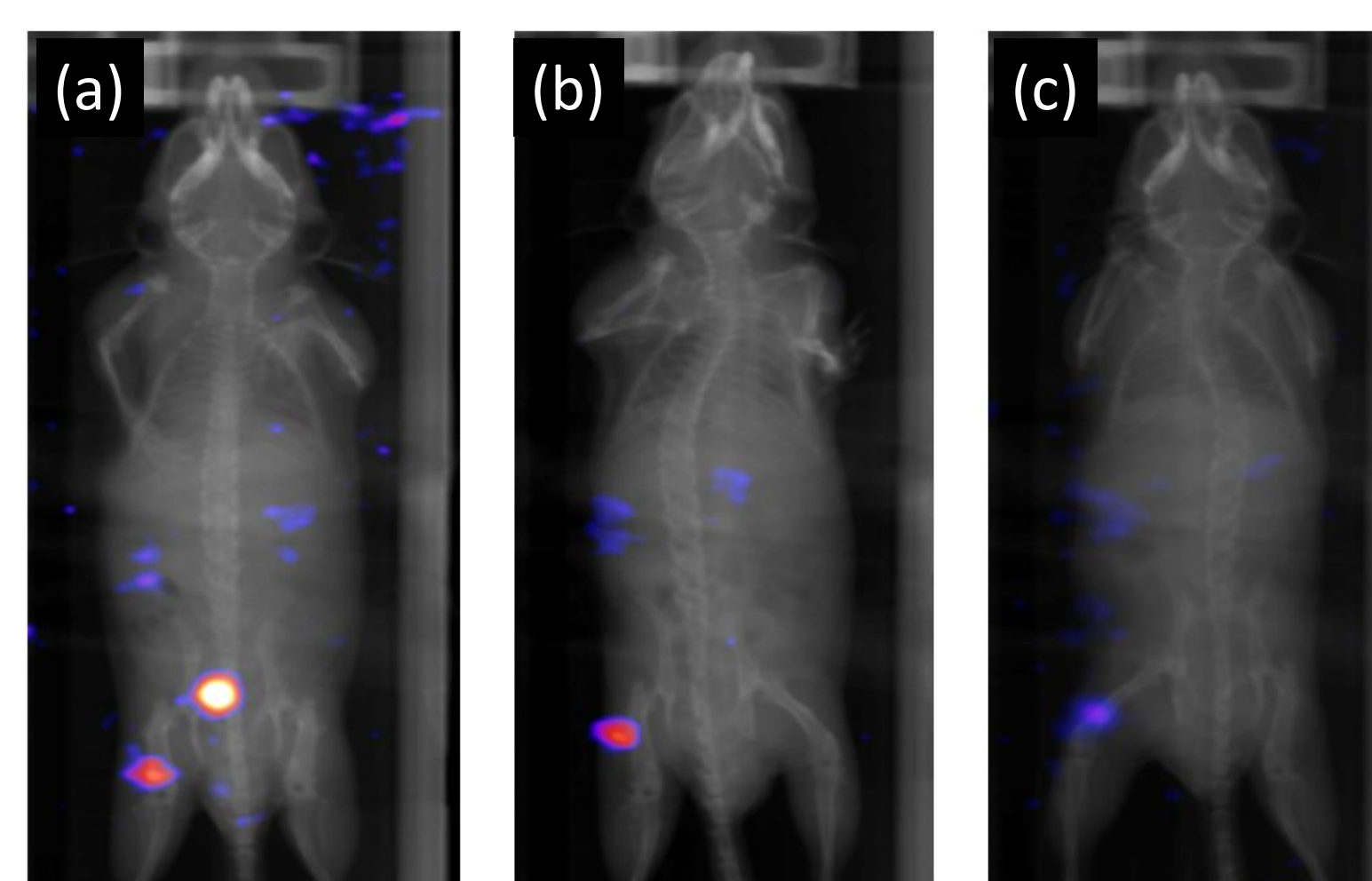


Figure 5: SPECT/CT imaging of a healthy male C57Bl/6J mouse, intramuscularly injected with 50 uL (0.37 MBq) of NPs suspension $[^{111}\text{In}]\text{-(3)}$ the left leg is injected with 1.5 mg Au/mL NPs at (a) 2 hrs p.i. (40 min scan) and (b) 4 hrs p.i. (1 hr 10 min scan) and (c) 24 hrs (1 hr 30 min scan)

