National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

BBSRC/NC3Rs joint call: development of next generation NATs awards

Developing the next generation of NATs

Professor Clare Blackburn, University of Edinburgh – A high-throughput-compatible animal-cell-free miniaturised thymic organoid model for thymus biology studies and *in vitro* T cell production

This award aims to create an animal-cell free miniature "organoid" of the thymus to replace the use of mice to study thymus biology and produce T cells.

The thymus is a highly specialised organ which produces T cells. Researchers study the thymus to understand how the immune system declines with age and to produce T cells for use in immunotherapy. Currently researchers rely on thymic tissue collected from mice. Given the small size of the thymus, this requires large numbers of mice to be bred and culled. Professor Clare Blackburn has developed a method to generate induced thymic epithetical cells (iTECs) by direct reprogramming of fibroblasts or pluripotent stem cells in the lab. TECs are critical to drive the development of different types of T cells and Clare aims to include the iTECs in a miniature organoid of the thymus to create a physiologically-relevant *in vitro* model. The new model will allow researchers to produce human T cells in the lab without the use of animals and support high throughput screening of drugs to boost thymus function.

Professor John Brameld, University of Nottingham – *In vitro* digestibility - reducing animal use whilst meeting the demand to evaluate alternative proteins

This award aims to develop an *in vitro* model of digestion to test dietary protein quality and replace post-mortem sampling of animals to evaluate new animal feeds.

In line with the global increase in animal agriculture, there is increasing interest in under-utilised crops as high quality and sustainable alternative protein sources for animal feed. However, their digestibility and quality must be evaluated before large scale use. Currently animals are fed a new diet before post-mortem sampling for amino acids in the intestines. Professor John Brameld aims to validate a lab-based model of digestion which will be faster, higher throughput, require less expertise and replace the use of animals. Screening new protein sources *in vitro* before feeding to animals also reduces the possibility of adverse reactions for live animals. John's technique will be useful for anyone working in the field of new protein sources including animal nutritionists and food and plant scientists in addition to those developing alternative protein sources and agricultural feeds in academia and industry.

Dr Anthony Buckley, University of Leeds – RoboHog: developing an *in vitro* gut model system of the porcine hindgut

This award aims to replace the use of pigs in microbiome studies with a benchtop *in vitro* scale model of the porcine gut.

The variety of microbes which live in the gut and intestines are important for food digestion, immunity and overall health. Optimising the microbiota of animals in agriculture can increase sustainability and animal wellbeing whilst reducing costs and environmental impacts. This can be achieved through careful selection of feed and supplements, however, the results of dietary changes are currently tested in live animals. RoboHog is an *in vitro* system which contains three zones, each individually controlled to mirror different aspects of the pig gut. Professor Anthony Buckley aims to develop and validate this physiologically-relevant model to investigate porcine microbiology, replacing pigs in studies of novel animal feeds and pathogen growth and treatments. Through industrial partnerships, RoboHog has the potential to establish more sustainable farming practices and tailor animal nutrition to refine the health and wellbeing of pigs in agriculture.

Dr Gyorgy Fejer, University of Plymouth – Developing a complex *in vitro* airway model to study respiratory viral pathogenesis, lung macrophage function and herpesviral vaccine vectors in pigs

This award aims to replace the use of pigs in respiratory infection research using an *in vitro* macrophage system.

Bacterial and viral respiratory infections in livestock pigs are a concern for their health and welfare and a potential economic burden for farmers. Alveolar macrophages (AMs) are immune cells which patrol the lung, engulf foreign microbes and alert the immune system. Scientists are researching AMs to develop more effective control strategies and vaccines to tackle respiratory infection in pigs. Current experiments either involve infection of live pigs or use AMs collected from pigs in an air-liquid interface (ALI) model of the airway. In previous NC3Rs-funded work, Dr Gyorgy Feijer developed a method to grow unlimited numbers of pig AM-like macrophages *in vitro* and shared this technique with other researchers, creating a repository of transgenic AM cell lines to support bacterial respiratory infection research. Building on this success, Gyorgy will now use these cells in an ALI model to replace the use of live pigs or pig-derived AMs. He will demonstrate the utility of the model by studying the replication of, and immune response to, two swine viruses, and test a new type of pig vaccine.

