IN VIVO TRACKING OF STEM CELLS IN MUSCLE REGENERATION THROUGH MULTIMODAL IMAGING

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BACKROUND & 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 for 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}In]In(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.

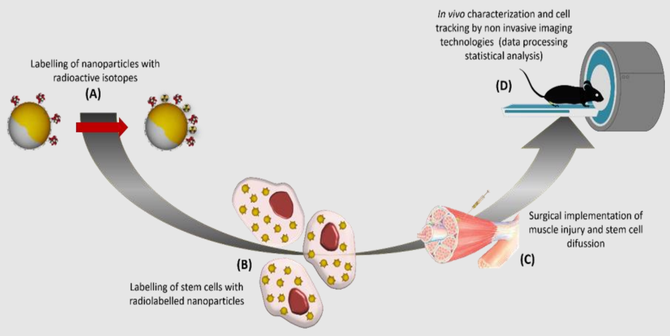


Figure 1: Schematic illustration of our approach.

METHODS & MATERIALS:

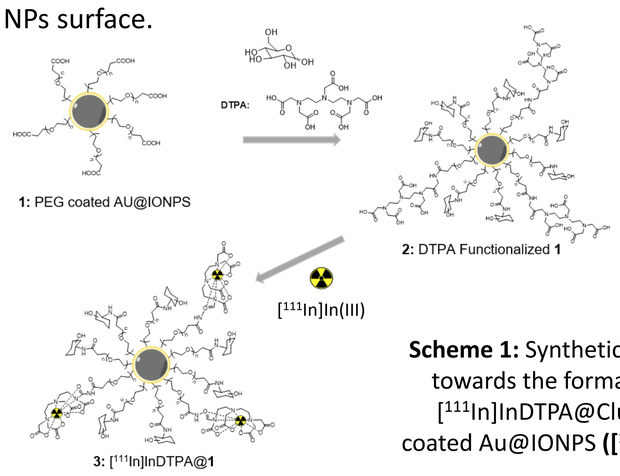
- Biocompatible and functional nano-based multimodal imaging agents were developed in order to enable non-invasive monitoring of living stem cells in small animal models through SPECT and CT imaging.
- The *in vivo* platform and methodology to track stem cells fate was established based on Au@IONPS (gold coated - magnetic core NPs manufactured by Bar-Ilan University (1)).
- The radiochemical testing was based on the development of radiochemical protocols for Au@IONPs, the QC of the resulted products through radio-TLC and kinetic stability assays.
- Optimization of [^{111}In]InDTPA-Au@IONPS uptake in PLX-PAD cells constituted the *in vitro* part of the platform.
- Muscle Injury performed in healthy Wistar Rats a day prior to imaging according to a method developed by *Marrota et al.*(2)
- Administration of [^{111}In]InDTPA-Au@IONPS performed intramuscularly at the point of the injury.
- The SPECT and CT imaging studies were performed on the systems by Molecubes.

Provider	System	Modality	Resolution (in mm)
Molecubes	γ-CUBE	SPECT	0.6
	x-CUBE	CT	0.05

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

RADIOCHEMISTRY:

PEG coated Au@IONPS **(1)** studied for their ability to be labelled with Indium-111. Diethylenetriaminepentaacetic acid [DTPA] was used to chelate the radioisotope on the NPs surface.



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

The labelling protocol was optimised aiming the highest possible radiochemical incorporation in combination with good kinetic stability. The results showed that a successful labelling procedure was established **(Figure 2)**.

