Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 22

Projects with a primary purpose of: Translational and Applied Research – Human Musculoskeletal Disorders

Project Titles and keywords

1. **Enhancement of Bone Repair and Bone Ingrowth**
    - Bone, biocompatibility, repair, ingrowth

2. **Mechanisms of skeletal repair and pain**
    - Fractures, bone, joint, pain

3. **Joint tissue remodelling in health and arthritis**
    - arthritis cartilage joint ageing

4. **Cell-matrix interaction in health and disease**
    - Integrin, basement membrane, regeneration, stem cell

5. **New Treatments for Heterotopic Ossification**
    - Heterotopic ossification, trauma, treatment, bone

6. **Therapy of rheumatoid arthritis**
    - Rheumatoid arthritis, treatment, animal models

7. **Models and therapy for congenital myasthenia**
    - Myasthenia, NMJ, AChR, DOK7, β2-agonists

8. **Cartilage repair using circulating stem cells**
    - Cartilage progenitor

9. **Mechanisms and treatments of craniofacial birth defects**
    - craniosynostosis, cleft palate, FGF, Crouzon, craniofacial

10. **Cell-extracellular matrix interactions and diseases**
    - Liver, Tendon, Extracellular matrix, Fibrosis

11. **Connective Tissue fibrosis**

12. **Chorioallantoic membrane model for tissue engineering**
    - Chorioallantoic, skeletal, angiogenesis, tissue, engineering

13. **Non-coding genes in osteoarthritis and musculoskeletal ageing**
    - snoRNAs, osteoarthritis, ageing, murine, cartilage

14. **Controlling osteoarthritis progression**
    - Osteoarthritis, ageing, trauma, mouse

15. **Assessing biomaterial and cell transplant strategies for bone formation**
    - Biomaterials, Scaffold degradation, Bone formation, Stem cells

16. **Developing drugs to treat Myotonic Dystrophy**
    - Myotonic Dystrophy, drugs screen, animal model

Project 1	**Enhancement of Bone Repair and Bone Ingrowth**	
Key Words (max. 5 words)	Bone, biocompatibility, repair, ingrowth	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work to be carried out under this project licence will be done to improve the experience of patients undergoing joint replacement and bone repair surgery. It also aims to reduce costs for the Health Service due to shorter stays in hospital. Currently there is a large unmet need for the provision of cost effective bone repair therapies; developing technologies and products to enhance bone repair that reduces pain and suffering to patients and decreasing the financial burden on the healthcare system. These technologies and products will help patients worldwide to regain normal lives in terms of their ability to carry out everyday functions which had been prevented either by degenerative joint disease or by trauma. The economic benefits would include fewer days lost at work, fewer hospital days and reduced care costs.	
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 first area is the evaluation of safety of new types of materials to be used in orthopaedic surgery. The second area is the evaluation of effectiveness of new types of materials to be used in orthopaedic surgery for the repair of bones. The third area is the evaluation of effectiveness of new types of materials and surfaces to be used in orthopaedic implants for the repair of joints.	
What species and	Over the expected 5 year duration of the licence we	

approximate numbers of animals do you expect to use over what period of time?	anticipate using approximately 400 rats, 280 rabbits, 100 mice, 80 pigs and 680 sheep.
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 surgical procedures detailed in this licence will cause mild or moderate post-operative discomfort which will be controlled by analgesics and refinements made from our previous experience. The maximum level of severity will be moderate. The animals will be killed at the end of the protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals will only be used where there are no alternatives to the use of animals in order to answer the questions that the project requires. For example, bone repair is a complex process that cannot be modelled using computer simulations or cell cultures. There are no suitable non-animal alternatives to animal models for assessment of bone ingrowth into replacement joints. Bone ingrowth is a complex process involving cell signalling pathways and tissue interactions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statisticians will be consulted in the planning stage of in vivo studies to determine the minimum number of animals required to answer the question being asked. This will reduce the numbers of animals used in total without compromising the data/information obtained. Our own ethics group will assess all protocols and experimental design prior to the start to ensure a minimum number of animals are used to the meet the study objectives.
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.	The majority of protocols utilise sheep as this species has bones of the size that will allow implants suitable for humans to be used. Rats, mice or rabbits are suitable for the evaluation of technologies at the stage prior to production of implants designed for humans. We have a great deal of experience with many of the protocols described in this licence. This experience has led to refinements in surgical technique, analgesic regimes and post-surgical care. Gait analysis has been used to monitor recovery after surgery in sheep models and this analysis has been used to improve post-surgical care.

