Home Office

NON-TECHNICAL SUMMARY

Tissue response following synovial joint injury and potential for therapies to restore joint structure

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

Key words

Cartilage, Bone, Osteoarthritis, Cellular therapy, Biomaterials

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

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?

The overall aim of this project is to study the biological mechanisms that contribute to the repair response after joint surface injury. This data will provide a better understanding of the role that drugs, biological molecules, biomaterials and regenerative cell therapies can play in restoration of joint tissues.

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?

Arthritis is the biggest cause of pain and disability in the UK. The most common form of arthritis, osteoarthritis (OA) is believed to affect an estimated 8 million people in the UK, and worldwide this figure rises to 343 million. Any joint in the body can be affected by OA but it is more frequently observed in joints that support the most weight, such as hips and knee. A joint is formed in the body where bones meet and to ensure that they can move against each other, their surface is covered with a smooth, slippery but tough tissue, called cartilage. In OA the cartilage begins to thin and its surface becomes rougher meaning that the frictionless movement of the healthy joint is lost. These changes, together with others in the surrounding bone and lining tissue of the joint can cause pain and loss of mobility. OA is associated with age but younger people are also affected, often impacting on their ability to work, exercise and socialise. Whilst total joint replacement surgery is an option for end stage disease there are no widely available treatments that can be used to halt or ease the progression of OA. Microfracture is a procedure where the surgeon creates a controlled joint surface injury at the site of localised cartilage damage to induce a limited repair response. This approach is currently used as a treatment in a restricted number of patients but produces cartilage which does not completely recreate the original tissue. The goal of our research is to understand how this surgical approach can be refined to benefit a much wider group of patients and produce repair tissue that restores joint function and halts further disease progression.

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

This project will provide greater insight into the healing of bone and cartilage within a joint following injury and how the addition of drugs, cells or materials suitable for introduction to living tissue can improve the healing process. These new discoveries will be shared with others at local, national and international scientific meetings and in widely available publications. The data from this work will form the basis of future funding applications and where appropriate will support patent filings to protect intellectual property. In more detail, it is our expectation that our research will produce one new

therapy that can be advanced to a point where it can be investigated further in a large animal model. Over the longer term (5-7 years) we aim to develop at least one human clinical trial to determine the safety and success of the therapies developed in this project.

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

In the early stages of this project (years 1-3), researchers who focus on understanding tissue damage in the joint and the potential to improve the healing response will be the major beneficiaries of this work. These findings could also have broader significance to those researchers studying mechanisms of disease in the joint and also the wider research community investigating tissue repair following injury. In the medium term (years 3-5), the findings from this work will benefit clinical groups and industrial companies interested in the development of new treatment approaches for the restoration of cartilage and bone in the joint. In the longer term (5-7 years), this work will benefit patients with localised cartilage loss that have been recruited to clinical trials. Ultimately (7-10 years) we hope that larger numbers of patients will benefit from the therapies developed from these studies once they have been shown to be safe and effective.

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

The discoveries from this work, which may include unsuccessful findings, will be shared in the first instance with our research networks. These groups meet regularly and include researchers from broad academic disciplines including life sciences, medicine and engineering as well as complementary areas of biological research. Our results will be disseminated more widely through presentation at national and international research conferences, through publication in peer-reviewed journals and where appropriate submission to "publish, then review" journals such as eLife or bioRxiv, an open access preprint repository.

Species and numbers of animals expected to be used

- Mice: Mice: 3700

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.

This project will use adult mice only. Mice are the lowest experimental species to humans with comparable organ systems and folding limbs that support body weight. This means that our studies on joint surface injury and healing will be performed in a tissue and mechanical environment that provides useful information directly relevant to humans. In addition, mice strains with specific genetic alterations could be used in our work to help identify the detailed mechanisms that are responsible for the healing of bone and cartilage in the joint.

