ESRF	**Experiment title:** Tracking therapeutic cells using k-edge imaging in a murine stroke model	**Experiment number**: MD1011
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Report:

Study #2- Non-invasive bi-color imaging of therapeutic cells embedded in a hydrogel and transplanted in the inflamed rat brain

Introduction:

Stroke is one of the most common causes of death in the western world. The administration of therapeutic cells directly into the brain infarct cavity at the chronic stage of ischemic stroke is able to promote brain regeneration and to rescue motor function.[1,2] However, one of the limitation of the intracerebral delivery of cells is substantial cell death. To overcome this problem, therapeutic cells can be embedded and delivered in a protective hydrogel.[3] A common requirement for the development of regenerative therapies for ischemic stroke is a non-invasive mean to visualize both cells biodistribution and hydrogel following their delivery. Our purpouse is to develop selective bi-color imaging of the regenerative medicine therapy, i.e. specific imaging of therapeutic cells on the one hand and of the cell-embedding hydrogel on the other. We plan to investigate two innovative strategies: K-edge imaging using spectral photon-counting CT (SPCCT)[4] and K-edge imaging using synchrotron radiation. K-edge imaging with Synchrotron-produced x-rays (SXR) will serve us as a gold standard for validation of the recently developped SPCCT approach (project supported by a H2020 grant in which we are partners, http://www.spcct.eu/).

Materials & Methods:

For our experiment, different bio-compatible metal-based nanoparticles were considered as contrast agents: gold-nanoparticles (AuNPs, Pr Cormode, University of Pennsylvania) for therapeutic cells labeling, and gadolinium-nanoparticles (GdNPs, Pr Parola, ENS, France) or iodine-nanoparticles (INPs, Pr Chevalier, LAGEP, France) for hydrogel labeling. We prepared phantoms for a range of concentrations of these nanoparticles to test for techniques sensitivity and obtain calibration curves for quantification analysis. Phantoms containing a combination of contrast agents were also prepared. Cells were labelled in vitro with the AuNPs and then intracerebrally (IC) delivered a hydrogel labelled with GdNPs (n=3) or INPs (n=3) in a

rat model of neuroinflammation. Animals were in vivo imaged by SPCCT the day after injection and 5 to 6 days after, and then in vivo imaged by SXR betweend 9 to 10 days post-injection. To perform K-edge imaging of a given element, an absorption image is first acquired just below the binding energy of the K shell electrons (Au: 79.7 keV; Gd: 49.2 keV; I: 32.2 keV) and then just above the binding energy of the electrons of the element of interest (Au: 81.7 keV; Gd: 51.2 keV; I: 34.2 keV).[5] The subtraction of these two acquisitions produces a specific image of the element. The imaging set-up at ID17 consisted of the fixed exit monochromator coupled with the PCO-edge camera (21 µm spatial resolution).

Results:

Our results show that specifc in vivo k-edge imaging of multiple elements was feasible using the ID17 imaging set up, providing proof of concept of bi-color k-edge imaging by SPCCT and SXR for intracerebrally delivered gold-labelled cells embedded within gadolinium-labelled or iodine-labelled hydrogel. In the Figure, SPCCT and SXR images for gold-labelled cells embedded in an iodine-labelled hydrogel are shown. The analysis of the regions of interest (volume and contrast agents concentration) for the different imaging techniques are ongoing, and will be compared to the contrast agents quantification on tissue brains samples by mass spectrometry (ICP-MS).

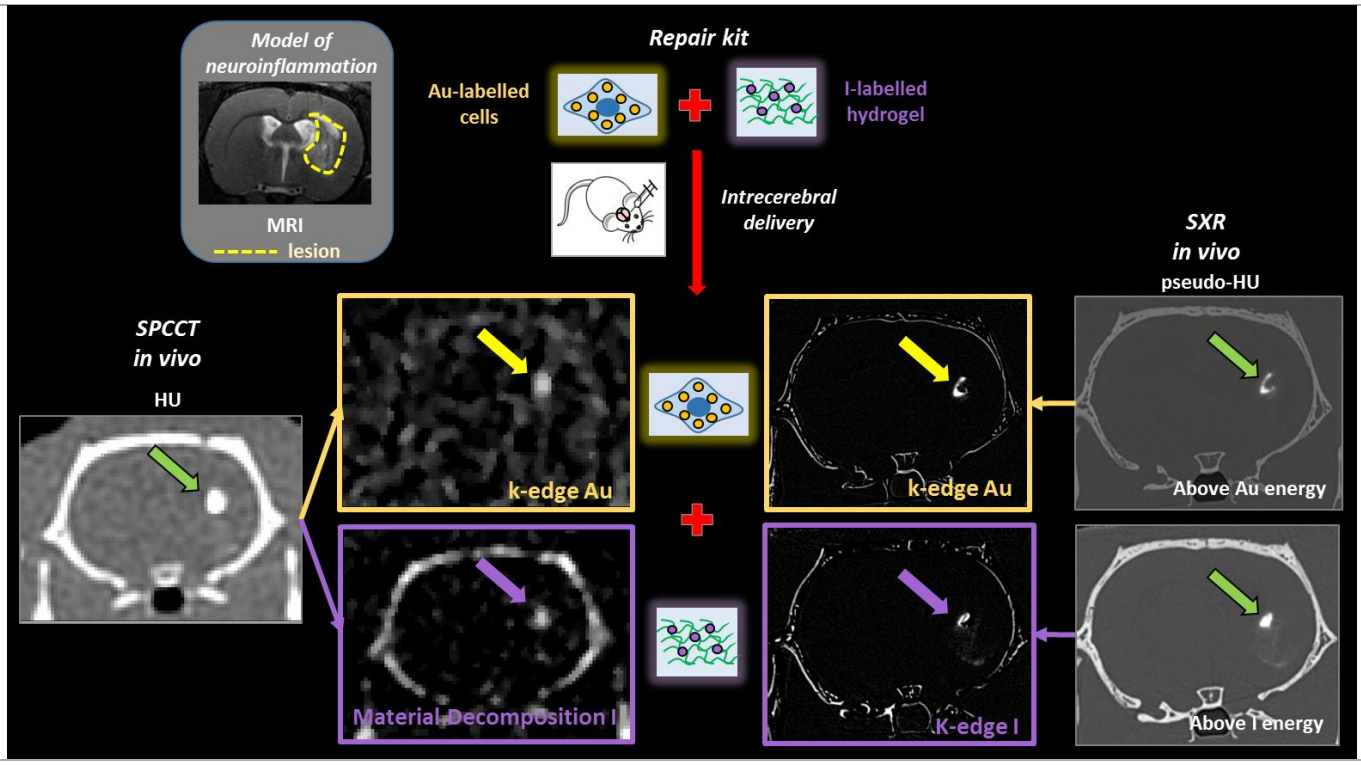

Figure. Proof of concept of in vivo specific bi-color imaging with SPCCT and SXR. Rats with a brain lesion are injected with a neuro-regenerative "repair kit" composed of therapeutic cells embedded within a hydrogel. For this rat, cells were labelled with gold, and hydrogel with iodine. SPCCT imaging is shown on the left: conventional attenutation image, and material-specific images (k-edge reconstruction for Au and material decomposition for I). On the right, SXR imaging: images acquired just above the k-edge energy of Au and I, and corresponding material-specific images (substraction between the images acquired just above the k-edge energy and those acquired just below the k-edge energy, for each material). HU: Hounsfield Unit; Au: gold; I: iodine; MRI: magnetic resonance imaging, SPCCT: single photon counting computed tomography; SXR: synchrotron-produced x-rays.

Conclusions & Perspectives:

These results provide a reference for the validation of k-edge imaging with SPCCT, a new technology with high potential for *in vivo* molecular imaging with CT. In vivo K-edge imaging of multiple elements was successfully performed with synchrotron radiation. The next step is to confirm these pilot results and to rigorously evaluate and validate our innovative approaches by including more live animals for quantification studies and statistical analysis.

Aknowledgements:

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