WESTERN SYDNEY
UNIVERSITY

School of Science

Summer Scholarship Research Program 2020

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Project 94: Recycled water and soil interactions: supporting a cool green city through regenerative agriculture, high quality recreational areas, and a healthy blue green grid

Supervisor(s): Jason Reynolds - j.reynolds@westernsydney.edu.au
Principal Supervisor

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Second Supervisor

Project description

This proposal seeks to investigate the potential for improved blue and green space management using recycled water in the rapidly urbanising Western Sydney region. An investigation into different soil landscapes and their interaction with recycled water will provide information on how soils behave in response to the use of recycled water. This information can then be used when planning the application of recycled water to new locations. We anticipate that this work will lead to further collaboration with Sydney Water and additional research funding, as well as provide a great opportunity for the student to network with an important industry partner.

Project Aims

The urbanisation of western Sydney presents challenges to how communities will source food and interact with the environment on catchment-level scales. With climate modelling indicating a warmer and drier western Sydney region, the application of recycled water to land areas may be of benefit to blue and green landscape management, agriculture, and horticulture. Field and lab-based investigation of recycled water and soil interactions are required to ensure future land management is appropriate for maximising nutrient capture in soil and minimising losses due to leaching, runoff and greenhouse gas. This project will generate impact through benefits to landscape productivity and resource sustainability within the western Parkland City.

Specifically, the student will undertake:

1. Soil sampling and classification across a range of sites in Western Sydney
2. Conduct chemical and microbiological analysis of soils
3. Apply treated recycled water to the soils and investigate the chemical and biological changes.

Project Methods

This proposal will investigate the four locations in to enable comparisons and it is anticipated that the soil sampling program will take up to three days at each site as soil profiles are described and soil samples collected.

This proposal includes surface soil sampling intervals of three composites samples per hectare and soil cores for lysimeter work collected at three per site. Recycled water samples will be collected from irrigation discharge points. Bulk water samples (for lysimeters) will be collected every second week over the 60-day experimental period at an approximate volume of 100L per site.

2.1 Field sampling and analysis of soils and waters

Soils and recycled water: understanding existing sites

- Soil taxonomy: Classification across sites with profile samples collected and tested at WSU for pH, texture, colour, biodiversity, bulk density, physical properties (including available water capacity), and potential contaminants.
- Nutrient and carbon accrual: Using data from soil taxonomy and analytical work soil carbon partitions and soil nutrient levels will be quantified. This will be used to illustrate soil nutrient and carbon increases in response to irrigation and the potential for soils to increase nutrient levels. Nutrient testing will include, phosphorous, nitrogen, and carbon along with a broad elemental analysis. Contaminant analysis will include
- Environmental microbiome: Identification of dominant microbial species and investigate differences between irrigated and non-irrigated locations. Traditional microbiological techniques only isolate and culture a small proportion of the microorganisms in soil samples which provides incomplete information about total soil biodiversity with cultivation-independent metagenomic approaches proposed in this work, the analysis of soil microbial communities makes it possible to capture the genomic information of even low-abundance populations and to reveal the multiple activities in soil. It is a useful tool to help us understand the soil microbiome and can provide information on the biogeochemical interactions of the soil-recycled water-plant system in relation to nutrient and contaminant cycling.

2.2 Greenhouse based lysimeter studies

Recycled water and soil cores: manipulating inputs and outputs

- Soil cores preparation: Intact soil cores will be collected at dimensions of 0.2m wide and 0.3m deep which incorporates the active root zone, majority of soil carbon storage, and majority of the soil biodiversity. These cores will be held in PVC pipes in upright positions and maintained under greenhouse conditions.
- Recycled water application: Interactions of recycled water with soil cores collected from the site will be explored by adding set volumes of recycled water at set intervals. Collection trays underneath the soil cores will collect drainage water. This work will be undertaken at WSU greenhouse facilities.
- Lysimeter soil and water: The soil lysimeters, drainage water, and input water will be analysed to understand physical, chemical and biological changes. Testing will include pH/EC/DO/pe, nutrients (N,P), DOC, DIC, alkalinity, elemental suite (ICP-MS), antibiotic (e.g. ciprofloxacin), medicinal (e.g. pseudoephedrine), illicit (e.g. cocaine and benzoylecgonine), and emerging contaminants (e.g. PFAS suite).

