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NON-TECHNICAL SUMMARY

Understanding regulation of cartilaginous tissue formation, joint disease and regeneration

Project duration

2 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

Cartilaginous tissue, Development, Cell phenotype, Joint disease, Therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

This project aims to understand the role different genes play in regulating the formation of cartilage within joints, as well as in maintaining joint health vs disease during ageing. The information gained will be used to help develop novel approaches that can be used to prevent degeneration or promote tissue repair.

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?

Joints, such as those in the knee and the intervertebral discs in the spine, play vital roles in allowing pain-free mobility. Conditions affecting these tissues, such as osteoarthritis in the knee/hip and chronic back pain caused by breakdown of tissue (degeneration) in the intervertebral discs, have a huge impact on societies around the world. For example, research by the World Health Organisation has shown that chronic low back pain is the leading cause of years-lived-with-disability globally and the cost of back pain in the UK is estimated to be between £14 and £28 billion/annum (1-2% of gross domestic product). Likewise, osteoarthritis currently affects around 528 million people worldwide, an increase of 113% since 1990.

The mechanisms that underpin the development and maintenance of healthy cartilage versus disease are not well understood. As a result current treatments only treat the symptoms rather than the underlying disease and hence offer poor long-term efficacy. Research is thus required to better understand how healthy tissue is formed and what changes to cause joint disease, with a long-term aim of developing new treatments.

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

1. Improved academic understanding of the mechanisms which underpin healthy development and maintenance of cartilaginous tissues such as those in the knee/hip joint and intervertebral disc in the spine

2. Improved academic understanding of how disruption or loss of pathways that maintain cartilage tissue health might lead to joint disease

3. Development of novel approaches that can be used to prevent joint disease or promote cartilage regeneration

The knowledge will be shared through academic publications and presentations at national and international conferences.

Where appropriate knowledge will be shared with the public or patients through engagement activities.

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

The research will directly benefit the research group by enabling us to advance our understanding of genes and pathways identified in previous studies on cartilage tissue health. Other researchers both within the establishment and at other institutions will benefit through new opportunities for collaboration.

Knowledge will be shared academically at conferences during the project. These conferences are annual, so it is envisaged that data will be shared from year 2 onwards.

Findings will be submitted for publication as soon as practically possible. Ideally this will be from year 2 onwards, but may not be until the project has been completed.

Wherever possible, negative/null results (e.g. where genes are found not to play an important role in tissue formation, health or degeneration) will be published as this knowledge is important for other researchers to know to prevent similar work being repeated in the future.

In the long-term the findings from the study may be used to develop novel therapies which will directly benefit patients suffering from common and debilitating joint conditions such as osteoarthritis and back pain. Findings, such as the identification of key genes that prevent or delay disease progression, may take many years to reach patients, but we work closely with clinicians to ensure help identify potential opportunities for clinical translation of the research.

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

Key findings will be presented at conferences and submitted for publication in open access journals. This will include negative/null findings wherever relevant.

Any large datasets generated during the study will be made available to others by submission to publicly-accessible repositories.

The models generated in the project will open up opportunities for collaboration with other experts in the field.

We will engage with relevant patient groups and clinicians to ensure the clinical relevance of the research aligns to their priorities and needs.

We will also engage with the public through a combination of public engagement activities organised with the establishment, and through the dissemination of outputs through the media in conjunction with our Media Relations team.

Species and numbers of animals expected to be used

- Mice: 2000

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 allow for genetic manipulation, enabling the study of specific genes and pathways within a specific tissue. This approach is not possible in other species. By targeting specific genes and pathways within certain cells in mice, we can study their role in promoting formation and maintenance of healthy cartilaginous tissue and whether their loss results in joint disease. The study will also enable us to identify cells and pathways that can be targeted for prevention of degeneration or regeneration of cartilage tissue.

To achieve this work we will study mice across life course from birth to old age.

Target genes will be knocked out in specific cartilaginous tissues at defined ages to explore the function of our protein of interest in controlling either healthy tissue formation of maintenance of healthy tissue versus initiation of changes that lead to joint disease.

