Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 6

Projects with a primary purpose of: Basic Research – Musculoskeletal System

Project Titles and keywords

1. **USING ZEBRAFISH TO MODEL OSTEOARTHRITIS**
   - Zebrafish, joints, development, ageing

2. **Establishment of the vertebrate body plan**
   - Vertebrate body-plan embryogenesis signalling development

3. **Critical genes in osteoarthritis**
   - Critical, genes, osteoarthritis

4. **Role of microRNAs in musculoskeletal tissues ageing**
   - Ageing, microRNA, sarcopenia, osteoarthritis, osteoporosis

5. **Validating computer models of mastication**
   - mastication, biomechanics, computer modelling

Project 1	**USING ZEBRAFISH TO MODEL OSTEOARTHRITIS**	
Key Words (max. 5 words)	Zebrafish, joints, development, ageing	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to learn more about the cellular processes occurring in joints that lead to the development of osteoarthritis. Currently we have a very limited understanding of the disease at a cellular and genetic level. In particular we want to know how joint cells interpret mechanical forces, and switch on relevant genes to control cell behaviour. We then want to understand the roles played by 'osteoarthritis susceptibility genes' so that we can identify new therapeutic targets that might be used to promote joint repair.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We envisage that our programme of work will lead to improved understanding of the genetic and cellular processes involved in joint formation and maintenance and how these are influenced when mechanical loading is abnormal or when osteoarthritis susceptibility genes present. In so doing we hope to identify treatments that could be used to promote joint healing and repair.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use zebrafish for the whole of the project. We anticipate using a maximum of 6000 over the 5 years of the project, many of these will be breeding colonies of zebrafish that carry fluorescent reporter proteins, which are not expected to experience any suffering or	

	ill effects.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The types of adverse effects that we expect to see for the larval fish might include alterations to jaw shape and jaw function. We will carefully monitor these fish to ensure that they can feed even if their jaw shapes are abnormal. Occasionally when we are using a new drug or generating a new genetic modification a small number of larval fish may develop adverse effects such as heart oedema or brain malformation, these fish will be killed as soon as these defects are identified. For adult and ageing fish we expect the adverse effects to be the onset of joint conditions that resemble human osteoarthritis. These would be likely to cause stiffness in some joints (those of the jaw and the spine) which could in turn lead to reduced swimming performance and reduced speed of the fish. We will monitor the behaviour of the fish daily and any fish that can no longer maintain their position in the water will be killed under terminal anaesthesia. By carefully monitoring fish to ensure that that they do not exceed these end points we expect these studies to be of mild severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The joint is a complex 3-dimensional structure containing many different cell types and which is subject to frequent movement, as such there are very few non-animal models that exist and none that model all aspects of joint cell behaviour. We therefore need animals to understand what happens to the different cell types during common human joint disorders such as osteoarthritis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed computational models that allow us to model the effects of altered muscle or joint shape on the mechanics of the joint, and through using these we have been able to reduce the numbers of zebrafish larvae used by around 25%. For those experiments that require animals we perform power calculations to define the minimum numbers required to achieve defined levels of statistical significance and we consult with statisticians and other biologists when planning experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We have chosen the zebrafish as most refined animal model available (as worms and flies do not have a skeleton). By using the translucent zebrafish in which we express fluorescent proteins in cells of interest we

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	can non-invasively watch many cellular processes in the skeleton, allowing us to collect dynamic data with minimal surgical intervention. To minimise suffering and discomfort the fish will be monitored daily and when there is any concern advice will be sought from the named veterinary surgeon and/or the Named Animal Care Welfare Officer and appropriate action taken.

Project 2	**Establishment of the vertebrate body plan**	
Key Words (max. 5 words)	Vertebrate body-plan embryogenesis signalling development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	My group studies the roles that a particular “family” of genes play in setting out the basic body plan in early embryos, in particular how the developing spinal cord and surrounding tissues become organised and distinguished from each other.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Genes in the family we are working on have been implicated in a number of inherited and sporadic disorders of human development, in particular the muscular dystrophies and myopathies. Their inappropriate expression (in the wrong place or at the wrong time) may contribute to the formation of some cancers. We therefore expect that our fundamental research will shed more light on these disorders, perhaps leading to novel means by which they might be treated or prevented in the longer term. To that end, we have entered into a collaboration with clinicians, with a view to improving the diagnosis of human spinal disorders.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year life of the project we will need 2500 mice and 100 rats. Our experimental work will focus on early mouse embryos, harvested from pregnant females after they have been killed humanely. Over the five years, we expect to need about 1000 of these animals. To generate them, we shall have to breed the various genetically altered mouse lines in-house, which accounts for the “use” of the remaining 1500 mice. The rats will be used as a source of serum, which is a component of the special media in which early mouse embryos are cultured.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We need to breed mice carrying these genetic alterations, but we do this in a way that minimises the chances that live-born animals with welfare problems are produced, while keeping the coloaies as small as is practicable. In a small number of cases, we will administer agents to pregnant mice to trigger genetic alterations in the target embryos, but these too are expected to cause no significant harm to either the females or the embryos. Rats will be deeply and terminally anaesthetised, in order to allow us to collect blood in a procedure that will cause no suffering or distress.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much of the knowledge obtained from research carried out in other vertebrate species, such as chick and mouse, can be extrapolated to human embryo development and can contribute to the understanding of the basis for many pathological conditions. The nervous system, even at the very early stages that we study, is very complex and organised in three dimensions. Our culture systems retain this organisation for a short period of time, but not indefinitely, so we need a constant supply of tissues from which to derive them. There is therefore no alternative to using living animals at the present time, The culture systems require very special media in which to bathe the tissues and cells. Rat serum is still an essential component of these media, though a