Project 2	**Mechanisms of skeletal repair and pain**	
Key Words (max. 5 words)	Fractures, bone, joint, pain	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The number of fractures worldwide increases dramatically due to the ageing population and the consequent rise in skeletal diseases such as osteoporosis and osteoarthritis The process of fracture healing is both long and arduous and inflammatory nociceptive pain is also associated with skeletal tissues damage and the resulting inflammatory process. The ultimate goal of our programme is to reduce fracture risk in the ageing population, to improve fracture healing and to minimise bone pain.	
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)?	This project will provide information on the basic science that uriderpins skeletal remodelling and repair processes, their modulations by mechanical and biological factors and their interactions. We expect to determine the mechanical and biological environments that promote optimal blood flow to bone, cartilage and bone formation and repair. This is a timely project due to the massive clinical need to address fragility fractures in the elderly and in skeletal diseases. Bone pain is also a serious complication after fractures and new therapeutic options for treating and preventing skeletal pain are required.	
What species and approximate numbers of animals do you expect to use	We will use a maximum of 2900 mice and rats, mainly mice (2000). Rodents are appropriate species because their fundamental skeletal biology is very similar to humans. Well-established models of diabetes,	

over what period of time?	osteoporosis and osteoarthritis that develop the full spectrum of characteristics of these diseases are available in rodents The numbers of rodents to be used are based on experience and calculations which we have undertaken that estimate the minimum number required to detect statistically valid differences between groups.
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?	All surgical procedures (ovariectomy, neurectomy, induction of osteoarthritis and fractures) to be undertaken are performed in rodents and do not exceed “moderate” in severity. We have developed over the years appropriate animal models of skeletal diseases and protocols that aimed at investigating the remodelling and repair responses of skeletal tissues to their mechanical and biological environment. The surgical procedures may however cause pain, including neuropathic pain, but in all cases, animals will be given pain killers. Rodents will also be repeatedly given substances by injection or have devices implanted under the skin or into their abdomen which will slowly release substances. They also be repeatedly anaesthetised for x-rays or other forms of imaging and may have their bones mechanically loaded under anaesthesia. Some animals will need to drink more and will urinate more due to developing diabetes. Animals may also become obese from being fed high fat diets. Rarely, some animals will be kept until they are old. All animals having these potentially painful procedures will also receive pain relief drugs for as long as necessary. All these experiments are performed by appropriately trained experimenters and are essential for the success of this project. Animals will be killed by a Schedule I or non- Schedule I method where necessary at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many aspects of our work are being achieved through the use of laboratory based in vitro cell and tissue cultures, which allow identification of the most likely treatments to be validated in vivo. This replacement reduces the numbers of animals used. The integrated physiological environment of the living animal is still essential to elucidate the patho-physiological mechanisms of fracture healing prior to application in advancing clinical management of fractures in both veterinary and human patients.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim to reduce the numbers of animals we use. Power analyses are always applied in order to identify the minimum number of animals that we need to use in order to answer the specific question being posed. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce further the numbers of animals. Our techniques and appropriate training of experimenters will also minimise the number of animals used.
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.	Our surgical models are at the moderate severity level, with appropriate protocols and end points to manage pain and infection. We will use anaesthetised animals for all surgical models and procedures. The strategy used would minimise the animal suffering which will be limited in our studies by our strict monitoring of severity limits. Our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia using pain killers and close monitoring of behaviours to detect and reduce any suffering. The animals will be killed immediately after the last point of testing or as soon as social behaviour, grooming, weight loss and/or wound healing indicate that the animal is suffering.