Opportunity for Skill Development

Skill sets in soil classification and site assessment are highly valued in the job sector at present. These skills will be provided throughout this project to the student. This coupled with advanced analytical techniques in soil chemistry and soil microbiology provide a leading edge to any student transitioning to paid work opportunities or looking to branch into higher degree research.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Students need to have completed undergraduate level studies in first year chemistry and biology. Knowledge and/or experience in soils is highly valued.

Project 95: Platypus Dinner: The Macroinvertebrates of Cattai and Little Cattai Creek Catchments

Supervisor(s): Michelle Ryan - m.ryan@westernsydney.edu.au
Principal Supervisor

Ian Wright - i.wright@westernsydney.edu.au
Second Supervisor

Project description

In June 2020 Environmental DNA (eDNA) samples were collected at 18 sites in the Cattai and Little Cattai Creek catchments, north west Sydney. These eDNA samples were analysed to determine if platypus were present at these locations. Eight out of the 18 sites had platypus DNA present. While a short habitat and water quality assessment was carried out at the time there is a lack of habitat and water quality data to suggest why platypus are using some creeks in the catchment over others.

This summer project will look at “platypus food”, macroinvertebrates (commonly known as water bugs), to help piece together why platypus are present and some sites in the catchment and not others. Macroinvertebrates make up the majority of platypus diet, but they are also useful bioindicators of long term creek health. As there are low numbers of platypus in the Sydney Basin it is important to use bioindicators to understand the current state of the ecosystems in which the platypus reside.

This project will see the student collect, identify and analyse the abundance and diversity of macroinvertebrates to help understand the distribution of platypus in the Cattai and Little Cattai Catchment. This project will contribute data to a larger project that is looking to determine the current status and population health of platypus in the Sydney Basin.

Project Aims

The aim of this project is to:

- Determine the difference in macroinvertebrate assemblages and diversity at sites where platypuses are present and sites where platypus are not present.
- Evaluate the health of a number of creeks in the Cattai and Little Cattai catchments using macroinvertebrates as bioindicators.
- Develop skills in identification of aquatic macroinvertebrates
- Develop skill in multivariate data analysis

Project Methods

Eighteen Sites in the Cattai and Little Cattai catchment have already been established as sample sites and the student will revisit these sites and complete the following for data collection and analysis.

At each site a number of water quality parameters, such as turbidity, pH, temperature, nitrates, phosphates, electrical conductivity and dissolved oxygen will be measured and recorded using field meters. On site, macroinvertebrates will be sampled using the methods outlined in the New South Wales Australia River Assessment System which is a widly accepted method of macroinvertebrate collection in NSW (Davies et al. 2009, Wright & Ryan 2016). Samples will be live picked in the field and preserved in ethanol where they will be transported to the laboratory to

be identified. Macroinvertebrates will be identified using published keys (e.g. Gooderham 2002) to family level (Wright & Ryan 2016).

Macroinvertebrate biotic indices (a number that allows for the raw data to be simplified to show different habitat preferences and pollution tolerances) will be calculated for each sample (Davies et al. 2009, Wright & Ryan 2016). This will allow for easy comparison between sites. Samples will be analysed using multivariate analysis software (PRIMER). Multivariate analyses have been demonstrated to be a sound technique to evaluate the ecology of macroinvertebrates in freshwater systems (Davies et al. 2009).

Opportunity for Skill Development

The student will develop a number of broad skills such as working independently, time management and scientific writing, however, there is also the opportunity for the student to develop a number of advanced skills that will place them ahead of other graduates from their cohort. Students will develop field working skills including assessing risks, collecting physical and biological water samples. Students will also develop an excellent understanding of using a dichotomous keys to identify individuals. These skills directly transfer to skills that may assist the student to gain a role in the biological laboratories at Sydney Water, NSW Water, Office of Environment and Heritage who routinely use biological indicators to assess water quality.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Students applying for this project should be enrolled in an environment (any), animal science or zoology degree. Students would be at an advantage if they have undertaken a unit which has required field work or the use of dichotomous keys to identify organisms (such as Management of Aquatic Environments).