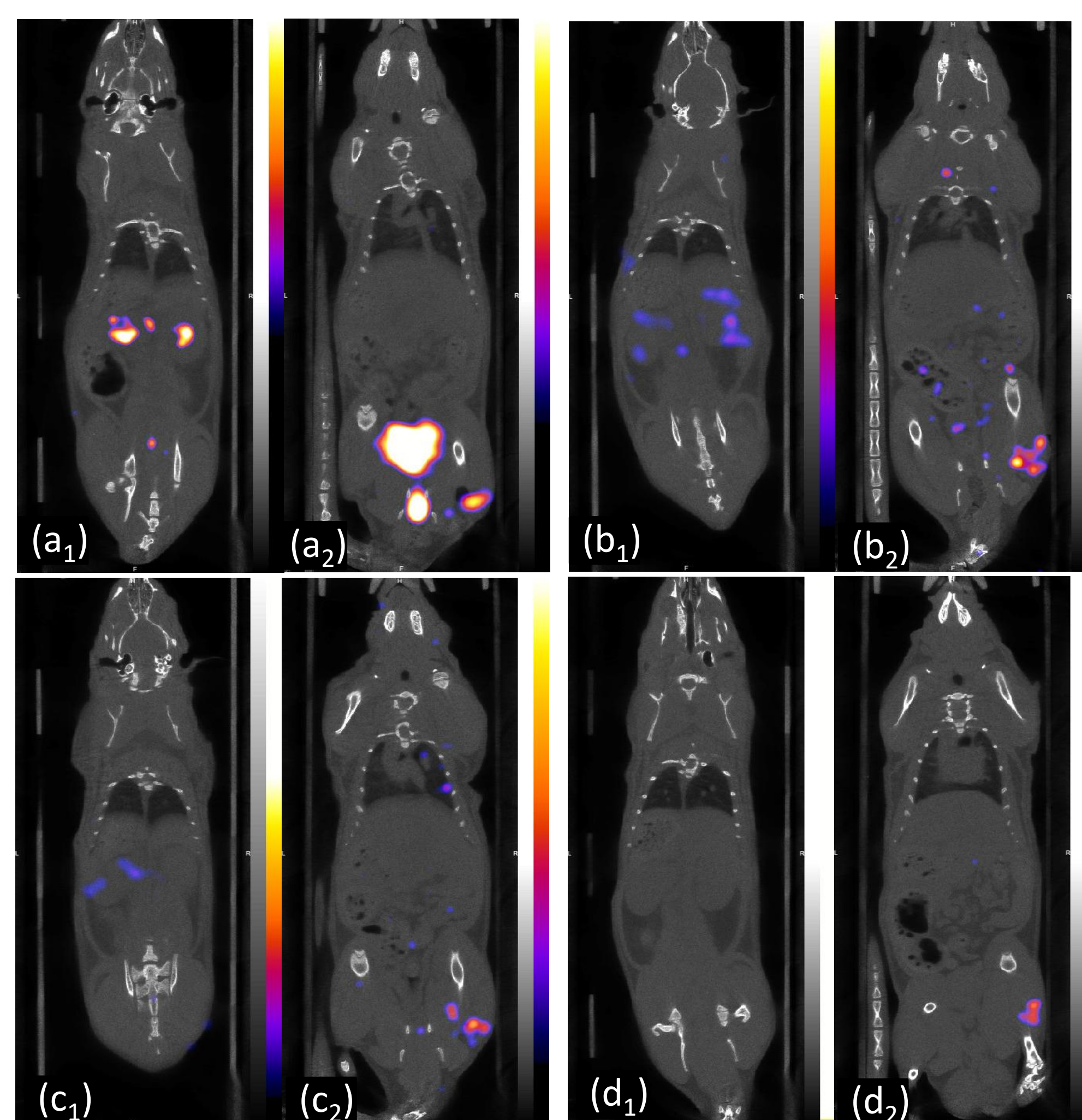


Figure 6: SPECT/CT imaging of muscle injured rat. The medial gastrocnemius of male rats was injured one day prior to the imaging experiments and $[^{111}\text{In}]\text{InDTPA}$ 3.27 MBq (100uL) of $[^{111}\text{In}]\text{InDTPA}$ and imaged at (a) 2 hrs p.i., (b) 4 hrs p.i., (c) 24 hrs p.i. and (d) 48 hrs p.i.; Coronal plane images of (i) kidneys, (ii) muscle injury