Home Office

NON-TECHNICAL SUMMARY

Models of tissue injury, regenerative repair, and reconstruction in the limb and appendages

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Injury, Wound healing, cell therapy, biomaterials, tissue engineering

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, embryo
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to study how animals that have an enhanced healing ability, respond to injury to their limbs, digits and ears. This will allow us to understand how these tissues regenerate, and where possible capture these mechanisms to use in regenerative biomaterials to repair wounds and create new tissue.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

If we can understand how certain mammals are able to regenerate and heal better, and understand how new tissue is formed, after injuries to their limbs, we can improve on our interventions such as surgery, and treatments to restore form and function after trauma, disease, or cancer excision. As surgeons, we see these demands on the NHS, and perform repairs and reconstructions on a day to day basis. Improved healing or engineered tissues that are biocompatible, to replace missing tissues would represents a major shift in surgical care.

What outputs do you think you will see at the end of this project?

The hope of this project is to understand how tissues can be regenerated or replaced to develop new therapies towards the clinic. This may be by development of a new biomaterial therapy or engineered replacement tissues for patients. We would seek to publish these innovations throughout the course of this project, like we have done in the past. There is much learning to be had from this work and as we have found in the past, when new discoveries are made, we can use the information to modify surgeries, generate high impact publications, use the data to apply for further grant funding, and develop new therapies and patents.

Who or what will benefit from these outputs, and how?

Patients who sustain traumatic injury or organ damage will be the beneficiaries of these outputs in the long term. All parts of the study are designed to have line of sight to patient benefit through our clinical group. This includes rapid translation of the science to change surgical practice (which we have previously demonstrated), but also development of new novel therapies, such as biomaterial and cellular therapies. Our work on engineering blood vessel networks, has the potential to allow for new tissue and possibly replacement organs to be engineered that would have a significant impact on people’s lives, with conditions or injuries requiring tissue replacement therapies. We would aim to have successful clinical impact within 10 years.

How will you look to maximise the outputs of this work?

All projects are collaborations between scientists and clinicians, from the biomaterial, cell biology and translational science field. Knowledge will be disseminated through publication and international presentation at both clinical and scientific conferences. Positive results towards clinical benefit will be subject to further grant funding and opportunities to translate wherever possible, this may be in tandem with industry partners.

Species and numbers of animals expected to be used

- Mice: 3500
- Rats: 700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice and rats are being used in the adult stage of their life stage. The justification is based on the fact that these are the lowest sentient mammals, that have anatomical structure and biological processes that are similar to man. Hence the study of injury and repair on these animals provides us with invaluable insight on how they can better heal. Our surgery and anaesthesia can be safely performed on animals of this age and size.

Typically, what will be done to an animal used in your project?

Our study will broadly cover three areas of wound healing interest.

1. How do animals that regenerate, heal wounds of the digit and ear?

2. Can we produce regenerative therapies from our understanding of this process?

3. Can we engineer vascularised tissue/organs that are stable and functional?

We will be breeding animals that have specific genetic changes that will allow us to examine particular areas of wound healing that may allow us to understand how to improve on the healing process. This would require animals that heal better or heal worse, or have genetic characteristics that enable us to understand and visualise the healing process better.

Testing of these animals to have the correct genes will occasionally require us to take a small blood sample from the tail or a small sample from the ear which may cause transient pain.

Some animals will undergo chemotherapy to clear their bone marrow and allow for bone marrow to be transplanted which will allow us label cells in their bone marrow or change the characteristics of their

bone marrow cells which may alter how they heal. This would allow us to examine the importance of bone marrow in the healing process. The bone marrow in these animals is always restored to bring the animals back to health.

Within each study, animals will be subject to injuries that are simplified versions of injuries seen in the clinical setting, and then observed for how they heal. This will involve either a cut across a finger or ear where there is no loss of tissue, or excision of tissue across the finger or ear, where there is loss of tissue. Injuries will be studied in animals that can regenerate, and by understanding how they heal better, we will try to harness the biology and apply them to biomaterials as a potential therapy. These injuries are small (several millimetres) and cause minimal pain, distress, and disturbance to the animals. As such these small injuries can be performed on the left and right paws or left and right ears without much disturbance, hence allowing for comparisons of how treatments work without using more animals and without more suffering.

In addition, we will be using our understanding of healing to generate new tissue. This will involve combining surgery, biomaterials, and cell therapies to enhance tissue growth and development into replacement tissue that may be used to transplant to other animals as a replacement for damaged tissues.

These experiments will be conducted to observe the full healing process, which is typically 3 months, and the number of procedures will be kept to the minimum required for us to obtain a meaningful understanding of the biology (usually 1 but up to a maximum of 2 per animal). The information collected has to be of sufficient quality so that we can reliably develop therapies for patients

What are the expected impacts and/or adverse effects for the animals during your project?

We aim to use only animals that are healthy in our study but in generating animals used for tracing of particular cells of the bone marrow, this necessitates chemotherapy and bone marrow transplantation. This makes animals susceptible to infection and we try to reduce the chances of this by acidified water, antibiotics and clean caging.

For these studies we will be performing very simple injuries on the digits or ears. These simple injuries can cause pain, bleeding and impact on mobility, but all of this is transient and allow for quick recovery. Having developed these procedures, it is rare to see anything more than transient discomfort in the animals or abnormal behaviour.