Project 3	**Joint tissue remodelling in health and arthritis**	
Key Words (max. 5 words)	arthritis cartilage joint 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
	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)	Rheumatoid arthritis and osteoarthritis (CA) significantly burden patients and healthcare services alike and of the l3million UK arthritis sufferers, 0.5million receive disability allowance (2.4biIlion in 2001). CA alone results in 2O6milIion working days lost at a cost of £l8billion, with annual NHS costs in excess of £1 billion which are increasing every year. Worldwide, 355million people have arthritis and with ageing populations, the World Health Organization indicates by 2025 degenerative bone and joint disorders will be the most common cause of physical disability (25% of all incapacitating conditions). Current front-line therapies are expensive and fail to halt disease in many patients such that there is an acute need for new drugs that more effectively block cartilage destruction. There are currently no treatments for CA despite more than I in 8 people suffering from CA in the UK, and the project objectives are to gain a better understanding of the biological processes underpinning joint biology in health and disease, and to use this information to identify and validate new targets and drugs for the development of new therapies for joint disorders.	
What are the potential benefits likely to derive from this project (how science could be advanced or	We aim to determine the biological processes that occur during disease that lead to increased amounts of the enzymes that destroy cartilage. Blocking the action of these enzymes should prevent cartilage damage,	

humans or animals could benefit from the project)?	and the scientific findings will be valuable in the design of new drugs that allow us to control the production of these enzymes in our joints. This will significantly benefit all arthritis sufferers by preserving cartilage, reducing pain thus increasing mobility and quality of life. Such treatments could also be used in companion animals such as cats and dogs who also get arthritis as they age.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice, typically C57B1L6 mice as it is a very common strain used in many laboratories, and many of the genetically-modified mice are on this background which minimizes numbers. To regenerate/resurrect mouse strains, we propose to use up to 16 mice/yr which would include the transfer of frozen embryos. Breeding of mice will represent the bulk of mouse numbers, and in order to generate the numbers of mice of the correct genetic background (or 'genotype') that are male (female mice cannot be used for the arthritis models but will be used for breeding purposes), we will use up to 800/yr. For the 'induced' models of arthritis, the mice will typically be 10-weeks old, and our experiments could involve up to 320 /yr whilst the spontaneous model will use up to 80 mice/yr. We anticipate that we will use mice over the full duration of the license although the timeframe of each model is limited, typically to 8 weeks after the induction of arthritis although longer for the spontaneous model since arthritis induction takes up to 20 weeks.
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 will induce arthritis in the mice via the use of (i) intra-joint injection of viruses engineered to express certain proteins, (ii) injection of collagen or antibodies to collagen, (iii) surgically destabilizing the joint, (iv) a strain of mouse that spontaneously develops arthritis over time. All model will develop arthritis in a single knee joint (except the spontaneous model), with the intention that the mice will develop a moderate level of arthritis by the end of the experiment in all the models. The viruses we may use are not otherwise harmful to the mice, and no adverse effects have been found. In animals where treatments will be assessed, these will be administered when disease severity is mild with the expectation that treatments will prevent the disease developing into a moderate disease severity. All animals are monitored for general health, well-being and mobility by trained staff, and all concerns are referred to the Named Veterinary Surgeon (NVS) who will stop any experiment should animals fail to respond to recommended treatments or are distressed by any