	number of research groups are attempting to identify the key factors so that these could be supplied in some other way. Should a satisfactory artificial substitute to rat serum be developed, we will of course switch to it. Where possible we use early chick embryos, but the use of genetically altered mice allow specific manipulation of a gene or gene elements and the subsequent examination of gene activity in a complex physiological environment not possible by other means. They provide a valuable method of understanding the function of particular genes in the development of a specific tissue(s) of interest. The "Notch" signalling pathway that we focus our research on is very closely related in mouse and man, making the targeted genetic alterations in mice a very useful tool in which to address questions of potential importance for human welfare
2. Reduction Explain how you will assure the use of minimum numbers of animals	We shall ensure the welfare of the adult breeding animals is our priority and we will simultaneously ensure we establish the smallest possible breeding programme that will still provide embryos of the required genotype. Likewise, we shall reduce the number of rats required for serum harvest by collecting as much blood from each animal as possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We do not expect any significant welfare problems in our mouse breeding programmes. If we need to give dams compounds to change gene expression in the foetuses, we do this in water or by dosing orally whenever possible, rather than by giving an injection. We will collect blood from rats under deep and terminal anaesthesia, thus ensuring that they experience no distress at all.

Project 3	**Critical genes in osteoarthritis**	
Key Words (max. 5 words)	Critical, genes, osteoarthritis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Osteoarthritis is a disease associated with ageing. We know that cartilage is degraded in osteoarthritis but we are not completely certain how it starts or how it progresses. This project will be investigating the involvement of certain genes in osteoarthritis. We are proposing to test how important these genes are in the onset or maintenance of the disease. In this project we will generate genetically modified animals in which we will delete or add a specific gene. The animals will be allowed to age to test if they naturally develop osteoarthritis compared with normal animals. Alternatively, some animals will undergo a procedure to induce osteoarthritis by injury to one knee and examine how fast osteoarthritis develops compared with non-injured knees, in the absence or presence of a particular gene. We will image the limbs of the animals to check on progress throughout the experiments.	
What are the potential benefits likely to derive from this	By determining the role of a particular gene in a disease or ageing, we create a target by which we	

project (how science could be advanced or humans or animals could benefit from the project)?	can halt or promote the gene depending on its role in the disease process. The information and new knowledge generated is likely to underpin the development of improved treatments and prophylaxis for human patients and animals with different types of OA
What species and approximate numbers of animals do you expect to use over what period of time?	We are using mice that are genetically altered for specific genes in order to understand their effect in osteoarthritis. We are estimating to use 14275 mice in the next five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In ageing mice, there may be clinical signs that include altered gait, loss of fur and skin irritation, dental abnormalities and perhaps tumour development. The injury model may involve surgery where the adverse effect may include lack of healing and breakdown of wound, which is rare. We will monitor these at all time and if any animal does not recover, it will be humanely killed. The procedures do not exceed moderate severity because we set a humane end point and none of the procedures impede the animal mobility to have access to food or drink. At the end of the experiments the animals are humanely killed and their joints are examined for the extent of osteoarthritis compared with controls.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Osteoarthritis is a complex disease with many cells involved so cannot be replicated in cell culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are using cartilage from abattoirs to test compounds before the use of animals, which will reduce the number of mice. We are also introducing imaging and gait analysis that can monitor the mice throughout the procedure and eventually minimise the amount that need to be killed at each time point to analyse the tissue. We have consulted a statistician to ensure we are not using the right number of mice to get the proper results.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are easy to manipulate genetically and can provide a large number of animals that can be used. Therefore they are best suited for this project. In every experiment, we have utilised the most refined gold standard methods that we have either developed ourselves with our collaborators or has been reported in the literature before and we used it ourselves. In this project we outlined the expected adverse effects that we have seen from experience or what we would expect knowing the genes that we are involved and the protocols that we follow. We have detailed the way we would recognise these effects and outlined a procedure to deal with them. When recovery from such adverse effect is not achieved, the animals involved are humanely killed at the earliest point possible.

