Home Office

NON-TECHNICAL SUMMARY

Mechanisms of organ development and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

organ, morphogenesis, epithelium, optogenetics, mechanics

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

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 further knowledge about the mechanisms of organ development and how these might relate to disease.

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?

Most organs in the body arise from simple tube-like structures, made from specialised cells called epithelial cells. These cells all point towards the fluid filled cavity (lumen) found at the centre of the tube. The strict organisation of epithelial cells into this rosette-like formation is critical for the function of the organ. If cell organisation is disrupted either during development or later in life, it can lead to disease. Therefore, understanding the fundamental mechanisms driving epithelial tube/cavity development is very important for the appropriate design of bioengineering strategies to grow organs synthetically. It is also important for identifying potential causes of epithelial organ disease so that these might be better targeted therapeutically.

Many factors are important for the normal development of epithelial tubes/cavities. Both chemical signals and mechanical environment can interact to determine the behaviour of cells during organ development. We plan to use a technique called optogenetics, which allows researchers to use light not only to see individual cells and their internal components as they build organs but also to manipulate them. This will allow us to change the signalling within particular cells and test the consequences on the mechanics and development of the whole organ. One of the organs that we will look at is the early brain of the zebrafish, which arises from an epithelial tube called the neural tube. This is a good model for the part of the human neural tube where it is common for birth defects such as spina bifida to occur. We hope that better understanding of the mechanisms behind neural tube formation will provide clues as to how these diseases can be avoided in future.

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

The overall goal of this work is to further the knowledge that our lab has uncovered about the fundamental processes that are necessary for normal organ formation within vertebrate animals and how these might relate to disease. This will provide important knowledge for future bioengineering strategies. In addition, this work should provide clues as to what happens to cells at the onset of diseases such as epithelial cancers and neural tube disorders, which may influence therapeutic strategy in the long term. Our work will also further develop an important bioscience tool - the ability to reversibly manipulate proteins and signalling within single cells within a whole organ using light (optogenetics). This can be used for a wide range of biomedical research.

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

The knowledge gained from our research will enable us and other researchers to better design bioengineering approaches with the long-term aim to grow organs outside the body. The knowledge gained will also benefit researchers in the field of organ disease to better understand how the mechanisms of organ development might link to initiation of disease. In the long-term this might lead to more targeted therapeutic approaches. In the short-term, the further development of technology, especially of optogenetic techniques *in vivo* (in the living animal) will benefit a diverse range of research groups, who can use this technology to facilitate a wide range of scientific research.

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

We widely share the results of our work via regular presentation of our data at international conferences. We share the techniques used by contributing to international practical workshops. We upload our data on pre-print servers and publish our results in open-access journals. We upload our datasets on open-access sites and share reagents and code used in publications via e.g. online depositories. We also share experimental outputs of publications such as plasmids via open access physical depositories and genetically modified lines of fish via e.g. sperm freezing.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 10,000

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 will use zebrafish embryos for this research because they are transparent and develop rapidly. The neural tube in the brain is formed over approximately 14 hours. This means that we can put the in-tact embryo under a specialised microscope and can easily image the whole process of vertebrate epithelial tube formation in their brain within the course of one experiment. The early zebrafish brain is also formed via a similar process to lower spinal cord formation in mammals, making it a good model of this process. All of our experiments will be carried out on embryos under 5 days old, which do not require a licence from the Home Office. To generate these embryos, it is necessary to generate and maintain a breeding population of adult fish, which does require a licence.

