Exploiting 3D in vitro models to advance diffusion magnetic resonance imaging (MRI) as a probe of cellular and tumour microstructure

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RESEARCH SUMMARY:

There is an unmet clinical need for early detection and characterisation of tumours, residual disease and metastases using non-invasive imaging methods.

Diffusion magnetic resonance imaging (MRI) exploits the motion of water molecules to sensitise the MRI signal to sub-voxel cellular and microstructural properties [Norris 2001, Taouli 2016]. Diffusion MRI is an essential component of clinical MRI in oncology for lesion detection and response assessment including quantitative analysis via the apparent diffusion coefficient (ADC) [Winfield 2019a]. ADC relates to tumour cell density but is also influenced by other factors including the extracellular matrix (ECM) and necrosis, leading to a lack of specificity [Winfield 2019b, Winfield 2021, Reeves 2023]. The lack of specificity impairs the ability of diffusion MRI to distinguish, for example, cellular tumour from fibrosis in stromal dense tumours [Muraoka 2008] or detect tumour infiltrating immune cells in some tumour types [Surov 2023]. Clinical studies to elucidate these factors are limited by complexity of the tumour micro-environment, practical constraints on in vivo measurements, spatial heterogeneity, and often lack a ground-truth. There is a clinical need for more specific diffusion MRI measurements that can inform on cellular/microstructural properties such as cell sizes, cell density, membrane integrity, properties of the ECM and infiltration by tumour cells or immune cells [McLaughlin 2020]. More advanced models of diffusion MRI offer scope to probe these cellular/microstructural properties [Mitra 1995, Fieremans 2010, Panagiotaki 2014, Jiang 2017]. By employing a ‘bottom up’ approach using controlled systems and a ‘ground-truth’ from digital pathology we will determine which models are the most suitable for oncological applications.

The aims of this project are to develop experimental diffusion MR methods to probe cellular/ microstructural properties using controlled systems (cell suspensions, cell pellets, spheroids, organoids) alongside digital pathology. The project will follow an iterative process of biological development, MR experiment and analysis, pathology and modelling in systems of increasing complexity. The study will test the hypothesis that advanced diffusion MR can non-invasively determine cellular/microstructural properties such as cell sizes, cell density, membrane integrity, ECM properties and infiltration.

SPECIFIC AIMS:

- Develop experimental diffusion MRI methods in cell suspensions using samples that recapitulate relevant cellular/microstructural properties.
- Evaluate models of the diffusion MRI measurements in cell suspensions and evaluate modelled values against digital pathology measurements.
- Use high-resolution diffusion MRI of cell pellets, spheroids and organoids to evaluate diffusion MRI models against digital pathology measurements.
- Select appropriate diffusion MRI models for clinical applications by simulation of motion of water molecules in samples (cell suspensions, cell pellets, spheroids, organoids) using digital pathology images and evaluate against experimental results.

The team has expertise on MR systems at a wide range of field strengths (1.5T-11.7T) with clinical and pre-clinical measurement capabilities, enabling complementary methods and enhancing translation. The team has expertise in using a variety of cell lines, which are available for the project. Immortalised cell lines and patient-derived organoids representing ovarian high-grade serous cancer will be used as an example cancer system as it is a cancer of unmet need, which displays complex spatial heterogeneity with peritoneal dissemination in advance stages with ECM remodelling and variable immune invasion of tumours [Ploski 2021, Clark 2022, Cunnea 2023].

The project is divided into three work-packages (WP):

<u>WP1. Development of diffusion MRI methods to evaluate cellular/microstructural properties in cell suspensions.</u>

We will produce suspensions of immortalised cell lines in gels suitable for diffusion MRI experiments. Sample preparation will be based on previously reported methods [Lundberg 1994, Katashima 2013] and extended to evaluate cellular/microstructural properties and treatment effects. Samples will recapitulate relevant cellular/microstructural properties to evaluate the influence of cell sizes, cell density, membrane integrity and ECM on diffusion MRI. Physical (heat, ultrasound) and chemical treatments will be used to alter cell membrane integrity. Other agents (collagen, fibronectin, other ECM proteins) will be added to alter ECM composition and structure. Tumour cell lines with altered expression of factors implicated in the ECM will be compared to parental cell lines. Digital pathology will provide ‘ground-truth’ readouts of cell sizes, cell density and structure/properties of the extracellular space. Diffusion MRI measurements will use a clinical high-field (3T) MRI scanner at RMH to estimate ADC and time-dependent diffusion measurements using short and long diffusion times. Measurements will be extended using ultra-high-field NMR using diffusion-ordered spectra at variable diffusion times and echo times to probe diffusion coefficients and relaxation times of water molecules in different compartments. Models will be fitted to the diffusion MRI measurements and modelled values (e.g. cell density) evaluated against digital pathology measurements.