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

The generation of genetically modified mice will involve natural mating of animals. The mice will be kept in standard conditions for up to 15 months.

To induce tissue-specific gene knockout, mice will be treated with tamoxifen. This will be administered either orally or by injection.

To establish whether the genetic modification has been successful, mice will be tested using one of the following approaches: ear biopsies, blood sampling, hair sampling, or mouth swabbing. This will be done once, unless there are technical problems which mean it has to be repeated.

Mice will then be maintained over a period sufficient to assess the success of gene targeting and age-related changes in cartilage tissue structure/composition, such as degenerate changes.

As well as examination of joint tissues after humane killing, tissues may also be examined using: non-invasive imaging methods, such as x-ray and MRI, and range of motion joint analysis, no more than once per month; and by observational gait analysis (a maximum of once per week).

Control mice, e.g. without gene knockout, will be included for comparison in all elements of the project. Wherever possible, tissue from control mice will be used across different experiments.

Where required, animals with altered immune status will be housed in a barrier environment.

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

No adverse effects are expected as a result of the breeding of genetically modified mice.

Mice treated with tamoxifen can show a modest weight loss of <15%. This has been commonly observed and is transient and does not extend beyond the period of active dosing.

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)?

100% of mice will be genetically modified and the breeding programme is a mild procedure.

50% of mice will experience a maximum of moderate effects, through tamoxifen treatment or development of joint disease. The remaining 50% of mice will experience a maximum of mild effects.

No animals will experience severe effects.

What will happen to animals used in 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?

Cartilage tissues are complex, containing multiple regions that each have different structures that evolve over time. Such complexity cannot be replicated using in vitro models and human tissues are difficult to obtain at defined stages of development, maturation and healthy ageing.

A mouse model is essential for us to achieve our aims. Mouse joint structures and cartilage tissue composition are similar to humans and have been widely used as models for joint development, maturation and age-related joint diseases. They also grow, age and develop joint diseases relatively quickly, allowing investigations over relatively short timeframes (up to 15 months).

Mice are also the ideal species for studying tissue-specific, targeted genetic modification. They will enable us to study the effect of editing specific genes and pathways on tissue health and disease progression over extended periods which is also not achievable in vitro, or using human tissues.

We will be able to establish healthy tissue formation, maintenance and development of joint disease at defined times using a range of non-invasive imaging techniques and end-point assays, all of which are

routinely available. Such an approach would not be possible with human tissue.

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

We have already undertaken extensive investigation of human tissues from early fetal development, childhood (healthy tissue) and adulthood/ageing (diseased tissue). These investigations have enabled us to identify key genes/proteins which may be important for regulating healthy tissue formation versus joint disease. However, such investigation of human tissues does not allow function to be established.

To establish function of specific genes and to develop novel therapies we have considered:

In vitro investigations using human cells in which specific genes or pathways are edited.

Non-protected chordates such as zebrafish.

Computer modelling.

Why were they not suitable?

Human tissue does not allow gene function to be investigated and we have no control over the ages of samples studied, or their relative health or disease status. This makes investigations complicated, in particular where adult patients may have multiple other conditions in addition to joint disease.

In vitro models using human cells in which specific genes or pathways are edited can be used as a partial replacement used for screening before moving in vivo, but do not fully replicate the complex environment of cartilage tissues or the long-term changes that occur during development or joint disease.

Non-protected chordates such as zebrafish are useful for studies where genes or pathways are edited, but the size and structure of the cartilage tissues in such species do not accurately model human tissues, limiting the applicability of the knowledge gained.

Computer models have recently been developed to model some aspects of cartilage tissues. However, these models lack the complexity required to study age-related changes in tissue structure. They are also not suitable for the study of specific genes and pathways in the context of cartilage tissue development or degeneration.

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 estimate that up to 2000 mice will be required for our project. These will be used in our breeding programme to generate mice where our specific genes of interest are knocked out, to study their role in cartilage tissue formation, maintenance of healthy tissue and development of joint disease, as well as to develop novel therapies.

