Using Surface Plasmon Resonance Excitation to Determine Real-Time Distributions of Gold Nanoparticles in Live Cells

Contact: frederick.currell@manchester.ac.uk

A. McCulloch
School of Physics, Queen's University Belfast.
L. Bennie, J.A. Coulter
School of Pharmacy, Queen's University Belfast.
S.W. Botchway
STFC, Rutherford Appleton Laboratory.
F.J. Currell
Dalton Cumbrian Facility, University of Manchester

Introduction

Heavy atom nanoparticles are increasingly studied for their potential use in cancer therapy. When localised at a tumour site nanoparticles have been shown to increase the success of traditional radiotherapy due to their dose-enhancing properties[1]. As such nanoparticles provide a pathway to better patient outcome using equipment already in place at many medical facilities.

Of all the nanoparticles currently studied gold nanoparticles, AuNPs, are of specific interest due to their low toxicity and their ability to be functionalised easily. Functionalisation adds an extra dimension to the benefits of nanoparticle therapy; for instance the Au-DTDTPA nanoparticle also acts as a contrast agent[2] while modifying AuNPs with a compound such as RALA may yield nuclear-targeting capabilities[3].

Understanding the physically driven dose enhancement provided by AuNPs is crucial in order to progress toward patient trials. To determine this knowledge of the location of the nanoparticle is key, with AuNPs internalised in the cell nucleus for maximum enhancement being the ideal scenario. Previous studies[4] have shown the colocalization of AuNPs with nuclei in fixed cells. This study aims to build upon this work using live cells, treated with AuNP solutions and imaged in real-time in order to investigate the dynamics of association and uptake of AuNPs.

Technique

Fluorescence lifetime imaging microscopy (FLIM) is traditionally used to image fluorescently tagged biological samples; where the lifetime of the fluorescent decay is used to identify the material and the signal strength to infer the relative amount present.

The same system may be used to induce two-photon excitation of gold nanoparticles via surface plasmon resonance (SPR), thus creating a signature photon emission burst. Figure 1 shows how this appears for a single pixel containing both AuNP and fluorescing cellular material.

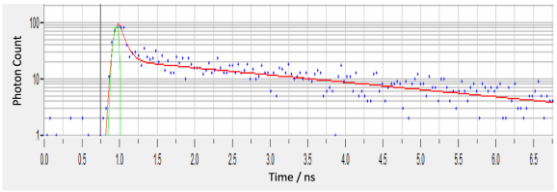

Figure 1: Typical photon decay curve obtained when performing FLIM in this study. The short sharp peak at early time points is characteristic of surface plasmon resonance from the AuNP and the long, slow tail off afterwards due to fluorescence from cellular material.

Analysis of the photon decay curve for every pixel in an image allows the location of AuNPs in a sample to be determined relative to stained cellular structures. In this study a 600nm, 0.6 mW laser pulse of length ~200 fs was used to probe the sample.

Live cell samples were imaged using this technique at 3 minute intervals after AuNP solution was applied. During photon collection the imaging plane is rocked through a small distance of $\pm$ 2 μm in the z-plane, sacrificing some z-resolution in order to avoid AuNPs moving in and out of the imaging plane. To minimise environmental stress on the live cells during the study steps were taken to maintain a suitable temperature and gaseous environment in the imaging chamber.

Results and Discussion

This study investigated the AuNP uptake of the human prostate cancer cell lines DU145 and PC3 with a variety of functionalised AuNPs. By combining all images taken in a data set movies were created showing, for the first time, the real-time behaviour of AuNPs upon

application to cells. Shown below in figures 2 and 3 are a selection of images from two such data sets.

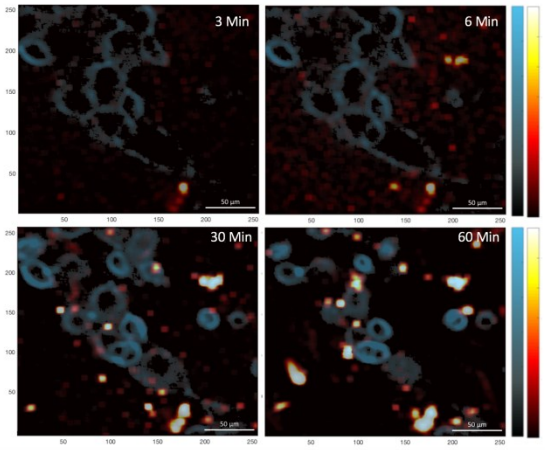

Figure 2: FLIM images of DU145 live cells with cell membrane stain (blue) 3, 6, 30 and 60 minutes after treatment with RALA-modified AuNPs (red-gold).

