KING'S College LONDON

Multiscale Models for Life Centre for Doctoral Training

Project Catalogue

Dear prospective MM4L student,

Welcome! Here is our guidance on choosing a PhD project.

This PhD project is a 3.5-year commitment with major impact on your training and future career. Please read this carefully to ensure that you can make an informed decision about your PhD.

Deciding on Projects and Learning New Things

Here you find the catalogue of PhD projects that you can choose from. Students often want to continue in the same field as their prior training. We strongly recommend that you do something different instead. To decide on a project, consider the learning opportunities that are central to a PhD:

- **Try something new** – such opportunities are rare, and they truly enhance your learning.
- **Broaden** your knowledge and experiences.
- **Maximise learning opportunities.** Don't do more of the same. Move outside your comfort zone. That's how **breakthroughs** happen.
- Be open to **new concepts and experiences** which you may encounter.

You won't know if you'll love it unless you try it! Your experience and education are still relatively narrow compared to the breath of MM4L. Many cutting-edge projects available in MM4L lie outside mainstream topics because of their interdisciplinary nature and originality. Don't reject them out of hand just because they don't lie in your comfort zone.

We recommend that you speak to **at least 3** (and ideally more) co-supervisors about their projects to get a broad sense of what we offer and learn about the breath of interdisciplinary biosciences. Even if you don't select a project, the meeting will be an important educational opportunity where you will learn about a new field from a 1-to-1 meeting with an expert. **You should indicate your 3 project selections on your application form.**

If awarded a studentship: Making Choices About Your PhD and Your Future

Your success in the PhD depends mostly on your environment as well as your working relationship with your supervisors and lab colleagues. **This criterion is far more important than the actual research topic.** (Do not ever pick a project where you love the scientific topic but cannot get along with the supervisor. A passion for skydiving should not override the lack of parachutes.)

You must find out as much about the research and training environment as possible to ensure that you can make an informed decision about where to pursue your PhD.

With over a decade of PhD training experience, the MM4L leadership team recognises that every supervisor-student relationship is unique. A perfect environment for one student might not work at all for another. **It is therefore critical to select a PhD that fits you.** A good fit ensures that you will be trained and supported to bring out your best, whereas a bad fit could compromise your training and affect your wellbeing.

Only accept a project that you are confident will lead to a PhD that fits you. There is **no commitment** to accept a project even if:

- You met with the supervisor(s).

- You indicated a preference at any time.
- You devised the project with the supervisor(s) or the supervisor(s) customised a project for you.

If you do not feel that there is a good fit, it is **totally ok** to decline and choose something else that you feel is a better fit, even if it might be awkward. It is critical to select a PhD that will allow you to flourish.

From our experience with hundreds of PhD students, these are the key factors in the success of a PhD – find out about them:

- The **most important factor** is the supervisor-student relationship.
- Lab/industry environment including people, equipment, and other resources.
- What you will learn (e.g., transferable skills, independence, confidence-building, how to fail and cope with failure).

Meeting with Supervisors and Lab Members

You must schedule meetings with both supervisors **before selecting a project.** The **goal** of these meetings is to set accurate expectations for you and your supervisor. You can thereby determine if the supervisor aligns with your expectations, and vice versa.

During the meeting, you should ask lots of questions, including:

- Get details about the project **again**. Determine precisely what you will do each year and get details of the research plan.
- **How does your project fit in with the rest of the lab?** Will you collaborate with others on one project, or will you have your own niche? How will credit be shared in publications and patents?
- Is the project as **advertised?** Science moves fast; some things can change since the indicative project was advertised.
- Find out about the **lab** and the **people** in it. How many are there and who will be supervising you on a day-to-day basis.
- Are **equipment and reagents in place** for the research?
- **Who will train you? How much time** will they devote to you? Can they train you **from scratch** if you lack certain skills or experience?
- What is the **role of each supervisor?** You will have two supervisors at King's. Depending on the project, you may also have a supervisor from an industry partner. Is it clear how they will work together to supervise your project effectively? **Ask all supervisors.**
- If your project involves **industry collaboration**, you can ask to see the contract agreement with the industry partner to look at resources and in-kind contribution as well as agreements regarding publication, your thesis, confidentiality, and Intellectual Property. You should also ask if there will be an **industry placement**, for how long, when, what will you be doing, and how will it contribute to your PhD training and thesis?

Tell your supervisors the following:

- Explain your **background and ability** so that they understand your training needs. Be sure that they are committed to provide the training that you need.
- Explain your **disabilities or conditions**. Ask them how they will accommodate you. The offer cannot be withdrawn because you have a disability or condition.
- Institutions (and your supervisors) have a legal requirement and moral responsibility to make reasonable accommodations to every student. There are many resources including additional funding for this purpose. But accommodations can only be made if you disclose your disability or condition.

You should also meet with students and/or postdocs from the lab, **without the supervisor present**. Ask about:

- **The supervisor's personality and working style.** How do they interact with and supervise people in the lab?
- **Lab culture.** Is the lab very social or very business-like? Do people tend to work individually or collaboratively? How much help and guidance can you expect from others in the lab?
- If you will be trained by people other than the supervisor, find out **how much training and time** they will devote to you.
- **Verify** everything the supervisor told you about the availability of reagents and equipment, your role in the project, and how credit will be shared.

If logistically feasible, also consider visiting the lab to see the actual space.

Equality, Diversity, and Inclusion (EDI)

MM4L is highly committed to EDI during recruitment and supporting our PhD students. We also ask students to consider the multifaceted aspects of EDI when deciding on a PhD project.

- Supervisors have **diverse** traits – they belong to different ethnicities, genders, sexual orientations, religions, ages, and may have disabilities or currently be pregnant / on maternity leave.
- Be aware of your **unconscious bias** and take that into consideration when selecting projects and supervisors.
- Be **inclusive** – make reasonable accommodations for any traits or conditions supervisors might have.

Great PhD projects can be found in all sorts of labs. The extra effort to look at a broad range of labs will pay off.

Questions?

For additional information, please contact Dr QueeLim Ch'ng (queelim@kcl.ac.uk) or Prof Karen Liu (karen.liu@kcl.ac.uk). Academics on the MM4L team will be happy to address any questions and provide personalised guidance.

Table of Projects

Project Reference: MM4L1

Modelling biomechanical contributions to chemotherapy resistance

Co-Supervisor 1:	Maddy Parsons, Randall Cell & Molecular Biophysics
Field of Expertise:	In Vitro Modelling; Molecular Biology; Translational Medicine
Co-Supervisor 2:	Rachel Bearon, Mathematics
Field of Expertise:	Computational Modelling; Bioinformatics; Translational Medicine
Contact:	maddy.parsons@kcl.ac.uk, rachel.bearon@kcl.ac.uk

Objectives

Breast cancer is the most common form of cancer in the UK, with ~55,000 diagnoses and 11,500 deaths from this disease per year. Whilst >90% of patients diagnosed with early disease survive more than 5-years, many patients with more advanced disease or specific cancer subclasses do not respond to therapy. This is because these tumours are complex and different in each patient, and because we have a poor understanding of the biology. Our recent data indicates that the biomechanical properties of breast cancer in patients directly correlates with response to chemotherapy. However, how tumour cells from different patients alter their mechanical stiffness in response to chemotherapy, and how this alters efficient chemotherapy uptake remains unclear. This project aims to combine patient-derived organoid models, advanced imaging, computational analysis, and mathematical modelling to understand how altered biomechanical properties of tumours and complexities of contributions from other cell types affects chemotherapy delivery and cancer cell survival. Data arising from this project will provide insight into contributions from the tumour microenvironment to therapeutic response and provide novel drug discovery approaches and model for treatment of cancer patients.

Workplan

The project will comprise wet lab experiments, computational analysis and advanced mathematical modelling to develop a more holistic understanding of how the biomechanical properties of the tumour microenvironment affect breast cancer cell response to treatment. The project will comprise 3 overarching aims:

1. Multimodal imaging of drug uptake and functional response in breast cancer organoids.

The student will learn how to culture human patient-derived breast cancer organoids and embed in collagen gels of increasing stiffness to recapitulate the tumour microenvironment. Organoids will be treated with fluorophore-labelled chemotherapy agents (taxanes, cisplatin) separately or combined and imaged daily using non-invasive fluorescent probes to mark nuclei and cell membranes. Live high-resolution imaging over treatment time courses will be used to detect uptake of drugs and subsequent functional responses. Orthogonal imaging approaches such as spatial mass spectrometry will be applied to detect spatial drug uptake/proteome changes at fixed time points and correlate with function. Computational image analysis pipelines will be developed to correlate drug uptake/exposure with spatial responses depending on biomechanical properties of ECM.

2. Computational modelling of drug uptake in response to biomechanical environment.

The students will develop a computational workflow to capture the spatial-temporal uptake of drugs within organoids and cancer cell responses (proliferation, invasion etc). This framework will be parameterised and tested against data from the above experimental pipelines, and the impact on model parameters of the biomechanical properties of the ECM will be rigorously quantified through in

silico simulations, the computational framework will provide predictions for more complex settings (e.g., co-culture), and enable the iterative testing of emerging concepts to inform experimental design.

3. Define stromal cell contributions to drug response.

To expand on the tumour-only models, experimental data will be gathered to incorporate inclusion of stromal cells (fibroblasts, immune cells) and imaging conducted to understand how these cells influence responses to biomechanical properties of the matrix and ensuing functional ednpoints. Resulting data will be incorporated into models to provide more complex frameworks to understand multi-cell interactions and predictive modelling of changes to cell density, stiffness and functional outcomes in response to standard of care therapies. Models will be further refined through incorporation of clinical metadata to determine contributions from any genetic mutations or other clinical features to explore heterogeneity in cell response to treatment.

The outputs arising from this project will provide robust experimental and computational pipelines to understand complex interactions within the tumour microenvironment and contributions from biomechanical and cell-specific interactions in therapeutic response. We anticipate this will be broadly applicable to other tumour types and provide open access computational models for broader testing of drug response in both academic and industry drug discovery settings. Importantly, the project will equip the student with excellent, highly desirable skill sets at the interface between advanced experimental and data/computational domains, enabling them to be fluent in both biological and computational languages.

Project Reference: MM4L3

Intestinal exosomes as key transporters across the intestinal barrier

Co-Supervisor 1: Joana F Neves, Centre for Host Microbiome Interactions

Field of Expertise: Stem Cell Biology; Translational Medicine

Co-Supervisor 2: Driton Vllasaliu, Institute of Pharmaceutical Science

Field of Expertise: In Vitro Modelling; Molecular Biology

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Objectives

Transport of nutrients, bacterial products, allergens and drugs across the intestinal barrier is key for a healthy gut and its dysregulation can lead to local and systemic diseases. The key players in this process are still being elucidated. A possible candidate are exosomes, which are membrane-bound extracellular nanovesicles secreted by most cells. Much attention has been recently devoted to exosomes as they play an important role as mediators of intercellular communication, having a distinct ability to carry small molecules, proteins, and nucleic acids to recipient cells. However, their role on intestinal pathophysiology, via intestinal mucosa cell-cell communication is unknown.

This project will build complex organoid models and use interdisciplinary approaches to characterise intestinal epithelial derived exosomes and determine how they modulate epithelia and immune cell function, which are key for intestinal health.

Objective 1: To develop an in vitro model of human intestinal organoid monolayers co-cultured with lymphocytes.

Objective 2: To characterize exosomes produced by intestinal epithelial cells in physiological conditions and after challenge with bacterial metabolites.

Objective 3: To determine how exosomes impact epithelial and lymphocyte function in vitro and in vivo in physiological conditions and in the context of intestinal inflammation.

Workplan

Work Package 1. Establishment of a human intestinal organoid monolayer-immune cell co-culture system.

Human intestinal organoids will be cultured on Transwell filters following methodology which Vllasaliu has successfully developed recently (Zhang et al. Molecular Therapy Nucleic Acids. In Review). Cells will be imaged daily (light microscopy) to monitor growth and transepithelial electrical resistance (TEER) will be used to indicate tight junction formation. The monolayers allow for easy access to the basal (immune cells site) and apical (bacteria/metabolites/drugs site) of the intestinal epithelial cells permitting easy manipulation of these cultures.