Outcome: Experimental assessment of diffusion model parameters in cell suspensions and evaluation of models compared with ground-truth digital pathology measurements.

WP2. High-resolution diffusion MRI in cell pellets, spheroids, and organoids.

Samples will be constructed with increasing complexity (cell pellets/spheroids/organoids) to extend methods from WP1 into more clinically-realistic systems. Samples will be grown and embedded in gels or ECM materials for diffusion MRI using methods developed at ICR to enable imaging of single cell pellets/spheroids/organoids. Ultra-high field MRI systems will be used to enable high spatial resolution required (7T pre-clinical MRI scanner at ICR; pan-London 7T clinical MRI scanner where ICR is a partner). Diffusion MRI will be used to estimate ADC and time-dependent diffusion measurements using short and long diffusion times. Initially cell pellets and spheroids grown from single immortalised cell lines will be investigated and extended to organoids and co-cultures of spheroids or organoids with normal and cancer associated fibroblasts/stromal cells and/or immune cells to create more complex/realistic samples. Drug treatments for ovarian cancer will be performed with standard-of-care chemotherapies (carboplatin, paclitaxel) or targeted therapies (PARP inhibitors). Radiotherapy treatments will be administered using a cell irradiator. Samples will be embedded and sectioned for digital pathology analysis aligned with the MR imaging plane to assess cell sizes, cell density and identify other cells/materials present (e.g. fibroblasts, collagen, endothelial cells) and responses to treatment.

Outcome: Experimental assessment of ADC estimates and time-dependent diffusion measurements in cell pellets, spheroids, and organoids, and comparison with ground-truth measurements from digital pathology.

WP3. Simulating motion of water molecules using digital pathology images and comparison with experimental results.

Digital pathology images from WP1-2, including annotations identifying cells and other materials, will be used to simulate motion of water molecules in biological structures, including intra/extracellular contributions, exchange between intra/extracellular compartments and ECM properties. Cell suspensions will be simulated initially and extended to more complicated structures (cell pellets/spheroids/organoids). Simulations will investigate the influence of cell sizes, cell densities and membrane permeability on diffusion MRI properties, and results will be evaluated against experimental results from WP1-2.

Outcome: Selection of best diffusion model following evaluation of simulated and experimental diffusion coefficients in biological structures.

WP1 will be conducted in Year 1, providing a foundation in biological methods (growth of cell lines, production of cell suspensions) and MR methods (acquisition, analysis) and will build foundations in digital pathology.

WP2 will be conducted in Years 2 and 3. The student will develop more advanced tissue culture methods using spheroids and organoids and will develop more challenging MR acquisition methods for diffusion MRI of individual spheroids and organoids.

WP3 will be conducted in Year 3 and part of Year 4. The student will develop a deeper understanding of diffusion MRI models via modelling using digital pathology images.

The student will be based at ICR/RMH (Sutton), where MRI experiments will be undertaken. The student will spend time in Dr Cunnea/Professor Fotopoulou's lab at ICL throughout their studentship.

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PERSON SPECIFICATION:

This project is suitable for a talented graduate or undergraduate student with physics, engineering, chemistry, or a related background. The standard minimum entry requirement is a relevant undergraduate honours degree (First or 2:1). We particularly welcome British applicants from Black and ethnic minority backgrounds, as they are underrepresented at PhD level within Imperial College and The Institute of Cancer Research.

The studentship will be registered at the Institute of Cancer Research with affiliate status at Imperial College London. The student will have access to both institutions and benefit from the world class research infrastructure and expertise across the two institutions. The student will become a member of the CRUK Convergence Science Centre PhD cohort which is a

unique group of students working across distinct disciplines to tackle the big problems in cancer. A unique convergence science training programme will provide the skills and language to navigate different disciplines.

FUNDING AND DURATION:

Studentships will be for four years commencing in October 2024. Successful candidates will undertake a four-year research training programme under the guidance of the supervisory team. Students will receive an annual stipend, currently £23,000 per annum and project costs paid for the four-year duration. Convergence Science PhDs cover tuition fees for UK students only. Funding for overseas fees is not provided, international students are invited to apply subject to outlining how they will meet the difference in tuition fees.