Investigation of manganese as a radioprotective agent in human cancers

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Certificate of Authorship

I, Thomas Edward Lockwood, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy: Science, in the Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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List of Publications

- Lockwood, T. E.; Westerhausen, M. T.; González de Vega, R.; Röhnelt, A.; Bishop, D. P.; Cole, N.; Doble, P. A.; Clases, D. Low background mould-prepared gelatine standards for reproducible quantification in elemental bio-imaging. *The Analyst* **2019**, *144*, 6881–6888.
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- González de Vega, R.; Lockwood, T. E.; Xu, X.; Gonzalez-deVega, C.; Scholz, J.; Horstmann, M.; Doble, P.; Clases, D. Analysis of Ti and Pb-based Particles in the Aqueous Environ-ment of Melbourne (Australia) via single particle ICP-MS. *Analytical and Bioanalytical Chemistry* **2022**, submitted for publication.

Abstract

Manganese complexes are effective and catalytic scavenges of $O_2^{\bullet -}$, mimicking the behaviour of superoxide dismutase (SOD). These complexes even replace typical SOD in certain extremophile bacteria, providing resistance to huge doses of radiation. Recently, manganese concentrations have been shown to correlate with inferred radiotherapy resistance in human tumours. Radiation plays an important role in the treatment of cancer, but there is still no reliable way to predict an individual tumour's response to therapy. Imaging of manganese in tumours using laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) could provide a novel way of predicting radioresistance and informing treatments, but currently implemented methods have some significant shortcomings. Measurement of manganese using ICP–MS is difficult due to the low concentrations in tissues and high background from polyatomic interferences. Existing LA–ICP–MS image processing software usually falls into one or more of the following categories: hard to use, limited in compatibility or unverifiable due to a closed-source nature. This thesis addresses these shortcomings by developing low-background standards and open-source software for both single cell (sc) and LA–ICP–MS. These new tools are then used to interrogate the metal contents of skin, testis, pancreas and brain tumours, and compare them to the inferred radioresistance of the cancers. Radioresistance is also investigated in a breast cancer cell model using ultraviolet (UV) radiation as a gamma surrogate.

Moulds were used to reproducibly prepare gelatine standards with controllable thickness, and surface characteristics that were improved over traditional methods. To prepare the standards: heated gelatine was pipetted into laboratory-made and commercial moulds, dried, frozen, de-moulded and dehydrated. When compared with traditional gelatine and homogenised tissue standards, the new standards had improved analytical figures of merit. Background signals for transition metals were reduced by removing endogenous elements from the gelatine using a chelating resin.

Open-source LA–ICP–MS imaging software was created using modern design and visualisation philosophies, minimising errors in data interpretation. The new software was compatible with data

collected using ICP–MS from common vendors, and implemented calibration, drift correction, spike filtering, segmentation, overlays and an image calculator. Documentation and a simple graphical user interface eases adoption, contributes to ease of use and maximises productivity.

Transition metals were quantified in tissue microarrays of testis, skin and pancreatic cancer. Concentrations of Mn, Fe, Cu and Zn all correlated with the inferred resistance of the tumours. These correlations also existed in normal testis, skin and pancreatic tissues. In testis tumours with different inferred radiosensitivities, seminoma and nonseminomatous germ cell tumours, only Cu correlated with resistance.

Concentrations of metals were quantified in glioblastoma and meningioma, brain tumours with vastly different patient outcomes. Glioblastoma contained significantly more Cu than benign (grade I) meningioma, but similar concentrations of other transition metals. Previous studies have noted raised serum Cu in patients with glioblastoma and this could be linked to tumour angiogenesis. Concentrations of Mn were increased in grade II over grade I meningioma, but did not reach significance.

To aid in the investigation of single-cellular Mn concentrations software was developed for single particle (sp) and scICP–MS analysis, with multiple methods of thresholding and calibration. The code is open-source, highly vectorised and could process 10×10^6 points in 19 ms. The software was demonstrated using the analysis of TiO_2 nano particles in water, LA–spICP–MS of micro-plastics in soil and the measurement of C fixation in algae.

The radioprotective properties of inorganic Mn^{2+} were investigated using UV radiation and a breast cancer cell model. Toxicity of $MnCl_2$ was determined, with an median lethal dose of 1.2 mmol L^{-1} in 1 % fetal calf serum (FCS), an order of magnitude lower than in 10 % FCS. Cells were treated with 10 and 100 µmol L^{-1} $MnCl_2$ for 24 h before exposure to 0 to 400 J m^{-2} UV-C radiation. Similar viabilities were observed across the range of treatments; Mn had no protective effects. Uptake of Mn from the media was determined using acid digestion and scICP–MS with similar results, although low signal prevent accurate quantification via scICP–MS in untreated cells. Multi-modal distributions of Mn was observed in treated cells, information that was lost when digestion was performed.

The tools created during this thesis will assist in future investigations of manganese and radioresistance. The primary limitation of imaging Mn via LA–ICP–MS, standards with insufficient limits of detection, has been solved and the new software will accelerate LA and scICP–MS analyses. Further experiments with large sample sizes and radiotherapy patient outcomes are required to definitively conclude if manganese complexes contribute to tumour radioresistance.

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Abbreviations

ANOVA analysis of variance

BDD boron-doped diamond

CD133 prominin-1

CD30 tumour necrosis factor receptor superfamily member 8

CNS central nervous system

CRM certified reference material

CSC cancer stem cell

DAB 3,3'-diaminobenzidine

DMEM Dulbecco's Modified Eagle Medium

DNA deoxyribonucleic acid

DSB double-strand break

FCS fetal calf serum

FFPE formalin-fixed paraffin-embedded

FITC fluoresein isothiocyanate

GUI graphical user interface

H&E haematoxylin and eosin

HIF1 hypoxia inducible-factor 1

HPF high-power field

HRP horseradish peroxidase

ICP inductively coupled plasma

IHC immunohistochemistry

LA laser ablation

LD_{50} median lethal dose

LED light-emitting diode

LOD limit of detection

MRN MRE11-RAD50-NBS1

MS mass spectrometry

NBT nitro blue tetrazolium

NHEJ non-homologous end joining

NP nano particle

NSGCT nonseminomatous germ cell tumour

PAM polyacrylamide

PBS phosphate buffered saline

PFS progression free survival

PMMA poly(methyl methacrylate)

PNC particle number concentration

PTFE poly(tetrafluoroethylene)

RGB red-green-blue

ROI region of interest

ROS reactive oxygen species

RSD relative standard deviation

sc single cell

SEC size-exclusion chromatography

SIMS secondary ion mass spectrometry

SOD superoxide dismutase

sp single particle

TBS tris-buffered saline

TMA tissue microarray

TOF time-of-flight

UV ultraviolet

XOD xanthine oxidase