Home Office

NON-TECHNICAL SUMMARY

Gene regulation of cardiovascular disease and regeneration

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

VEGF (Vascular Endothelial Growth Factor), Atherosclerosis, Cardiovascular repair and regeneration, Cardiovascular Development, Angiogenesis

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Zebra fish (Danio rerio)	embryo, neonate, juvenile, adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 find out more about how our blood vessels and hearts work, and what goes wrong when diseases happen. Our goal is to find new treatments, such as gene or cell therapy, or find drugs that can help fix these problems.

A retrospective assessment of these aims will be due by 3 October 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

Coronary artery disease is a major cause of death and ongoing health issues for adults worldwide. It primarily occurs when arteries in the heart and brain become blocked, usually due to a combination of factors like atherosclerosis (plaque buildup) and thrombosis (blood clots). Understanding how cells and molecules contribute to plaque formation is crucial for developing treatments to prevent artery narrowing and blockage. Our goal is to study the role of specific molecules in atherosclerosis development within different types of cells in the plaque, such as macrophages, smooth muscle cells, and endothelial cells. Our previous research has shown that specific receptors to a growth factor are involved in atherosclerosis and could be potential targets for therapy. We aim to further understand how they affect plaque build-up using knockout animals or administering therapeutic molecules.

After a heart attack, mammals experience irreversible loss of cardiac muscle, resulting in a permanent scar that impairs heart function. However, in zebrafish, a temporary scar forms after injury, which is

gradually replaced by new, functional heart tissue. Studying zebrafish has been crucial for identifying the molecular pathways involved in this regeneration process. Previous research suggests that our receptors of interest also play a role in cardiac regeneration. We plan to continue investigating this by using zebrafish with altered gene expression to understand how those receptors influence the different stages of heart regeneration. This will help us uncover the specific cellular functions modulated by these receptors during the regeneration process.

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

This project aims to take what we have learned in the lab and investigate if it can be applied in vivo to study heart and blood vessel disease similar to those seen in humans. We want to find out if changing the expression of certain genes or blocking specific signals can protect against heart and blood vessel diseases. By studying mice and zebrafish, which have similar biological processes to humans, we hope to see if our ideas could eventually help patients.

We are particularly interested in looking at how changing the activity of certain genes and signals affects heart and blood vessel diseases. This could give us clues about new treatments for conditions like heart attacks and heart failure. We will also explore ways to reduce the buildup of artery plaque build-up and help the heart heal better after a heart attack.

If our experiments go well, we hope to publish our findings in a top scientific journal. This could pave the way for new treatments that might help people with heart and vascular diseases in the future.

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

Findings from this work will be made available to other scientists through publication in peer-reviewed journals. We will increase the shared knowledge of the scientific community in the fields of vascular diseases and regenerative biology. Under the previous project we published 3 papers (and 3 more are currently in preparation). Because we conduct basic research, clinical translation is further down the line however our work is essential to identify molecules and their receptors/effectors important for cellular signalling driving diseases and/or regeneration.

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

We will disseminate our research by attending and organising conferences/seminars nationally and internationally. Under the previous project, we presented our findings at 2 international and 2 national scientific meetings (2019-2023) and I was the co-organiser of a 2-day workshop focused on animal models of regeneration. Our field of research is vibrant and highly collaborative and we share resources effectively with one another.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 10,000
- Mice: 2,000

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.

We are interested in how vascular disease start or worsen, using special mice and fish that have been changed at the genetic level. These animals either have certain receptors or molecules related to blood vessel growth turned on or off. We might also give them drugs that affect the signalling of these receptors. Then, we put them on a high-fat diet to see how it affects plaque build-up in their arteries, which can cause vascular disease.

Vascular disease is complicated, involving different types of cells, and depends on the diet the animal eats. Among animal models, mice are the best for studying how artery blockages happen, and there isn't a better model available.