Project 96: Milky Way Spiral Arms and Mass Extinctions

Supervisor(s): Luke Barnes - l.barnes2@westernsydney.edu.au
Principal Supervisor

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Second Supervisor

Project description

Student will use the most up-to-date Milky Way model and solar orbit data in order to test the hypothesis that the Sun's galactic spiral arm crossings cause mass extinction events on Earth. To do this, we will create a new model of the Milky Way's spiral arms by combining a large quantity of data from several different surveys. We intend to combine this model with an elsewhere derived solution for the solar orbit to determine the timing of the Sun's historical passages through the Galaxy's spiral arms. We intend to design a new model with a symmetrical appearance, with the major alteration being the addition of a spur at the far side of the Galaxy. Furthermore, we will identify all known historical mass extinction events that might be explained by the motion of the Sun around our Galaxy.

Project Aims

- To create a new model of Milky Way and
- To examine if passage through the Milky Way Spiral Arms caused all known historical mass extinction events.

Project Methods

The student will follow the following steps, all of which will use standard software packages, but will require python scripting on a Linux machine:

- Learning to process and understand astronomy data
- Analyse all present models of the Milky Way
- Analyse all historical mass extinction on Earth
- Understanding the physics driving such a spiral structure of the Milky Way
- Writing a paper in an international peer-reviewed journal.

The work will also involve working with colleagues and other students at WSU, and will probably include joining the CSIRO Summer Students for lectures on astronomy.

Opportunity for Skill Development

Develop expertise in

- Astronomy techniques
- Managing and combining large data sets
- Use of High-Performance Computing
- Data analysis and modelling
- Writing a journal paper

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Knowledge of Python and Linux, or similar

Project 97: Using Theta Burst Stimulation Treatment with the Vestibular Ocular Reflex Protocol to alleviate symptoms in patients with Mal de Debarquement Syndrome (MdDS)

Supervisor(s): Cherylea Browne - c.browne@westernsydney.edu.au
Principal Supervisor

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Second Supervisor

Project description

Mal de Debarquement Syndrome (MdDS) is a rare neurological condition that affects the vestibular system and currently has no cure. Symptoms of MdDS include a continuous swaying, rocking, or motion-like feeling that typically arises following a motion experience such as traveling on sea, which leaves patients with a feeling of chronic “sea legs” or “sea sickness” though they are on stable ground. In addition to the unusual perception of phantom motion, clinical features include: chronic fatigue, cognitive slowing, visual-motion sensitivity, hypersensitivity to environmental stimuli, headache/migraine, unsteadiness, loss of coordination and anxiety. It is estimated to affect 0.04% of the world’s population, though many sufferers remain undiagnosed or misdiagnosed. As the research of MdDS is still in its infancy, the underlying cause and aetiology of symptoms is currently unknown, and thus treatments for this condition are often ineffective. Hypothesised to be a disorder of neuroplasticity, MdDS is believed to pathologically arise from the inability of the brain to inhibit mechanisms activated during motion exposure in order to prevent motion sickness.

Researchers in the US have shown that specific regions in the brain have altered activity in MdDS patients, i.e., the entorhinal cortex and the amygdala. Both areas are involved in learning and memory, with the former involved in spatial encoding and the latter emotional encoding and memory modulation, suggesting that a dysfunction in the two brain regions could play a role in the abnormal perception of constant motion. Recent studies point towards Theta Burst Stimulation (TBS) as a possible treatment, which is a revolutionary patterned form of repetitive transcranial magnetic stimulation that closely mimics the natural rhythms of neuronal activity. It is proposed that TBS can help reduce the associated symptoms of MdDS such as depression and migraine. Another treatment that is showing promise is the Vestibular Ocular Reflex (VOR) Protocol, which is a manual therapy that has been shown to greatly reduce patients’ motion perception by recalibrating a dysfunctional VOR. These treatments separately are not yet considered cures as patients still report some symptoms after treatment.

Project Aims

We aim to assess the effectiveness of a novel combination therapy in improving MdDS patient symptoms, we will conduct the following study: Using TBS Treatment with the VOR Protocol to alleviate symptoms in patients with Mal de Debarquement Syndrome (MdDS).

