



Home Office

## NON-TECHNICAL SUMMARY

# Circadian rhythms in health and disease of the musculoskeletal system

### 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

Osteoarthritis, body clock, exercise, therapy, ageing

### Animal types

### Life stages

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Mice

embryo, neonate, juvenile, adult, pregnant, aged

## 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 understand how daily rest and activity patterns regulate skeletal tissue functions and protect us from age-related diseases. We also aim to investigate whether we can utilize our intrinsic body clock mechanisms to enhance tissue repair and improve treatment options for skeletal conditions, such as osteoarthritis.

**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?**

Osteoarthritis is common and debilitating, affecting 60% of people over the age of 65, with currently no cure and no disease modifying drugs. Numbers of people suffering from OA will rise with increased longevity in the population and the disease represents a significant economic burden on the health service and is a major cause of decreased quality of life amongst the ageing population. There are currently no effective treatments for OA and joint replacement and analgesia are the only options for sufferers of OA. Therefore, new understanding of how cartilage tissue is maintained on a daily basis and how this process goes wrong with ageing is urgently needed. Body clocks are 24 hourly (circadian) rhythms driving key aspects of our physiology and behaviour, including the sleep/wake cycle. Recent research has demonstrated an essential role of the intrinsic clock timing mechanisms for normal function and structural integrity of the articular cartilage and other connective tissues. The program of work proposed here will help us understand how connective tissues such as those in the articular cartilage in our joints are maintained by our body clocks and daily rest/activity cycles, and why loss of this temporal control mechanism contributes to the development of osteoarthritis and other age-related diseases. The new knowledge gained in this project will help us understand why we develop this disease when we get older, and how we may be able to utilise the body clock mechanism to optimize treatment timings or design novel therapeutic approaches.

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

The research outputs of this project will include:

(1) In depth understanding of how connective tissues in our skeletal system are formed and maintained on a daily basis, despite the routines of wear and tear associated with loading and activity. It is currently unknown whether and how exercise can reset our body clocks in the skeletal system. Proving our hypothesis in vivo in mice will allow us to identify a novel disease modifying intervention (timed daily exercise) for age-related skeletal diseases, such as osteoarthritis. These non-invasive approaches are likely to bring significant clinical benefits to patients experiencing osteoarthritic pain. This information will open new research avenues in the field of connective tissue research, arthritis, exercise biology and sports medicine.

(2) A mouse model of osteoarthritis (OA) where clock genes of interest are selectively deleted in the cartilage of adult mice.

(3) Data from our mouse model showing how body clocks influence the onset and progression of OA, both spontaneously and following surgical destabilisation of the knee joint. These will include imaging data and joint 'damage scores' to demonstrate whether OA develops more quickly and with greater severity in our model compared to controls, data on joint mobility and data on the levels of molecules that are associated with pathways that promote or inhibit the onset and development of OA.

(4) Insights into the mechanisms by which body clocks have protective effects in joint tissues and how our drug candidates harness these mechanisms in the treatment of OA.

We will publish our findings in open access peer-reviewed journals making them available to the OA research community.

We will present our findings in the form of posters and oral presentations at conferences.

The project will support our development of a disease-modifying treatment for OA.

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

These outputs have the potential to benefit patients with OA - a very common and debilitating joint disease that affects millions of people worldwide - by improving our understanding of the disease process and, ultimately, by supporting the development of new disease modifying treatment and interventions.

The outputs will also be of value to scientists who are conducting basic and clinical research in OA by providing new insights into the regulation of disease-associated mechanisms. This research will also shed new light on exercise biology and sports medicine.

We will also engage with media outlets (including newspapers, radio and television networks) to publicise our major findings and maximize impact of this work.

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

We will maximise the outputs by disseminating the outcomes of our work through conferences, seminars and publications (of both successful and unsuccessful approaches). We will also engage with OA patients and their clinicians to ensure that our work to develop disease-modifying drugs or interventions (e.g. timed exercise) for OA is aligned with their priorities and needs. We will also collaborate with other researchers by making our knock-out mouse models available to other researchers.

### **Species and numbers of animals expected to be used**

- Mice: 5000

## 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 utilise mice as a model of human osteoarthritis. We will also conduct treadmill exercises using young adult mice to investigate effects of exercise training on their skeletal circadian rhythms.