Dr Fabrice Gielen, University of Exeter – A high-throughput spheroid fusion platform for the templated-assembly of 3D neuromuscular junctions

This award aims to replace some rodent models of the neuromuscular junction (NMJ) with a 3D human organ-on-chip system.

NMJs are the points of contact between neurons and muscles and are critical to neuromuscular development. Mouse models are routinely used for research but murine and human NMJs have key differences, including their size, complexity, differences in synaptic protein expression and being prone to age-related degeneration. These differences affect the translatability of research using mice to develop drugs to treat human neuromuscular disorders. Dr Fabrice Gielen aims to produce a human 3D *in vitro* model of the NMJ by fusing hundreds of neuronal and muscular organoid spheres in a microfluidic chip. Each chip is expected to hold up to 400 functional NMJs formed by human neurone-muscle spheroid pairs. This model will produce data more representative to humans faster than mouse experiments it aims to replace and has the potential to accelerate the development of new drugs for neuromuscular disorders.

Dr Nicholas Hannan, University of Nottingham – Understanding mechanisms driving lung disease caused by environmental particulate matter

This award aims to develop an animal-free multi-cell type model of lung alveoli to replace the use of animals in respiratory pathology experiments.

Research into respiratory disease has increased significantly over the last 30 years, in line with increasing incidence due to inhaled chemicals such as cigarette smoke and environmental pollution. Animal inhalation studies are widely used as current cell-based models focus on lung epithelial cells and do not capture the whole lung organ response to inhaled substances. Dr Nicholas Hannan aims to combine human alveolar epithelial cells derived from stem cells with macrophages, fibroblasts and endothelial cells to create an immune competent model of lung alveoli. The multiple cell types will be suspended in a synthetic hydrogel to recapitulate tissue stretching, which is important for lung function. This model will encompass the cellular interactions and signalling, immune environment and extracellular matrix modifications which contribute to respiratory pathology, allowing researchers to replace the use of mice in some respiratory studies. To help other scientists replace animals, Nicholas will develop a training course on the model and host a conference on animal-free models of human disease.

Dr Alison John, Imperial College London – Developing a lung organoid signalome for real-time analysis of senescence-associated cellular cross talk

This award aims to develop an *in vitro* system for real time visualisation of senescence-associated cell signalling in the lung to replace some mouse models of ageing.

Repeated exposure to inhaled agents including tobacco smoke, allergens and pollution damages the lungs over time. Accumulated damage and ageing reduce the ability of cells to respond to challenges, until they become senescent. Senescent cells are unable to divide, repair themselves or die. They induce neighbouring cells to become senescent and their accumulation is linked to diseases of ageing, including chronic lung disease. Research in this area predominantly uses transgenic mice and involves procedures to induce lung injury such as smoke inhalation. Dr Alison John aims to replace these animal models with a “signalome” of 3D lung organoids containing multiple cell types which are individually fluorescently barcoded. The signalome cell signalling can be visualised in real time to investigate the pathways which lead to senescence, how senescent cells alter neighbouring cells and induce pathological changes which lead to disease. This research could help to identify novel targets for senotherapeutic drugs to treat age-related diseases.

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Dr Sarah Jones, Manchester Metropolitan University – A human *ex vivo* model of haemostasis: A replacement for rodent tail bleeding assays

This award aims to replace rodent tail bleeding assays by developing a human *ex vivo* model of haemostasis.

Haemostasis is the tightly regulated process of blood clotting to prevent life threatening bleeding or clotting and involves the blood vessel wall, cells in the blood and proteins circulating in the plasma. Research largely relies on the rodent tail bleeding assay, in which the tail is amputated and the time to stop bleeding is measured. These experiments are highly variable meaning large numbers of rodents (10 to 20 per experimental group) are required to obtain reproducible data. Dr Sarah Jones aims to develop an alternative haemostasis model using human placentas donated after birth. The placenta will be perfused with human blood and punctured with a needle, mimicking blood clot formation in a human blood vessel. In addition to measuring bleeding time, high resolution microscopy will be used to visualise clot structure, composition and morphology. This will allow Sarah to investigate the regulation of blood clotting in human tissue at a cellular and molecular level for the first time. Sarah also aims to develop cold storage and cyropreservation methods for placental tissue to allow more researchers to take up this model.