Blood or intestinal harvested lymphocytes, such as Innate Lymphoid Cells, will be added to the basal side of the epithelial cells. The Neves lab has successfully established 3D cultures of intestinal organoids with immune cells (Jowett et al, Nature Materials 2021 and Cell Reports 2022) and will use this knowledge to develop the 2D organoid monolayer immune co-culture model. The growth and phenotype of cells in co-culture will be monitored by imaging and TEER measurement.

At the end point, transmission electron microscopy (TEM) will be employed to reveal the overall morphology, including the presence of microvilli. Then phenotype of epithelial and immune cells will be determined by several methods including, immunocytochemistry, flow cytometry and RT-PCR.

Work Package 2. Characterization of intestinal epithelial cell-derived exosomes.

Exosomes are produced by all cell types and our preliminary data confirms their production by human intestinal organoids. The supernatant of the organoid monolayer cultures will be harvested and exosomes isolated using established ultracentrifugation-based approaches. Exosomes will be characterised using state-of-the-art nanotechnology and analytical tools, including for size, number, surface charge, the expression of key exosomal protein markers and protein composition via proteomics. These measurements will be done at steady and after challenge of the organoid monolayers with bacterial metabolites (such as butyrate).

Work Package 3. Determination of the impact of intestinal exosomes on epithelial and immune cells.

Exosomes produced by monolayer organoids and metabolite challenged organoids will be isolated as above and added to the immune-organoid monolayer cultures. The impact of these exosomes on epithelial and lymphocyte phenotype will be determined as described in work package 1.

To test these effects in vivo, fluorescently labelled exosomes will be administered into wild-type mice by oral gavage, and their biodistribution, as well as impact on intestinal barrier function and immune and epithelial cell phenotype will be determined at steady-state and in intestinal inflammation (DSS-induced colitis).

Exosomes are a great candidate to delivery new therapies. Thus, its crucial to understand their physiological role and how they are modulated by the intestinal environment such as the microbiota (and their metabolites) and any impact they might have on crucial intestinal players such as the epithelial cells and the intestinal lymphocytes.

Overall, this project will build a new intestinal model that will allow to dissect the role of intestinal exosomes and by using a combination of in vitro and in vivo systems it will explore exosome functions in the gut in health and disease.

Project Reference: MM4L4

A dynamic map of intra-mitochondrial viscosity and diffusiveness in healthy, diseased, and aged neurons

Co-Supervisor 1:	Alessio Vagnoni, Basic & Clinical Neuroscience
Field of Expertise:	Animal Modelling; In Vitro Modelling; Molecular Biology; Live cell imaging, Optogenetics
Co-Supervisor 2:	Simon Ameer-Beg, Comprehensive Cancer Centre
Field of Expertise:	Physics, engineering, optics, bioimaging and biophysics
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Objectives

1. To measure the viscosity of the mitochondria in different neuronal compartments (for example, cell bodies, axons, synaptic terminals) ex vivo in Drosophila. This will generate the first map of mitochondrial viscosity within living neurons in an organismal context.

2. To characterise mitochondrial viscosity as they are transported within the axons of neurons. This will show whether different populations of mitochondria, for example stationary vs motile, are characterised by a specific viscosity profile.

3. To test whether mitochondria under tension, usually observed during mitochondrial dynamic events, adjust their microviscosity profile. We will specifically model a pre-mitochondrial division state by inducing mitochondrial stretching through optical trapping as well as using appropriate Drosophila genetic backgrounds.

4. To analyse intra-mitochondrial protein diffusion and correlate the extent of mitochondrial content mixing with the viscosity state of the organelle. Diffusion will be at first measured through micro-rheology experiments by engineering a new genetically encoded mitochondrial probe.

5. To test whether viscosity of different mitochondrial population is affected in specific genetic backgrounds modelling neurodegeneration and accelerated ageing.

Workplan

1. The student will first learn how to culture the fruit fly Drosophila melanogaster and to obtain a 'larval fillet' preparation amenable to high-resolution live microscopy. Drosophila motor neurons will be stained through bathing in a solution of the molecular rotor FMR-1 and imaged ex vivo on a Leica Stellaris Dive FALCON commercial system in the Centre of Excellence for Fluorescence Lifetime Imaging within the Microscopy Innovation Centre as well as a bespoke Massively Parallel Fluorescence Lifetime Imaging (mpFLIM) microscope in the Ameer-Beg lab.

Mitochondrial viscosity will be measured by FLIM to exploit the direct correlation between viscosity and lifetime of the FMR-1 dye. Because mitochondrial morphology and function are known to be partly dependent on the subcellular location of the organelle, the student will focus on understanding if mitochondrial viscosity is compartment dependent. They will measure the viscosity in the cell soma, at the synaptic terminals and in the proximal, mid, and distal portions of the axons. This initial characterisation will provide a novel map of neuronal mitochondrial viscosity and function as essential benchmark for future studies.

2. The student will move onto more detailed analyses by characterising the viscosity of moving and static mitochondria in the axons of neurons in real time. Because mitochondria transported in opposite directions (towards the cell body and synaptic terminals, respectively) are thought to be functionally

distinct, this viscosity analysis will be further stratified by studying mitochondria that move in an anterograde and retrograde direction within axons.

3. We hypothesise that the dynamic state of mitochondria correlates with a specific viscosity profile. To directly test this hypothesis, the student will use Drosophila genetic backgrounds in which fission and fusion proteins are depleted to trigger elongated or shortened mitochondria, respectively, and measure their viscosity. The student will also directly be able to apply force on the mitochondria to induce membrane stretch and measure the viscosity in this context. This innovative approach will be achieved by using a force calibrated optical tweezer system (Impetux SensoCell), unique in the UK, integrated in a SWARM FLIM system within the Ameer-Beg lab.

4. Molecular diffusion is correlated to viscosity by the Stoke-Einstein equation. The student will perform micro-rheology experiments by developing a new genetically-encoded mitochondrial targeted nanoparticle probe (mito-GEM) to characterise protein diffusion in the matrix of the mitochondria located in different neuronal subcellular locations. This will be based on our previous work with the cytoplasmic GEM sensor (https://www.biorxiv.org/content/10.1101/2023.09.18.558344v1) coupled to single particle tracking to measure cytoplasmic diffusiveness. If we are not able to track the GEM particles in the constrained space of the mitochondria, we will use FRET analysis of a soluble GFP reporter.

5. The student will be able to test whether Drosophila strains expressing human proteins causing ALS, Parkinson disease and Alzheimer's disease display compartment-dependent and strain-specific mitochondria microviscosity profiles. Fly neurons overexpressing progerin will be used to model accelerated ageing. If time allows, these studies can be expanded into an in vitro human model of neuronal ageing already in use in the Vagnoni lab.

Project Reference: MM4L5

Building physics-informed Neural Networks to bridge the gap from micro- to macro- modelling of human cortical folding development

Co-Supervisor 1:	Katie Long, Developmental Neurobiology
Field of Expertise:	In Vitro Modelling; Development; Stem Cell Biology; Biomaterials
Co-Supervisor 2:	Emma Robinson, Biomedical Engineering
Field of Expertise:	Computational Modelling
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Objectives

The shape of the human brain has long been linked to its function, with changes in shape often associated with changes in cognition. Subtle changes in shape, often called malformations of cortical development (MCDs), can occur in many neurodevelopmental disorders, suggesting regulation of brain shape is an important developmental process. MCDs are thought to be associated with disruptions to the processes of cell proliferation and apoptosis, cell migration, and post-migrational development.

However, how the human brain develops to the right shape, and what goes awry in MCDs is not fully understood. There has been an understanding that the process of cortical folding results from biomechanical tensions as the cortex expands. However, a complete model of the process remains elusive. Plausible theories include more rapid growth of the cortex relative to sub-cortical white matter [Tallinen 2014], and the impact of axonal tension as neuronal connections form [Van Essen 2020]. But these are incomplete and can only partially replicate the patterns of cortical folding observed in live human brains.

This project combines cellular and Physics-informed neural network (PiNN) simulations to bridge the gap between in vitro/ex vivo and in silico tissue models of cortical folding to macroscale observations from whole brain imaging.

Workplan

Overall, the objective is to build models that incorporate biomechanical constraints, using PiNNs to make predictions that can be tested in the tissue/cell-based model systems. The cellular models will use induced pluripotent stem cells (iPSCs) from individuals with typical and atypical brain shapes, grafting of these cells into human cortex explant cultures. The fetal tissue that the Long lab works with is during the critical window of fetal brain development (12-20 pcw) where many of the cell behaviours listed above occur (proliferation, migration and apoptosis), providing a unique model to investigate changes in cell behaviours within a physiological tissue environment. Imaging and analysis of the differences in cellular processes will be combined with computational modelling to understand how altered cell behaviours in development lead to changes in brain shape.

Machine-learning will be used to optimise biomechanical models of cortical growth and deformation [Da Silva 2021] from simulated data that will learn to mimic the behaviour of the tissue cell models. In this way it will be possible to train an AI to design entirely new models of tissue folding that much more closely reflect the in vivo patterns of cortical folding that we observe from longitudinal fetal MRI data. The overarching objective will be to see if we can simulate (in silico and in vitro) the development of cortical malformations such as polymicrogyria and lissencephaly.

This information will be used to inform the wet lab side of the project, which will build tissue models (Long et al, 2018) in which these biomechanical parameters can be tested. For example, would altering tissue stiffness result in abnormal folding of the cortex? We can also combine the tissue with

neural cells derived from patient iPSC lines. These will be selected from patients with known structural malformations of the cortex, or can alternatively be manipulated to express mutations known to result in cortical folding malformations. The biomechanics of these cells in the tissue environment, for example their migration speed and direction, could then be used to inform the in silico models to investigate if this would lead to the structural change seen in the patient MRI.

Tallinen, Tuomas, Jun Young Chung, John S. Biggins, and L. Mahadevan. 2014. “Gyrification from Constrained Cortical Expansion.” Proceedings of the National Academy of Sciences of the United States of America 111 (35): 12667–72. doi:10.1073/pnas.1406015111.

Van Essen, David C. 2020. “A 2020 View of Tension-Based Cortical Morphogenesis.” Proceedings of the National Academy of Sciences of the United States of America 117 (52). Proceedings of the National Academy of Sciences: 32868–79. doi:10.1073/pnas.2016830117.

Long KR, Newland B, Florio M, Kalebic N, Langen B, Kolterer A, Wimberger P, Huttner WB (2018) Extracellular matrix components HAPLN1, lumican and collagen I cause hyaluronic acid-dependent folding of the developing human neocortex. Neuron 99: 702-19.

Silva, Mariana Da, Carole H. Sudre, Kara Garcia, Cher Bass, M. Jorge Cardoso, and Emma C. Robinson. 2021. “Distinguishing Healthy Ageing from Dementia: A Biomechanical Simulation of Brain Atrophy Using Deep Networks.” In Machine Learning in Clinical Neuroimaging, 13–22. Springer International Publishing. doi:10.1007/978-3-030-87586-2_2.

Project Reference: MM4L6

Tissue-scale mechanics in pancreatic cell fate decision and shape acquisition

Co-Supervisor 1:	Francesca M Spagnoli, Gene Therapy and Regenerative Medicine
Field of Expertise:	Animal Modelling; In Vitro Modelling; Development; Stem Cell Biology; Translational Medicine
Co-Supervisor 2:	Sergi Garcia-Manyes, Physics
Field of Expertise:	Physics, Mechanobiology
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Objectives

Spatiotemporally regulated reciprocal interactions between epithelial cells and surrounding microenviroment(s) are essential to build functional organs but also to maintain adult organ homeostasis. The pancreatic microenvironment is composed of multiple cell types, including mesenchymal cells, endothelial cells, neural and immune cells as well as the ECM. The interplay between pancreatic epithelium and its microenvironment is fundamental for the development of a proper functional pancreas, including cell differentiation and growth. Despite the relevance of such tissue interaction(s), very little is known about the different sets of cellular interactions that occur in the developing pancreas between pancreatic cell types and surrounding cells as well as ECM components. Moreover, it is unknown whether microenvironmental signals establish 'niches' with distinct mechanical properties, which in turn might control different aspects of pancreatic tissue formation. In this project, we aim at understanding how mechanics regulate cell behaviours in the context of pancreatic development and how to use these insights to help guide the strategy and design of functional engineered tissues.