In some animals we will be implanting biomaterials that may have a biological effect. They are all tested to be biocompatible and should allow for better healing. However, they may rarely cause tissue reactions that are usually transient. We will provide pain relief to limit the symptoms from this. We also have vascular tissue engineering model that hopes to grow new tissue and organs. Rarely the cells may grow into benign tumours. If this occurs and limits the function of the animals, the experiments will cease.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

90% of the mice bred on the licence will be expected to experience sub threshold harms with 10% possibly experiencing mild harms for differences in their genetic make up.

100% of mice undergoing bone marrow transplantation will be expected to experience no more than moderate harms.

100% of mice undergoing surgical procedures which include; 1. injury and repair, or 2. Vascular tissue engineering or 3. Vascular tissue transplantation will be expected to experience no more than moderate harms

100% of rats undergoing surgical procedures which include; 1. injury and repair, or 2. Vascular tissue engineering or 3. Vascular tissue transplantation will be expected to experience no more than moderate harms

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

After tissue injury there are changes in blood flow, inflammation, the formation of blood vessels and tissue remodelling, including interactions between numerous cell and tissue types. No in vitro cell culture can study these interactions in concert.

Which non-animal alternatives did you consider for use in this project?

We have tried to mathematically model these changes to replace the use of experimental animals and are exploring ex vivo models using whole limb perfusion, and organs on a chip technology that will run in parallel to these studies. If specific questions can be answered and validated on these systems the use of animals can be reduced and replaced.

Why were they not suitable?

Mathematical models are not able to model the complex interactions of systemic inflammation in a spatial temporal fashion. The information acquired in vitro and in silico is insufficient to allow progression to trial in human patients.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the numbers of animals used based on our current throughput of animal experiments, what is technically feasible to perform, and the planned scientific questions we are planning to answer over the next 5 years. We have considerable experience in planning the right numbers of animals for the research questions in order to maximise use, and minimise waste. This is also considered in the context of our current group make up and the projects which are funded. The minimal number of animals are used to allow us to answer the posed scientific question.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We are constantly evaluating ways to obtain the most information from the smallest number of animals, and this includes using more sensitive methods of quantification, using numerous modalities of analysis on one sample and ensuring the experiments are well-designed to minimise animal waste. All animal procedures are only conducted once protocols have been discussed with in-house animal facility expertise, including the vet, and signed off. This is supported by pilot experiments to ensure feasibility prior to embarking on bigger studies. We are always looking for other models that can potentially provide us with the same information that does not harm animals including experiments that can gain the data direct from patients. Animal experiments are only considered when the information cannot be obtained from other publications or the clinical setting.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will always conduct experiments with the necessary pilot studies so we are in the optimal condition to proceed with larger numbers of animals. We are now also taking more of a systems biology, and non-destructive sample approach to allow us to generate far more information from each animal than previously possible. This is done with comparison to known datasets and in silico models which reduces animal numbers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We have developed the simplest injury and repair models that create the least disturbance and suffering on the animal which still provides us with useful information to take back into the clinic

Where abnormal behaviour is observed, this usually indicates that the animal is suffering and as such we limit any further involvement of the animals in the experiments. We will monitor for any suffering and where necessary intervene with pain relief, water and food. If this is insufficient, the use of animals in study will end and they will be humanely killed.

We will use models developed in the mouse and rat which are the lowest sentient animals for the purposes of mammalian study. Mice are used for their genetic tractability that allow us to examine the role of inflammation after injury, and rats are used because some regenerative biomaterials cannot be fabricated to a small enough size and resolution to be used in the mouse. The engineering of vascular tissues in the mouse is unique to our group and will be used in our studies to engineer tissue for replacement tissues and organs.

Why can’t you use animals that are less sentient?

Mice and rats are the least sentient animals that have relevant mammalian biology and also, they have anatomical structures that are similar in composition and configuration to man. As we are interested in how the body heals after injury, it is important that we study how these animals recover as you would expect humans to do the same.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All injury models are developed with clinical scenarios in mind and each surgical procedure has been refined to its simplest form

All surgeries are designed to be quick, precise, and optimise workflow in theatres. Sterile equipment is used throughout. Complications are carefully monitored for and are fortunately rare. We continue to ensure all procedures lie well within the moderate severity range. The experimental models outlined are well validated, reproducible and consistent injuries, with the minimum amount of pain, suffering, distress and lasting harm. We have many years of experience in refining our techniques and all team members have skill sets attained through extensive training and/or clinical experience. For precision all procedures are performed under high magnification.

In addition to our surgical models, our use of bone marrow transplant models have undergone refinement by moving away from harsh radiotherapy based bone marrow depletion, to using chemotherapy based depletion which results in far more reliable and less harmful bone marrow depletion. We monitor these animals carefully and provide acidified water, irradiated diet and prophylactic antibiotics to minimise chances of infection.

Suffering is minimised through careful monitoring and by use of analgesia, and good husbandry. We have reduced variability in the surgeries over the years that allows us to obtain meaningful,

reproducible results that will continue to be refined. We also ensure careful training of all our staff to be mindful and skilful at surgical procedures and animal care.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We use guidance from PREPARE and ARRIVE to ensure the standards of our animal experimentation and reporting allow for the quality, reproducibility and translatability.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We regularly receive updates from our establishment about new and novel surrogates to animal experimentation. We regularly keep up to date with published activity and calls form the NC3RS and have applied for previous awards. In addition, we actively are trying to develop our own systems that do not use living animals, such as ex vivo perfusion and organs on a chip technology, or perform investigations whenever possible on patients themselves, and perform scientific outcome measures on tissue from patients via our biobank.