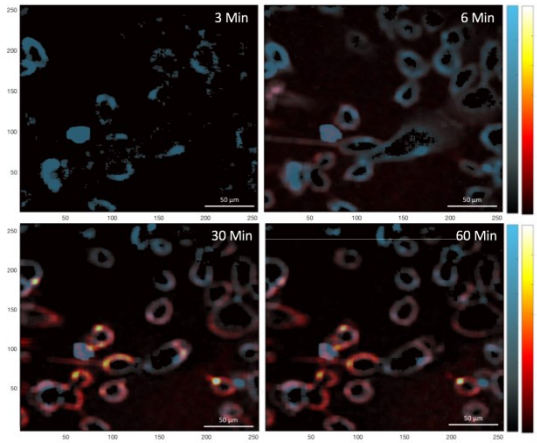

Figure 3: FLIM images of PC3 live cells with cell membrane stain (blue) 3, 6, 30 and 60 minutes after treatment with the Au-DTDTPA functionalised nanoparticle (red-gold).

From the figures above, and also from the additional data not shown here for brevity, it is clear that in both the cases of RALA and DTDTPA modified AuNPs associate with the cell membrane on a timescale of minutes. This association is particularly rapid and intense for the Au-DTDTPA nanoparticle preparation, with strong association happening in less than 30 minutes.

Imaging was continued for up to approximately 2 hours in each case after which the cell samples began to become stressed even with the countermeasures in place to avoid detrimental environmental pressures. This meant that whilst strong membrane association was observed neither data set unambiguously demonstrated evidence of cellular uptake over the imaging time period.

This observation however has led to the hypothesis that the cell membrane is being seen to act as a bottleneck during AuNPs journey inside the cell. Large numbers of nanoparticles build up on the outside of the cell membrane before being endocytosed in smaller numbers which may not necessarily be detectable using this technique. This is further reinforced by complementary fixed cell studies performed at much later time points which show AuNPs in large numbers inside the cell membrane as shown in figure 4.

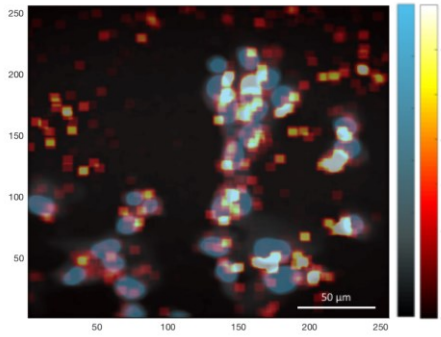

Figure 4: FLIM image of DU145 fixed cell sample 24 hours after treatment with RALA-modified AuNP solution.

Ongoing Work

Currently work is being undertaken to perform further quantitative analysis on these data sets. The aim is to provide a physical measure of the increase in membrane association in time for each formulation of nanoparticle.

In addition, as can be seen in figure 2 at later timepoints, the RALA-AuNP preparation used undergoes a certain degree of self-association upon application, possibly due to the non-optimum treatment environment. This observation has proved useful and has prompted further work into creating a more stable version of this preparation for further improved radiosensitisation.

As discussed one limitation of this technique is maintaining a suitable environment for the live cell samples and thus imaging at time points beyond 2-3 hours has proven difficult. Moving forward, investigating the uptake dynamics in the 3-24 hour time window would be highly valuable in probing the hypothesised bottleneck behaviour. One possible method to achieve this could be to allow for rest periods in the experiment during which the live cells may be allowed to return to their incubator to re-stabilise.

Conclusions

A new method for investing the uptake dynamics of a nanoparticle in live cell samples in real-time has been demonstrated. Cell membrane association has been shown for multiple formulations of functionalised gold nanoparticle as can be seen above. Further refinements to this technique allowing imaging to further time points have also been suggested.

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References

1. Hainfeld, J. F., Slatkin, D. N. & Smilowitz, H. M. The use of gold nanoparticles to enhance radiotherapy in mice. *Phys. Med. Biol.* **49,** N309–N315 (2004).
2. Butterworth, K. T. *et al.* Preclinical evaluation of gold-DTDTPA nanoparticles as theranostic agents in prostate cancer radiotherapy. *Nanomedicine* **11,** 2035–2047 (2016).
3. Mccarthy, H. O. *et al.* Development and characterization of self-assembling nanoparticles using a bio-inspired amphipathic peptide for gene delivery. *J. Control. Release* **189,** 141–149 (2014).
4. McQuaid, H. N. *et al.* Imaging and radiation effects of gold nanoparticles in tumour cells. *Sci. Rep.* **6,** 19442 (2016).