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

In a typical experiment, the mouse will undergo a single surgical procedure to create a joint surface injury in the knee of the animal. This surgical procedure will be completed under general anaesthesia so that the animal will remain in a state of sleep/unconsciousness throughout. The mouse will then be kept alive for up to eight weeks before being humanely killed and tissues harvested for further study. In approximately 30% of the animals a modified material that can be safely introduced into the body will be included at the site of injury to assess its impact on repair. In a further 30% of the animals, a drug or therapy will be administered (orally or by injection, either into a vein, into the body cavity or directly into the joint space of the knee) to determine its potential to modify joint repair following injury. Cells as a therapy will be administered on a single occasion into the joint space whilst drugs may be used on multiple occasions before (for example to target the function of a particular cell type) and/or after the surgical procedure (to directly impact on the healing response). Approximately 15% of animals will have imaging (like x-ray analysis) performed at single or multiple time points whilst the injury is healing. This may include specialist imaging such as magnetic resonance imaging (MRI) to identify cells that have been labelled to allow their visualisation. Detailed analysis of the MRI data will detect altered signal from the labelled cells revealing their specific location within the joint structure. In some experiments, the surgical procedure will expose the mouse knee but not include making a joint surface injury. These so-called, sham-operated mice will allow us to understand the impact of the surgery alone on the biology of the knee joint.

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

The procedure to induce a joint surface injury is generally (in 95% of animals) well tolerated. We anticipate that 100% of the animals that undergo this procedure will have pain, lameness and joint swelling but this will rapidly improve over 48 hours after which the animals will not show any clinical signs. During this initial 48 hour period animals will receive pain relief. In some cases though, animals may continue to show or develop during the experimental period reduced movement, weight loss, reduced food intake or an abnormal coat. If after interventions, such as treatment with antibiotics or high energy soft food, these clinical signs persist then the animals will be humanely killed.

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)?

Mouse: Mild 10%

Mouse: Moderate 90%

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

- Killed
- Used in other projects

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?

Prior to performing experiments in animals we conduct detailed laboratory studies of the human cells that we judge important in the healing of joint surface injuries, identifying their characteristics and response to factors likely found in the wound environment. However, it is not currently possible using laboratory based approaches to reproduce both the mechanical environment and the complex organisation of tissues and cell types that occur through time in response to injury in the joint. Several of the cell types that are implicated in the repair response only have access to the damaged tissues via blood vessels and so we need a functioning heart and circulatory system to investigate these responses in a way that is clinically relevant and meaningful. Our earlier experience with the experimental approach described here has provided us with a good overview of the healing events after osteochondral injury in the mouse. It has also illustrated the robustness and quality of the data from our studies providing insight into the activity of some cells and the molecules that they may respond to or produce during osteochondral repair. However, further detailed questions remain concerning the function of key cell types following synovial joint injury and the efficacy of therapies. It is these questions that drive the breadth of our planned studies.

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

Approximately 80% of our research is conducted in the laboratory to study individual human cell types both alone and in simple model systems of two or three different cell types. This work includes using the latest cell printing and other manufactured cell culture environments. These studies directly contribute to the replacement of animal studies by identifying the cell types, treatments and time points after injury which should be the focus of our investigations.

Why were they not suitable?

Even the most advanced laboratory cell culture environment cannot reproduce the mechanical forces and complex tissue and cell interactions that occur within a sophisticated anatomical structure such as the joint. For this reason it remains necessary to address the unanswered questions surrounding the healing response in the joint using animal studies.

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?

The number of animals has been estimated based on our experience of using these and similar experimental approaches over the last five years. For each of the studies that we wish to perform we are able to predict the number of animals that will be needed to give us reproducible and reliable information. We also understand the lengths of time that our studies need to be performed over to give clear answers to our research questions. This enables us to predict how many experiments can be accomplished over the time given to our studies.