Professor Martin Knight, Queen Mary University of London – Production of a human growth plate organ-chip model of skeletal development

This award aims to develop a human cell-based skeletal growth plate organ-on-a-chip to replace the use of rodents in some studies of development, ageing and disease.

The growth plate is an area of tissue near the ends of children's long bones that determines skeletal development. *In vivo* studies on the growth plate typically use rodents by developing transgenic models to replicate diseases such as dysplasia or by surgically destabilising the knee joint under anaesthesia. *In vitro* models currently do not replicate some key features of the growth plate, such as the gradient between bone and cartilage and the presence of blood vessels. This limits the replacement potential of these models. Professor Martin Knight will use human bone marrow-derived stem cells with a gradient of differentiation cues to develop an organ-on-a-chip model of the growth plate, including a vascular channel to mimic blood vessels. To encourage uptake of the model, Martin will use commercially available cells and has partnered with Emulate, an organ-on-a-chip biotech company, who will publish protocols and characterisation data for use by other researchers.

Professor Mark Lewis, Loughborough University – A platform to investigate multi-tissue crosstalk mediated by exercise induced soluble factors released from human skeletal muscle

This award aims to develop a human multi-tissue model to investigate the impact of exercise on connected organ systems, replacing the use of mammals in some exercise studies.

Exercise helps prevent the risk and severity of many diseases, including type 2 diabetes, cardiovascular diseases and dementia. The impact of exercise varies from person to person making it difficult to understand specifically why exercise is clinically beneficial. Mammalian models are used to determine the impact of exercise on organs such as the heart, pancreas, brain and bones. Studies in rodents are also typically performed in models of relevant diseases, which may require transgenic animals to be bred. Professor Mark Lewis and colleagues have previously developed an *in vitro* model of human skeletal muscles, which regenerates function after injury. Mark will now use this model with protocols to mimic the impacts of exercise on the skeletal muscle and measure the signalling molecules released, specifically exerkines. He will then expand the model by adding bone to create co-cultures and administering exerkines to determine the impact of exercise and demonstrate the utility of this model in investigating tissue interactions.

Professor Roisin Owens, University of Cambridge – A novel approach for modelling the healthy nose-brain axis *in vitro*

This award aims to replace the use of mice in some studies of the nose-brain interface by coupling nasal organoids with bioelectronic sensors.

The nasal epithelial barrier and commensal bacteria that colonise the nasal passage prevent harmful pathogens from entering the body. Both the nasal epithelial barrier and the nasal microbiome change with age, making elderly people more susceptible to central nervous system infections. To understand the impact of ageing on the nose-brain interface, mice are imaged using MRI while the animal is under anaesthetic to analyse the brain. Professor Roisin Owens and colleagues will further develop the current *in vitro* models available for studying the nose-brain interface by using an e-Transmembrane device. Microbiome and tissue biopsies from human sinonasal surgeries will be cultured as organoids on electroactive scaffolds integrated in the device so that barrier integrity and neuron firing can be analysed in real time. The device also contains multiple chambers, enabling drug uptake into the olfactory epithelium to be studied increasing the number of *in vivo* studies this model could replace.

Dr David Richards, University of Exeter – A novel in silico framework for early mammalian embryo development

This award aims to replace the use of mammalian models in some embryogenesis research studies by developing a mathematical modelling framework of the mammalian embryo.

Understanding the early stages of embryogenesis in mammals is key to advances in fertility, IVF treatment and conservation of endangered species. *In silico* studies can be used to model complex processes and reduce the number of *in vivo* studies performed but high quality data from human embryos has not previously been available to build computational models. *In vivo* studies predominantly use embryos from mice but can also use embryos from rabbits and livestock. Using new human cell data from collaborators, Dr David Richards will develop a computational model that replicates various stages of embryogenesis including cell division, differentiation and apoptosis. David will also incorporate data from mice, rabbits and livestock further expanding the number of *in vivo* studies that could be replaced with the computational model. He will work with project partners in IVF clinics to ensure the model is applicable to human development and build confidence in the model prior to dissemination.