For studying how tissues repair themselves, we use genetically modified zebrafish that are good at healing naturally. Zebrafish are perfect for studying how the heart can regrow because the complexity of the cardiac tissue is very similar to the mammalian heart. Additionally, the cellular processes stimulating the growth of new blood vessels are comparable to those activated in human tissue repair.

We can even track specific cells using genetically modified fish that express fluorescence in certain types of cells, making it easier to understand how particular cell types behave during regeneration.

When it comes to studying how blood vessels repair themselves, we use zebrafish embryos. We create a small injury to their blood vessels with a needle, and then we observe them as they heal over the next few days. They are transparent and easily observable under the microscope, allowing us to track how vascular cells proliferate and heal in real time.

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

- To study the development/regression of vascular disease, genetically modified mouse models will be fed a diet high in fat to induce the build-up of plaque. Depending on the model, the loss of expression of specific genes can either be constitutive (our preferred option) or inducible. The latter option will be chosen if the loss of the gene is detrimental during development and must be delayed to minimise the suffering of the mouse. This is typically done by injections or oral administration, which will be refined and kept to a minimal number of occurrences. The mice will not be subjected to any surgical intervention.

- To study heart regeneration, we routinely use the cryoinjury model of cardiac myocardial infarction. To do so, we briefly anaesthetise the fish and apply a probe made up of a fine metal filament that has been cooled down in liquid nitrogen. The probe is in contact with the cardiac tissue only for a few seconds and the fish is then placed back into its tank, without the need for suturing. The overall surgical intervention does not last more than 5 minutes and the fish resumes swimming after less than a minute. No further procedures are necessary until the end of the experiment.

- Vascular regeneration is investigated following caudal fin amputation, which consist of cutting up to 50% of the fin. Similarly, the fish is under anaesthesia only for a few minutes and resume swimming shortly after being placed back in its tank. The fish might be anaesthetised again to quantify the size of the regenerated tissue but no further surgical intervention will be conducted until the end of the experiment.

- To study vascular repair, we use zebrafish embryos which are briefly anaesthetised to restrict their movement. It is to note, that, contrary to adult fish, the embryos do not rely on gills for breathing as oxygen freely diffuses through their skin. With the discrete prick of a fine needle, we disrupt the vascular network of the embryos. They are then placed back to water without anaesthetics and regain normal activity quickly. We then observe the subsequent repair by observing them under the microscope, for which they must be anaesthetised again, however, no further surgical intervention will be conducted until the end of the experiment.

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

- Few adverse effects (e.g., skin irritation) are expected from the atherosclerosis study (plaque build-up in the arteries) as the mice are not subjected to any surgical intervention. The mouse genetic models we are planning to use are not known to have adverse effects. Atherogenic diets are expected to increase body weight.

- Fish undergoing cardiac cryoinjury will feel only transient moderate to severe pain, which may be managed with the administration of analgesics. The fish resume swimming shortly after the surgical intervention and previous behavioural monitoring indicates that most fish experience only moderate pain. However, it is still to be determined which analgesic agent is best to alleviate the effects of the surgery on the fish.

- Fish undergoing fin amputation will experience mild to moderate pain which may be managed with the administration of analgesic agents. No other detrimental effects have ever been observed in the past or are expected.

- The damage inflicted by the needle to the vascular network of the embryo has little observable detrimental effect. The damaged tissue starts the healing process almost immediately. The embryos are closely monitored, and previous experience showed high survival rate. Nevertheless, it is unknown and currently debated if the zebrafish embryos experience pain at this early stage of development. Repeated anaesthesia at later developmental stages would induce mild distress.

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)?

- Vascular disease/ atherosclerosis study (mouse model): maximum expected severity will be moderate and only attained if unexpected adverse effects occur, such as skin irritation due to the diet. However, most of the animals under this protocol, will be classed under mild severity.

• Heart regeneration study (Adult Zebrafish): 90% of fish under this protocol are expected to experience moderate severity, 10% of fish might experience transient severe pain due to lack of efficient analgesic cover.

