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Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023042

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Experiment title	PROBING METALLODRUG ACTION ON HUMAN CANCER CELLS BY QSM
MRF Instrument	**QSM - Quantum Scanning Microscope**
Access Route	Direct Access
Science Areas	Biology and Bio-materials, Chemistry, Medicine
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 2
Previous GP Number: no
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

Cancer is a leading cause of death worldwide, with 19.3 million new cases in 2020 from which more than 50% die from the disease. Triple-negative breast cancer (TNBC) represents 15-20% of all breast cancers. Osteosarcoma, in turn, is the most frequent primary sarcoma with a higher incidence for 10 to 16 year-olds. Cisplatin (cis-Pt(NH3)4Cl2) was the first inorganic drug introduced to the clinics, mainly against solid tumours. However, its clinical application is still restricted by dose-limiting deleterious side effects and acquired resistance, as well as by a lack of specificity against several cancer types. Improved chemotherapeutic approaches against TNBC and osteosarcoma are therefore an urgent clinical need, targeting malignant cells with minimal damage to healthy tissues. Quantum scanning microscopy is a cutting-edge technique which allows to attain detailed topographic images of even heterogeneous samples, at the sub-cellular level, with high sensitivity and imaging speed. The main goal of this study is to obtain high spatial resolution images of human cancer cells upon drug administration, allowing to attain an accurate description of the drug impact on cellular morphology and biochemistry.

Publications

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IM@IT E-platform: No

Instruments	**Days Requested:**
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Sample record sheet

Principal contact Professor Maria Paula Marques, University of Coimbra - Molecular Physical-Chemistry R&D U
MRF Instrument **QSM - Quantum Scanning Microscope** **Days Requested:** 2
Special requirements:

SAMPLE

Material	human breast cancer cells with and without drug	human osteosarcoma cells with and without drug	DNA human cancer cells with and without drug
Formula	cis-Pt(NH3)2Cl2, Pd3Spd2Cl6	cis-Pt(NH3)2Cl2, Pd3Spd2Cl6	cis-Pt(NH3)2Cl2, Pd3Spd2Cl6
Forms	Solid	Solid	Solid
Volume	100 ml	100 ml	100 ml
Weight	100 mg	100 mg	100 mg
Container or substrate	non-coated glass windows (Crystran, 1x13 mm)	non-coated glass windows (Crystran, 1x13 mm)	non-coated glass windows (Crystran, 1x13 mm)
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	room temperature - K	room temperature - K	room temperature - K
Pressure Range	atmospheric pressure - mbar	atmospheric pressure - mbar	atmospheric pressure - mbar
Magnetic field range	up to 50mT - T	up to 50mT - T	up to 50mT -
Standard equipment	None	None	None
Special equipment	No	No	No

SAFETY

Prep lab needed	Yes	Yes	Yes
Sample Prep Hazards	No	No	No
Special equip. reqs	No	No	No
Sensitivity to air	No	No	No
Sensitivity to vapour	No	No	No
Experiment Hazards	No	No	No
Equipment Hazards	-	-	-
Biological hazards	cytostatic	cytostatic	No
Radioactive Hazards	No	No	No
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	Removed By User	Removed By User

PROBING METALLODRUG ACTION ON HUMAN CANCER CELLS BY QSM

Scientific context and expected results

Cancer is a leading cause of death worldwide, with 19.3 million new cases in 2020, from which more than 50% die from the disease [1]. Triple-negative breast cancer (TNBC) represents 15-20% of all breast cancers, being more prevalent in premenopausal young women [2]. Due to its aggressiveness and metastatic potential, it has a high risk of recurrence leading to a substantial mortality in the first 5 years after diagnosis. The overall survival is 13.3 months, and less than 30% of the cases survive longer than 5 years [3]. Osteosarcoma, in turn, is the most frequent primary sarcoma, with most cases developing between the ages of 10–16 years [4,5]. Cisplatin (*cis*-dichlorodiamine platinum(II), *cis*-$Pt(NH_3)_2Cl_2$) was the first inorganic drug introduced to the clinics, effective mainly against solid tumours [6,7]. However, its clinical application is still restricted by dose-limiting deleterious side effects and acquired resistance, as well as by a lack of specificity against several cancer types (*e.g.* metastatic) [8]. Therefore, improved chemotherapeutic approaches against TNBC and osteosarcoma are an urgent clinical need, targeting malignant cells with minimal damage to healthy tissues. Numerous cytostatic drugs have been developed over the years, namely Pt- and Pd-based agents introduced upon the discovery of cisplatin [8-14]. In particular, Pt(II) and Pd(II) polynuclear chelates with polyamines constitute a specific class of DNA-damaging agents that have been found to lead to an enhanced therapeutic effect, through interactions with DNA (and other targets such as intracellular water) not available to conventional Pt-drugs [9-11]. The activity of this type of polynuclear complexes was found to be mediated by selective covalent binding of the metal centres to DNA bases (mainly the purines at their most nucleophilic nitrogen atom, N7), yielding long-range intra- and interstrand adducts responsible for cell growth arrest and apoptotic death. Extensive studies have been performed by the team on this specific class of DNA-targeting agents [15-21] (Figure 1), particularly on a trinuclear Pd(II) chelate with spermidine ($Pd_3Spd_2Cl_6$, $Spd=H_2N(CH_2)_4NH(CH_2)_3NH_2$) which has shown promising antineoplastic effect towards TNBC and osteosarcoma cells (ongoing studies).