	of the procedures in accordance with standard protocols. All animals will be humanely sacrificed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal studies have been performed in the arthritis field for some time, Although our group and others have made significant progress in understanding the underlying biology of disease, we cannot yet fully translate this into clinical trials. Indeed, current therapies are not universally successful. It is imperative such animal studies are performed to further our knowledge of disease processes by translating laboratory-based observations to living animal. Furthermore, mouse models of arthritis represent an important link between preclinical evaluations and human clinical trials, whilst access to genetically-modified mice makes the mouse ideal to perform these limited but important analyses. Such studies are informed by extensive in laboratory-based research, and we will continue to use this to restrict animal use to only experiments that have compelling, supporting data from the laboratory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To generate statistically robust data to meet our objectives, power calculations are used to help determine minimum animal numbers. The most important effect we wish to measure is reduction in disease severity in mice treated with a compound, or in genetically-modified mice compared to normal mice. Assuming disease incidence is 100% in our models, and assuming a moderate disease score is reduced by 50%, then power calculations indicate a maximum group size of 10 mice per treatment will generate statistically significant data with respect to disease score in all models. It is envisaged mice generated from the breeding of specific genetically-modified mice will produce sufficient mice to both maintain each strain and for the assessment of arthritis. However, breeding colonies will be monitored carefully to avoid over-production, and animals of specific strains will be used by more than one research project whenever possible. Breeding colonies not required in the short/medium term will be stored as frozen embryos/sperm to minimize continued animal production.
3. Refinement Explain the choice of species	Arthritis models in mice have been used extensively. Most models cause disease in a single joint (100%

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.	incidence), and in models where incidence is lower we will adopt new methods that improve incidence and thus keep numbers down. Disease severity will be moderate in all cases with the intention to reduce this either in genetically-modified mice or in mice treated with drugs. Live imaging work also helps reduce animal numbers since repeated evaluations of the same mouse can be performed over a time course. Pain relief is routinely administered post-operatively. Due to the mild to moderate disease severity expected, it is our experience that a need for further pain relief is unlikely, and will not form part of our routine protocols unless specified by the Named Veterinary Surgeon. Disease severity will only reach moderate thus reducing/negating the requirement for further pain relief during the disease progression phase. The majority of genetically-modified mice bred under the authority of this project have no clinically deleterious consequences. Any pain associated with procedures such as embryo transfer will be minimized by appropriate use of analgesics.

Project 4	**Cell-matrix interaction in health and disease**	
Key Words (max. 5 words)	Integrin, basement membrane, regeneration, stem cell	
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)	Our aim is to provide meaningful insight into tissue maintenance in health and disease with an emphasis on skeletal muscle to gain insight into potential therapies for muscle wasting in disease and ageing. Skeletal muscle has its own stem cell, which has the capacity to renew lost muscle either upon injury, in muscle wasting diseases or in daily wear and tear. These stem cells are crucial that muscle mass is built after birth but then they become dormant in adults. When there is an injury, signals are generated that instruct the stem cells to divide and repair the damaged muscle. Much is known about factors located in the stem cell nucleus. However, very little is known what role the environment outside of the stem cell plays in controlling its function in the repair process, and how this information is then translated into the cell interior, which is also called stem cell niche, influences the stem cells during the repair process. Using genetically altered mice, we will investigate the role of the environment in muscle regeneration, and evaluate the contribution other adult stem cells present in skeletal muscle for their contribution to skeletal muscle regeneration.	
What are the potential benefits likely to derive from this project (how science could be	We hope to further our understanding of the role the local environment of muscle stem cells plays in development and disease progression and/or	