Project 4	Role of microRNAs in musculoskeletal tissues ageing
Key Words	Ageing, microRNA, sarcopenia, osteoarthritis, osteoporosis
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

As the ageing population continues to increase, it is important to address ageing-related health issues. There is currently no effective treatment for most musculoskeletal (MSK) disorders, such as sarcopenia, osteoporosis or osteoarthritis that lead to loss of muscle, bone and cartilage deterioration and lead to frailty, which increase in prevalence during ageing. Cellular senescence is an established common mechanism associated with dysfunction of tissues during ageing. Senescent cells do not play their required role in the tissues and are considered pathogenic. We and others have shown the presence of senescent satellite cells, mesenchymal stem cells and chondrocytes and the associated MSK deterioration during ageing. Moreover, depletion of senescent cells in mice leads to improved function of various tissues during ageing. The mechanisms associated with senescence and functional deterioration of MSK tissues (muscle an bone wasting, joint degeneration) with ageing are not fully understood, however it is accepted these are multifactorial, with genetic and epigenetic mechanisms involved. microRNAs (miRNAs, miRs) are a class of small RNA molecules regulating gene expression.

miRNA regulate the balance and function of the MSK tissues and their expression is altered with ageing, however the relevance of these changes is still unknown. The overall aims of this project are to (1) identify and model changes in miRNA:target interactions in senescent cells of the MSK system, conserved in humans and mice, during ageing resulting in MSK deterioration (2) establish the potential of miRNA-based interventions in restoring the functionality of MSK cells undergoing

senescence (cellular ageing) and therefore in preserving MSK homeostasis and preventing MSK disorders during ageing.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

There is currently no treatment available to reduce the loss of skeletal muscle mass that occurs during ageing, cartilage degeneration leading to osteoarthritis and bone loss associated with osteoporosis. Identifying the mechanisms by which microRNAs regulate disrupted process in muscle, cartilage and bone during ageing, will have fundamental implications for the design of interventions to prevent or reverse the age-related decline in musculoskeletal tissues in the ageing population and are directly translatable (microRNA-based therapeutics are already in clinical trials, phase II for liver cancer). Understanding the processes by which frailty develops in the elderly will undoubtedly lead to the development of intervention strategies and significantly improve the quality of life of a large proportion of the population. Wild type and genetically modified mice (premature models of ageing) will be used. The numbers are approximately 168 adult mice and 168 old mice to be treated with microRNA mimic, antagomiR or saline over the period of 3-6 months in total.

What types and approximate numbers of animals do you expect to use and over what period of time?

The expected number of animals used in the experimental protocols is several hundred mice over 5 years. These will be adult, old and genetically modified animals.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The expected adverse effects, although very rare, may be associated with muscle or joint injury protocol and resulting (sporadically) change in movemement. These will be monitored for through observing the mice daily, weighing the mice. Any significant changes or moderate changes lasting more than 3 days will result in animal cull by schedule 1 and tissue collection. At the end of the protocols, mice will be culled using schedule 1 or terminal anaesthesia.

Application of the 3Rs

Replacement

Mice have similar muscle physiology to human muscle and the availability of genetically altered mice provides definitive data necessary to achieve the objectives for this study. A number of lower order species were considered but they are not relevant to the study of skeletal muscle dysfunction related to this project.

Skeletal muscle consists of terminally differentiated multinucleated and innervated cells. Regeneration of damaged muscle is a complex process involving clearance of necrotic tissue from the site of damage by the immune system, activation of resident stem cells, differentiation proliferation and fusion of myoblasts and maturation into mature muscle. Cell culture of muscle is possible by proliferation of muscle stem cells. These only remain viable for 10 - 14 days although this alternative will be used whenever possible. Osteoarthritis is a disease of the whole joint: cartilage, bone and synovium. Ageing is a whole organism phenomenon. The ability to examine the effect of age on muscle structure and function is not possible within the limits of cell culture and due to the critical role of environment of the cells in muscle ageing. However, isolated primary muscle fibres and immortalised muscle cell lines will be studied used where appropriate.

Reduction

The proposal has been designed with the help of statistical advice to minimise the number of mice used. Calculations suggest that n=12-18 per time point will be necessary to achieve statistical significance

Refinement

Most of our work will be carried out in mice. Mice are chosen for these experiments due to the similar physiology between mouse and human musculoskeletal system. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study.

The mouse is relatively short-lived, therefore the effect of age on musculoskeletal structure and function can be fully documented over a relatively short timescale.