• Fin regeneration study (Adult Zebrafish): 90% of fish under this protocol are expected to experience mild severity, 10% of fish might experience transient moderate pain due to lack of efficient analgesic cover.

• Vascular repair study (Zebrafish Embryos): As stated earlier, it is difficult to ascertain pain in this model. However, because of the invasive nature of the injury which will repair over the following period, a maximum severity to be described as moderate is to be expected.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 3 October 2029

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 have learned a lot from studying cells in dishes about how our growth factors and corresponding receptors of interest work together. Now, we need to check if what we found in those experiments holds true in living organisms. In all the protocols used, alternatives are not available that replicate the response of, for example, the heart or a blood vessel, to injury, or that reproduces the complex cellular environment of a live tissue during repair.

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

If cell culture/co-culture experiments are useful to investigate up to a couple of cell types simultaneously and helpful to dissect and investigate specific signalling pathway by silencing gene expression or chemical inhibition, the build-up of plaque in arteries results from the interaction of several cell types, including the circulatory immune system.

However, when possible, we will use alternative cell culture and ex vivo models as much as possible for pilot work preliminary to animal studies, and for more extensive analyses and dissection of

signalling mechanisms.

Why were they not suitable?

Cell culture and ex-vivo explants do not recapitulate the complex environment of the biological response to injury. For example, inflammation brought up by immune cells requires a fully functional circulatory system. Additionally, the complex microenvironment made up of extra-cellular matrix and factors secreted by several cell types and their finely orchestrated interactions are only possible in living organisms. Additionally, because we investigate disease progression and regeneration of full organs such as blood vessels or the heart, the time frame of the response is long and relies on a succession of timely orchestrated processes. For example, during zebrafish heart regeneration, all phases, from inflammatory phase (characterised by the primary immune response), the reparative phase (with the activation of the epicardium (the outer layer of the heart) and revascularisation of the heart) and finally the regenerative phase (proliferation of cardiac muscle cells) must occur sequentially to allow successful regeneration. Similarly, in vascular disease, the development of the plaque is dependent on several factors (including blood flow), cells types, and their secreted factors and a complex environment. Such complex environments cannot be modelled in vitro and using ex-vivo models.

A retrospective assessment of replacement will be due by 3 October 2029

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

I have estimated those numbers on previous experience, my previous PPL and animal returns from the past 5 years. I have considerably reduced the number of animals as I have limited the number of protocols from my previous project licence.

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

I routinely use the NC3R's Experimental Design Assistant to design in-vivo studies when applying for grants. Although useful in understanding the final number of animals used for the experiment, the

NC3R's EDA unfortunately cannot predict or take into account the animals that are generated via breeding but are not of the correct genotype and therefore that cannot be used. However, if not useful for preliminary studies of my own, I systematically offer the carcasses of those animals to colleagues who need tissues for their own studies. In our institution, communication for sharing resources is efficient and facilitated by the animal technical team.

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

I have over 10 years of experience in breeding animals for research, including mice and zebrafish. I systematically and carefully monitor the number of breeding cages to manage our resources fully. We visit the animal units regularly and perform inventories to understand the breeding performance and monitor the fertility of our experimental animals. Additionally, we genotype our animals to ensure the correct breeding are in place and to minimise the number of animals generated. For zebrafish, homozygous lines are kept to produce experimental animals and age-matched wild-type animals are used as control. When possible, and to minimise genetic drift, homozygous lines are outcrossed yearly.

If no data can be extracted from the literature, pilot studies are performed on a restricted number of animals. This allows us to understand the amplitude of the effect of a specific treatment, and informs us on the number of total animals required to attain sufficient power for our experiment.

Finally, as mentioned above, the sharing of tissues is highly facilitated by excellent communication between colleagues and the animal technical team.

A retrospective assessment of reduction will be due by 3 October 2029

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 mouse model of atherosclerosis (blood vessel plaque build-up) is very well refined and has been used extensively in the field. The mice are not expected to be in any pain during the whole timescale of the experiment. They are left to exhibit normal behaviour and care is taken to enrich their environment to minimise harm.