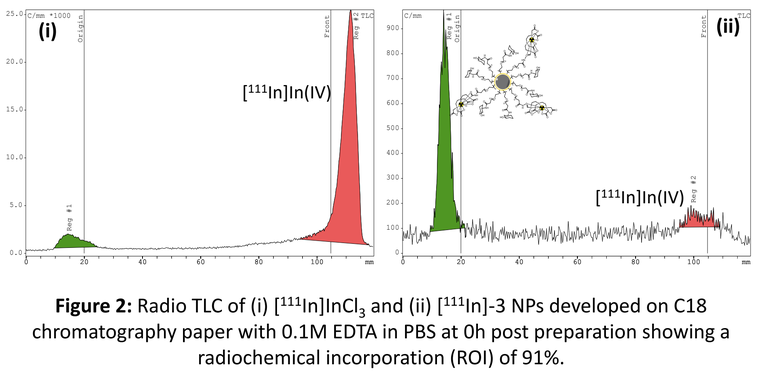


Figure 2: Radio TLC of (i) [^{111}In]$InCl_3$ and (ii) [^{111}In]-3 NPs developed on C18 chromatography paper with 0.1M EDTA in PBS at 0h post preparation showing a radiochemical incorporation (ROI) of 91%.

Kinetic stabilities of [^{111}In]-3 NPs. NPs were incubated at 4°C, 24°C, 37°C and in Human Serum Albumin (HSA), showing an adequate stability up to 24hrs post preparation.

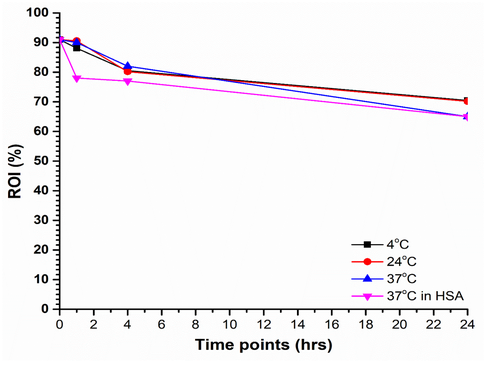


Figure 3: Graphical representation of the kinetic stability assays performed at different temperatures and in different solvents

IN VITRO CELL UPTAKE:

Exposure of PLX-PAD cells to **[^{111}In]-3** NPs in saline solution was explored and resulted in the successful staining of cells (**Figure 4**).

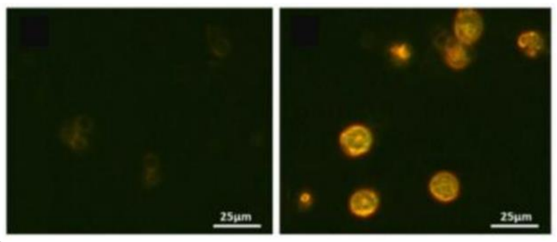


Figure 4: Microscope Images of NP stained cells.

Control Sample (No NPs) Cell stained with **(1)**

IN VIVO IMAGING:

Muscle injured male Wistar rats were imaged using different contrast agents to study the ability of the NPs to track stem cell. The first experiment was performed with [^{111}In]InDTPA as a control of the radiolablled NPs (**figure 5**) the second one the *in vivo* biodistribution of **[^{111}In]-3** NPs when administered intramuscularly (**Figures 6**). The last group demonstrates the in vivo fate of the stem cells (**Figures 7**).

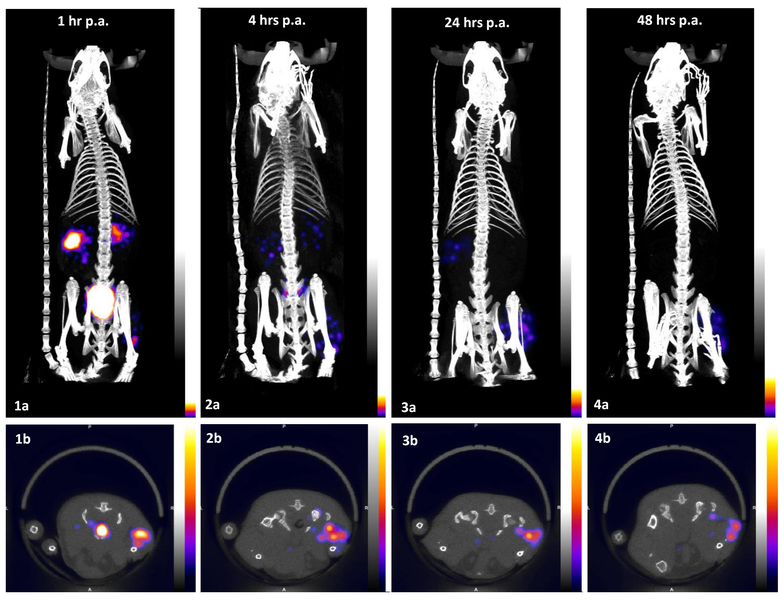


Figure 5: SPECT/CT imaging of medial gastrocnemius injured male Wistar rats. Then 3.27 MBq (100uL) of [^{111}In]InDTPA were administered and imaged at (1) 1 hr p.a., (2) 4 hrs p.a., (3) 24 hrs p.a. and (4) 48 hrs p.a.; (a) 3-D rendering, (b) axial plane images of the muscle injury.

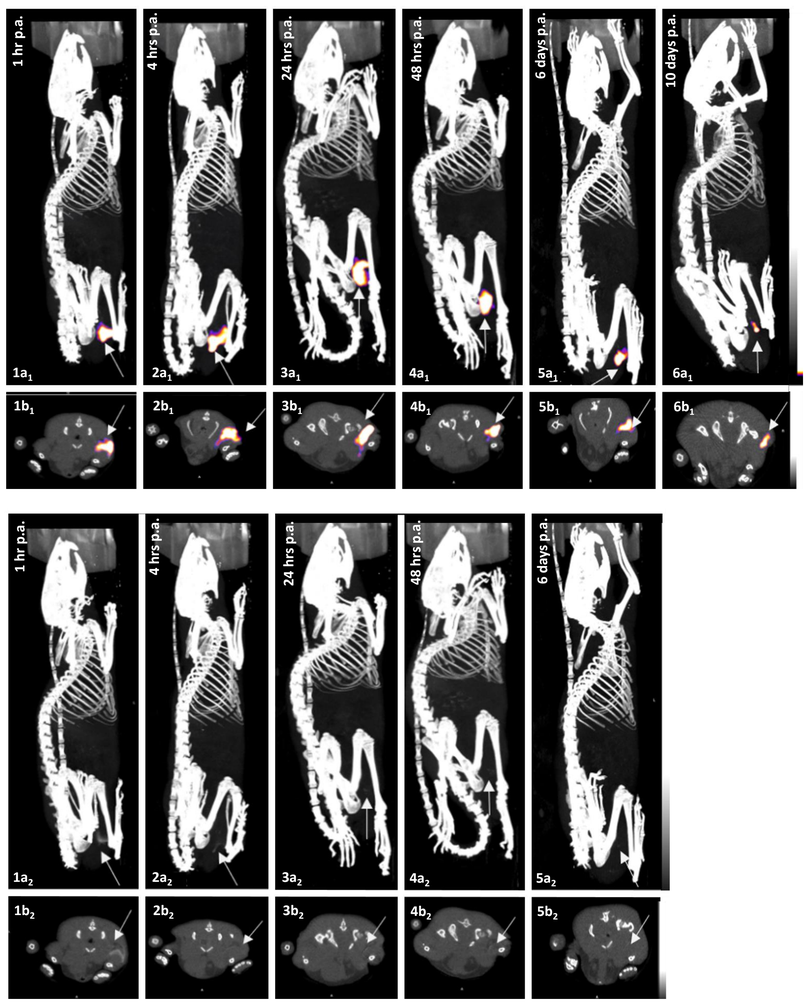


Figure 6: SPECT/CT and CT imaging of medial gastrocnemius injured male Wistar rats. 7.5 MBq (100uL; 13mgAu/mL) of [^{111}In]-3 NPs were administered and imaged at (1) 1 hr p.a, (2) 4 hr p.a., (3) 24 hrs p.a. and (4) 48 hrs p.a; (5) 6 days p.a. and (6) 10 days p.a. (a) 3-D rendering and (b) axial plane images of the muscle injury.