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

The adult zebrafish will be housed in a dedicated aquarium within the department, run by trained staff. We will generate genetically altered zebrafish by introducing modified genetic material at the 1 cell embryo stage and growing these embryos to adulthood. In order to know which fish contain genetic alterations we sometimes need to carry out genetic analysis via e.g. cutting a small portion of the fish's tail fin under general anaesthetic and analyse the genetic code inside this tissue. The fish is then kept in a separate tank with fresh water and the fin then regrows relatively quickly (within approximately 2

weeks). Where appropriate, other methods of genotyping may be used, such as swabbing the surface layer of the skin. Adult fish will be maintained until a maximum of 30 months of age (although we aim to only keep adult fish until 18 months of age in the majority of cases). During this time, adult fish will be bred in specialised breeding tanks to enable the production of genetically altered zebrafish embryos. We very occasionally need to anaesthetise fish for the collection of eggs and sperm. At the end of the protocols fish will be humanely killed or supplied to other project licences or recognised establishments with the authority to breed and maintain genetically altered zebrafish of this type.

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

We do not usually expect there to be adverse effects to adults caused by the genetic alterations that we introduce. However, for example in the case of introducing optogenetic genes, it is sometimes possible that, due to basal activity of these genes, some adverse effects might arise in the fish. If this occurs, we would expect such effects to be mild (such as thinner bodies). However, it is possible that sometimes moderate effects might occasionally arise (such as significantly bent body-axis, which might effect swimming). It is also possible that the survival of larvae to adulthood might not be as high in some genetically modified lines when compared to wild type lines. If moderate effects were to occur, we will humanely kill the affected fish. We do not expect there to be any adverse effects from breeding the zebrafish. It is unlikely but possible that fish might develop an infection following removal of a small part of the tail fin, in which case we will humanely kill the fish. For both genotyping and sperm/egg collection, it is possible that fish may not recover from anaesthesia but this is very unusual (less than 1%).

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)?

Zebrafish. Mild: 90%. Moderate: 10%

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

- Used in other projects
- 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?

This focus of this project is understanding how organs are built and how disease is initiated inside a living animal. It is important to use an animal rather than looking at cells in culture because the physical forces and interactions between cells are very different in a culture system. Therefore, it is

only possible to fully understand the cellular behaviour during organ development by looking inside an in-tact animal. However, we are able to carry out some of our research using cells grown outside an animal (cell culture) – for example we have recently published a paper using cell culture to determine how cell-cell contact is involved in initiating cell polarisation and have accordingly reduced the number of animals used in our research. As we investigate the animal model alongside this culture system, we will learn whether more of our work can be carried out in culture.

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

We have introduced the use of cell culture models in our lab, which we have already used to replace some of our animal experiments. This resulted in the discovery that the centre point of epithelial organs is directed via aligning adhesions between cells with the plane of cell divisions. Carrying out this work in culture reduced our anticipated animal usage in our last licence by roughly 25%.

Why were they not suitable?

It is not possible to entirely replace animal experiments for this project since it is important to test whether the principles identified in cell culture are the same *in vivo*. This is especially important when considering the mechanical aspects of organ formation, which will be dependent on the physical environment of the organs.

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?

All of our experimental work will be carried out in zebrafish embryos younger than 5 days old (which are not protected under The Animals (Scientific Procedures) Act 1986. Animals older than 5 days old will only be used for establishing genetically altered zebrafish for subsequent breeding. The number of adult animals used is therefore solely related to the numbers required to maintain sufficient breeding stocks of animals. We have listed the maximum number of adult zebrafish that we would use for breeding purposes over 5 years to be 10,000. I calculated this based on requiring approximately 120 fish per new generation of fish for each genetically modified line. We will make a new generation for each line every year (5 generations per line). In addition, when generating new genetically modified lines, the F0 embryos need to be genotypically screened. Therefore, approximately 200 additional fish may be required per new line at the F0 stage to find appropriate founders to generate the F1 generation. Based on raising 15 established lines and generating 4 new genetically modified lines over the course of the 5 years, this makes approximately 10,000 adult fish. In addition, we have listed a maximum number of 2000 of these fish that might be kept on an alternative breeding protocol, depending on their genetic background.

However, we anticipate that the numbers that we will actually use should be significantly lower, since we are committed to actively reducing animal usage (see below).