Use of a mouse model will allow us to carry out genetic modification to generate cartilage-specific gene knockouts in order to specifically explore our protein of interest in the knee joint during the development of OA. The generation of the gene knockout will require a breeding programme, which will use pregnant mice, embryos and all life stages from birth to adult mice.

Cartilage-specific gene knockout will be stimulated in young adult mice at the point when they have a mature musculoskeletal system. We will use these knockout mice to explore the spontaneous development of OA over a timeframe of up to 18 months.

We will also use the DMM (destabilisation of the medial meniscus) model of knee OA, where surgical destabilisation of the knee joint causes OA to develop over a few months. This model is used by many researchers in the OA field and is well accepted as providing informative data on disease-associated pathways and the effects of therapeutic interventions.

**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. Some mice will be kept for up to 18 months without joint destabilisation surgery and then humanely killed so that joint tissues can be examined for molecular and cellular changes.

Mice with their clock molecules genetically engineered to carry either a light-emitting molecule or a deletion will undergo graded endurance training using a multi-user motorised treadmill, until running continuously for up to 60-mins per day, up to 5 days a week for up to 4 weeks. Each mouse will only go through one regimen of exercise. Animals will be acclimatised to both the environmental conditions (such as the mechanical noise of the motor) and to treadmill running which will gradually increase to the designed duration and speed. On completion of the exercise regime, mice will be killed for tissue collection and downstream analysis of circadian rhythms.

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

To induce OA, mice will undergo a precise surgical procedure (under anaesthetic) to destabilise the knee joint. Animals will be humanely killed at a series of endpoints after surgery (up to a maximum of 20-weeks) so that joint tissues can be examined for molecular and cellular changes.

Some mice will be treated by a series of injections (up to a maximum of 1x/week, max 12 injections) with our drug candidates to improve disease outcomes.

As well as the endpoint examinations of knee joint tissues, the development of OA might also be examined using non-invasive imaging methods, such as x-ray and MRI (no more than once per month, max. 12 months), and by observation of the animals' mobility (a maximum of once per week).

Control mice, e.g. without gene knockout, will be included for comparison in all elements of the project.

### **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.

Adverse reactions are not anticipated for the treadmill running regimes we plan to use. Habituation to treadmill training may induce mild physiological and psychological stress. If an animal refuses to run during the habituation steps, it will be removed from the study.

Short-term treatment with Tamoxifen can result in some weight loss; these effects will be short-lived.

Following joint destabilisation surgery, mice might experience some inflammation, discomfort and ultimately arthritis in their joint - i.e. a swollen joint and possibly limping. Our previous work in the DMM model has shown that, although we can see changes in the joint tissues at the endpoints of experiments, mice show very little sign of discomfort.

Injection with our drug candidates is not expected to have any adverse effects, based on our previous work.

### **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)?**

The breeding programme is a mild procedure. No animals are expected to experience adverse effects.

The treadmill exercise is a mild procedure. No animals are expected to experience adverse effects.

The effects of tamoxifen treatment may be mild or moderate; this will be minimised by optimising the dose and route of delivery. Most animals will experience some effect.

The effects of DMM surgery is moderate. All or most animals will be affected.

The spontaneous development of OA in our gene deletion mice may be mild or moderate. We do not yet know the extent to which OA will develop in these mice.

#### **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?**

We are studying the complex biological process by which the articular cartilage is maintained through daily wear and tear, and subsequently can become degraded in osteoarthritis. The onset and progression of OA in humans is very variable. We consider that this is due to differences in intrinsic protective pathways within the joint. We have identified circadian timing mechanism in the cartilage as a critical regulator to inhibit OA-associated processes.

We are now at the stage of directly determining whether circadian rhythm disruption are pathogenic factors in the aetiology of OA and for these studies, there is no alternative but the in vivo model. Defining the molecular mechanisms involved and how they influence joint structure and function will improve our understanding of OA and support our development of new disease-modifying drugs. We make extensive use of cell culture models to study individual aspects of the process, but the holistic and complex process of connective tissue functions, including the aspects of the complex series of events leading to cartilage degradation associated with osteoarthritis cannot be studied in vitro.