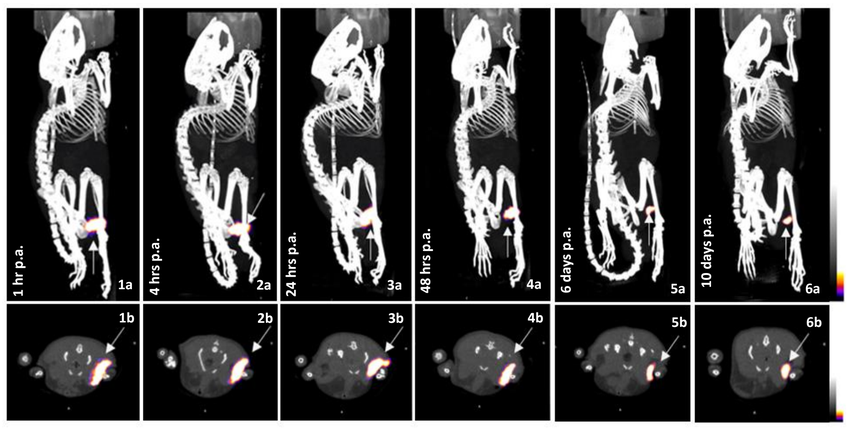


Figure 7: SPECT/CT imaging of medial gastrocnemius injured male Wistar rats. 1.3 MBq (100uL; ~4.4*10^6 cells/mL) of stem cells labelled with [^{111}In]-3 were injected and imaged at (1) 1 hr p.i., (2) 4 hr p.i., (3) 24 hrs p.i., (4) 48 hrs p.i, (5) 6 days p.i., and (6) 10 days p.i; (a) 3-D rendering (b) Coronal plane, and (c) axial plane images of the muscle injury.

The theoretical activity that was expected to be found in each animal was calculated for all the time points and was compared with the actual activity measured to confirm that **[^{111}In]-(3)** are not released from the cells *in vivo.*

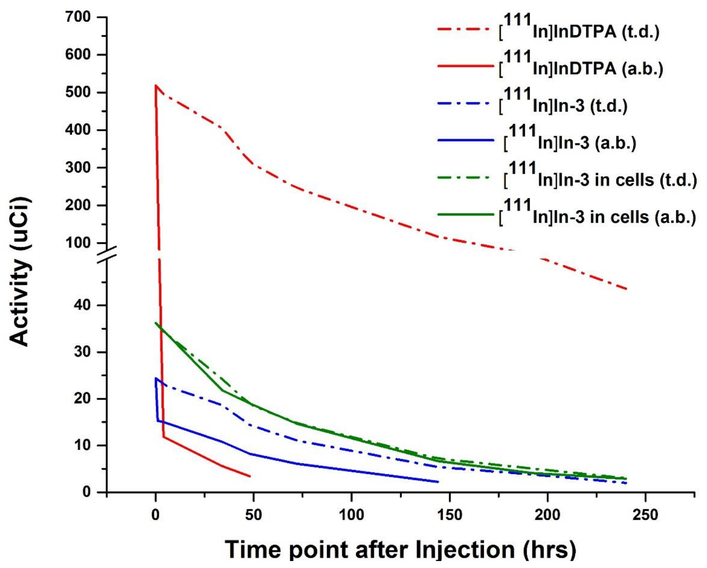


Figure 8: Schematic comparison between theoretical decay of the injected dose (t.d.) and the actual biodistribution (a.b.) of the administered dose for each group.

OVERVIEW OF RESULTS:

- Successful labelling >95%
- Sufficient cell uptake of NPs in PLX-PAD cells
- Simultaneous SPECT and CT imaging in muscle injury model rats of **different administration groups.**
- Fast clearance of NPs, shown *via* both SPECT and CT monitoring test
- 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. Radiolabelled Au@IONPs have been uptaken by stem cells and used in muscle regeneration scheme for the first time.

Imaging studies showed a clear signal increase in the point of injection, but at the same time all the obtained results suggest that when the substances are administered on their own (not up-taken by cells), they follow a gradual clearance from the body. Whilst, when they are administered within the cells they are staying at the region of the cells, which allows us to test the stem cell fate i*n vivo*.

REFERENCES:

1. O. Betzer, R. Meir, T. Dreifuss et al., *Scientific Reports*, 2015, **5**, 15400.
2. Marrotta M et al., *International Journal of Sports Medicine,* 2016, **37**, 386.

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