Dr Victoria Salem, Imperial College London – Developing a human vascularised pancreatic islet on a chip

This award aims to develop a vascularised “islet-on-a-chip” to enable human tissue to be cultured *in vitro* and replace the use of animal tissue in longitudinal imaging studies in pancreatic islet function research.

Pancreatic islets are groups of cells that release hormones to maintain blood sugar levels. In diabetes, these cells become damaged or dysfunctional. Human islets cannot be biopsied or imaged for research due to the location of the pancreas, and islets post-mortem begin to deteriorate in *in vitro* culture after 24 hours. Islets can be isolated from rodents or, due to the small size of the animals, radiological imaging can be used in some studies. To extend the life and utility of donor human islets, Dr Victoria Salem will use blood outgrowth endothelial cells to develop a vascular network to sustain the islets *in vitro*. Multiple chip designs will be used to allow tissue to be sustained until it vascularises or so an islet can be placed into a pre-existing vascular network. Victoria will build confidence in the model and demonstrate its utility by determining islet function in health and disease.

Dr Kirill Volynski, University College London – Novel *in vitro* platform to study molecular mechanisms of neurotransmitter release and synaptic plasticity

This award aims to replace some live imaging studies of neurons by developing an *in vitro* model of neurotransmitter release.

Rapid neurotransmitter release from nerve terminal vesicles is controlled by calcium signals, which results in neuronal firing. This process is disrupted in many neurological disorders. The mechanisms of calcium-controlled neurotransmitter release are predominantly studied in brain tissue or in neuronal cells isolated from embryonic mice, using a combination of electrophysiology and fluorescent imaging techniques. Transgenic animals are also bred to determine how these mechanisms are disrupted in disease. Professor Kirill Volynski will expand on a platform recently developed by his collaborators at the University of Yale. The existing platform consists of a single vesicle fusion assay and Kirill will develop protocols to mimic the calcium signals that result in vesicular neurotransmitter release. This will allow researchers to control the variables and precisely define the components of the platform enabling mechanistic studies.

Dr Qibo Zhang, University of Surrey – Developing a next generation *in vitro* 3D immune organoids system for studying vaccine-induced immune response and immune-ageing across the life-course

This award aims to develop an *in vitro* test for vaccine-induced immune responses using human lymphoid tissue replacing the use of some animals in vaccine immunogenicity testing.

There are few *in vitro* models currently available for vaccine efficacy studies and as a result vaccines are tested in challenge studies comparing immunised and control animals to confirm disease protection. Such protection can be predicted by immune cell activation and production of immune effector molecules, including antibodies. Dr Qibo Zhang and colleagues have previously showed that respiratory infection vaccines are able to induce increased immune cell and antibody responses in adenoids and tonsils, also termed nasopharynx-associated lymphoid tissue (NALT). NALT can be isolated from tonsils removed during routine surgery and, working with an industrial partner, Qibo will develop a 3D culture system to sustain NALT *in vitro*. He will build confidence in the model by investigating the changes in immune responses and vaccine-induced immune protection levels caused by ageing.

Enhancing the capacity and confidence in existing NATs

Dr Emma Blain, Cardiff University – Testing and validation of an *in vitro* 3D human chondrocyte model to replace animal use in mechanobiology research

This award aims to demonstrate a 3D *in vitro* chondrocyte model is fit-for-purpose by modelling mechanical strain, replacing the use of animals in some studies of joint ageing and disease.