The use of a mouse model is essential to achieve our aim. Mice have a musculoskeletal system that is similar to humans and are well established as informative models of OA. Mice are also the ideal species for genetic modification. The generation of a cartilage-specific gene knockout for our proteins of interest in young adult mice will allow us to specifically define the effects of clock genes within the knee joint. Previous studies have already shown protective effects of circadian rhythms in mice and we are now uniquely placed to conduct the in vivo studies necessary to prove the causal link between chondrocyte circadian disruption and OA disease phenotypes and unravel the underlying mechanisms.

Mice mature much more rapidly than humans, so we will be able to look at the age-associated development of OA in our model over a relatively short timeframe (up to 18 months). In addition, surgical destabilisation of the knee joint in mice causes rapid onset of OA and is a very well accepted model of post-traumatic OA in humans. In these mouse models we will be able to use non-invasive methods to assess changes in joint mobility and structure during the study timeframes. At the study endpoints we will also be able to quantify joint damage and determine the expression of genes and proteins implicated in OA. In the joint destabilisation model we will be able to gather data for very early time points (from 6 hours after surgery) through to a maximum of 20 weeks. Evaluation of precisely defined time points, from the point of onset and throughout the progression of OA, could not be achieved using human tissues.

Before in vivo studies, we will test potential therapies in cell culture models to check that they have the expected effects in improving circadian rhythms and modulating the expression and activity of the clock related chondro-protective pathways.

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

We have considered using clinical knee tissue samples from people with late-stage, symptomatic OA that are collected during joint replacement surgery, or early stage OA. We have already used these to explore how our protein of interest influences the expression of other proteins known to be associated with cartilage damage and how our drug candidate acts to suppress cartilage breakdown. We have also considered the use of cell culture models of cartilage, which we have used previously to generate mechanistic data.

### **Why were they not suitable?**

The proposed work requires a model system that provides an accurate representation of the whole knee joint and where we can carry out analyses at precisely defined time points that represent OA onset through to late-stage disease. Human clinical samples are not suitable because they would not allow us to carry out genetic manipulation. Furthermore, there are no good diagnostic markers for early OA, and patients are seen by clinical teams at variable times following joint injuries, so it is impossible to identify patients at specific stages of the disease. Cell culture models could provide the option of genetic manipulation to knockout the protein encoding our gene of interest. However, OA is a disease of the whole joint and disease progression depends on the mechanical loading of the joint, which can only be achieved in a live animal model.

## **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 that up to 5000 mice will be involved in our breeding programme that will generate the genetically modified mice to be used for clock gene reporters and cartilage-specific knockout of our genes of interest. 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.

We will use up to 500 mice for our studies on treadmill exercise. This is based on various timing and frequency of exercise protocols.

We will use up to 1000 mice for our studies on the spontaneous development of OA. In addition, we estimate that up to 400 mice will be used in the DMM model of OA. This is based on: (1) the different experimental time points we will use (in order to explore the effects of our protein of interest throughout the early stages of OA development), and (2) previous work by ourselves and collaborators using the DMM model, which suggests that each experimental group will need to include 10-12 mice in order to generate informative data. Among these mice for spontaneous or induced OA studies, we have estimated that up to 200 will be treated with Tamoxifen to stimulate cartilage-specific gene knockout. This is based on genetic targeting of 2 different clock genes.

**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 our own and collaborators labs and taken the advice of a statistician to reduce the number of mice being used in each experimental group. We will continually review the outcomes of experiments and use our data to optimise animal numbers as the project progresses.

By using both male and female mice in the spontaneous OA and DMM-induced OA studies we will reduce the numbers 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 OA is common in both women and men.

**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. We will also draw on our established local expertise for tamoxifen induction to ensure minimal animals are needed for the generation of cartilage-specific gene knockout.

We will follow the PREPARE guidelines (Smith et al 2017) to ensure the quality, reproducibility and translatability of our animal experiments.

To ensure efficient breeding, we will minimise the number of animals kept "on the shelf" and will breed, as far as possible, on demand. We will also have regular reviews of the colony performance. We have already optimised genotyping protocols.