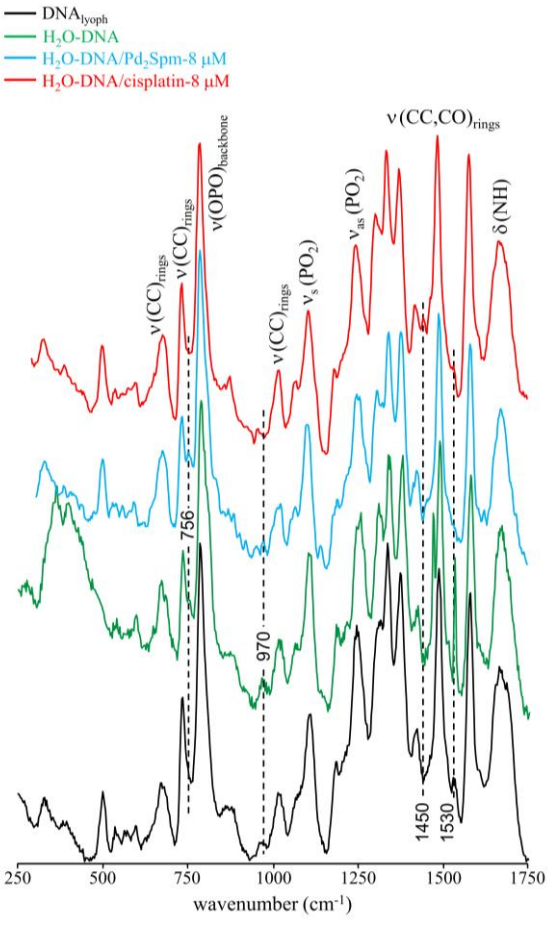

Figure 1 - Raman spectra (250 - 1750 cm^{-1}) of DNA_{lyoph}, H_2O-DNA_{hyd}, untreated and upon incubation (for 48 h) with cisplatin-8 µM or Pd_2Spm-8 µM [19].

The development of improved pharmacological agents requires information at the cellular and subcellular levels – evaluation of *in vitro* biodistribution and assessment of metabolic impact being key for a successful rational drug design. The main goal of this study is therefore to obtain **high spatial resolution images** of human cancer cells, upon drug administration, allowing to attain an accurate **description of the drug impact on cellular biochemistry/metabolic profile**, as well as the cellular response to treatment.

Imaging a biological sample through quantum optics has remained a challenge, mainly due to the intrinsically weak signal measured and the fragility of the quantum states of light. Quantum scanning microscopy (QSM) is a cutting-edge technique which allows to attain detailed topographic images of even heterogeneous samples such as eukaryotic cells, at the sub-cellular level, with high sensitivity and imaging speed [22, 23]. As a next-generation scanning probe magnetometer based on diamond technology, it allows to detect small changes in local magnetic field with high spatial resolution of a few tens of nm. A nitrogen-vacancy (NV) centre in the diamond lattice is used to detect magnetic field variations that can often also be reflected in a change of the fluorescence from the NV.

The QSM technique is an ideal tool to attain this goal, and unique in the QZabre-LLC, Zurich, Switzerland, offering this capability.

The proposed experiment has a twofold purpose:

A) to obtain images of cellular response to the tested anticancer agents, and assign this response to individual chemical components – biomarkers of drug action.

B) to assess the drugs' distribution within the cell, from the metabolic changes detected at the subcellular level, upon drug exposure.

Both goals will be attained concurrently since the topographic images are expected to yield accurate information on the **drug's bioavailability** and **cytotoxic profile** *via* their **effect on the cellular biochemical fingerprint**. The results thus gathered, will allow to achieve a reliable information on the impact of the potential anticancer compound $Pd_3Spd_2Cl_6$ on MDA-MB-231 and MG-63 cells, as well as on DNA, these results being compared to the effects observed for cisplatin.