advanced or humans or animals could benefit from the project)?	prevention. The primary aim of the project is to unravel fundamental basic mechanisms of skeletal muscle regeneration, which have not been studied so far. In the longer term, there is a potential benefit that new drugs and/or new therapeutic strategies based on this work can be developed to combat muscle wasting in disease and in ageing.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 12300 mice, comprising a mixture of genetically altered and wild-type mice over the duration of this project.
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 expect no more than moderate discomfort from our procedures, transgene inductions, substance administration, muscle regeneration, cell transplantation or glucose tolerance studies Adverse effects like premature death or side effects due to the procedures will be avoided by humanely killing the animals before the onset of adverse effects and by following aseptic techniques. At the end of all procedures animals will be killed according to a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The exact regulation of muscle development and regeneration can only be achieved in vivo. In vitro cell culture models do not reflect the mechanical strength exerted on the tissue and the interaction with other cell types. We are using ex vivo and in vitro models for our analyses, but ultimately defined cell-matrix interaction is only achievable in an animal model. Similarly, elucidating the final adult stem cell function can only be tested conclusively in an animal model. The mouse is therefore a useful model to characterise complex cellular processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use several measures to reduce animal numbers. When possible we use homozygous mutant mice for mating and use as much tissue from animal as possible for our research and/or collaborators. We also have established immortalised cell lines as in vitro culture models for studying basic mechanisms and ex vivo methods are used to analyse basic concepts of adult stem cell function.
3. Refinement Explain the choice of species and why the animal model(s)	The advantage of using the mouse as model organism is its usefulness for genetic manipulation. The mouse therefore provides an essential tool for the molecular analyses in this project, which may be

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.	tested in future in human conditions for their usefulness. Our protocols are routinely examined to ensure they are current best practice. Where surgery is required we employ analgesia to ensure minimal discomfort. We use humane end-points for all studies.

Project 5	**New Treatments for Heterotopic Ossification**
Key Words	Heterotopic ossification, trauma, treatment, bone
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):

There is a condition known as heterotopic ossification which is where bone grows into muscles and around joints in patients who have suffered serious injury. In particular, soldiers who suffer very severe injuries in battle have a very high chance of developing this problem. The ways in which we currently try to prevent this problem from occurring do not work very well and can cause other problems. Once the bone has formed, the only way to remove it is by an invasive operation.

We are developing a new way to prevent and treat this problem. Our idea is that it might be possible to dissolve the hard component of the extra bone or even to prevent the hard component from forming in the first place. This is a completely new idea. We have done experiments in the laboratory using simple chemicals to prove that the core idea works. However, we do not know whether or not the concept will work in a living body.

There are two objectives of the animal experiments. First, we need to set up a way in which we can reliably cause the animals to develop the problematic bone in their soft tissues. This has been done before so we have a clear plan for how to recreate it in our setting. What we will add at this stage is a comprehensive assessment of the bone formed and the ability to compare this with samples of heterotopic ossification from human patients with this problem. The second objective is to treat the animals with our experimental chemical in order to test whether or not it can prevent the problematic bone from forming or even if it can dissolve it once it has formed

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)?

Human patients who develop heterotopic ossification can suffer from a range of problems including pain, stiffness of joints, and breakdown of the skin over the new

bone. Given that many patients who have had limb amputations due to severe trauma will end up with heterotopic ossification in their stumps, these symptoms can make it very difficult or impossible for them to use artificial limbs. Furthermore, for those patients who have symptoms that cannot be managed any other way, surgery need to be performed that is painful and carries its own risks. Surgery often delays the rehabilitation of these patients also. If we could develop a treatment that works better than anything that currently exists then we could lessen the suffering of these patients and potentially remove the need for surgery.

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

We propose to use approximately 540 adult rats for this work over approximately 5 years.

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?

We propose to perform operations on the rats under anaesthetic. We will cut the tendon on the back of one of their legs and then sew the skin together. Previous studies have shown that the rats are able to move around afterwards with no difficulty. There are able to feed, drink, and look after themselves with no observable problems. From the evidence we have researched, it seems that the most appropriate severity category for these experiments is “moderate”.

Application of the 3Rs

Replacement

The experiments described in this work are part of a larger body of work that has the objective of developing a working treatment for human patients. While we have shown that the treatment works on simple chemicals in the laboratory, the only way to see if it will work in a living animal is through testing it on a living animal. There are no substitutes currently in existence that can recreate the complexity and pathology of this problem.

Reduction

We have selected the most reliable way of causing the animals to develop heterotopic ossification. This will reduce the number of animals required at every stage. Also, we will take a staged approach to the experiments. By using only small numbers of animals at each stage and using the data to inform the planning of the next stages we will keep the numbers as low as possible.