## 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 use genetically modified mouse models to allow selective deletion of clock genes in the cartilage of young adult mice. This model will be used to explore the effects of the gene deletion on the onset and progression of knee osteoarthritis (OA). We will determine whether OA develops spontaneously in the knockout mice over time (up to 18 months); in this case we anticipate that any OA-like symptoms will be mild and slowly progressing. In genetically engineered mouse lines where there is an absence of any spontaneous OA phenotypes, we will initiate OA by surgical destabilisation of the knee joint in the DMM (destabilisation of the medial meniscus) mouse model. This is a very well characterised model, which we have used previously. We have observed that whilst mice develop OA-



like damage to their cartilage, they exhibit little or no evidence of discomfort/pain over the timeframe of our proposed experiments.

Healthy mice enjoy running and it is an enriched experience for them to do so. Healthy wild type mice under standard animal house conditions will 'run' for 8-15 km per day in a running wheel. Indeed, we will use wheel running to confirm intact behavioural circadian rhythms in young adult mice with cartilage tissue-specific deletion of clock genes. However, voluntary running (e.g. through running wheel) is not suitable training apparatus for the specific aim in this project as previous work has shown that running wheels produce a heterogeneous response in their level of running. In addition, mice will not normally run at daytime. As such, treadmill is the methodology of choice because it is the only regimen that simultaneously allows for precise control of time of exercise, its intensity and volume.

### **Why can't you use animals that are less sentient?**

OA is a complex disease that involves the whole joint and is influenced by factors including joint mechanics as well as cellular and molecular processes. In order to explore the onset and development of human OA 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. In addition, there are well-established methods for genetic modification and for surgical induction of OA in mice - both of which are essential for the proposed project. For the treadmill exercise study, again we need adult mice that have a mature musculoskeletal system that is similar to an adult human.

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

Potential refinements include habituation for treadmill, increased monitoring, post-operative care and pain management.

The major welfare cost to the clock gene knockout mice in this study is the development of OA-like symptoms. Since the purpose of this project is to study OA in the mouse knee joint, this welfare cost cannot be changed. However, any apparent pain associated with the onset of OA will be controlled by the use of appropriate analgesics as advised by the NVS with animals monitored to ensure adequate dosing.

With regards to the Tamoxifen induction of cartilage-specific gene knockout in our mouse model, we will continue to optimise the least harmful way of administering Tamoxifen in terms of the route, dosage, duration and intervals, whilst still ensuring effective knockout (determined by genotyping). Tamoxifen treatment can cause some weight loss but these are typically transient.

With regards to the DMM surgical model of OA, we will continually monitor our procedures for pre- and post-operative care to ensure that these are optimised. For example, using sterile techniques to prevent infection at surgical sites and making adjustments to food, bedding and enrichment and using postoperative analgesics if required. In addition, we will carry out pilot studies and order our experiments so that the data we gather can be used to refine the numbers of animals being used at each stage, i.e. to ensure that all experiments are informative, but without using more animals than necessary. 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.

With regards to the treadmill exercise experiments, experience from a local colleague shows that adverse reactions are not anticipated for the running regimes we plan to use. Habituation to treadmill training may induce mild physiological and psychological stress. To mitigate this, animals will be acclimatised to both the environmental conditions and to treadmill running. Animals will be monitored for adverse signs such as fatigue and indications of injury, which if they occur the exercise will be halted.

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

We will use published documents recommended by NC3Rs to ensure that we are using the most refined approaches in all our experiments.

We will closely follow the best and refined practice on GA breeding, Aseptic surgery, Prepare guidelines, Pilot studies, Systematic review, Enrichment, Social housing, Tube handling and 3Rs assessment, as listed on the NC3Rs website.

With regards to DMM model of OA, we will regularly review the scientific literature for work by other researchers in the field to identify opportunities for refinement. In terms of treadmill exercise, we will closely review the scientific literature for similar work from other groups to refine our protocols.

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

We are registered with NC3Rs and subscribe to their monthly newsletters, which will keep us informed regarding advances in the 3Rs. We also have access to an NC3Rs Regional Programme Manager. We will utilise the NC3Rs Experimental Design Assistant as well as seeking guidance from our local NVS and NACWO to inform the implementation of any advances that can be applied to our project.