Workplan

The Spagnoli laboratory has longstanding interest in intrinsic and extrinsic mechanisms regulating pancreatic cell identity. They set up ex vivo and in vivo models to study the composition of the pancreatic microenvironment in the mouse embryo and human tissue. They focus in particular on the pancreatic mesenchyme, defined its heterogeneity at the transcriptome level and identified a putative "niche" controlling pancreatic differentiation. The Garcia-Manyes laboratory focuses on mechanobiology across different length-scales. They use a combination of bespoke nanomechanical techniques at the single molecule and at the single cell level to interrogate how mechanical forces affect protein folding and how cells react to mechanical forces.

The proposed multidisciplinary project will build on our previous findings and combine our complementary expertise. We aim at resolving the full spectrum of cellular interactions in pancreatic tissue across space and time and how they provide mechanical cues to aid in controlling cell and tissue structure and function.

Specific goals of the project are:

1) To spatiotemporally resolve mechanical properties across mouse pancreatic embryonic tissue and organ cultures using atomic force microscopy (AFM) combined with fluorescence microscopy.

2) To integrate mechanical properties with microenvironmental features, including cellular interactions, ECM and signaling molecules distribution. To this aim, we will use spatial transcriptomic datasets of mouse pancreatic embryonic tissue recently generated in the Spagnoli lab.

3) To characterize the functional properties of selected putative mechanical “niches” that will be defined in aims 1 and 2 using mouse pancreatic embryonic organ cultures and synthetic iPSC-derived models.

Overall, these studies will shed light onto how tissue-scale mechanics is involved in controlling cell fate acquisition and morphogenesis during organ formation.

Project Reference: MM4L8

Many ways to grow a bone: an evolutionary development and mechanical simulation investigation into the growth and development of sesamoids bones

Co-Supervisor 1:	Michael Berthaume, Engineering
Field of Expertise:	Computational Modelling; macroevolutionary modelling
Co-Supervisor 2:	Abigail Tucker, Centre for Craniofacial & Regenerative Biology
Field of Expertise:	Stem Cell Biology; Development
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Objectives

Sesamoids are variably present bones located in tendons and ligaments, often near joints. They form in embryonic development in response to both molecular and mechanical (i.e., muscular) cues. As they grow, they function as "pulleys", redirecting muscle forces and alleviating stresses in the tendons/ligaments. In humans, the presence/absence of knee sesamoids can influence susceptibility to knee pain, osteoarthritis, and affect knee surgeries.

Recent evolutionary models on primate knees have suggested there is more than one way to grow and evolve sesamoids. For this project, the student will combine developmental biology and evolutionary biomechanics to reconstruct the evolution and formation of knee sesamoids in placental mammals. To do so, the below objectives will be followed:

1) Analyse developmental series of foetal knees of a variety of mammalian species, including mouse and human, using molecular and morphometrics approaches, complemented by data gathered from the mammal collections at the Natural History Museum.

2) Model the growth, development, function, and evolution of sesamoids in the knees of mice and humans using biomechanical, finite element, musculoskeletal, and statistical models. Models will be validated using morphometric data.

Workplan

The following workplans (WPs) will be conducted:

WP0 Literature review (months 0-3): For the first three months, the student will conduct a literature review. Papers will include existing hypotheses concerning sesamoid growth, development, and evolution in mammals, bone growth, development, and biology, and biomechanical modelling of bone.

WP1 Development (months 3-15): Embryonic and foetal knees will be analysed by microCT and using thin sections to visualise development of knee sesamoids with a focus on mouse and human tissue (provided by the Human Developmental Biology Resource). The mouse data will be compared to knees from CACNAS1 mutant embryos, which have a defect in muscle contraction, to understand how force impacts sesamoid formation. In these mice some sesamoids fail to form while others appear to initiate as normal, highlighting the complex nature of the mechanical and chemical signalling environment. Tissue will be analysed for changes in gene and protein expression.

WP2 Deep time evolutionary modelling (months 6-24): A systematic review of sesamoid presence/absence in mammals will be produced, combining published data and collections at the Natural History Museum. An updated phylogeny will be created. Phylogenetic comparative methods will be used to investigate the tempo and mode of evolution of knee sesamoids in mammals, and to investigate if sesamoid presence/absence is correlated with any ecological parameters, such as mode of locomotion.

WP3 Evolutionary biomechanics (months 12-36): Two sets of simulations will be run. First, thin sections from WP1 will be used to construct 2D finite element (FE) models of knees. Muscle forces, estimated using muscle cross-sectional area will be applied to the model to simulate muscles contracting in utero, and iterative simulations will be used to model sesamoid growth. Two model geometries will be used. First, where sesamoid ossification centres are attached to primary bones (e.g., as is the case with the patella and femur). Sesamoid detachment and creation of the synovial joint will be simulated. Second, tendons without sesamoids will be modelled to investigate if strains necessary for sesamoid formation occur independently in the tendon.

In the second set of simulations, species of interest identified from WP2 will be identified and chosen for musculoskeletal modelling. Using geometrical data derived from microCT scans, changes in lever arm mechanics of the muscle through knee flexion will be examined.

This project is feasible for a PhD studentship as background work has already been completed, including applying for permissions to access relevant foetal knee collections and a systematic review of sesamoids in placental mammal knees (conducted in 2020, to be updated during project).

The research will shed light on the functional role of sesamoids in mammals, and explain how differences in knee sesamoid anatomy occur in humans. Such knowledge can impact our understanding of human knee health, with the potential to develop therapeutics and physiotherapies that can alleviate knee problems, such as osteoarthritis, by incorporating an understanding of sesamoid anatomy.

Project Reference: MM4L9

Modelling Oral Lubrication Mechanisms: Bridging Simulation and Sensation

Co-Supervisor 1: Sorin-Cristian Vlădescu Engineering

Field of Expertise: Computational Modelling; Development; Biomaterials

Co-Supervisor 2: Guy Carpenter, Centre for Host Microbiome Interactions

Field of Expertise: Biomaterials

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Objectives

Meat and dairy alternatives are humanity's top lever to fight climate change, reducing greenhouse gases 11 times more efficiently per investment dollar than zero-emission cars. A major biological barrier to the widespread adoption of plant-based diets is astringency, a loss of oral lubrication while consuming plant-based proteins, leading to lower palatability.

Our current research into plant-based astringency reveals that particles within protein preparations can have a large (30%) effect in causing delubrication of the salivary film on the tongue, leading to the perception of dryness. Our previous work on carbonated beverages also revealed bubbles can affect taste and mouthfeel by affecting the salivary film on the tongue. In this project we believe the properties of the particles and their interaction with the salivary film on the tongue can be harnessed to create novel fat replacement additives. It is known that fat affects mouthfeel and is used in food products to reduce astringency, given the strong correlation between fat percentage and measured friction.

This project brings together a multi-disciplinary team to analyse particle movement under shear conditions to create models which can predict lubrication profiles for in-vivo testing. The aim is to characterise the mechanisms through which particles influence sensory response.

Workplan

The aim of the proposed experimental work is to determine the mechanisms affecting oral lubrication by foods. Specifically, to understand the astringency (reduced oral lubrication) or creaminess (increased oral lubrication) behaviour of different particles, we will measure how the particles behave in vitro, then model mechanical forces induced by particles and finally test different particles in vivo to determine how they are perceived inside the mouth and how humans sense movement at mucosal surface level. We will study how fat molecules behave inside the oral cavity and how their mechanical behaviour can impact the perception of various food products. The project aims to achieve the following deliverables:

WP1: Direct observation of the flow and deformation inside the oral contact of the plant proteins and fats. We will employ a unique, newly designed experimental setup mimicking the physical and mechanical characteristics of the human tongue. Its components include: (a) 3D-printed biomimetic tongue (already developed at King's College), (b) custom-designed oral tribometer simulating complex, real-life tongue movements, (c) optical microscopy combined with fluorescence detection of salivary and food proteins by prior labelling. Particles to be studied will start with fat liposomes, then compare them to polystyrene, silica or mica particles (with different charges) and then plant proteins.

WP2: Computational modelling in the context of taste and oral tribology. FEA (Finite Element Analysis) will be employed for the first time as a tool to study tissue deformation inside the oral cavity. We will begin by creating a digital representation of the oral tissues (i.e., the tongue and the palate), discretising the geometry into interconnected elements to approximate the continuous behaviour of the oral tissue.

The FEA model will provide detailed information about the stress, strain, and deformation patterns within the oral tissues. This information is crucial for understanding how different particles entrapped inside the oral contact (information collected under WP1) impact the tissue properties and geometries, and how external forces influence the mechanical response at mucosal level. This will help understand how tissue properties affect food breakdown and bolus formation, ultimately impacting taste perception.

WP3: Integration with Computational Fluid Dynamics (CFD). The FEA model of the oral tissues will be integrated with fluid dynamics simulations for saliva flow and mass transfer models for flavour release, to create a comprehensive understanding of the entire oral processing system of various particles. This will shed light on how cells perceive movement at mucosal surfaces under real life scenarios, when mucins bind to oral surfaces.

WP4: Determining correlations between computational models and microscopy observations, and taste/ sensory assessments from a human panel. Currently, food product development is primarily informed by humans evaluating the taste, texture, mouthfeel etc. of proposed formulations. However, this approach is time-consuming, expensive, and dependent on subjective reporting. Determining correlations between computational models (informed by scientific measurements) and taste perception would allow food manufacturers to decrease reliance on human testers, lowering costs and increasing speed to market by employing a lab-first approach.

Project Reference: MM4L10

Establishing a model of perfused microvasculature

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Co-Supervisor 2:	Pablo Lamata, Biomedical Engineering
Field of Expertise:	Computational Modelling; Bioinformatics
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Objectives

Human organoids have emerged as a promising platform to bridge preclinical research with clinical trials. Employing human blood vessel organoids (BVOs) as a model of human microvasculature, we have previously shown that metabolic perturbations can initiate a mechanotransduction cascade and trigger structural remodelling. Blood flow is an important determinant of vascular structure in vivo, but the interplay between flow, metabolism and mechanotransduction in the microvasculature is not well understood.

Objective 1. Establish a microfluidic platform for BVOs.

We will establish a microfluidic system to culture BVOs under flow conditions. BVOs will be exposed to laminar flow and the impact of vascular perfusion on the vascular structure will be assessed. Further experiments targeting PFKFB3, an activator of glycolysis, will elucidate the role of flow in the metabolic reprogramming of the microvasculature.

Objective 2. Generate a 'digital twin' model of the microvasculature.

Computational analysis integrating the vascular features under flow and static conditions with functional information from the key metabolic and proteomic hubs will be used to generate a 'digital twin', i.e. a model of the microvasculature. Computational simulations will then be used to interpret experimental findings, to investigate the key triggers of microvascular dysfunction, and to generate novel hypotheses about the underlying mechanisms.

Workplan

Objective 1. Establish a microfluidic platform for BVOs.

BVOs will be generated using the Wimmer et al protocol. To determine the effect of laminar flow on the microvasculature, BVOs on day 17 of differentiation will be plated on gelbrin hydrogels in microfluidic-based perfusion bioreactors. The gelatin-fibrin substrate that can support strong attachment of organoids under flow conditions will enable a single BVO to be plated in each well in a µ-Slide Spheroid Perfusion Uncoated bioreactor (ibidi). Gelbrin gels will be used to coat the niche area of the wells. For these experiments BVOs with diameter 500µm ± 50 will be used. A total of 21 BVOs in organoid media will be plated in each bioreactor and allowed to attach overnight. The following day, flow will be applied for 5-7 days. Different flow rates, ranging from (200-600mBars) will be used. Flow through the vasculature will be visualised using 40 kDa fluorescein isothiocyanate (FITC)-labeled dextran beads. Functional human microvasculature will be assessed by hCD31, PDGFRβ and FITC labelling. To evaluate the BVO perfusion in response to metabolic stress, BVOs will be incubated with EBM2 for 24h presence or absence of PFK15 (2.5µM) and/ or CTGF (50ng/ml). Treatment with TNFα (10ng/ml) will be used as a positive control for increased vascular permeability.

