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NON-TECHNICAL SUMMARY

Dissecting Vertebrate Heart Development

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Heart development, Cardiac Defects, Trabeculation, Morphogenesis, Cell biology

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, 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?

To understand the cellular, molecular and physical mechanisms driving vertebrate heart development and function.

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?

During embryonic development, heart is the first organ to develop and function, as this is crucial for the survival of the embryonic life. As the embryo grows, to support its physiological demands, the developing heart acquires numerous specialized structures to function optimally. Defects in this process of heart development lead to heart disease at birth, a leading cause of morbidity and mortality worldwide. Therefore, in order to understand the causes of heart developmental defects, it is crucial to understand the cellular, molecular and physical mechanisms underlying heart development. The project's findings will hugely advance our understanding of cardiac defects, thus facilitating the diagnosis and discovery of potential therapies.

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

The outputs of this work include:

1. A novel understanding of the underlying cellular processes and physical factors like blood flow that contribute to cardiac trabeculation – a process crucial for heart function.

2. A better understanding of how genetic factors like Erbb2, Notch and Taz signalling contribute to cardiac trabeculation.

3. A better understanding of the interplay between genes and mechanical signals regulating heart morphogenesis.

4. A better understanding of how organs develop inside a growing embryo.

We expect to publish these new findings in peer-reviewed journals.

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

This project aims to uncover the cellular and molecular events underlying trabecular morphogenesis – a process through which the heart increases its muscle mass for optimal functioning . These findings will be of fundamental importance to the cardiovascular research community, and hugely advance our understanding of cardiac defects, thus facilitating the diagnosis and discovery of potential therapies. Understanding the interplay between cellular processes and molecular signals is important to reveal the key design factors that regulates the formation of a robust complex organ like the heart. We expect these findings will potentially provide a rationale for tissue-engineering efforts and regenerative medicines.

The tools generated during this project, for example zebrafish transgenic reporters of signalling pathways' activities or transcription factors' expression will be of enormous benefit to the research community as they can be used in numerous other projects where a visual readout of signalling is required. Further, the knowledge generated during this project will inform the wider community of development biology researchers interested in organ development.

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

To maximize the output of the work, we will publish research papers in peer-reviewed journals or preprint servers. We will present our work at both big international meetings and smaller workshops. We will strive to highlight unsuccessful approaches for the benefit of other researchers.

We will collaborate extensively, both internally and externally and this provides another avenue to share details of approaches that were ultimately sub-optimal and how methods were improved.

If we develop new methods, we will publish specific protocols papers.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 95,500

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 aims to understand the cellular, molecular and physical mechanisms driving vertebrate heart development and function. For this we use zebrafish as our model organism. We have chosen this system because of the rapid embryonic development ex utero and optical transparency which allow a direct monitoring and visualization of development, morphology and physiology in the living organism. Also, during the first week of development, zebrafish can survive through passive diffusion of oxygen thus completely bypassing the need of a functional cardiovascular system. This is a unique advantage, as it enables us to use experimental tools compromising cardiac functions while keeping the animal alive, which is otherwise impossible to achieve in other model systems because of early lethality. Thus, zebrafish presents itself as an ideal system to study heart development. Most of our objectives will be achieved by working on zebrafish embryos that are less than 5 days old. To understand the later stages of heart development, we will need to work on juveniles.

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

We will generate reporter lines expressing biosensors for different signalling pathways or labelling various cellular compartments like the membrane. We will also generate lines in which we have deleted or mutated specific protein components of signalling pathways required for heart development.

These transgenic lines will mainly be studied at the level of embryos and larvae less than 5 days old, and in some cases, we will observe them at the juvenile stages to understand how heart develops and matures.

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

We do not expect any adverse effects for most zebrafish experiments as we are mainly working with transgenic lines that have no negative effect on development or we are monitoring mutant larvae younger than 5 days old. For a small proportion of experiments, when we need to observe the effects of mutations in animals beyond 5 days old, there may be some developmental abnormalities. If we observe abnormal behaviour, such as reduced swimming activity or retreat to dark tank corners, the fish will be 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)?

Moderate severity is expected for about 10% of fish.

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?

The heart is the first organ to develop and function during development to sustain the embryonic life. In the most common type of human birth defect (congenital heart diseases), this process of heart development is compromised resulting in structural abnormalities and defective cardiac function. We want to understand the regulatory mechanisms underlying heart development, as we believe this will hugely improve our understanding of cardiac disease and facilitate diagnosis and treatments. We have chosen to work on animals because in order to get a comprehensive and accurate understanding of these regulatory mechanisms, we need to study the heart while it is growing and developing inside an embryo.

Our model system is Zebrafish, which offers several distinct advantages as a powerful vertebrate model organism to study embryonic cardiac development, as explained below:

1. During the first week of development, zebrafish can survive through passive diffusion thus completely bypassing the need of a functional heart. This allows us to use tools ablating heart functions which is otherwise impossible to achieve in other model systems because of early lethality.

2. The optically transparent zebrafish embryos allows us to study dynamic developmental events in a living embryo.

3. In addition to short generation time, zebrafish embryos develop externally which makes them highly accessible to various manipulations.

4. Recent development of novel genome editing tools (such as the CRISPR/Cas9 system) have made it possible to generate mutant zebrafish lines in a highly efficient manner and the technology is easy to implement.