Refinement

Rats have been chosen because the scientific evidence shows that they can be made to develop heterotopic ossification highly reproducibly and they have a bone structure closer to humans than mice. Also, as they are physically bigger than mice so the operation to cause the bone formation is technically easier and the amount of bone that forms is greater so is easier to detect.

Project 6	**Therapy of rheumatoid arthritis**	
Key Words (max. 5 words)	Rheumatoid arthritis, treatment, animal models	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	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)	Tumour necrosis factor (TNF) blocking drugs are a major advance in the treatment of rheumatoid arthritis and are now widely used in the clinic. However, TNF inhibitors does not cure the disease and therefore years of therapy may be required, resulting in high costs and the potential for serious infections, cancer and other adverse outcomes. In addition, about a third of patients fail to respond adequately to anti-TNF therapy and in a significant proportion of patients who initially respond well, there is a progressive loss of efficacy. In this project we aim to devise novel strategies to treat rheumatoid arthritis with the ultimate aim of achieving long-term disease remission. In healthy individuals regulatory T cells prevent and control autoimmunity and inflammation. However, in rheumatoid arthritis these cells are defective and unable to control disease. Under our previous license we showed that this defect is caused by epigenetic DNA methylation, a process which controls gene expression and also contributes to the development of cancer. Hence, an important aspect	

	of this project will be to evaluate the potential of inhibitors of DNA methylation to induce long-lasting disease remission.
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)?	Rheumatoid arthritis affects 1% of the world's population and causes chronic pain, disability and premature death. Current drugs do not cure the disease and require continuous treatment to prevent relapse. Furthermore, most drugs weaken the immune system and have harmful side-effects. This research aims to develop new treatments that provide long-term reductions in disease activity without the need for continuous drug administration. We also predict that new drugs will be identified that are effective in reducing pain and helping patients to lead more active and productive lives and our aim is take at least one of the drugs developed from this license through to clinical trials in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	8000 mice over 5 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?	Mice will be bred and maintained. A proportion of the mice will be immunised with an antigen in order to provoke an autoimmune response. Up to 50% will develop arthritis of moderate severity and these will be treated with promising anti-arthritic drugs. Adverse effects include pain, which will be controlled by the administration of analgesia, skin ulceration at the injection site and reduced movement. Mice may also experience diarrhoea during irradiation and reconstitution of the bone marrow or when kept in a germ-free environment. Some of the drugs may have unexpected adverse effects and these will be monitored carefully. The mice will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Animal models are necessary because arthritis is not a static process confined to a single tissue and culture techniques do not offer a realistic alternative. Rather, the inflammatory process is dynamic and

use non-animal alternatives	highly complex, involving trafficking of cells from distant sites to the joint via the circulation. Hence, modelling the effects of treatment on arthritis must at some stage involve whole animals, rather than isolated tissue extracts.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of animals will be minimised through careful planning of experiments and by the use of cell based culture systems whenever 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 will use a mouse model known as collagen-induced arthritis which is less severe than other models and bears most similarities to human disease. Collagen-induced arthritis has previously been shown to be predictive of human rheumatoid arthritis. To reduce pain during arthritis, analgesics will be provided and subcutaneous injection on the flank reduces the risk of ulceration. The duration of active arthritis will be kept to a minimum (10 days) and severity limits will be in place to ensure no mouse suffers unduly. Mice with arthritis will be provided with easier access to food and supplemental bedding will be provided. Irradiation will be sub-lethal and split into two smaller doses to reduce the likelihood of adverse effects and mice will always be reconstituted within 24 hours. The use of germ-free mice avoids the use of antibiotics.