Milestone: Establish a perfusion bioreactor for BVOs.

Outcome: Obtain functional readouts for the BVO permeability.

Objective 2. Generate a 'digital twin' of the microvasculature.

Our aim is to generate an in-silico representation of the microvasculature using the structural and functional readouts obtained from BVOs. This model will incorporate features associated with several aspects of vascular rarefaction such as lower vessel density, shorter vessel length, centrality, reduced interconnectivity, incomplete loops, broken branches, decreased pericyte coverage, but also vascular landmarks- vascular end and vascular bifurcations. This will enable us to construct a collection of topological 'vascular network' models. We will then enrich the model with function, and based on the capillary flow we will test the hypothesis that 'poor structural vascular network' leads to functional impairment. The model will also be used to investigate the mechanistic link between metabolic rewiring and structural and functional alterations.

Image J and Matlab software programmes will be used to quantify the vascular network features mentioned above. In the second stage, the functional data obtained in objective 1, using the bioreactor for BVO perfusion and flow patterns at baseline and following stimulation, will be incorporated to the model to produce a simplified version of a 'digital twin' that could be used to identify microvascular dysfunction and predict microvascular perfusion.

Milestone: Establish a computational model of vascular rarefaction

Outcome: Create a computational tool to interrogate the link between structural remodelling in the microvasculature and impaired flow.

Project Reference: MM4L11

Building a multiscale model of cardiac fibrosis and treatment response

Co-Supervisor 1: Alkystis Phinikaridou, Biomedical Engineering

Field of Expertise: Animal Modelling; Translational Medicine; Medical Imaging

Co-Supervisor 2: Gilbert Fruhwirth, Comprehensive Cancer Centre

Field of Expertise: In Vitro Modelling; Molecular Biology; cell tracking; Animal Modelling

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Objectives

Scientific basis:

Heart failure (HF) affects 64 million people globally and causes sudden death. Cardiac fibrosis (CF) drives HF creating a need to model CF and therapeutically target it. There is currently a lack of a model to study cardiac fibrosis and the effects of novel therapeutics. Thus, building an imaging data-driven model of cardiac fibrosis development and aetiology and how can it be impacted with novel cell-based therapies as anti-fibrotic treatment is needed.

Current imaging technologies do not directly measure CF and no specific antifibrotic drugs are available. Secreted matricellular proteins have been shown to inhibit CF and preserve cardiac contractility in vivo by inhibiting TGFβ signalling. An engineered effector T cell immunotherapy targeting myofibroblasts was reported to treat cardiac fibrosis in mice. Moreover, regulatory T cell (Treg) therapy has been proposed as a candidate to counteract cardiac inflammation- induced CF.

In this project we aim to develop advanced models to diagnose CF and quantify the anti-fibrotic effect of cell-based therapies. To achieve this aim this project will employ a multiscale approach (from cells, to tissue to organ) to directly model the cardiac fibrosis in vivo (Phinikaridou-lab) and use a novel Treg-based therapy to deliver an anti-fibrotic protein (Fruhwirth-lab).

Workplan

Specific objectives for each year:

(1) Build the in vivo animal model of CF and use fibrosis-specific MRI-probes to quantify the extent of cardiac fibrosis (Year 1-1.5).

(2) Build an imaging-based data model of cardiac fibrosis aetiology and progression (Year 1.5-2.5).

(3) Establish an in vivo traceable Treg-cell therapy (by PET imaging) to deliver a known anti-fibrotic protein (AF-Tregs) (Year 2.0-2.5).

(4) Interfere with cardiac fibrosis by administering anti-fibrotic agents including AF-Tregs in vivo and use the cardiac fibrosis model (from Aim 1) to predict therapy efficacy (Year 2.5 to 3.5)

Techniques

- Mouse husbandry, handling, and surgical techniques.
- In vivo PET for cell tracking and MRI to measure cardiac function and fibrosis.
- Tregs cell isolation, purification, and culture
- Build a primary component analysis (PCA) model of the evolution of cardiac fibrosis at a voxel-by-voxel bases using MRI images and treatment response.

Project Reference: MM4L12

Host environmental impacts on bacterial methylation profiles in the human gut microbiota

Co-Supervisor 1:	Jordana Bell, Twin Research & Genetic Epidemiology
Field of Expertise:	Computational Modelling; Bioinformatics
Co-Supervisor 2:	David Moyes, Centre for Host Microbiome Interactions
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Objectives

As part of a recently awarded ERC/EPSRC funded grant we are undertaking novel characterization of bacterial DNA methylation in the adult human gut microbiota, to explore its regulatory potential for bacterial gene function, and its relevance to human diet and health.

The proposed PhD project will integrate within the existing framework to tackle the following specific objectives:

1. Apply and compare multiple analytical pipelines to quantify bacterial DNA methylation profiles of candidate bacterial species in the human gut microbiome, based on long-read DNA sequencing data.

2. Characterise variability in the bacterial DNA methylation of candidate species in human cohorts.

3. Explore if host environmental factors, such as medication use, leave a signature on the gut bacterial methylome.

4. Test whether particular bacterial DNA methylation signals are associated with microbially derived metabolite levels.

Outcomes. This project will characterise human gut bacterial DNA methylation as a novel tool to uncover molecular pathways mediating microbiome impacts on human health. The results have potential to uncover novel mechanisms of bacterial gene function regulation across the entire microbiota, rather than in individual bacterial isolates as has been studied to date.

Workplan

Like eukaryotic genomes, bacterial genomes are subject to epigenetic modifications, specifically DNA methylation. Bacterial methylation (BACMETH) was identified in the 1960's and has historically been associated with bacterial DNA restriction-modification systems, which protect bacterial cells from foreign DNAs. More recently DNA methylation has been shown to play important roles in multiple aspects of bacterial biology, including protection against phages, timing of DNA replication, transposition and conjugal transfer of plasmids, DNA repair and cell partitioning, genome stability, and regulation of gene expression. Bacterial DNA methylation includes 3 types of modifications - N6-methyladenine (6mA), 5-methylcytosine (5mC), and N4-methylcytosine (4mC), of which 6mA is most prevalent and has been linked to gene expression regulation. Genome-wide changes in BACMETH of individual bacterial isolates have been observed in response to changes in nutrient content of growth medium, and our data in oral pathogens align with these results (Costeira et al. 2023, mSystems).

Recent developments in long-read sequencing allow for detection of multiple DNA methylation base modifications across the bacterial genome, using for example, Oxford Nanopore Technology (ONT) sequencing. However, to date only two studies have explored DNA methylation in the vertebrate gut microbiota in 2 infant human gut microbiota samples.

As part of ERC/EPSRC funded efforts we are undertaking novel characterization of BACMETH in the adult human gut microbiota. The proposed PhD project will integrate within this framework to specifically explore how host environmental exposures, such as medication use, relate to bacterial methylation in the human gut.

Year 1-2. Compare methods to quantify bacterial methylation and apply these to data from the TwinsUK cohort.

We will apply and compare multiple analytical pipelines to quantify BACMETH profiles of candidate taxa in the human gut microbiome based on ONT sequencing data. Once a consensus pipeline is reached, this will be applied to ONT data from TwinsUK cohort samples. The aim will be to characterise variability in BACMETH of candidate taxa in TwinsUK.

Year 2-3. Explore if host lifestyle and environmental factors, such as medication use, leave a signature on gut BACMETH.

The project will explore existing data on host lifestyle and environmental exposures, such as smoking, exercise, and medication use in TwinsUK. We will carry out large-scale association analyses to identify links between candidate gut BACMETH signals and host environmental factors such as medication use.

Year 3-4. Test whether bacterial methylation signatures are associated with microbially derived metabolite levels; PhD project write-up.

Candidate BACMETH signals will be compared to select microbially derived metabolites in stool and plasma samples from TwinsUK cohort participants. The aim is to identify potential functional read-outs of BACMETH.

The proposed PhD project will focus on analysis of bacterial DNA sequencing data from human stool samples from TwinsUK. The cohort is one of the most deeply phenotyped studies in the world, with multiple layers of existing biological data spanning host genetic, epigenetic, transcriptomics, metabolomic, and proteomic data, as well as gut metagenome profiles. The project will be computational, based on the analysis of novel and existing datasets.

Project Reference: MM4L14

Multi-omics modelling to understand the genetic and molecular mechanisms of Type 2 Diabetes

Co-Supervisor 1: Kerrin Small, Twin Research & Genetic Epidemiology

Field of Expertise: Molecular Biology; Bioinformatics

Co-Supervisor 2: Oliver Pain, Basic and Clinical Neuroscience

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Objectives

Type 2 Diabetes (T2D) and obesity-related traits are global epidemics. In the UK alone, ~4 million people are living with diabetes and 10% of the NHS budget is spent on diabetes. A limitation to early detection and treatment of T2D is its diverse clinical presentation and response to treatment. Improved methods for stratification of patients would have a large clinical impact.

Genome wide association studies (GWAS) have identified genetic variants associated with T2D, and there is much excitement in using Polygenetic Scores (PGS) to predict risk of disease from genetic data, and to identify the best course of treatment for individuals once they develop disease. Several studies have identified clinical subtypes of T2D that differ in their clinical presentation and underlying genetic signals, and there is evidence these subtypes act via molecular processes in different tissues. A key tissue for several subtypes is adipose tissue (fat), a dynamic endocrine organ.

This project will model molecular processes in human adipose tissue via multiple layers of molecular data from the TwinsUK cohort (transcriptomics, epigenetics, metabolites). It will integrate these molecular models with genetic risk of T2D to identify key molecular processes underlying disease development, and their impact on clinical symptoms.

Workplan

This project will leverage deep 'omic data derived from adipose tissue and clinical data from large biobanks to model molecular process in human adipose tissue, and use these models 1) to identify the molecular signatures of T2D subtypes in this key metabolic tissue and 2) to determine if the predictive utility of PGS are increased by joint modelling of genetic and tissue relevant 'omic data. It will involve both analyses of large datasets and exploration of new modelling techniques.

Year 1: In order to identify genes mediating risk of Type 2 Diabetes in adipose tissue we will perform Transcriptome-Wide Associations Studies (TWAS) in large biobanks, such as UK Biobank. We will examine each T2D subtype separately to determine if different genes mediate the each subtype. Given the high sex-specificity of gene expression in adipose tissue, we will run models incorporating male and female specific adipose gene expression signals. This aim will use established TWAS methods to impute the adipose transcriptome into biobanks by leveraging a new dataset describing the genetic regulation of gene expression in 2,400 adipose tissue samples generated by Co-Supervisor Small.

Year 2: Evaluation of new computational methods integrating genetic risk of Type 2 Diabetes and 'omics data. The unique molecular insights provided by large-scale TWAS can be leveraged in combination with high-resolution individual-level transcriptomic data to calculate Polytranscriptomic Scores (PTS). Recent research has demonstrated PTS can enhance prediction of disease risk and clinical characteristics. We will develop novel approaches for the calculation of PTS models and evaluate their predictive utility for T2D and obesity-related traits.

Year 3: We will extend the methods from Year 1 and 2 into additional layers of adipose molecular 'omic data, including epigenetics and metabolomic data collected in the TwinsUK cohort. We will model

each ‘omic individually, and then jointly in a multi-omic framework. This aim will expand our understanding of the molecular networks that mediate the genetic risk of T2D and its subtypes.