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

Our previous studies have provided us with a clear understanding of the magnitude of effect that is biologically meaningful and its variation within the experimental data. This means that in the experiments that we perform, each experimental group will contain the minimum number of animals to provide biologically meaningful data. When a new treatment, method of imaging or adaptation of the surgically created injury is introduced, it is first investigated in pilot experiments that contain fewer animals. This approach allows us to understand the effectiveness of the intervention and quality of the data to decide if the group sizes in these experiments should be recalculated. We will use the NC3Rs Experimental Design Assistant and other online tools to help us in confirming that our experimental approach is reliable and likely to provide reproducible data.

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 use a number of complimentary approaches to reduce the number of animals needed in our work:

1) Pilot experiments will be performed with fewer animals when a new drug, method of analysis or modification of surgical practice is introduced. This will ensure that subsequent larger studies can be designed to be as accurate as possible.

2) In studies where the effects of a new treatment or sham surgery versus injury are evaluated, the mice will be randomly allocated to control and treatment groups. In addition, where possible, any treatment will be administered to the animals in such a way that the person delivering it is not aware of whether it is the active treatment or inactive control substance. When the assessment of healing is based on microscopy, "blinded" observers will score the specimens and in experiments analysing large datasets bioinformaticians will analyse the data with no prior knowledge of the experimental groups. The data will then be matched back to the original experimental groups at the end of the analysis, so avoiding bias.

3) Where appropriate we will share excess breeding animals with other researchers and use any animals culled as tissue donors for use by our group or by other researchers.

4) In studies that use human cells as a therapy, multiple studies using the same human cells will be performed. This approach minimises variation introduced by using different human donors and reduces the number of animals used.

5) In some studies, non-invasive imaging techniques will be used to follow aspects of the experimental outcomes of our work through time in individual mice. These approaches mean that fewer animals will be required as it removes the need to cull animals at distinct timepoints.

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.

The principal experimental approach that will be used in these studies is a surgically created joint surface injury model. This approach has been refined such that it causes the least pain and suffering to the animals. The surgical procedure itself is expected to result in lameness which will improve within 48 hours. The recovery from surgery will be rapid and progressive with no deterioration in lameness expected to occur. This model is not expected to result in persistent clinical signs that fail to respond to treatment (such as pain relief medication or antibiotics). If however, clinical signs do not resolve animals will be humanely killed and no animal will experience lasting harm.

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

Our work focuses on the joint, a complex part of the anatomy which undergoes significant change throughout development and into adulthood. Adult mice are the least sentient animal that allows us to perform these investigations. The response to injury and the repair processes that follow take days and weeks to develop and for this reason it is impossible to perform these studies under terminal anaesthesia.

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

All animals used in our studies will have a minimum period of 7 days acclimatisation to their environment, including social group, prior to undergoing a surgical procedure. In these experiments each mouse receives a single tissue defect in one knee only which minimises initial pain, discomfort and lameness that the animal might experience. Immediately following the procedure the animals will be housed with access to warmth, post-operative bedding and other nesting materials and wet mash food on the floor to minimise the need for movement. Working with the staff at our animal facilities there are thorough procedures for the post-operative monitoring of all experimental animals, including the use of score sheets to record animal health status, discomfort, lameness and weight. The major clinical impact of our surgical procedure is initial lameness which can be clearly identified by observation but the close monitoring of bodyweight will also help ensure that humane endpoints are adhered to. Oral pain relief medication, that is flavoured to make it more pleasant-tasting, will be given to the mice for several days following surgery.

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

Best practice guidelines including those published by the Laboratory Animal Science Association (LASA), e.g. record keeping, performing surgery, education and training, and reporting of experimental results will be followed in our work. Specific guidance on the principles of surgery will be followed from LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery - A report by the LASA Education, Training and Ethics section (E Lilley and M. Berdoy eds.). The health status of all animals will be supported by guidance on body condition scoring e.g. Ullman-Culleré MH, Foltz CJ. Lab Anim Sci. 1999 Jun;49(3):319-23. In our studies we will also follow the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines on experimental conduct, including study design, avoiding bias and statistical analysis of results.

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

I will continue to review relevant publications, guidelines and best-practice information. This will include resources from The National Centre for the 3Rs (NC3Rs) and practical guidance from the Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA).