



Gold nano-particles as x-ray CT contrast markers for cells *in-vivo*

C. J. Hall¹, E. Schultke², B. Juurlink, R. Menk³, F. Arfelli⁴, L. Rigon⁵, A. Astolfo⁴

¹Monash Centre for Synchrotron Science, School of Physics, Monash University, ²Dept of Anatomy and Cell Biology, University of Saskatchewan

³Elettra, Sincrotrone Trieste S.C.p.A., ⁴Dept. Of Physics, University of Trieste.

ABSTRACT

In biomedical research any technique which allows the identification of cancer cells or therapeutic pluripotent cells *in vivo* is being employed in an escalating effort to both understand and combat disease. Most of the methods are limited to the analysis of tissues or cells *ex-vivo* and are not capable of producing *in-vivo* images. We believe the use of x-ray tomography for pre-clinical longitudinal studies of disease progress, and of cell therapy efficacy would be embraced if an appropriate cell marker was available.

One such potential marker is gold nano-particles (GNPs). Initial work has shown that cells can be rendered visible in X-ray microCT if they have a number of GNPs incorporated within them. Studies to date have shown that the number is entirely compatible with the normal function of the cell. Some 26,000 GNPs of an average of 50nm in diameter have been taken up by the cells and shown not to affect their proliferation. Both C6 glioma and murine olfactory ensheathing cells have been successfully imaged in the head, liver and spine of rats using both synchrotron and micro-focus x-ray sources in CT. Some results are shown and the prospects for future development are discussed.

BACKGROUND

- Why try to track cells *in-vivo*?
 - To inform disease progression studies
 - To help develop cell therapies
- How can this be done?
 - One potential method is: **precision x-ray tomography**
- What do we need in order to do this?
 - An x-ray source and instrumentation of an appropriate quality
 - A contrast enhancement technique to render the marked cells visible.

We have the source and instrumentation already in the form of the Australian Synchrotron and local expertise in x-ray imaging. What is now required is development of the contrast agent.

OBJECTIVE

We are seeking to develop cell markers which will provide single cell visualisation *in-vivo* using x-ray computed tomography. The markers and associated imaging technique should permit longitudinal studies on small animal models of both diseases and cell therapies.

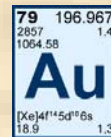
METHODS

Gold colloids have been used to produce probes in optical and electron microscopy for many years.

- Gold colloids are readily made in the laboratory.
- With care and purification a tight distribution of particle size and shape can be obtained (monodisperse).
- Proteins readily bond to their surface through electrostatic bonds.

Why is pure gold a good material to use as a cell marker?

- Gold metal nano-particles (GNPs) of the size used for marking are inert.
- Metallic gold particles of this size are non-toxic and biologically stable.
- Incorporating the GNPs within the cells has no measurable effect on cell proliferation.



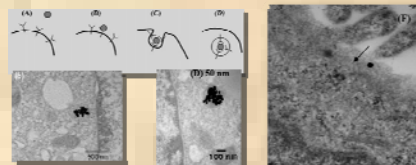
At x-ray energies of 24 keV the attenuation length of soft biological tissue is **24 mm**

The attenuation length of gold at the same energy is **11 microns** (>2000 times shorter)

⇒ even at large dilutions the gold may act as a good absorption marker.

Note: The gold K shell absorption edge is at **80.7 keV**. The L III edge is at **11.9 keV**. The K shell fluorescence photon energy is **68.8 keV**.

Cellular endocytosis of Gold Nano-Particles (GNPs)



Courtesy Warren Chan, University of Toronto, Institute of Biomaterials and Biomedical Engineering

Synchrotron x-ray CT

The medical physics beam line at the Elettra synchrotron (Trieste, Italy) was used for these preliminary studies. SYRMEP provides bright monochromatic beams of low divergence well suited for x-ray CT.

Elettra synchrotron, Trieste



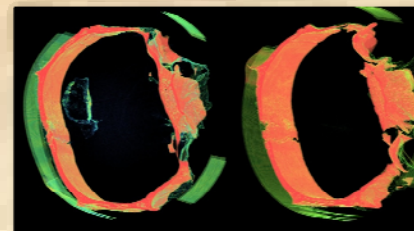
SYRMEP beam line



RESULTS

First images from synchrotron x-ray CT

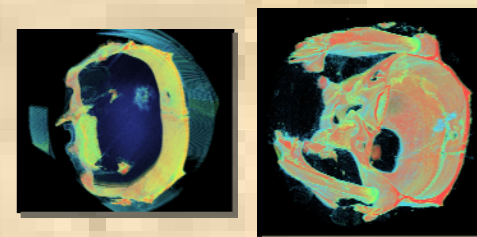
- Two rat brain images are compared below. In one the injected glioma cells were loaded with GNPs. The other was used as a control. The same number of glioma cells were injected, but they were unmarked.



Gold loaded glioma cells Unmarked glioma cells



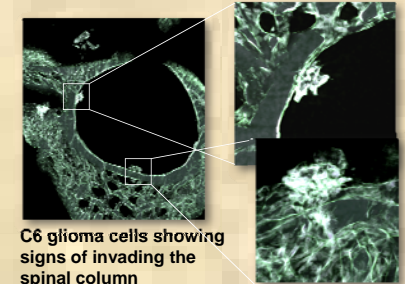
Comparison of a slice through the x-ray CT image with an hematoxylin and eosin (H&E) stained tissue section of the brain.



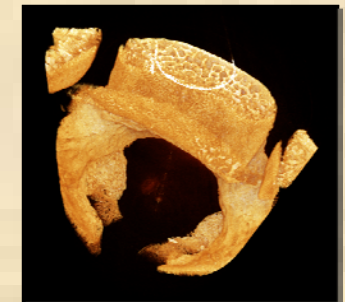
Two further synchrotron x-ray CT images of GNP marked lesions due to induced glioma in rat brains.

RESULTS

- Glioma cells were also injected into the centre of the spinal cord. The cells were shown to have migrated to the outside of the cord and even to have started invasion of the bone of the spinal column.



C6 glioma cells showing signs of invading the spinal column



A spinal column with marked cells was imaged on a laboratory micro CT system at La Trobe university. Although the marked cell contrast is not as strong. The lesion is readily visible.

With thanks to Dr. Rob Norman for collecting the data, and Prof Andrew Peele at La Trobe University for the use of the microCT facility.

CONCLUSIONS

- Gold nanoparticles of ~50 nm in diameter are readily incorporated into C6 glioma and olfactory ensheathing cells. (OECs).
- No detrimental effects of marking on the cells have been observed so far.
- The x-ray properties of the GNPs means that the position of the cells are visible in an x-ray micro CT image.
- Synchrotron monochromatic X-ray CT images provide the best contrast, but lab based micro CT works too.

For additional information please contact:

Chris Hall
School of Physics / Monash Centre for Synchrotron Science
Monash University, Clayton
Chris.hall@sync.monash.edu.au