Dr Emma Blain and colleagues have previously developed a 3D model of cartilage-like tissue using human immortalised chondroprogenitor cells. The cells produce a highly organised tissue with the correct composition to support mechanical function. This model should be able to replace animals in studies understanding how chondrocytes sense and respond to mechanical signals. These *in vivo* studies predominantly use rodents where a knee is surgically destabilised under general anaesthetic to create an uneven load upon the joint. Emma will apply varied mechanical strains to the 3D *in vitro* chondrocyte model and use RNA sequencing to identify transcriptomic changes in the cells as a result of the mechanical strain. She will compare this data to existing *in vivo* loading data building confidence in the technique and disseminate to researchers by hosting training sessions in her laboratory.

Professor Sue Kimber, University of Manchester – Advanced human pluripotent stem cell kidney organoid model for investigating development and disease

This award aims to further develop a micro-organoid model of the kidney using human pluripotent stem cells (hPSCs) to replace the use of mice in kidney development and disease studies.

Current kidney organoid models lack macrophages which play a key role in the development of the fetal kidney to promote blood vessel development and kidney outflow. This limits their physiological relevance and uptake by the kidney research community. Professor Sue Kimber and colleagues will generate macrophages from hPSCs and incorporate them into kidney organoids, which they will then scale down to create “micro-organoids”. In collaboration with industrial partner CELLINK, a 3D bioprinting company, the kidney micro-organoids will be bioprinted, to increase reproducibility, and cultured under flow to increase physiological relevance. Sue will build confidence in the model and demonstrate its utility by performing detailed functional studies on the role of macrophages in human kidney development.

Dr Tilo Kunath, University of Edinburgh – Establishment of a cryo-bank of lineage-committed neural progenitor cells produced from engineered human pluripotent stem cells

This award aims to establish a human-induced pluripotent stem cell (iPSC)-derived neural progenitor cell bank enabling non-stem cell specialist researchers to take up the method and replace the use of *in vivo* models in neuroscience research.

There are a number of barriers to the uptake of iPSC technology, including the expense, time and expertise needed to establish stem cell culture and differentiation within a laboratory, which can limit the replacement potential of this method. Dr Tilo Kunath has developed a protocol to differentiate iPSCs along neural cell lineage pathways and freeze them in a progenitor cell state. This commits the cells to producing a specific cell type upon thawing without the need for collaborating research groups to have specialist stem cell expertise. Tilo and colleagues will now create the “Edinburgh Progenitor Cell Bank”, a quality-controlled bank of frozen progenitor cells to distribute to neuroscience researchers in academia and industry.

Professor David Lee, Queen Mary University of London – Engineering circadian biology into induced pluripotent stem cell organ-on-a-chip models

This award aims to introduce circadian rhythmicity to induced pluripotent stem cell (iPSC)-derived organ-on-a-chip models to replace the use of mice in drug evaluation studies.

Dysfunction in the internal body “clock”, or circadian cycle, is a causal factor in ageing and many diseases. Over 80% of proteins that are druggable targets are affected by the circadian clock and are likely to benefit from timed administration. Drug efficacy studies are usually performed in mice, however mice are nocturnal and poorly predictive of human circadian biology and this contributes to late-stage failure in drug discovery programmes. Current organ-on-a-chip models used to evaluate drug efficacy do not incorporate circadian rhythms, limiting the replacement, therapeutic and commercial potential of the technology. Professor David Lee and colleagues will luminescently tag key genes that control circadian rhythms in human iPSCs before differentiating into endothelial and smooth muscle cells and growing them in an organ-on-a-chip device. David will demonstrate the utility of the model in drug evaluation by inducing circadian rhythms in the cells and monitoring emitted luminescence.

Dr Joana Neves, King's College London – Human organoid model to generate mucosal immune cell populations

This award aims to establish an organoid model to generate human innate lymphoid cells (ILCs) in sufficient numbers to replace the use of mice to study ILC function.

Human ILCs exist in small numbers, making it difficult to obtain enough cells to study their role in maintaining health and fighting infection. ILC research is currently carried out using pooled mouse ILCs harvested from a number of animals to provide a large enough number for experiments. Dr Joana Neves has previously developed a protocol to generate large numbers of human ILCs by co-culturing ILC precursors from human blood with human induced pluripotent stem cell-derived mucosal organoids. Joana will now characterise the organoid-generated ILCs by comparing phenotypes to *in vivo* cells, building confidence in the model and increasing its adoption by the immunology research community.