We aim to:

- Measure the effectiveness of TBS alone for treating MdDS
- Measure the effectiveness of VOR alone for treating MdDS
- Measure the effectiveness of combined TBS and VOR for treating MdDS

- Measure anxiety and depression levels before and after treatment using gold standard questionnaires
- Measure posturography (objective symptoms) before and after treatment
- Measure subjective perceptions of symptoms before and after treatment

Project Methods

All patients will be required to complete an intake questionnaire, a Hospital Anxiety and Depression Scale (HADS), a Dizziness Handicap Inventory (DHI), a Misery Scale and a Visual Analogue Scale (VAS) questionnaire. They will also be required to complete a symptom diary one week before and four weeks after treatment week, and will have a follow-up 3 months later.

TBS Treatment:

The TBS treatments will be administered by Mr Rocco Cavaleri. One session per day of TBS will be administered over the target site (Dorsolateral Prefrontal Cortex).

VOR Treatment:

The VOR treatment will be delivered by Dr Cherylea Browne, which consists of optokinetic (OKN) stimulation while seated in a chair in a darkened OKN drum, specifically built for the experiment. A full-field OKN visual stimulus will be projected on the drum walls, filling the visual field of the patient, including peripheral vision. Patients will be seated at 60 cm from the wall of the drum.

Posturography Measurements:

Using a custom program designed for a Wii Fit Balance board, posturography measurements will be taken three times each day; before the TBS treatment, after the TBS treatment and after the VOR treatment.

Opportunity for Skill Development

If the main project goes ahead, the student will have the opportunity to develop patient communication skills, data collection skills, learn how to obtain posturography measurements, observe Theta Burst Stimulation set up and administration, and observe Vestibular Ocular Reflex protocol administration.

If COVID-19 restrictions are still in place, the student will have the opportunity to develop a patient survey, be involved in participant recruitment, patient communication skills, data collection skills, and learn how to clean and analyse large data sets.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

We are interested in recruiting a student enrolled in the Bachelor of Physiotherapy course, who is interested in novel vestibular rehabilitation techniques for those with neurological disorders. Preferably someone who is in the later stages of the course i.e. 2nd -4th year students.

Project 98: New methods to predict GI for the Australian rice and biscuit industries

Supervisor(s): Marion Gaborieau - m.gaborieau@westernsydney.edu.au
Principal Supervisor

Patrice Castignolles - p.castignolles@westernsydney.edu.au
Second Supervisor

Project description

We developed some new methods to monitor the production of (bio)ethanol through a fermentation process. This method was then applied to food, namely breakfast cereals and it was adapted to monitor other processes such as the functionalisation of chitosan films for cell culture or macromolecules dissolution. This method is now applied to rice as part of the PhD work of James Lee (ARC Industrial Transformation Training Centre for Functional Grains) and to biscuits in collaboration with the company Arnott's. In this work, a simplified version of the method will be tested on known rice and biscuit samples.

Project Aims

1. Observe whether conductivity is changing during the in vitro digestion of different cooked rice's
2. Observe whether conductivity is changing during the in vitro digestion of different biscuits
3. Compare the potential changes of conductivity with the changes in sugar released by the digestion

Project Methods

The experiments include three main steps:

1. Sample preparation,
2. Conductivity measurement,
3. Data analysis.

In terms of sample preparation, the rice will be cooked in tea balls following a protocol previously developed within a GI taskforce (composed of NSW Department of Primary Industries, Charles Sturt University, Southern Cross University and Western Sydney University researchers). The biscuits will be roughly broken into small pieces.

In terms of conductivity measurement, the conductivity detector will be connected to an Agilent capillary electrophoresis instrument. The detector will be calibrated with the fluid of interest (gastric or intestinal fluid). The sample will be placed in a vial together with the relevant fluid and if relevant the appropriate digestion enzymes. A small volume will be continuously transferred to the conductivity detector through a capillary.

The data analysis will consist of plotting the evolution of conductivity with digestion time and compare this with the evolution of sugars released already determined in our team.

The whole sequence with the three steps will be first demonstrated to the student by the supervisors on a given sample (one type of rice or biscuit) in a given fluid (gastric or intestinal). This training can be done while the student is on zoom if laboratory access was still restricted. The sequence will then be applied to other samples or in another fluid by the student (or the supervisors for the laboratory part if laboratory access was still restricted).