Project Reference: MM4L15

Modelling the dynamics of gene regulatory networks controlling lineage dependent patterned cell death within developing neural circuits in Drosophila

Co-Supervisor 1: Darren Williams, Developmental Neurobiology

Field of Expertise: Animal Modelling; Development; Stem Cell Biology; Molecular Biology

Co-Supervisor 2: Alessia Annibale, Mathematics

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Objectives

Nervous systems contain a diversity of cell types intricately organised into complex functional networks. During neurogenesis developmental processes ensure that specific neuronal subtypes are generated in appropriate numbers at the right locations.

Within insect nervous systems segmentally repeated populations of 60 neural precursor cells, called neuroblasts generate the majority of neurons in the CNS. These uniquely identifiable neuroblasts divide repeatedly producing pairs of neurons, we call A and B cells. Remarkably precise 'neuroblast specific' fate-based decisions control whether the A or B neuron dies.

This patterning dictates the number of specific neuronal cell types available for building circuits and determines segment-specific and sex-specific differences in circuitry as well as evolutionary novelties in other species. This precisely patterned fate, of cell death, is controlled by two genes called reaper and grim, which are under complex transcriptional control.

Our project aims at building a mathematical model to describe the gene regulatory networks underlying these precise lineage-specific deaths, reveal key nodes within the network and then test them using sophisticated in vivo Drosophila genetics. Our goal is to uncover the 'transcriptional logic' of cell death and ultimately apply this model to other species where different patterns of cell death generate species-specific circuit motifs.

Workplan

1. The student will perform analysis of RNAseq data from doomed and non-doomed neuronal populations along with ATAC-seq and CUT&RUN chromatin profiling from the cis-regulatory regions of Reaper and Grim, the two proapoptotic genes we know to be responsible for lineage-specific cell death.

2. Machine learning tools will be used to infer gene regulatory networks that are death specific in different neuroblast lineages within the insect nervous system.

3. Non-equilibrium statistical mechanical techniques will be used to analyse the dynamics of gene expression on such network models. This mathematical modelling will allow us to predict key nodes within gene regulatory networks responsible for driving cells towards death.

4. Predicted molecular key nodes will then be experimentally tested. This workflow will include mosaic clonal loss of function experiments removing candidate transcription factors and determine changes in expression levels of Reaper/Grim and death in vivo. Alongside this CRISPR/Cas9 will be uswed to remove binding sites in Reaper and Grim locus determine changes in expression levels and death in vivo.

5. The data will then be used for further refinement of the mathematical model and tested with application to RNASeq from a closely related wingless species where patterns of PCD are different. https://elifesciences.org/articles/59566

Project Reference: MM4L16

A multi-omic study of Clonal Haematopoiesis-derived leukocytes post-myocardial infarction using a patient-derived xenograft model

Co-Supervisor 1:	Daniel Bromage , School of Cardiovascular and Metabolic Medicine & Sciences
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Co-Supervisor 2:	Lynn Quek, Comprehensive Cancer Centre
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Objectives

Myocardial infarction (MI) elicits a dysregulated inflammatory response that exacerbates cardiomyocyte loss, maladaptive left ventricular (LV) remodelling, and the development of heart failure (HF). Post-MI inflammation activates tissue repair but excessive inflammation exacerbates aberrant remodelling.

Clonal haematopoiesis (CH) is a novel risk factor for CV disease. Individuals acquire mutations in blood cells, including immune cells, without apparent blood disorder. Multiple large studies have identified CH as a public health risk as it is associated with increased risk of HF and death, possibly by altering the inflammatory functions of myeloid cells.

We have shown that monocytes from patients with CH, particularly those with DNMT3A mutations, are common in patients with MI. Recovery of LV ejection fraction (LVEF) was significantly worse in these DNMT3A-mutant individuals, which skew monocytes towards a pro-inflammatory phenotype. Compared to non-CH monocytes, genes associated with inflammation were upregulated, while antigen presentation and interferon responses were downregulated.

We want to study how CH affects ventricular remodelling by developing and characterising a murine xenograft MI model using CH patient-derived monocytes. Using this model, we will investigate cell-cell interactions between CH cells and tissue-resident macrophage populations, which are spatially heterogenous and known to regulate the cardiac response to MI.

Workplan

Hypotheses:

i) CH patient-derived monocytes migrate to infarcted myocardium and promote exaggerated inflammation;
ii) Inflammation varies depending on mutations and spatially restricted resident cells;
iii) CH monocytes worsen maladaptive ventricular remodelling and HF compared with non-CHIP monocytes in xenograft models.

Our research aims are:

Aim 1: Generate and characterise a CH patient-derived murine xenograft MI model.

To generate a humanised xenograft model, haematopoietic stem progenitor cells (HSPCs) from CH and non-CH individuals will be injected into NBSGW/NSG-SGM3 immunodeficient mice. Upon engraftment, mice will have circulating mutant human leukocytes, including monocytes detectable by flow cytometry (FACS). We can also detect mutations by PCR in mouse blood. Upon stable engraftment, the student will compare baseline cardiac dimensions and function using

echocardiography (LV end diastolic volume, global longitudinal strain, and LVEF) in CH and non-CH mice.

Aim 2: Multi-omic characterisation of extent and quality of the inflammatory response after MI in xenografted mice.

To assess the post-MI inflammatory response and interactions of CH cells with tissue-resident populations, xenografted mice will be subjected to left coronary artery (LCA) ligation. To reveal temporal dynamics of inflammation, FACS on myocardial digests will quantify and phenotype myeloid cells in the infarct zone at 1, 3 (inflammatory phase) and 7 days (proliferative phase) post-MI. As CH skews monocytes towards an inflammatory, classical phenotype, we expect a more cellular and prolonged inflammatory response in CH mice. To resolve interactions between infiltrating CH cells with tissue-resident cells and spatially restricted effects, we will use cutting-edge, spatially resolved RNA-sequencing (Curio Seeker). The student will be trained in computational analysis of these data using established pipelines, with support of the Hub for Applied Bioinformatics (PI: Alessandra Vigilante).

Mutant monocytes from CH patients may influence 'bystander' non-mutant cells through aberrant cytokine production. The student will analyse collagenase-digested cardiac tissue of CH and non-CH mice using ELISA for IL-1b, IL-6 and CXCL2 (implicated in our previous experiments), and any other significant cytokines from spatial RNA-seq experiments.

Aim 3: Assess the impact of CH-derived monocytes on ventricular remodelling and heart failure.

To determine the functional significance of CH monocytes, the student will characterise ventricular remodelling, comparing LCA ligated or sham, CH and non-CH-engrafted mice. Cardiac function can be studied using 3D-speckle tracking echocardiography at 1 week (for early changes) and 4 weeks (the standard to observe remodelling after murine MI). After 4 weeks, excised hearts will be examined for fibrosis and remodelling markers. As mice with CH monocytes may have worse maladaptive ventricular remodelling, they will compare CH and non-CH mice after re-perfused infarction or sham to assess potential differences in infarct size. If time permits, we aim to pursue cardiac organoid models available via our collaborators in the BHF Centre of Research Excellence, to serve as a high-throughput method for targeting the novel pathways and cell-cell interactions we identify.

This project will provide invaluable knowledge on CH-mediated dysregulation of post-MI inflammation and identify potential therapeutic targets. The long-term vision is to investigate novel anti-inflammatory drugs to prevent progression to heart failure after MI.

Project Reference: MM4L17

Modelling gene regulatory network dynamics during lineage reprogramming of glia into neurons

Co-Supervisor 1: Benedikt Berninger, Developmental Neurobiology

Field of Expertise: Animal Modelling; In Vitro Modelling; Development; Stem Cell Biology;

Co-Supervisor 2: Alessia Annibale, Mathematics

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Objectives

Transcription factors can trigger the conversion of brain glia such as astrocytes into neurons, opening new avenues towards brain repair. When successful, this process appears to involve intermediate states resembling those found in natural neurogenesis. However, the process can go awry resulting in failed reprogramming. This suggests that the conversion process requires specific dynamics in order to lead to successful fate conversion.

The present project aims at developing a mathematical model describing the molecular trajectory of glial cells undergoing transcription factor-induced reprogramming into neurons. The specific goal is to determine the gene regulatory networks that define distinct stages along the trajectory and mathematically model their temporal dynamics. This in turn will lead to prediction of key nodes underlying network dynamics which will be tested by perturbation studies in experimental in vitro and in vivo models of glia-to-neuron reprogramming. Conversely, identification of these critical nodes will help determining molecular roadblocks that result in abortive reprogramming and predict molecular strategies how to overcome these roadblocks.

Workplan

1. The student will perform analysis of single cell OMICS data sets (single cell transcriptomics and chromatin accessibility) data of in vitro and in vivo lineage conversion of mouse astrocytes into induced neurons following forced expression of proneural transcription factors such as Ascl1 and Neurog2.

2. Machine learning tools (e.g. autoencoders) will be used to infer gene regulatory networks that are able to mimic the conversion process.

3. In turn, non-equilibrium statistical mechanical techniques will be used to analyse the dynamics of gene expression on such network models. This mathematical modelling will allow us to predict molecular key nodes in gene regulatory networks responsible for network dynamics and thus successful as well as abortive reprogramming.

4. Predicted molecular key nodes will then be experimentally validated by perturbation experiments of glia-to-neuron reprogramming by CRISPR/Cas9 or RNA short hairpin technology in vitro cultures of mouse astrocytes and glia-to-neuron conversion in the mouse brain in vivo.

5. This may allow for 2nd generation of single cell OMICs data (i.e., scRNA-seq and ATAC-seq following molecular perturbation).

6. The data will then be used for further refinement of the mathematical model.

Project Reference: MM4L18

Combining modelling and in vivo approaches to understand cell fate decisions in the nervous system

Co-Supervisor 1: Andrea Streit, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Development

Co-Supervisor 2: Alessia Annibale, Mathematics

Field of Expertise: Computational Modelling; Mathematics

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Objectives

The nervous system is arguably the most complex organ in our body with thousands of different cell types with different functions. Yet, during embryonic development, it arises from only three distinct cell populations. The neural plate gives rise to the central nervous system, while neural crest cells generate the peripheral nervous system along the entire body axis and placodal cells the sensory nervous system in the head. All three populations come from a pool of common progenitors that is set aside early in development. How do these progenitors become different from each other and acquire their unique identities?

Ultimately, the identity of a cell is defined by the genes it expresses. While the genes are critical to determine a cell's functional properties, non-coding regulatory regions are key to control where and when these genes are expressed. Thus, to understand the molecular mechanisms involved, we must integrate both gene expression and their control.

The objectives of this project are:

i) to use mathematical modelling to predict key regulators of cell fate choice based on existing large data sets;
ii) design experiments to test these predictions in vivo, and feedback these results into the model.

The project will provide new mechanistic insight into how cell identity is established in the nervous system. It will equip the student with an interdisciplinary skill set to answer fundamental questions in biology.

Workplan

This project aims to understand how neural, neural crest and placodal cells diversify. To begin to address this we have surveyed how gene expression changes from progenitor to definitive neural, neural crest and placodal cells at single cell level and identified the non-coding regulatory regions that control these changes. Now, we will combine mathematical modelling and in vivo experiments to explore the underlying gene regulatory networks and molecular mechanisms.

The first aim is to use a combination of statistical mechanical and machine learning techniques to predict a gene hierarchy as cells diversify, link transcription factors and their targets and predict how these factors drive cell fate choice.

The second aim is to design functional experiments in the chick embryo to test predictions and probe the molecular events, as well as improving the predictions made by modelling.

Together, this project will provide new understanding of the fundamental principles that govern cell fate choices. However, similar principles also lie at the heart of understanding homeostasis, disease, regeneration and repair. Our findings will therefore have a much wider impact.

The project will be co-supervised by Alessia Annibale, an expert in complex systems modelling, Grace Lu, an expert in bioinformatics of gene regulatory networks, and Andrea Streit, an expert in development of the nervous system and cell fate decisions. Throughout the project, the student will be exposed to an interdisciplinary research environment taking advantage of this supervisory team with cross-disciplinary expertise. They will be trained in different approaches for network modelling, in experimental design and development and experimental approaches to test model predictions.

