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NON-TECHNICAL SUMMARY

Control of matrix homeostasis in health and disease

Project duration

5 years 0 months

Project purpose

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

Key words

collagen, immune cells, fibroblasts, fibrosis, therapy

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

To investigate the molecular and cellular mechanisms of collagen homeostasis.

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?

Collagen accounts for approximately 25% of protein mass in the body, and is the most abundant protein that is deposited outside the cell (i.e. extracellular matrix), with a primary function of providing a physical scaffold. Abnormal production or removal leads to diseases like fibrosis (overabundance of collagen fibrils; accounts for 45% of all deaths in the developed world) or poor wound healing (insufficient collagen production). Thus understanding how cells control this normal balance is crucial to finding new efficient therapies.

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

Collagen is the most abundant protein in the human body, providing structure to organs with very different functions (e.g. tendons vs lungs). It forms what is called the extracellular matrix, which is a proteinous scaffold outside the cell providing support as well as influencing cell behaviour. Collagen deposition is a tightly controlled process; dysregulation underpins many pathologies and age-related conditions, including fibrosis and heart disease. Despite collagen's fundamental importance, therapeutics for diseases associated with collagen have been lacking, due to conceptual hurdles in understanding how collagen is assembled/removed.

Closing these knowledge gaps will form the majority of outputs at the end of this project.

The major output from this project will be the knowledge of how collagen deposition is controlled at the molecular level, by coordinated actions of different types of cells (fibroblasts, immune cells) that play major roles in health and disease. This research will further our understanding of fibrotic responses, and identify new targets for treating lung fibrosis. It will also deepen understanding of other conditions heavily involving the immune system and matrix, e.g. wound healing and cancer metastasis. Additionally, it may also provide understanding on how to treat collagen-associated rare diseases (e.g. osteogenesis imperfecta, Ehlers-Danlos Syndrome), which usually manifests as genetic musculoskeletal defects. The findings of this work will be disseminated in peer-reviewed publications, to scientific audiences at conference meetings, and to the lay public via forums such as Twitter and presentations/conversations at public or patient engagement events.

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

My research is of particular interest to the academic community: it will tackle fundamental yet understudied questions in basic biology, towards an improved understanding, in an unbiased manner, of the protein networks involved in controlling how collagen are processed in fibroblasts and immune cells (e.g. monocytes, macrophages). This knowledge can then be applied to understand the disease-progression mechanism of collagen pathologies. It will also define the roles of monocytes and macrophages in collagen deposition. As such, several academic disciplines will benefit from this research in the 4-5 year time mark:

1) Researchers interested in matrix biology functions. There is a distinction between the secretion of collagen and the assembly of collagen into a scaffold, and they are separately controlled by the cells. The molecular insights that will be answered in this research will change the way matrix biologists associate collagen production to collagen functionality.

2) Clinical scientists working general fibrosis research. This work will greatly advance molecular knowledge on how a collagen scaffold is controlled, opening new avenues to treatment.

3) Immunologists studying how the matrix influence immune cells response and vice-versa. This research will identify the roles of monocytes and macrophages in collagen trafficking.

4) Academic clinicians who work on relevant diseases that are known to be exacerbated by collagen dysregulation, such as cancer, osteoarthritis, and cardiovascular diseases.

As molecular mechanisms are elucidated, new targets for therapies to treat collagen pathologies will be revealed, thus in the mid- to long-term (5-15 years) these targets will be able to be further tested with the aim to develop them for clinical application that will benefit patients.

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

All data accrued in the project will be disseminated regardless of the outcome. We will publish the research output, as well as scientific approaches, in a timely manner in high impact open access journals to ensure the research will have the greatest impact. Where appropriate, the manuscript will be deposited on preprint servers (e.g. bioRXiv) to maximise availability to everyone. We will also attend and present research findings regularly, at scientific conferences to share and discuss results with our national and international colleagues, and establish new collaboration to maximise research impact. Effective communication with established collaborators and any new potential collaborators will be ensured, in the means of online joint-lab meetings and delivery of seminars to a wide audience beyond this institution. We will also promote the research findings and provide lay summaries on Twitter, and present at internal and external seminars, and engage with the public about the research (e.g. outreach activities). We will also engage with with small focus groups who may benefit from our ongoing research (e.g. patients groups).

Species and numbers of animals expected to be used

- Mice: 2000 over 5 years.

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.

In this project I will breed two mouse models. These are a well-established Per2::luc mice that have an internal reporter for circadian studies, and Nluc::col1a2 mice that have collagen-I tagged at a specific region (N-propeptide) endogenously.

Although most animals exhibit circadian rhythms, many of the tools and approaches for circadian studies were developed in mice. The circadian clock mechanism is highly conserved between mice and humans, and there is also much similarity between the physiology of the two species, thus mice are a good model organism to define processes that could then be inferred in humans.

As the Nluc::col1a2 mice are a new mouse strain, we will be characterising the strain at all ages to understand how the N-propeptide controls collagen homeostasis. For all other experiments, we will be using 12 week-old or older mice, to ensure that effects observed are comparable to that of an adult human patient.

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

The mice will be bred and maintained using standard protocols. All mice to be used for data gathering will be humanely killed. Mice will also be genotyped, which will most likely be done by ear biopsy, or by sampling either the blood or hair, or by mouth swabbing.