Opportunity for Skill Development

The student will acquire or strengthen both laboratory and soft skills:

- Working with food in a chemistry laboratory (working safely despite handling a common food, following preparation protocols to avoid contamination),
- Operating advanced analytical instruments (capillary electrophoresis, contactless conductivity detection),
- Data analysis using Excel and more advanced graphing software (OriginPro),
- Team work - the student will be part of a large team beyond the supervisors: visiting PhD student James Lee, Prof. Christopher Blanchard (Charles Sturt University) and his team at the ARC Industrial Transformation Training Centre for Functional Grains, Dr Rachelle Ward at the NSW Department of Primary Industries, colaborators at Arnott's,
- Presentation as part of the scholarship program but also to the research team, including by zoom,
- Writing skills: beyond writing the report, the student will be invited to participate in the writing of any peer-reviewed manuscript in which their work would be included. The publications cited above in the project description include 4 WSU undergraduate project students as co-authors for their undergraduate project work.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

The student must be a 2nd or 3rd year BSc or BMedSci student.

Project 99: Inhibiting the reproductive cycle of SARS-CoV-2 virus by binding of novel drugs

Supervisor(s): Roland Gamsjaeger - r.gamsjaeger@westernsydney.edu.au
Principal Supervisor

Liza Cubeddu - l.cubeddu@westernsydney.edu.au
Second Supervisor

Project description

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel highly infectious RNA virus that belongs to the coronavirus family (for a recent review see for example Wang et al., Int J Antimicrob Agents: 105948, 2020). The World Health Organization has declared the ongoing outbreak to be a global public health emergency. Based on recent research, SARS-CoV-2 has high transmissibility and infectivity, and a low mortality rate compared to severe acute respiratory syndrome and Middle East respiratory syndrome coronaviruses (SARS-CoV and MERS-CoV, respectively).

Replication of the viral genome is a fundamental step in the virus life cycle (see e.g., White et al., RNA Biol. 8(2):182–183, 2011). The protein, SARS-CoV Non-structural protein 9 (Nsp9), was found to be essential for SARS-CoV-1 virus replication through its ability to bind RNA (Friedman et al., J Virol 86:884-897, 2012). Homologs of the Nsp9 protein have been identified in numerous coronaviruses including SARS-Cov-2 (Nsp9COV19), human coronavirus 229E (Nsp9HcoV), avian infectious bronchitis virus (Nsp9IBV), and porcine epidemic diarrhoea virus (Nsp9PEDV).

Three-dimensional structures of Nsp9 from these viruses have been determined by X-ray crystallography. Moreover, a recent paper deposited on the bioRxiv preprint server (Littler et al., DOI- 10.1101/2020.03.28.013920) describes the structure of Nsp9 from the novel SARS-CoV-2 virus, although this has not been peer-reviewed to date. Interestingly, despite the major role that Nsp9 plays in viral replication, its binding to RNA is very weak (high µM to low mM range; Egloff et al., Proc Natl Acad Sci USA 101:3792-3796, 2004 & Sutton et al., Structure 12: 341-353, 2004).

Our collaborator Prof Derek Richard from the Queensland University of Technology (as part of CARP Pharmaceuticals) has sucessfully tested several novel drugs for their efficiency to combat SARS-CoV-2 virus in infected live cells These molecules are currently undergoing further testing in mouse models. The molecular details of the mechanism of these drugs are centred on their ability to bind, and thus inhibit the function of Nsp9. This project will focus on using NMR spectroscopy to structurally characterise drug binding to Nsp9 with the aim of determining the affect the drug has on the interaction of Nsp9 to RNA.

Project Aims

1. Recombinant expression and purification of Nsp9 protein in Escherichia coli
2. Drug binding experiments using NMR HSQC experiments with Nsp9 protein
3. HSQC titration experiments with addition of drug and/or RNA to Nsp9 protein

Project Methods

The Nsp9 DNA construct provided by CARP Pharmaceuticals will be transformed into E. coli BL21 cells. The student under the supervision of the applicants or any HDR students will then use standard methods available in our lab (cell cultures, Ni/NTA beads and size exclusion chromatography) to express and purify Nsp9 (Aim 1). We have already complete resonance assignments of Nsp9 at this point and the student will use these two in combination with HSQC experiments (800 MHz spectrometer based at the University of Sydney) to determine drug and RNA binding to Nsp9 (Aims 2 and 3).

Opportunity for Skill Development

- Student will develop a wide range of laboratory skills using cutting edge equipment.
- Student will learn how to work independently and as part of a team.
- Skills relevant to further research studies such as Masters, PhD will be acquired.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

- Student is expected to be pro-active and diligent.
- Student is required to have basic molecular biology and protein knowledge.
- Student should have completed Functional Proteins and Genes as well as Molecular Biology.
- A final year student is desirable due to the high-level equipment being used and the potential to carry out further research studies (Masters).

Project 100: Bioinformatic identification of protein biomarkers for efficacy of novel anti prostate cancer drug c2

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Principal Supervisor

Shadma Fatima - shadma.fatima@inghaminstitute.org.au
Second Supervisor

Project description

This project aims to discover biomarkers which indicate the efficacy or resistance of a new anti-prostate cancer drug. The ten-year survival rate from diagnosis of prostate cancer is 98%, but this apparently positive statistic is misleading since it is the third most common cause of cancer death. This year approximately 20,000 men in Australia will be diagnosed with the disease. Prostate cancer is a slowly progressing disease, but once it enters the final stages treatment options are extremely limited. In 2020 the disease will kill an estimated 3300 Australian men. Diagnosis of prostate cancer can be difficult and often requires an invasive biopsy of the prostate gland. Treatment options include radiotherapy, chemotherapy, surgical prostate removal, androgen deprivation therapy, or just regular monitoring, depending upon the level of severity.

A new drug has been developed, designed to treat advanced prostate cancer. The cyclic peptide c2 (Fig. 2) shows great effectiveness in animal models (Fig. 1) and a first in human clinical trial has demonstrated oral absorption and negligible toxicity. However, its mechanism of action is not fully understood and ways to measure the drug's biological effectiveness (or tumour resistance) need to be developed.

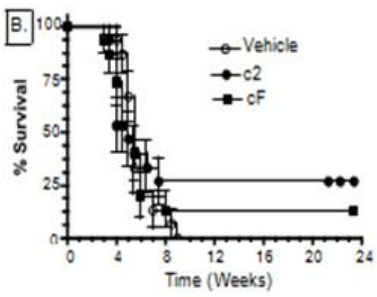

Fig. 1 Animal response to c2

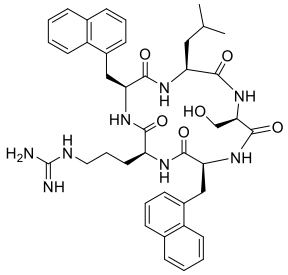

Fig. 2. Structure of c2 drug

In this project, three different stages of prostate cancer cells will be grown in the lab and treated with the c2 drug. The PC3, DU145 and LNCaP cells will be harvested and the protein extracted. Analysis of the cellular proteins by LC-MS/MS technology combined with bioinformatic processing of data will enable the researchers to determine which proteins are changing significantly in concentration relative to untreated cells. This information will yield a list of protein candidates which will not only shed light on the mechanism of drug action but provide possible diagnostic/prognostic biomarkers to be used eventually in next-generation patient care. Such information will be a highly valuable complement to data obtained from a patient's circulating tumour cells obtained by liquid biopsy. It is hoped that the incorporation of this knowledge into new treatment biotechnologies will save the lives of more than 1000 Australian men each year.

Project Aims

This project aims to discover protein biomarkers in three different types of prostate cancer cell undergoing treatment with a novel anti prostate cancer drug. It is hoped that such information will enable clinicians to take a sample of blood from a patient and tell:

1. Whether the patient has prostate cancer,
2. If so what stage the disease is at and
3. How well the patient's cancer is responding to treatment.

Project Methods

1. Grow PC3, DU145 and LNCaP prostate cancer cell lines in suitable media
2. Treat each cell line with a suitable concentration range of anti-prostate cancer drug c2 (or related)
3. Harvest cells from treated and untreated paired experiments, remove lipids and extract proteins
4. Digest proteins with the enzyme trypsin
5. Analyse resulting peptides using LC-MS/MS
6. Using bioinformatics software, process the raw data such that proteins whose concentration changes most significantly are discovered in each cell line
7. If time permits, relate such potential biomarkers to biochemical pathways in order to gain insight into the drug's mode of action

Opportunity for Skill Development

- Cell culture work
- Protein extraction and digestion
- Use of complicated, modern analytical instrumentation (LC-MS/MS)
- Use of bioinformatics software
- Awareness of prostate cancer research in the literature

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Interest in research. Preferable background in chemistry, biochemistry or cell biology, but not essential.

Project 101: Establishment and analysis of a 3D spheroid model of Glioblastoma cell lines for application in drug discovery

Supervisor(s): David Harman - d.harman@westernsydney.edu.au
Principal Supervisor

Shadma Fatima - shadma.fatima@inghaminstitute.org.au
Second Supervisor

Project description

Patients with the brain cancer glioblastoma multiforme (GBM) face one of the poorest outcomes of all cancers. Despite being identified one hundred years ago, there have only been four drugs and one device ever approved by the FDA for the treatment of this cancer. Current brain cancer models, that are used to discover new therapies rely heavily on using a few well-characterised cancer cell lines grown flat on plastic dishes as cell cultures. These models are limited because these flat cultures do not mimic well a typical brain tumour microenvironment inside a patient. The failure of new drugs with promising preclinical data to translate into effective clinical treatments may relate to the use of simplified 2-dimensional in vitro GBM cultures. The aim of this short project is to establish and validate a work-flow to develop 3D tumoroids derived from GBM cell lines that will recapitulates key histological features of in vivo derived GBM tumour and its molecular profile. Briefly, we will use a unique bioprinting platform (Incucyte), developed here in NSW, that allows us to grow spheroids inside a gel in 3 dimensions with tissue-like structures that will better imitate natural GBM tumours. This means we can derive invivo like tumours from GBM cell line in a petri dish and can screen new drugs and can determine the DNA, RNA and protein profiles of treated and untreated GBM cultures to validate the effectiveness of the drug.

The Ingham Institute is one of two beta-test sites in Australia for the recently developed Rastrum 3D gel bioprinter, a technology discovered, developed, and commercialised in NSW by CONCERT member Institutions (Inventia Ltd, https://vimeo.com/246901350). We will culture U251 and U87 GBM cell lines as 2D and 3D spheroids. Spheroid growth will be monitored using the Incucyte imaging system. We will then treat 2D and 3D tumoroids with therapeutic doses of temozolomide and standard cell viability assays (Promega) will be done to check the differences in effectiveness and IC50 dose estimated at 2D and 3D culture. The protein extracted from treated and untreated 2D cell culture will be subjected to Furthermore the proteomic profile of the treated and untreated culture will be assessed LC-MS/MS technology combined with bioinformatic processing of data to determine the changes in proteomic profile happened due to changes in the culture conditions and will also shed light on the mechanism of drug action. This project will help in validation of the 3D GBM spheroid model as a platform to screen new drugs. Success will open a pathway where patient derived GBM cells will be cultured invitro as, have their tumour molecular profile determined, potential treatments identified and tested and this information provided to treating physicians within a few weeks of surgery to allow them to incorporate it into the patient treatment plan.

Project Aims

1. To validate invitro grown 3D spheroids from GBM cell lines as a platform to screen new therapies for GBM treatment.
2. To check the differences in GBM drug concentration and effectiveness in 2D and 3D models if any.

3. To identify differences in protein markers in treated and untreated 2d and 3D GBM models.

Project Methods

1. Grow colonies of GBM cell lines in suitable media.
2. Rastrum Manual printing kit will be used for 3D printing (2x106 cells seeded).
3. Treat each colony with a suitable concentration range of temozolomide.
4. Perform cell viability assay, determine IC50 for both 2D and 3D cell cultures
5. For proteomics assays harvest cells from treated and untreated paired experiments, remove lipids and extract proteins
6. Digest proteins with the enzyme trypsin
7. Analyse resulting peptides using LC-MS/MS
8. Using bioinformatics software, process the raw data such that proteins whose concentration changes most significantly are discovered in each cell line
9. If time permits, relate such potential biomarkers to biochemical pathways in order to gain insight into the drug's mode of action

Opportunity for Skill Development

- Cell culture work, Incucyte platform
- Protein extraction and digestion
- Use of complicated, modern analytical instrumentation (LC-MS/MS)
- Use of bioinformatics software
- Awareness of GBM cancer research in the literature

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Interest in research. Preferably chemistry, biochemistry or cell biology background but not essential.