We will strive to minimize the number of procedures performed on animals deemed to be sentient by doing most of our work on zebrafish embryos and young larvae, before the onset of independent feeding. For some of the experiments, where we need to analyse later stages of heart development, we will need to work on juveniles. We will also use adult or juvenile fish for the fin clipping for genotyping, transient warming to induce expression of transgenes, and gamete harvesting. Concerning the genotyping protocol, mating will be performed as often as possible followed by analysis of phenotype to determine the genotype of the fish as an alternative to fin clipping.

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

When needed and when suitable, we will strive to complement our in vivo analyses with tissue culture models, organoids and/or mathematical models.

Why were they not suitable?

We can only acquire a limited amount of information about heart development from in vitro systems. The heart’s development is inherently linked to its function (heart-beat) and physical cues like blood-flow. These parameters cannot be recapitulated by in vitro systems, thus severely limiting the relevance of the information derived from them.

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 calculated the numbers of fish required based on the numbers of mutants and transgenics that we are currently maintaining, plus those that we need to generate to be able to achieve the aims of the

project. We are currently maintaining around 40 transgenic lines and 5 mutant lines. In the next five years, we will generate around 25 new transgenic lines and 5 new mutants. We have also analysed each step of the protocols and have estimated the numbers based on the experimental needs.

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

We regularly review our zebrafish stocks and cull any that are no longer required. For all zebrafish lines, we plan to freeze sperm to archive the line. This will allow us to maintain only those lines in the aquarium that we are using in on-going experiments. We will also share our transgenic lines and exchange transgenic lines with other zebrafish labs, in the UK and elsewhere. This will help us to minimise the number of mutant and genetically modified strains that we keep in our own aquarium. Moreover, the stocks of adult mutant fish that we will keep will almost all be heterozygotes carrying recessive mutations and thus phenotypically normal. In addition, because we will share the fish aquarium with a number of other labs, we ensure that multiple workers perform their experiments on the same day to maximise the use of the embryos from a given batch of fish.

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

We estimate that we will use 400 fish per line over 5 years for maintaining them.

To generate new transgenic lines, the estimated numbers of fish are based on established published protocols, statistics on transgenesis's efficiency from ours and other laboratories. Typically for one transgenic line we will raise 100 F0s, which will be screened by crossing them and subsequently screening their progeny for the transgene. Typically, 20-30 of the adult F0s will be founders. We will choose 2-3 of these and raise their progeny; the others will be culled. For each ‘family’ we will be raising between 50 and 80 fish which will be genotyped to identify heterozygous carriers of the transgene. The transgenic lines will be then maintained as for other wild type and mutant lines, which corresponds to about 2-4 tanks of around 20 fish each.

We will use a similar strategy to generate mutant fish by CRISPR-Cas9 technology. To produce one mutant line we raise 200-250 founder fish, which are phenotypically normal after microinjection of the CRISPR/Cas9 constructs. To screen for germline transmission and loss-of-function alleles the F0s will be crossed to wild type fish, and their progeny will be screened as embryos for the presence of the mutation. We will pick 2-3 positive founders and raise 200-250 progeny. The others will be culled. The F1s will be screened for the heterozygous mutation by fin clipping. Heterozygous F1s will be further outcrossed to wild type fish to segregate away any non-specific mutations and in-crossed to other transgenic lines (mutant lines to generate double mutants, GFP-transgenic reporter lines). We will raise 100-150 F2s from these crosses and screen them for the desired heterozygous mutations. The mutant line will be then maintained as for other wild type and transgenic lines, which corresponds to about 2-4 tanks of around 20 fish each.

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 use the zebrafish model for the heart developmental work, as these embryos develop ex utero and can be manipulated genetically and are transparent, and thus ideal for imaging.

We will minimize suffering by paying attention to the fish population's general health, by paying attention to water quality, feeding regimes, and fish population density in each tank. We will check all breeding stock daily and cull any that show signs of significant illness or deformity. Where surgical or other potentially distressing procedures are required, e.g. fin clipping, we will perform them under general anaesthesia with analgesia both pre and post fin clip. Any fish or fish larvae showing signs of distress on recovery from a surgical or other procedure will be killed promptly by an approved method.

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

We use zebrafish embryos for our developmental work, and most of this is on fry less than 5 days old, which are deemed non-sentient and before the onset of independent feeding. To understand the later stages of heart development, we will have to work on juveniles. In this case, we will mostly use non-invasive imaging and where possible, we will imply terminal anesthesia and analgesics

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

We will achieve most of our objectives using non-invasive microscopic techniques, which does not cause any pain or stress. Surgical procedures like fin clipping will be performed with suitable anaesthesia and animals will be monitored post-surgery to ensure that they recover well. We will also use suitable analgesia for all surgery. Any novel chemical substance will be tested in a small-scale pilot study for toxicity. We will standardize our imaging experiments on small-scale to first identify the optimal condition for imaging to ensure minimum suffering.

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

We are aware of NC3Rs. We also discuss with colleagues in other research groups new improvements that lead to refinement.

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

We will stay up to date via regularly communication with BRF staff, other scientists in the field and regular visits to the following website https://www.nc3rs.org.uk/3rs-resources.