The intravenous, intramuscular and intraarticular injections and the dose of microRNA mimics and inhibitors have been optimised by us (Figure 2). We will use the lowest dose and the minimal number of injections to obtain statistically significant results based on power calculations and previous data.

The joint injury models are well established and designed to minimise the suffering of the animals. Gait analysis will allow for comprehensive and clinically relevant phenotype analysis and will reduce the number of animals use through multiple

visualisation of each animal (as opposed to the need of culling more animals for each time point when using alternative imaging methods).

To minimise animal suffering, we will initially perform these experiments only in young mice and keep these animals for 28 days maximum post-operation. Analgesia will also be used when necessary. We will only proceed with the experiments in old mice when we are satisfied that the protocol used doesn't not cause any adverse effects or substantial disability. A number of check points for minimising animal suffering are included in the protocol design, including clear end points, shortest time necessary and minimal number of interventions. The applicants have considerable experience in all the techniques used.

The general experimental designs and methods of analysis of the results have been discussed with University's statistician.

Project 5	Validating computer models of mastication
Key Words	mastication, biomechanics, computer modelling
Expected duration of the project	1 year(s) 6 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The goal of this research study is to demonstrate that computer models are capable of accurately predicting the internal forces generated and sustained by the muscles and bones of rabbits while they feed. This will be achieved by comparing the numerical predictions from models to a new set of direct experimental measurements on rabbit muscles and bone. This will be pursued through three specific methodological objectives, namely to:

(1) collect in-vivo data on bone motion and muscle physiology on rabbit mastication.

(2) collect anatomical and image data on bone and muscle morphology in rabbits.

combine data from (1) and (2) to build and validate computer models of rabbit mastication.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The goal of this project is to demonstrate that computer simulation approaches can contribute significantly to reduction, replacement and refining of the use of animals in biomechanical research. Many experimental studies in animal biomechanics are highly invasive, causing pain and distress to the animals before they are euthanized. In theory, once of digital model has been created, computer simulation has the potential capacity to completely replace (or maximally reduce) the use of animals in that area of biomechanical research and/or medical device design. The anatomy and/or behaviour of a digital model can manipulated or altered near-infinitely without any harm or distress to a real animal. This can allow, for example: a model analysis to be extended to a different strain/breed of the same species (or a morphologically

similar species) by digital modification of the anatomy/behaviour; elements of anatomy to be modified in multiple ways (e.g. removal of teeth/bone) to examine the consequences of different surgical approaches; and for implant devices digitally inserted into models to examine their mechanical impact and performance, all without the need for any experimentation on real animals.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use up to 6 male New Zealand White rabbits in this pilot feasibility study. The study will take less than one year to complete. This data will guide the design of a larger study in the future.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The surgical procedures and associated experiment methods are of moderate severity. As with any surgery there is minor risk of complications such as skin irritation at suture sites. At the end of the experiments all animals will be euthanized using a Schedule 1 method due to the need to carry out medical imaging (CT, MRI) and anatomical dissection to build the computer models.

Application of the 3Rs

Replacement

To validate a computer model requires a large amount of data about the anatomy and mechanics of feeding used by rabbits. This data does not exist for rabbits, or indeed any other experimental animal. Therefore a systematic anatomical and biomechanical investigation of rabbit feeding is required in which all the primary determinants and measures of feeding are measured from a small cohort of rabbits. Constructing models from medical imaging data of those same rabbits can then directly and immediately validate computers simulation. Only in this way can models we truly validated and their potential for achieving 3R’s in future studies be demonstrated.

Reduction

As this is a pilot study aimed at demonstrating our capability of carrying out our experimental-computational workflow, we will restrict work to a maximum of six rabbits. We will review our data after completion of the work on three rabbits to evaluate whether any further experimentation is required to meet our stated objectives. If no further data is required then we will carry out no further experimentation on rabbits. We will also use our experimental data from these rabbits, in conjunction with the limited data already available in the literature, to guide

sample size considerations (i.e. power calculations) when designing the larger bodied of work in the future..

Refinement

Rabbits are the first-choice experimental animal for dental implant design studies because of their size and easy handling. According to international standards regarding species suitable for testing implants in bone, rabbits represent an important species. Although the rat is also a frequently used model, it is not really regarded as a suitable model for testing dental implants and bone remodeling due to significant differences in bone composition, healing, and anatomy to humans. Therefore because rabbits represent by far the most widely used species in this context we proposed to use them to demonstrate the capacity of validated computer modeling as a means of achieving replacement, reduction and refinement of animal use in future studies of dental surgeries and implant design. We have the facilities and expertise for housing this species. In this project we will use male New Zealand White rabbits because their large body size (~3kg) is more amenable to the surgical procedures and x-ray imaging than smaller breeds of rabbits.