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

Most of the mice will not experience any adverse effects. However one of our two strains will experience a moderate developmental phenotype which includes a domed head and short stature. However, with floor feeding, no additional adverse effects have been observed in our previous work. Some mice will experience an approximate 35% reduction of body size and weight, as a result of failure to grow to a full adult size (compared to wild type littermates). All mice will be closely monitored, continuously assessed for their body conditioning score (which gives a quantitative measure of body fat and muscle), and any general adverse effects beyond what is expected will lead to them being 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)?

No severities for the Per2::luc strain.

Moderate severities for the Nluc::col1a2 strain.

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?

This project aims to study the extracellular matrix in health and disease, with a specific focus on collagen-I. Non-animal models do not form an extracellular matrix that is present in animal models and so animals are required for this specific project.

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

1. in vitro 2D co-culture system.

2. in vitro spheroid cultures, which are 3D cell cultures.

Why were they not suitable?

All in vitro co-culture/spheroid culture systems only allows for 2-3 types of cells to be studied in terms of their interaction with one another, and lacks the complexity seen in animal models. Lack of blood flow to the cultures means that immune cell infiltration cannot be mimicked, and thus the dynamic nature of extracellular matrix regulation is lost in these non-animal systems.

Additionally, the use of animal models allow the discovery of new interactions central to matrix homeostasis. A great example is the creation of the Nluc::col1a2 mouse strain, where the in vitro cell system showed no defects on collagen deposition, but nevertheless the mouse has musculoskeletal defects, highlighting the dynamical nature of collagen homeostasis control that impossible to replicate in an in vitro system.

Once observations and subsequent hypotheses have been developed from the mouse studies, different types of cell culture systems will be used to establish the details of the molecular mechanism that drives these observations; these molecular mechanisms will then be verified in the mouse again to ensure the findings are correct even in complex organisms.

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?

For primary tissues and cells, and from prior studies, we estimate we will require around 100 Per2::luc mice per year to allow for circadian time course studies. Each mouse yields 40,000 alveolar macrophages which will only be sufficient for 1 or 2 in vitro/ex vivo experimentation.

To get statistically significant results in the Nluc::col1a2 mice we must perform a cross between heterozygous mice to wildtype mice to study the heterozygous phenotype genotypes.

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

The research team consulted with statisticians to advise on our experimental design.

Both genders of mice will be used, although recorded separately (in case there is any sex related variability in phenotypes), to ensure we are not biased towards any sexes in our reporting.

Primary measurements in this case is body weight, As we do not yet have data on variability of body weight of the het/homo lines at maturity, we utilised the best available data) to estimate the number we may require.

We will revisit power calculation again once more preliminary data has been collected, and reassess the sample size required. The number provided here is conservative and ignores litter to litter variability, so as to ensure we have sufficient data to draw meaningful conclusions.

For both strains, we have established a breeding plan that will yield the required numbers of mice with the smallest number of crossings.

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

Efficient breeding will be used to reduce the numbers of mice used in the experiments. Additionally, any tissues unused will be shared with other groups that are interested in circadian rhythm, or utilising primary cells for downstream in vitro studies. We have a track record of sharing tissues with other groups to maximize usage of the mouse.

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.

Mice will be closely monitored from birth to minimise animal suffering.

Floor feeding provides enrichment and allows shorter mice to reach food easily. With floor feeding, heterozygous Nluc::col1a2 mice can live up to a year without additional adverse effects. There are no malocclusions or noticeable behavioural differences between Nluc::col1a2 heterozygous mice and their littermates. Heterozygous mice do not have additional suffering; thus it is likely this mouse strain will allow valuable insights into how these rare diseases occur, while having minimal impact on the their overall well-being. Once pups are yielded they will be closely monitored, genotyped, and measured. Any mice with additional adverse effects will be humanely killed to minimise pain, suffering, distress, and harm associated with that particular genotype.

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

One of the key caveats of animal research is the use of immature animals which leads to incompatibility between what is observed in an animal to what is observed in humans, thus we are deliberating selecting mice of a more mature age to better mimic adult human (patho)physiology. Terminally anaesthetised mice will not allow for dynamic investigation of collagen homeostasis, and less sentient species (e.g. zebrafish) do not assemble collagen-I in the same way as mammals in their skeletons, which is the major matrix effector molecule that causes pathological progression.

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

Per2::luc mice are already a well-established mouse strain within the facility, and no additional procedures will be performed on these mice without a humane end point.

Nluc::col1a2 mice - post-genotyping, all mice will be monitored closely for adverse effects such as musculoskeletal development defects, dramatic loss of weight, and loss of appetite. We expect the heterozygous strain to have defects such as hydrocephaly and short stature, however with soft food the mice will not have any other adverse effects. Homozygous breeding programme will be closely monitored to ensure animals do not suffer due to the additional Nluc::col1a2 allele. This will be a general monitoring to ensure we are capturing any issues that we are not anticipating.

Animals in this project will have an humane end-point of around 12-14 weeks, with some that may extend to older age (e.g. 6-15 months) as long as there are no adverse effects on the health of the mice.

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

My ethos is to reduce waste, promote alternative, and increase reproducibility of my animal research, which is why I will continuously consult the NC3Rs website (https://nc3rs.org.uk/3rs-resources) for up-to-date best practice guidance, in particular with breeding and maintenance plans. Another website I plan to consult is the PREPARE guidelines (https://norecopa.no/prepare).

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

Continuous communication and consultation with the staff in the animal facility and with the NC3Rs through access to newsletters will ensure advances in 3Rs are captured. All lab members will be required to use the online resources available (with special emphasis on breeding and colony management) at the NC3Rs website (https://nc3rs.org.uk/breeding-and-colony-management) before beginning experimentation, and I expect all lab members to attend 3Rs workshops organised by the animal facility.