Experiment outline

The polynuclear $Pd_3Spd_2Cl_6$ chelate (prepared and fully characterised by the team) will be probed against TNBC and osteosarcoma cells, as well as cisplatin (as a reference, mononuclear, drug). Apart from the cells, DNA extracted from either of them (TNBC or osteosarcoma, respectively MDA-MB-231 and MG-63 cell lines) will be monitored upon drug exposure, as target models (commercial DNA being probed as a reference). QSM images will be acquired with various modes of operation: using an AFM mode the target area of interest will be identified and approached. Later, quench mode fluorescence spectroscopy as well as NV magnetometry will be used to try and discriminate drug-free against drug-exposed samples. Differences in magnetic response or fluorescence emission between both types of samples are sought, and will be correlated to drug impact.

The samples will be prepared by the users (at Coimbra University): (i) cells (with and without drug) will be cultured directly on non-coated glass windows (Crystran, 1x13 mm) and formalin-fixed ($5x10^4$ cells/ml); (ii) DNA will be extracted from both types of cancer cells following a procedure previously optimised by the applicants [12]. Either $Pd_3Spd_2Cl_6$ or cisplatin, will be added *in vitro* to either MDA-MB-231 or MG-63 cells, for 48/72 h, at concentrations within the corresponding IC_{50} values (4-14 μM, for MDA-MB-231 and 12-14 μM, for MG-63).

QSM data (single cell imaging *per* acquisition) will be obtained for the DNA and cell samples, at the QZabre-LLC, Zurich, Switzerland, using a Laser Scanning Confocal Microscope Leica TCS SP8 with DMI8 microscope and FCS Picoquant module with PMT detector for transmission imaging.

2 days are requested (including optimisation of acquisition settings), to measure:

- [MDA-MB-231] - 2 samples (control) x 3 cells *per* sample
- [DNA/MDA-MB-231] - 2 samples (control) x 3 cells *per* sample
- [MDA-MB-231+Pd_3Spd_2] - 2 concentrations – 6 samples x 3 cells *per* sample
- [DNA/MDA-MB-231+ Pd_3Spd_2] - 2 concentrations – 6 samples x 2 cells *per* sample
- [MDA-MB-231+cisplatin], 2 concentrations – 6 samples x 3 cells *per* sample
- [DNA/MDA-MB-231+cisplatin] - 2 concentrations – 6 samples x 3 cells *per* sample
- [MG-63] - 2 samples (control) x 3 cells *per* sample
- [DNA/MG-63] - 2 samples (control) x 3 cells *per* sample
- [MG-63+ Pd_3Spd_2] - 2 concentrations – 6 samples x 3 cells *per* sample
- [DNA/MG-63+ Pd_3Spd_2] - 2 concentrations – 6 samples x 3 cells *per* sample
- [MG-63+cisplatin] - 2 concentrations – 6 samples x 3 cells *per* sample
- [DNA/MG-63+cisplatin] - 2 concentrations – 6 samples x 3 cells *per* sample

References

[1] S.V.S. Deo *et al. Ann. Surg. Oncol.* 29 (2022) 6497. [2] Y. Li *et al. Breast Cancer Res.* 22 (2020) 1. [3] M.B. Zerdan *et al. Cancers* 14 (2022) 1253. [4] A. Abarrategi, *et al. Stem Cells Int.* 2016 (2016). [5] H.C. Beird *et al. Nat. Rev. Dis. Primers* 8 (2022) 77. [6] B. Rosenberg *et al. Nature* 205 (1965) 698. [7] B. Rosenberg *et al. Nature* 222 (1969) 385. [8] J. Reedijk, *Proc. Natl. Acad. Sci. USA* 100 (2003) 3611. [9] M.P.M. Marques *ISRN Spectroscopy* 2013 (2013) 1. [10] N. Farrell *Chem. Soc. Rev.* 44 (2015) 8773. [11] M.N. Alam *et al. Coord. Chem. Rev.* 316 (2016) 36. [12] A.L.M. Batista de Carvalho *et al. PCCP* 21 (2019) 4162. [13] M. Vojtek *et al. Drug Discov. Today* 24 (2019) 1044. [14] M.P.M. Marques *et al. Int.Rev.Phys.Chem.* 39 (2020) 67. [15] S.M. Fiuza *et al. Chem.Biol.&Drug Design* 77 (2011) 477. [16] M.P.M. Marques *et al. PCCP* 17 (2015) 5155. [17] A.L.M. Batista de Carvalho *et al. PLoS One* 11 (2016) e0167218. [18] A.L.M. Batista de Carvalho *et al. Faraday Disc.* 187 (2016) 273. [19] M.P.M. Marques *et al. Molecules* 25 (2020) 246. [20] T.J. Carneiro *et al. Pharmaceutics* 14 (2022) 259. [21] R.C. Laginha *et al. Int.J.Mol.Sci.* 24 (2023) 1888. [22] R. Tenne *et al. Nature Photonics* 13 (2019) 116. [23] C.A. Casacio *et al. Nature* 594 (2021) 201.