From experience and informal discussions with colleagues using similar models, the heart injury model is known to be tolerated by adult zebrafish. They experience a transient level of pain which can be managed via the administration of analgesics, so it does not exceed moderate severity; however the best analgesics regiment is yet to be fully defined, therefore a few animals might experience transient severe pain if the analgesia coverage is not fully efficient.

Similarly, from experience, the zebrafish fin amputation model is well tolerated and no adverse effects exceeding mild discomfort have been observed to date with analgesics administration.

Finally, the needle injury model to induce vascular repair in the zebrafish embryo is using the less sentient and more immature life stage. Although debatable if the embryos feel any pain at all, injuries are well tolerated, and embryos continue their development normally whilst repairing the wound. With this new PPL application, we wish to observe the repair process fully which includes the recruitment of immune cells but also the subsequent growth and reorganisation of blood vessels on a longer timeframe.

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

The development of plaque build-up to investigate vascular disease relies on a fully functional and a complex immune system, as well as a timeframe that is not compatible with less mature life stages. In fact, the mice must be specifically raised on a pro-atherosclerotic genetic background, as animals, in general, do not develop plaque naturally.

To investigate regeneration, animals must recover from the injury to allow the regeneration process to happen. For the zebrafish cardiac injury model, this occurs over a 90 days period following the surgery, and for the fin regeneration model, this is achieved over a 21 day period. Similarly, they rely on the interaction of several cell types, their secreted molecules and the deposition of a complex microenvironment that cannot be mimicked by less mature, less sentient life forms.

Similarly, bearing in mind the needle injury is carried out between 3- and 4-days post-fertilisation, the subsequent inflammation and vascular repair occur over a period that exceeds the time during which the zebrafish embryo is not protected under the Animals (Scientific Procedures) Act 1986 (ASPA).

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

With the help of our animal technical team, we implement rigorous monitoring of our animals, both in routine husbandry but also during post-operative care. All zebrafish surgeries occur in the morning to allow a long recovery period with staff on site to ensure the level of severity described in the project licence is not exceeded.

Additionally, as mentioned previously, we also aim to investigate what is the best analgesic regimen for the recovery of our zebrafish protocols, as to date, the standardised use of analgesic agents has not been demonstrated to efficiently alleviate pain for all our models.

Finally, when administrating drugs in mice we currently use intra-peritoneal (IP) injections. When/if possible, we will aim to administer drugs via oral gavage, although, it is again debatable if IP injections

are more or less stressful than oral gavage for the mouse.

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

I will consult published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

Other resources are available including guidance and publications from the NC3Rs and Laboratory Animal Science Association.

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

I stay informed on the published literature in animal welfare. In particular, we receive regular updates from our animal technical team and the Animal Welfare and Ethical Review Body (AWERB) about recent advancements in refining in-vivo methods and the 3Rs (Replacement, Reduction and Refinement). I regularly attend relevant seminars and conferences where information on animal welfare is disseminated (RSPCA meetings, online International Zebrafish Society (IZFS) seminars etc…)

Additionally, being a member of the relevant societies and attending local seminars, I have access to a wide range of resources and a network of colleagues working in the field, available for informal discussion and advice on best practice.

In our institution, we are very lucky to have access to a dedicated team of veterinarian professionals who are world-leading experts in animal ethics and welfare. I have been in regular contact with our Named Veterinary Surgeon (NVS) to refine our protocols and will continue to do so for this project. We also receive regular seminars organised by the NC3Rs Regional Programme Manager and our lab receive the NC3Rs newsletter. Finally, our animal unit management team offer regular PPL refresher courses and our training records are regularly checked to ensure our practical skills are up to date (e.g., Schedule 1 and era-notching/fin-clipping are regularly re-assessed).

A retrospective assessment of refinement will be due by 3 October 2029

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?