Project Reference: MM4L20

Multiscale modelling of neural crest cell behaviours in development and cancer

Co-Supervisor 1: Karen Liu, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Animal Modelling

Co-Supervisor 2: Rivka Isaacson, Chemistry

Field of Expertise: Molecular Biology

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Objectives

Neural crest cells are highly migratory: they travel throughout the developing embryo, eventually contributing to most organ systems and must readily adapt to dynamic changes in their microenvironment. Post-translational modifications such as phosphorylation mediate these responses through rapid and reversible control of protein activity, binding, and subcellular localisation. This project aims to decipher the mechanisms by which phosphorylation events by an important enzyme, glycogen synthase kinase 3 (GSK3) influence neural crest cell behaviours during embryo development and how they are misregulated in cancer. The host laboratories and key collaborators offer a wide range of research skills including microscopy, cell biology, biophysics and mathematical modelling. We will use an integrated combination of these techniques to identify and characterise key substrates of GSK3 at the organism, tissue, cell and molecular levels, with a particular emphasis on exploring the interaction of GSK3 with Anaplastic Lymphoma Kinase (ALK), which hyperactivates GSK3 in cancer settings.

Workplan

During the first 9 months the student will receive training from the Liu lab in tissue culture of neural crest cells and associated assays, and from the Isaacson lab on recombinant protein expression and purification to optimise production of GSK3 and ALK variants. This will set them on their way to a good set of results for the upgrade. Between 9 and 24 months they will establish proteomic profiling of GSK-3α/β in mammalian neural crest cells and use biophysics (microscale thermophoresis/isothermal titration calorimetry) and biochemistry (pull-downs) methods to determine whether ALK directly or indirectly regulates GSK-3 isomers. Subsequently, using tissue and cell methods they will build up a networked picture of interactions and phosphorylation events. This will be verified and iteratively explored at the molecular level and, through collaboration with the group of Izaak Neri in the disordered systems group in maths, will feed into a tuneable mathematical model of GSK3 signalling. In order to establish competence in the techniques the student will initially receive in person guidance for 100% of their laboratory time which will be decreased gradually. The objective is to allow the student to learn from errors and become skilled users capable of independent operation and troubleshooting. From 18 months onwards the student will continue to receive support and guidance with particular emphasis on experimental design and interpretation, dividing their time between Liu and Isaacson labs depending on their results and the directions the project takes which will be constantly monitored through lab meetings and thesis committee meetings. At such time as it is required the student will also receive training in NMR/EPR spectroscopy and X-ray crystallography from the Isaacson lab, and extensive data processing training for EM allowing them to model data at high resolution. The object is to provide sufficient room so that the student can benefit from guiding their own PhD but to provide support on an ad hoc basis. We will encourage continual writing up of results, hopefully for papers in addition to the upgrade report so that the thesis is not a panic at the end. At the start of the final year we will formulate a plan for the thesis and remaining experimental work and we will reserve the last three months solely for thesis writing.

Project Reference: MM4L22

Cancer neuroscience: probing brain – tumour interactions in live human brain tissue

Co-Supervisor 1: Gerald Finnerty, Basic and Clinical Neuroscience

Field of Expertise: Neuroscience; electrophysiology; imaging

Co-Supervisor 2: Graeme Stasiuk, Imaging Chemistry & Biology

Field of Expertise: molecular imaging; chemistry

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Objectives

Gliomas are the commonest type of tumour that start in the brain. Their prognosis is extremely poor. A major reason is that gliomas are very invasive. As a result, the current treatment, which involves brain surgery to debulk the tumour followed by radiotherapy and chemotherapy, does not extend life dramatically. New treatments are desperately needed.

Gliomas rarely spread outside the central nervous system. This suggests that there is something special about the brain environment for gliomas.

We aim to develop glioma models that give deep insights into how gliomas interact with the brain. The student will make glioma cells fluorescent with probes conjugated to quantum dots that bind to glioma specific markers on the cell surface, e.g. mutant EGFR. This will enable the student to study how the tumour cells grow and spread in brain tissue with live cell longitudinal imaging in either ex vivo "live" human brain tissue or in a rodent glioma model. Neural activity in the peritumoural cortex will be recorded electrophysiologically to determine whether brain activity affects growth of brain tumours. Finally, the student will manipulate the tumour microenvironment with existing or novel therapies to investigate their effects on glioma growth and spread.

Workplan

Year 1. Learn how to make quantum dot (QD) fluorophores emitting in the visible and NIR range and to evaluate functionalised QDs in a murine glioma model.

i) QDs

- synthesise fluorescent QDs based on InP/ZnS and Ag2S) via different methods, hot injection and microwave methodology. The nanomaterial will be coated in various ligands to prevent serum proteins from associating.
- functionalise QDs with a targeting motif for glioblastoma to create the fluorescent agent
- evaluate functionalised QDs in glioblastoma cell lines using FACS, MTS assays and confocal microscopy to show specific binding to the surface proteins on the cell. Test whether there is internalisation into the cell and if the agent has any toxic effects on the cell.
- Validate functionalised QDs on non-tumour cell lines.

ii) murine glioma model

- undertake all the Home Office training courses (module 1-4) and obtain a personal licence to handle animals and undertake the experiments.
- learn how to run preclinical IVIS scanner
- evaluate functionalised QDs in subcutaneous glioblastoma tumour models and measure uptake of the agent, biodistribution and pharmacokinetics.

Year 2: Learn how to collect blocks of live human tissue, prepare human brain tissue slices.

- The student will be trained in the Human Tissue Act and the provisions around it
- Learn how to collect blocks of live human brain tissue from the neurosurgical operationg theatre
- Incubate human brain tissue slice with quantum conjugated probes and perform live cell fluorescence imaging.
- Learn image acquisition and analysis of migrating tumour cells.
- Learn extracellular electrophysiological recording and quantification of brain activity.

Year 3: Manipulation of glioma cell growth and spread

- Use established therapies or novel agents to alter the growth and spread of glioma cells in murine glioma model and ex vivo live human brain tissue.

Project Reference: MM4L24

The Role of Mitochondria in Neurodegeneration Across Regions and Cell Types of the Human Brain

Co-Supervisor 1:	Alan Hodgkinson, Medical & Molecular Genetics
Field of Expertise:	Bioinformatics; Molecular Biology
Co-Supervisor 2:	Alfredo Iacoangeli, Biostatistics & Health Informatics
Field of Expertise:	Computational Modelling; Machine Learning
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Objectives

Human mitochondria operate system-wide to regulate key biological processes. Consequently, mitochondria have been implicated in a wide range of common diseases, particularly those that occur in high energy tissues such as the brain. However, it is often not known whether altered mitochondrial function occurs because of changing cellular environments that are driven by disease states, or whether mitochondrial dysfunction forms part of the causal pathway of the disease itself. In this project we will identify whether tissue and cell type-specific mitochondrial transcriptional processes play a causal role in neurological disorders such as Parkinson's Disease and ALS. To do this, well will utilise large quantities of gene expression data from interconnected regions and cell types of human brains to computationally model the genetic mechanisms that contribute to variation in mitochondrial transcriptional processes using complex machine learning approaches, before integrating protein and metabolite data to validate downstream impacts on mitochondrial function. Validated models will then be applied to large-scale independent datasets (such as UK Biobank) to identify which mitochondrial processes are casually associated with disease. This is important to understand, as it will allow a better focus on the biological pathways that could be targeted therapeutically to reduce disease risk.

The project will make use of existing large scale omics datasets to model the relationship between genetic data, mitochondrial transcriptional processes, and downstream functional outputs identified from protein and metabolite data from different regions and cell types of the human brain.

Aim 1: Identify genetic/molecular changes in the mitochondrial and nuclear genomes that influence key mitochondrial processes across cell types and study how they affect tissue functions. This will be achieved through processing bulk and single-cell RNA sequencing data from human samples of multiple brain regions and cell types, to extract mitochondrial transcriptional features, before identifying genetic mechanisms associated with variation in these events.

Aim 2: Based on the identified genetic/molecular signatures from aim 1, machine learning approaches will be designed and implemented to build models that are able to predict mitochondrial processes from genetic data alone. These models will then be tested in independent datasets to confirm their validity and to assess downstream biological implications using protein and metabolite data.

Aim 3: Validated models will then be used to impute mitochondrial processes into massive population and disease cohorts, such as UK Biobank (incorporating half a million individuals), and imputed values will then be compared to a range of neurological disorders and quantitative phenotypes that are associated with disease. In this way, we will identify mitochondrial processes that are casual of disease, which could then be targeted therapeutically to modulate disease risk.

Project Reference: MM4L25

Modelling the effect of genetic instability on cancer progression across scales

Co-Supervisor 1:	Eugene Makeyev, Centre fro Developmental Neurobiology, IoPPN
Field of Expertise:	In Vitro Modelling; Bioinformatics; Computational Modelling; Molecular Biology;
Co-Supervisor 2:	Claire Wells, Comprehensive Cancer Centre, Faculty of Life Sciences & Medicine
Field of Expertise:	Stem Cell Biology; Translational Medicine; Cancer Biology; In Vitro Modelling;
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Objectives

Cancer cells tend to accumulate mutations, increasing tumour heterogeneity and complicating therapeutic interventions. Such genetic instability is frequently observed in cancers with compromised homology-directed repair (HDR) of damaged DNA, including breast, ovarian, prostate, and pancreatic cancers. Previously, we identified a long non-coding RNA, PNCTR, which is significantly upregulated in many advanced and metastatic cancers (https://doi.org/10.1016/j.molcel.2018.08.041). We demonstrated that PNCTR promotes cancer cell survival by sequestering the RNA-binding protein PTBP1 within the membraneless perinucleolar compartment (https://doi.org/10.1080/19491034.2024.2306777).

Our recent data indicate that elevated PNCTR expression in cancer cells is associated with a genetic rearrangement of its locus, which correlates with defects in the HDR pathway. Notably, many low-grade breast cancers express PNCTR in a binary manner, with some cells expressing high levels of this RNA, while other cells within the same tumour express none. Combined with inter-tumour differences in PNCTR expression, this raises the intriguing possibility that the PNCTR-encoding DNA sequence is a hotspot of genetic instability, which may contribute to cancer progression and be leveraged for new therapies and diagnostics.

Here, we propose to develop and characterise cancer heterogeneity models by systematically analysing PNCTR expression in human cancers in vivo and modulating its expression in cancer cell and organoids in vitro.

Workplan

Building on our multidisciplinary expertise in bioinformatics, genomics, mammalian genetics, cancer biology, organoid cultures, and translational medicine (e.g., https://doi.org/10.1016/j.molcel.2018.08.041; https://doi.org/10.7554/eLife.99040.1; https://doi.org/10.1083/jcb.201501072; https://doi.org/10.1016/j.cellsig.2024.111233), we will pursue two strategic directions:

1. Understanding the impact of PNCTR locus instability in vivo (PhD year 1)

To examine the role of genetic instability in PNCTR biology at the in-vivo tissue scale, we will analyse RNA- and DNA-sequencing data from cancer databases (e.g., https://portal.gdc.cancer.gov), assessing PNCTR RNA abundance, locus structure, and potential alterations in HDR and other cancer-linked genes. We will focus on breast cancers, given their high incidence of HDR defects (e.g., BRCA1/BRCA2

mutations) and prevalence in the UK (56.8K diagnoses and 11.5K deaths annually; https://www.cancerresearchuk.org).

PNCTR expression differences across cells within the same tumour will be investigated by fluorescence in situ hybridisation (FISH) of tissue samples from King's Breast Cancer Biobank. Since the perinucleolar compartment has been linked to metastases (https://doi.org/10.1080/19491034.2024.2306777), we will immunostain the samples for relevant protein markers. We will additionally investigate possible correlation between PNCTR expression and T-cell infiltration and tissue rearrangement using immune and stromal/ECM markers. Targeted short- and long-read sequencing will allow us to link PNCTR tissue expression with its locus rearrangements.