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

Licenced animals older than 5 days old will only be used for establishing genetically altered zebrafish for subsequent breeding. Therefore, it is not possible to reduce the number of licenced animals used via experimental design. However, we still aim to reduce the number of unlicenced zebrafish embryos under 5 days old being used in our experiments via experimental design. There are several experimental design assistants and guidelines available to help us with appropriate design of each experiment. For example, the NC3Rs EDA (https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda) and PREPARE guidelines (https://norecopa.no/more-resources/experimental-design-and-reporting/).

We will also ensure that our publications conform to the ARRIVE guidelines: https://www.nc3rs.org.uk/arrive-guidelines.

For example, to make our experiments robust, we will control for variability in the following ways:

We will reduce environmental variability by carefully housing breeding adult fish in the dedicated zebrafish facility and by keeping genetic background constant within each genetically modified line of fish.

We will assess normal levels of variability within experiments via pilot experiments, allowing us to select appropriate statistical methods and number of embryos.

We will reduce bias by randomly selecting embryos collected from a pool of breeding adults and by assigning treatment and control groups in a way that is unknown to the person analysing the data (blinding).

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 several methods to reduce the numbers of adult animals used. First, we will share relevant fish stocks with other users within the facility. Second, we will try to limit repeated breeding to once per week to optimise breeding performance. Third, we will minimize the generation of transgenic lines and use wild type embryos wherever possible for our experiments. Fourth, we will freeze sperm from genetically altered lines of zebrafish for longer-term storage. We will also carry out efficient genotyping and raise fewer fish per generation wherever possible. Using these methods, we have already successfully reduced our animal usage in our current PPL, so far reporting far lower numbers than the maximum level on our licence.

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.

We will use zebrafish during this project, in order to establish and maintain breeding stocks of fish that we will use to produce the genetically altered embryos.

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

To understand how organs develop within vertebrate organisms it is necessary to use vertebrate animals. The zebrafish embryo is the most refined vertebrate model possible for the work that we propose. The zebrafish embryo is also an ideal model system for studying organ development, since they are small, transparent, develop rapidly and it is possible to alter their genetics in a reasonably straight-forward way. This means that they are an ideal system for using the optogenetic approaches that are integral to this project.

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

We don’t envisage any suffering in the vast majority of licenced animals beyond the mild procedures described above. Moderate effects might occasionally arise due to the genetic alteration of the fish, as described above. If that occurs, the animals will be promptly killed using a humane method. We will only use zebrafish embryos younger than 5 days old for our experiments, which are not yet capable of independent feeding. We will aim to reduce any potential suffering of these embryos by promptly killing them using a humane, approved method at the end of the experiments and, where possible, by anaesthetising embryos that are sufficiently developed to be capable of initiating movement during imaging (those above 18 hours old).

Adult fish will be housed in a dedicated centralised zebrafish facility, where they will be looked after by full time staff, who will ensure their welfare. Numbers of fish per tank, water quality and food quality and quantity will be carefully controlled.

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

Appropriate experimental design for our experiments in unlicenced embryos under 5 d.p.f. will be carried out, as described in the ‘Reduction’ section above. Licenced animals older than 5 days old will only be used for establishing genetically altered zebrafish for subsequent breeding. These will be housed in a dedicated centralised zebrafish facility, where they will be looked after by full time trained animal technicians, who will ensure their welfare, in line with their training on best practise. There are several resources to inform us about the current research on refinement of procedures (e.g. https://norecopa.no/species/fish/, https://nc3rs.org.uk/3rs-resources/zebrafish-welfare,

https://www.lasa.co.uk/current_publications/). These will be taken into account when deciding on the most appropriate method for procedures..

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

Advances in 3R tools are internally circulated. We can also access advances via the NC3Rs (https://nc3rs.org.uk/resource-hubs) and Norecopa website pages (https://norecopa.no/databases-guidelines). If scientifically appropriate advances in 3Rs arise in the course of the project, we will seek advice from the named veterinary surgeon and named animal care and welfare officer about whether and how to implement them.