Professor Jennifer Nichols, University of Edinburgh – Optimising human stem cell models to decipher signals and responses during organogenesis

This award aims to further develop “gastruloid” technology using human stem cells to replace the use of mice in early human developmental studies.

Gastrulation is the process where organs develop in an embryo following implantation in the uterus. Most research to understand the molecular processes of gastrulation uses mice, where embryos are removed from culled pregnant animals. Gastrulation can also be modelled *in vitro* using gastruloids. These are 3D cell cultures, which are derived from mouse embryonic stem cells (ESCs) and can be differentiated into tissues with a similar physical and molecular structure to embryonic tissues. The NC3Rs has previously invested in gastruloid technology with fellowship funding to Dr David Turner. David is now a co-investigator on the grant with Dr Jennifer Nichols. Jennifer has developed a protocol to generate gastruloids from human ESCs, increasing the relevance of this technology to human development. Jennifer and colleagues will now use the human model to determine developmental processes during gastrulation, focusing on gut and limb development, further expanding the replacement potential of gastruloid technology.

Dr Malgorzata Wiench, University of Birmingham – Epithelial barrier model: in silico modelling and high throughput assessment

This award aims to enhance the reproducibility and reliability of human epithelial organotypic rafts to increase their potential to replace animal models in oral mucosa and skin research.

Human skin and oral mucosa are protected from the external environment by multi-layered complex epithelia. These form a barrier against abrasion, toxins, infectious agents, water loss and UV radiation and are key for maintaining functional healthy tissues. The epithelial barrier can be studied *in vivo* or *in vitro* using raft cultures, 3D cultures where epithelial cells form multi-layered differentiating tissue. There are a number of barriers to uptake of epithelial organotypic raft cultures, including technical challenges, poor reproducibility and a lack of reliable high throughput systems. Many of these are due to variations in the air-liquid interface which results in inconsistent growth and stratification of cells. Dr Malgorzata Wiench has developed a Buoyant Epithelial Culture Device (BECD) where human epithelial rafts float on top of media and the air-liquid interface is automatically maintained. Malgorzata and colleagues will now further develop the BECD to increase throughput and extend functionality to assess epithelial integrity and barrier function building confidence in the model and increasing uptake.

Dr Robert Williams, University of Bath – Implementing an MEA platform in human neurones for studying age-related neural network dysfunction and testing dietary interventions

This award aims to combine human embryonic stem cell (ESC)-derived neurons and microelectrode array technology to replace the use of some mouse models in neural network dysfunction research.

Cognitive decline and other symptoms of brain ageing are caused by a loss of connections between cells resulting in communication failure known as neuronal network dysfunction. This is typically studied in mouse models, with experiments performed both *in vivo* and *ex vivo* using cells isolated from the animal. However here are clear species differences in the response of humans and mice to neuroprotective interventions. Professor Robert Williams will develop an *in vitro* platform for studying neuronal network dysfunction mechanisms using human ESC-derived neuronal cells in combination with microelectrode array technology to record the neuronal network that forms. Robert will demonstrate the utility of the model by inducing age-related changes in the neuronal networks and testing the effect of various metabolites added to the cultures on preserving neuronal connections.

Dr Beata Wojciak-Stothard, Imperial College London – Organ-on-a-chip model of pulmonary arterial cell-cell interactions

This award aims to further develop a human pulmonary artery-on-a-chip model to replace the use of animals in some vascular remodelling studies.

Pulmonary arteries transport deoxygenated blood to alveolar capillaries to collect oxygen and proper function is essential to sustain life. Dr Beata Wojciak-Stothard has developed a microfluidic organ-on-a-chip model of the human pulmonary artery using primary endothelial and smooth muscle cells. This has attracted significant interest from the pharmaceutical industry. Beata and colleagues will now expand the model to fully meet the needs of industry by incorporating other pulmonary vascular cell types into the model. They will also optimise the device for long-term cell culture enabling studies into angiogenesis and vascular modelling that also require animals, increasing its replacement potential.