This is based on: (1) the number of experiments we plan to use these mice for, and (2) the conservative assumption that each litter of mice we breed will include one mouse with the correct genotype.

For each gene of interest we estimate that up to 1000 of the mice that we generate in our breeding programme will be treated with the drug tamoxifen to stimulate the tissue-specific gene knockout. This is based on the different experimental time points we will use (in order to explore the effects of our protein of interest throughout tissue development, ageing and joint disease), and previous work by collaborators, which suggests that each experimental group will need to include 8 mice in order to generate informative data.

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

We have used the outcomes of work in publications and our collaborator's labs, and have taken the advice of a statistician to reduce the number of mice being used in each experimental group. We will use pilot studies to further adjust group sizes as the study progresses.

By using both male and female mice in our studies, and by utilising efficient breeding strategies, we will reduce the number of genetically modified mice that we need to breed. Furthermore, generating data in both male and female animals is important with regard to the translation of our findings towards human clinical studies, where joint disease is common in both women and men.

We will also ensure that tissues from control mice, e.g. without gene knockout, are used across different experiments.

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 continually review the outcomes of experiments and use our data to optimise animal numbers as the project progresses. For example, we will conduct pilot studies to optimise the protocol for tamoxifen induction of tissue-specific gene knockout and to determine the group sizes required in each study.

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.

This project will selectively knock out genes of interest in cartilage tissues of mice at different stages of development and ageing. We will use these models to explore the effects of the gene deletion on healthy tissue formation and maintenance during ageing versus spontaneous development of joint disease. The information will be used to develop novel therapies to treat joint disease.

Given the slow-progressing nature of joint diseases, some animals will be allowed to grow old to study the changes that occur in cartilaginous tissue structure and composition. Any effects are likely to be mild to moderate and we will minimise the time animals experience joint disease.

To induce or knock out gene expression in animals or to deplete specific cells, some animals will be given tamoxifen by mouth, injection, or through food. Any effects will be mild and transient and we will optimise the dose and duration of treatment.

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

In order to explore the onset and development of human joint disease we need to use a live animal model with a mature musculoskeletal system that is similar to an adult human. The adult mouse fulfils this requirement. Non-mammalian animals are limited in their use because their musculoskeletal structures are too different from the human and their lifespan is too short to allow age-related changes to be studied.

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

The major welfare cost to the mice in this study is the development of joint disease symptoms. Since the purpose of this project is to study the effect of genes in regulating formation and maintenance of healthy cartilaginous tissue versus joint disease, this welfare cost cannot be changed. However, we will not investigate genes which are known to have severe impacts on human health and will limit the time animals experience disease whilst giving analgesics where appropriate.

We will carry out pilot studies to determine the least harmful way of administering tamoxifen, whilst still ensuring effective knockout (determined by genotyping). We will preferably use an oral method of administration. Tamoxifen treatment can cause some weight loss and signs of general malaise such as loss of appetite, hunched posture and piloerection, but these are typically transient.

As well as collecting data on joint damage and gene expression profiles at the end of each experiment, we will use non-invasive methods (such as imaging and observation of animals' mobility) during the course of each experiment so as to maximise the information we obtain, but without any additional welfare costs to the mice.

Ageing animals will be carefully monitored by staff trained to work with ageing animals. Group sizes in ageing experiments will be increased to accommodate for loss of animals and to avoid single housing due to animal losses due to old age. Longer drinking spouts will be used, and animals will be monitored for adverse effects such as changes in weight, dermatitis, piloerection, paleness, changes in

mobility, lumps, eye defects, abnormal respiration, or stools. If these are observed animals will be treated accordingly, and animals that develop severe effects will be humanely killed.

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

We will abide by the recommendations of the joint working group on refinement (Morton et al, Laboratory Animals. 2001;35:1-41) and use published documents recommended by NC3Rs to ensure that we are using the most refined approaches in all our experiments. We will also regularly review the scientific literature for work by other researchers in the field to identify opportunities for refinement.

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

We will regularly check information on NC3Rs website, we’ve signed up to the NC3Rs newsletter, we will meet the establishment's 3Rs manager, and attend regional 3Rs symposia.