Systematic analysis of this data, alongside clinical attributes (e.g., cancer stage, BRCA1/BRCA2 status, hormonal type, demographics, treatment history, and survival rates), will illuminate the mechanisms behind PNCTR activation in cancer. Importantly, the new data will help us enhance current computational models for breast cancer recurrence prediction (e.g., https://doi.org/10.1186/s12911-023-02377-z), providing a clear pathway to medical translation.

2. Modelling PNCTR locus instability in vitro (PhD years 2-3)

Our preliminary data indicate that tumours acquire PNCTR-expressing cells through error-prone resealing of DNA double-strand breaks by non-homologous end-joining. To model this at the in-vitro cellular scale, we will introduce DNA breaks in PNCTR-negative BRCA1/BRCA2-wild-type breast cancer cells using CRISPR/Cas reagents designed based on our in-vivo data. PNCTR expression and the structure of its DNA locus will be then analysed by FISH, quantitative PCR, and sequencing. Further mechanistic insights will be obtained using inhibitors of different branches of the non-homologous end-joining pathway.

Since PNCTR is expressed in larger fractions of cells in advanced/metastatic compared to low-grade tumours, we hypothesise that PNCTR-expressing clones expand as disease progresses, due to an enhanced ability to survive and/or metastasise. As a bridge between the cellular and tissue scales, we will test this hypothesis in breast cancer organoids retrofitted with an optogenetically inducible PNCTR locus. Inducing PNCTR in subsets of cells, using photoactivatable CRISPR/Cas or Cre/Lox systems, will enable us to monitor temporal changes in organoid composition and compare the metastatic potential of PNCTR-positive and -negative cells within the same organoid using appropriate invasion and migration assays.

This direction will refine our understanding of the mechanisms and functional consequences of PNCTR expression in cancer and provide a valuable resource for future anticancer drug screens.

Outlook

PhD Year 3-3.5 will focus on completing experiments and thesis preparation. Overall, this project will immerse the student in cutting-edge biomedical research, equipping them with expertise in advanced computational and experimental approaches.

Project Reference: MM4L26

Molecular changes affecting muscle stem cell behaviour in ageing: a combined computational and experimental approach

Co-Supervisor 1:	Robert Knight, Centre for Craniofacial and Regenerative Biology
Field of Expertise:	Animal Modelling; Molecular Biology; Development; Stem Cell Biology;
Co-Supervisor 2:	Steffen Zschaler, Informatics (NMES)
Field of Expertise:	Computational Modelling; domain specific computational languages;
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Objectives

The goal of this project is to identify age-related changes that affect the function of muscle stem cells (muSCs). Specifically, we aim to relate age-associated molecular changes to altered muSC behaviour in vivo and identify potentially causal factors for ineffective muSC function in ageing. This will be achieved by combining in silico and in vivo experimentation to create simulations of muSCs, predict how ageing affects their function, them experimentally test this.

Workplan

Muscle stem cells (muSCs) are critical for regeneration and show a dynamic migratory response to tissue injury. How muSCs interpret signals from their local environment and other cell types to make decisions about migration, proliferation and differentiation is poorly understood. To determine how muSCs integrate signals to make fate decision will be investigated using a combination of in vivo imaging in a zebrafish model of tissue repair, molecular profiling and computational modelling. Bioactive molecules predicted to regulate muSC responses will be tested using pharmacological and transgenic tools and outputs compared against computational models to identify age-associated changes.

The programme of work will be performed as follows:

WP1: Use the zebrafish telomerase mutant to model ageing to relate age-associated molecular changes to altered muSC behaviour in vivo and identify potentially causal factors for ineffective muSC function in ageing.
WP2: modify a flexible computer model with a user-friendly interface to simulate and study cell behaviour dynamics for muSCs. Explore how well the model can simulate cell-shape feedback and cell-cell interaction feedback in response to altered cell signalling. This model will be used to make predictions about how changes to signalling molecules affect cell behaviour.
WP3: Use gene expression profiles of muSCs and muscle obtained from telomerase mutants to predict which molecular changes are occurring in ageing tissues. Using predictions from the computer model the aim is to test how novel candidate ageing-modifying molecules affect muSC function in vivo using pharmacological, transgenic, and CRISPR/Cas9 manipulation in zebrafish and capturing cell responses by live cell imaging.

Supervisor 1 (Robert Knight) will provide expertise in live cell imaging, molecular manipulation and use of zebrafish as a model organism.
Supervisor 2 (Steffen Zschaler) will oversee training in using and modifying a computational model for cell behaviour.

Project Reference: MM4L27

Structural homeostasis in developing tissues - holistic regulation of growth and death

Co-Supervisor 1: Richard Wingate, Centre for Developmental Neirobiology

Field of Expertise: Development;

Co-Supervisor 2: Jack Lee, Biomedical Engineering and Imaging Science, FoLSM

Field of Expertise: Computational Modelling; Biomaterials; Animal Modelling;

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Objectives

Our project seeks to understand how intracellular structural organisation translates into large scale tissue growth. This will be achieved by constructing and testing a series of computational predictive models of tissue growth based on in vivo imaging of the developing zebrafish. We will take advantage of a model system where intracellular proteins associated with cilia, centromeres, cytoskeleton, cell interfaces and filopodia can be imaged across the entirety of an intact living tissue. These events take place in a single-cell thick sheet of epithelial tissue that seals the internal cavity of the developing brain – the roof plate of the fourth ventricle. This unique model allows cellular processes to be correlated with overall changes in shape and tissue integrity under normal and experimental conditions.

To achieve this overall aim, we will 1) develop machine learning algorithms that can automatically segment time-lapse 4D movies of developing tissue. 2) analyse intracellular organisation in the context of patterns of cell division and extrusion (death), 3) generate computer models that faithfully capture cell behaviour over multiple scales (intracellular events to tissue shape). 4) Test the validity of models using a range of experimental in vivo approaches to manipulate the mechanical constraints on growth in zebrafish.

Workplan

The roof plate must shrink and stretch in response to the brain's growth. The outstanding advantage of our model is that we can image the entire organ including its boundaries enabling us to unite events across biological scale in an intact system. Using confocal microscopy we can monitor intracellular events, cell movement and tissue conformation in an intact in vivo system. The data we extract will feed new models of epithelial growth across scales.

The workplan comprise parallel and complementary in vivo and in silico approaches. We have extensive preliminary data that gives us confidence that the aims are achievable through a 36 month workplan with clear contingency for experimental setbacks.

There are four work packages. 1 and 2 will be completed in the first 12 months. 3 and 4 will be completed in the subsequent 8 months, leaving 4months for writing and 6 months for additional work as arises and contingency.

1. Timelapse recordings of normal zebrafish development. We will use our validated approaches to film intracellular and cellular events in the context of an intact roofplate. We will exploit a number of existing transgenic lines where intracellular proteins have been labelled with fluorescent markers to monitor cell events at high resolution.

2. Automated segmentation algorithms will be developed in parallel to process 4D movies to extract fluorescent signal with minimal background. Our model tissue is near optimal for manual

segmentation and will allow a rapid training and development of machine learning tools to extract data from time lapse recordings. Currently, cell size, centroids, shape parameters and boundaries are extracted frame-by-frame. Out tools will allow rapid processing of large data sets and the automated generation of data on cell shape and movement.

3. Computer models of cell behaviour will be constructed using the data extracted from normal development. We are particularly interested in modelling how tissue tension might regulate intracellular events. We propose to apply structural engineering modelling tools based on graphic statics to frame cellular processes within the holistic forces applied across a model surface.

4. Experimental manipulation of zebrafish development will allow us to test the validity of the predictive power of our models. This will be achieved by two contrasting approaches. Firstly, we will use genetic mutants in which the boundaries of the roofplate tissue have been manipulated to predict how geometry impacts on the regional distribution of cellular events such as intercalation, division and extrusion. Secondly, we will use direct mechanical manipulation to change the global parameters of growth. We have successfully trialled the use of agarose embedding over 6 hours to restrict the size of the developing zebrafish larva. We will also explore methods of applying discrete focal forces to the roof plate sheet of cells to marry predicted changes in cell behaviour form our model with observed events in vivo.

Project Reference: MM4L28

Using human retinal organoids to study functional and structural development of amacrine cells

Co-Supervisor 1:	Robert Hindges, Centre for Dev. Neurobiology, IoPPN
Field of Expertise:	Molecular Biology; Development; Animal Modelling; In Vitro Modelling;
Co-Supervisor 2:	Rachael Pearson, Department of Medical & Molecular Genetics, FoLSM
Field of Expertise:	In Vitro Modelling ; Development; Stem Cell Biology; Biomaterials; Translational Medicine;
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Objectives

The retina is a multi-layered structure, enabling the capture and processing of visual information before transmitting the resulting signals to specific targets in the brain. Amacrine cells (ACs), located at the interface between bipolar (BCs) and retinal ganglion cells (RGCs), are crucial to split the visual input into feature-specific information channels, including the detection of oriented shapes. We previously have shown in zebrafish that this is dependent on asymmetric AC subtypes, marked by the synaptic cell adhesion molecule Tenm3. We further found that the dendritic shape directly influences AC functionality. However, it is not clear how this shape is generated during development, for example if this is depending on the adjacent embryonic tissues.
The Aim of this project is to generate human retinal organoids (hROs) to investigate the developmental mechanisms underlying the generation of these ACs in vitro, without the full complement of embryonic signals. We will determine the localisation of TENM3-positive cells in organoids and follow their laminar arrangement. Finally, by creating a stem cell line expressing a fluorescent protein driven by the TENM3 promoter, we will be able to assess the generation of AC dendritic structures in live organoids and compare this to the observed steps in zebrafish.

Workplan

The work contains several overlapping steps, as the generation of hROs takes considerable time. The student will take advantage of the ongoing hRO production by the Pearson group, which will allow some of the basic work already to be done in year 1. The Pearson lab has well-established protocols for the generation of laminated hROs closely recapitulating normal human retinal differentiation. Most of the work will be carried out in the brand new, state-of-the-art CDN organoid facility, with associated technical staff. This also includes financial and staff support for the MM4L student while generating hRO.

The work is generally divided in three major packages:
- Investigating the distribution of TENM3-positive ACs during human eye development
- Generating gene-edited human stem cell lines to fluorescently label TENM3-positive ACs.
- Generation of hROs allowing the investigation of AC neurogenesis.

Year1:
- Using in situ hybridisation, we will determine the distribution of Tenm3-expressing cells during human eye development, in addition to other AC markers we have identified in zebrafish. The human fetal tissue for this is available through the Human Developmental Biology Resource (HDBR) already used by the CDN. The results will be compared to our data from zebrafish/mouse and will result in a comparative description for the existence of different AC subtypes.

- Using genome editing, we will insert a fluorescent marker into the locus of TENM3, to monitor and follow the generation of TENM3-positive cells during the development of the human eye, modelled in hRO.
- Introduction to organoid culturing.

Year 2:
- Culturing of hRO. These will be harvested at different time points and used for marker analysis for different cell types, including (TENM3+) ACs. Distribution of cells will be compared to results from human retinal tissue, as well as zebrafish/mouse.
- Start of using gene-targeted iPSCs for generating hROs.

Year 3 and 4:
- Morphological and functional analysis of TENM3-positive ACs in organoids, including the assessment of dendritic field and arborisation patterns, and presence of pre- and postsynaptic structures.
- Assessing the functional activity patterns of generated hROs using an existing Multi Electrode Array device (MaxWell).

This central part of the work will result in novel and exciting data to describe the generation of different retinal subtypes in vitro, in comparison to the in vivo situation. It will further uncover if the dendritic structure of amacrine cells, which is crucial for the cellular function, can be generated in an organoid or if additional in vivo context is required. This will have profound consequences for the efforts to generate accurate, fully functional retinal tissue for the study of human retinal disease and for therapeutic purposes after eye damage through injury or disease.

Fallback options: Should the generation of hRO, including the use of targeted cell lines, prove too time consuming or inefficient, we will use mouse organoids (from ES cells) as alternative model. Such organoids develop significantly faster and are well-suited to answer our fundamental questions on amacrine cell development.

Project Reference: MM4L29

Multimodal imaging of tissue response to bacterial infection and treatment with synthetic neutrophils

Co-Supervisor 1:	Adrian Najer, Institute of Pharmaceutical Science/Faculty of Life Sciences & Medicine
Field of Expertise:	In Vitro Modelling; Molecular Biology; Biomaterials; Synthetic cells;
Co-Supervisor 2:	Mads Bergholt, Centre for Craniofacial & Regenerative Biology/Faculty of Dentistry, Oral & Craniofacial Sciences
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Objectives

The global health crisis of antibiotic resistance (1.27 million deaths in 2019) requires new solutions to counteract bacterial pathogens. This includes a need for innovative applications against globally relevant infectious diseases, such as those caused by Staphylococcus aureus or certain haemolytic Escherichia coli strains.

Bottom-up synthetic biology allows the creation of advanced therapeutic systems, e.g. synthetic cells, for controlled antibiotic delivery or exertion of bactericidal action without antibiotics. We propose to formulate "synthetic neutrophils" with improved antibacterial activity, whilst omitting undesired components of endogenous neutrophils. This project leverages membrane-damaging exotoxin action to locally trigger the release of artificial neutrophil extracellular traps (aNETosis) from synthetic neutrophils, which will attack the bacteria together with antimicrobial drugs and an antibacterial enzyme cascade (free or as nanoreactors).

We will design an analysis framework using fluorescence and label-free Raman-based imaging to evaluate loading homogeneity and release from synthetic cells. Collaboration with the group of Dr Vincenzo Torraca (Department of Infectious Disease; 3rd supervisor), who is developing zebrafish models for bacterial infections (including the abovementioned infection models), will enable analysis of synthetic cell and wound-healing response in vivo. Whole tissue response to infection and treatment will be visualised by fluorescence- and Raman-based imaging and analysis methodologies.

Workplan

Aim1a (Najer)
Synthetic neutrophils will be assembled by co-encapsulating a polymer as artificial NETs (anionic or cationic polymer) together with the two enzymes glucose oxidase (GOX) and MPO (free or as nanoreactors; https://pubs.acs.org/doi/full/10.1021/acsnano.0c07459) and conventional antibiotics (controls will omit individual components). Assembly techniques will involve spontaneous hydration and microfluidics to form giant liposomes, hybrids or copolymer-only (PMOXA-b-PDMS-b-PMOXA) giant vesicles (ca. 10-20 µm diameter). Use fluorescence imaging for routine synthetic cell visualisation. Employ electron microscopy techniques to study synthetic cell architecture in detail (at the Centre for Ultrastructural Imaging).

Aim1b (Najer)
Synthetic neutrophil activation and aNETosis will be tested by adding commercial bacterial toxins (https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.202200036). Use fluorescence spectroscopy,

imaging and fluorescence correlation spectroscopy (FCS) to detect aNETs formation efficacy. The cascade reaction of the two enzymes converts glucose to highly reactive –OCl, a potent antibacterial chemical species that can be detected with a fluorescent probe.

Aim2 (Bergholt)
Analyse synthetic neutrophils using confocal Raman imaging. Develop a deep learning framework for Raman image analysis to enable label-free quantification of polymer/drug loading across the synthetic cell population. Employ the developed analysis technique to assess synthetic cell homogeneity and quantify toxin-triggered release.

Aim3 (Torraca)
Test the synthetic neutrophils in vitro using bacterial cultures of S. aureus/haemolytic E. coli strains (using available clinical isolates derived from diabetic foot patients). Find the required bacterial density to trigger synthetic cell activity to inform subsequent in vivo experiments. Next, evaluate synthetic neutrophil action after microinjection in vivo using zebrafish infection models (https://www.cell.com/trends/cell-biology/fulltext/S0962-8924(17)30181-2). Visualise the function of the synthetic neutrophils and evaluate the survival of zebrafish larvae. Use fluorescent mutant zebrafish lines to study immune cell response, such as macrophage/neutrophil infiltration in the infected tissue, with and without synthetic neutrophil treatment.

Aim4 (Bergholt)
Use the Raman-based imaging and analysis framework for fixed zebrafish from Aim3 to characterise the tissue response in greater detail (https://www.nature.com/articles/s41467-020-19827-1). This label-free technique will allow spatial detection of synthetic neutrophil components, e.g. aNETs, as well as endogenous tissue responses that cause changes in DNA/protein/lipid distribution in vivo in zebrafish larvae. Develop further the deep learning framework for Raman image analysis (Aim2) to assist in the automated analysis of zebrafish +/- infection and +/- synthetic neutrophil treatment. Track the wound-healing response by analysing whole infected/treated tissues at different timepoints.

Project Reference: MM4L30

Establishing the digital twin of the cardiac organoid: elucidation of cardiomyocyte mechanobiology in heart failure

Co-Supervisor 1:	Matthew Stroud, SCMMS Denmark Hill
Field of Expertise:	In Vitro Modelling; Stem Cell Biology;
Co-Supervisor 2:	Pablo Lamata, Dept. Digital Twins for Healthcare, BMEIS
Field of Expertise:	Computational Modelling;
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Objectives

Ventricular remodelling patterns in heart failure patients can guide diagnosis and treatment. These phenotypes have markedly different aetiologies: increased systolic vs diastolic stresses lead to concentric vs eccentric hypertrophy, respectively. Their cardiac myocyte and ECM remodelling pathways also differ: sarcomeres grow in parallel in concentric vs in series in eccentric hypertrophy, with a respective increase/decrease in collagen matrix deposition. However, the nature of the mechanical stimuli and the cellular mechanotransduction pathways responsible for these alterations remain obscure. The Objective of this Studentship is to develop a digital twin of a cardiac organoid to analyse changes in stiffness at the cellular and pericellular level. Thus, we propose the following Aims:

Aim 1: Establish a cardiac organoid to emulate the micro-mechanical environment corresponding to conditions that lead to adverse cardiac remodelling.

Aim 2: Reveal the molecular and cellular responses to these stimuli, in terms of: cardiomyocyte morphology; nuclear shape; cardiomyocyte and pericellular matrix stiffness.

Aim 3: Build the digital twin (the computational model) of the organoid to generate mathematical models that predict tissue growth and remodelling.

Aim 4: Design an experimental integrative approach combining Aims 1-3, as the groundwork towards model-guided cardiovascular tissue engineering.

Workplan

WP1: Establishing the cardiac organoid platform
iPSC-derived cardiomyocytes and fibroblast co-cultures will be seeded on PEG hydrogels that is interspersed with fluorescent microbeads on 4Dcell SmartHeart cardiac organoids. Fibroblast co-cultures enhance cardiomyocyte maturation and contraction, and microbeads provide fiducial markers for imaging matrix deformation during the contractile cycle.

WP2: Modulation of cardiac organoid contraction using mechanical and neurohormonal stimuli
The physiological stiffness of human myocardium is around 5-10kPa. Upon pathological remodeling and fibrosis, this increases by an order of magnitude to around 100kPa. To mimic this, the stiffness of the hydrogel will be modulated accordingly. Upon which, cardiomyocyte contraction will be stimulated using isoproterenol or Omecamtiv Mercabil or inhibited with Verapamil or Blebbistatin.

WP3: State-of-the-art lightsheet microscopy of beating cardiac-organoids
The SmartHeart system enables imaging-based functional readouts that afford precise spatio-temporal analyses required for accurate digital twin modelling in silico. Specifically, cardiomyocyte

contraction and relaxation velocities, contraction strength and relaxation, matrix deformation, as well as live calcium imaging can be performed to feed into the digital twin model (described in WP4). Live imaging will be performed using the state-of-the-art fluorescence lightsheet microscopy platform we have developed in-house. This enables rapid temporal acquisition (multiple images per contraction cycle) of large spatial volumes with minimal phototoxity. Furthermore, it allows simultaneous imaging of the matrix-embedded beads (for matrix deformation calculations), myofibril contraction (for contractility), and nuclear deformation (for mechanotransduction analysis). After live-imaging, cardiac organoids will be fixed and stained to interrogate cardiomyocyte alignment and sarcomeric organisation using super-resolution imaging and transmission electron microscopy.

WP4: Digital-twin modelling of the cardiac organoid
An in-silico replica of the experimental set up will be established to better interpret results and to generate the computational framework for predicting remodelling. To represent the mechanics of the organoid, we will follow a phenomenological approach where the interactions between cardiomyocytes, their pericellular environment and the extracellular matrix (ECM) will be modelled with a multiphasic, non-linear finite element model (FEM). Inverse data assimilation techniques will be used to estimate the constitutive parameters of the FEM model that best explain the observations. The main challenge will be the definition of the model complexity (e.g. number of phases, regional vs. global parameters) and choice of assumptions (e.g. fixing either the cellular or pericellular space parameters) that solve the parameter identifiability. The main opportunity will be the ability to enhance the experimental readouts and protocols (within WP2 and WP3) to improve the fidelity of the model and its parameters.

In summary, the complementary approaches between state-of-the-art imaging, cardiac organoid and digital twin modelling will enable the direct estimation of the regional cardiomyocyte stiffness, stress, work, and power. The project will deliver powerful biomechanical biomarkers to enable predictions about how cardiomyocyte morphology and function are affected in the different types of adverse cardiac remodelling.

Upon successful establishment of the cardiac organoid model, further steps will be to use patient-derived iPSC-cardiomyocytes from patients with cardiac laminopathies to tease out the pathophysiological mechanisms in play.

Project Reference: MM4L31

Modelling the biophysics of cell-cluster migration in breast cancer

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Objectives

Breast cancer is a leading cause of cancer-related deaths in women in the UK. Each year over 55,000 new cases are diagnosed, and over 11,000 patients die. These staggering numbers account for 15% of all cancer cases and 7% of deaths, making breast cancer the fourth most common cause of cancer-related death. Even though mortality rates are decreasing, they remain significantly higher in more deprived areas, and the number of annual cases is predicted to continuously increase over the next decades.

Metastasis is the process by which a tumour spreads from its original site and colonizes remote locations in the body, giving rise to secondary tumours. In breast cancer, it is one of the primary drivers of mortality. At the core of metastasis there is cell migration, as cancer cells break away from the primary tumour and make their way through the body. Traditionally, it was thought that this was accomplished by single cells, but recent results show that many metastases are seeded by cancer cell clusters. However, the mechanisms that lead to their formation, cohesion and migration are still poorly understood.

This PhD project aims to close this knowledge gap by investigating the physical forces that underpin cancer cluster behaviour. While it is common knowledge that any movement can be understood in terms of the forces that generate it, this approach has been critically overlooked when studying cellular dynamics. Cells are sophisticated force actuators, that sense the environment's stiffness, speed up and slow down, coalesce and disperse, and change their own elasticity to optimize movement. We will manipulate and study this rich phenomenology to discover the physical rules followed by cancer cell clusters.

Workplan

Aim 1) Developing an experimental approach to create 2D cancer cell clusters in vitro with precisely controlled shape and size.

Aim 2) Investigating how forces within these clusters regulate their detachment from bulk tissues and subsequent migration.

Aim 3) Testing this understanding by introducing controlled single-cell migratory cues using optogenetics and optoporation.

Experimental techniques: The student will work with 2D in vitro tumour models, traction force microscopy (TFM), and light-activated control of cell migration and cell transfection. These methods enable bottom-up reconstructions of cancer cell clusters and simultaneous measurement of cell forces during migration.

All techniques are already established in the supervisors' research groups. The student will receive hands-on training in hydrogel manufacture and functionalization, mammalian cell culture and transfection techniques, immunostaining, scanning confocal microscopy, substrate patterning and microfabrication.

Data analysis and computational skills: This project will generate large microscopy datasets for TFM analysis, cell shape and trajectory measurements, and quantification of cytoskeletal and cell-adhesion proteins.

The student will gain expertise in image analysis and become proficient in software and programming tools, including FIJI, MATLAB, Python, and Imaris.

Theoretical knowledge: This research sits at the intersection of cell biology, tissue engineering, and soft matter physics. While primarily experimental, the student will also engage with key theoretical concepts from these fields to interpret and contextualize their results.