Project 7	Models and therapy for congenital myasthenia	
Key Words (max. 5 words)	Myasthenia, NMJ, AChR, DOK7, β2-agonists	
Expected duration of the project (yrs)	5	
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)	Aim: To utilise animal models of inherited diseases of the neuromuscular junction in order to study the disease mechanisms and devise better therapies. Signaling between nerves is achieved largely at specialised sites called synapses. Congenital myasthenic syndromes are a group of inherited diseases that cause severe and sometimes life-threatening disability in children and adults and affect the synaptic connection between nerves and muscles, termed the neuromuscular junction This results in generalized fatigable muscle weakness. Many patients are wheel-chair bound, have a marked reduction in their quality of life and may require almost constant care. Understanding how the mutations cause disease enables us to give patients correct treatments and genetic counselling, and provides the knowledge base required for exploring new therapies. In some recently identified forms of the disease it has not been possible to establish precisely why the information transfer from nerve to muscle is defective.	

	In these cases we would like to study the process of defective information transfer in detail in animals in order to discover what is going wrong. Once this has been established, rational therapeutic approaches can be developped for these patients.
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)?	Some of the diseases of the neuromuscular junction respond partially to drugs that affect the transmission of information from nerves to muscles. However, because there are relatively few patients with these conditions it is not possible to do controlled trials. We make mouse models of these inherited disorders so that we can scientifically establish the most beneficial doses of the drugs and drug combinations, and to establish the effect they have on the neuromuscular junction if taken long-term. We are also in the position to investigate new drug treatments. Algorithms for current treatments now given to patients are based on knowledge gained from mouse studies over the last twenty years. There have recently been examples for disease models in mice where gene therapy using viral vectors has makedly enhanced neuromuscular junction function, could improved neurological signs. Gene therapy will likely become more important in future in humans, in particular for those genetic or degenerative neurological diseases for which no effective drug can be found, and the gene therapy studies may help develop these techniques for disorders with neuromuscular junction involvement.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 3400 mice over a five year period.
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?	Our animals will be used to test the optimum combination of current drug therapies and the potential of new therapies to alleviate the disease symptoms. Mouse models of myasthenia show the defining clinical feature for these disorders which is fatigable muscle weakness, meaning the more muscles are exercised the weaker they become. Muscles regain their strength after a short period of rest. In a standard cage mice will not be over-

	exercised and will appear normal, but will show weakness on prolonged exercise. Drugs used are chosen to benefit the mice, and the mice will undergo tests that measure grip strength and muscle fatigability, with the severity level of moderate. Mice will be killed at the end of the treatment trials and their muscles examined in detail to give additional information about how well the treatment is working.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project involves the study of the highly complex process of signalling across a synapse which involves many hundreds of proteins. To truly reflect this complex human disease and study the effectiveness of treatments it is necessary to have an in vivo model and mouse models are able to closely mirror these human genetic disorders.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all the diseases we study we first investigate in detail in cell culture systems how mutations within a gene affect function. The mutated genes are introduced in cells and the potential disease mechanisms investigated. While this does not truly reflect the complex process of synaptic transmission, it gives clues as to which treatments may be effective. Based on previous experience over many years we are able to accurately predict the minimum number of animals needed to give statistically significant results for each treatment that we trial. We have also developed neurophysiology techniques to monitor synaptic function of individual mice during a treatment trial which both reduces the number of animals required and increases the statistical significance of results obtained.
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.	This project will exclusively use mouse models. The mouse neuromuscular junction has properties that are very similar to human. We have exclusively generated mouse models designed to accurately reflect these human conditions. The models we have generated have been engineered (i.e. contain a fluorescent marker making the junction visible) so that experimental therapies can be efficiently monitored. All drugs or therapies that will be used are chosen for their potential benefit to the animals. Mild non-invasive methods such as inverted screen test have proved highly reliable and reproducible for testing fatigability. Functional testing of neuromuscular transmission in live animals reduces the numbers required to gain statistical significance

	and this is achieved by in vivo neurophysiology under recovery anaesthesia. Both the anaesthesia and recovery from anaesthesia have been modified to utilise drugs and husbandry that will minimise the after-effects of anaesthesia.

Project 8	**Cartilage repair using circulating stem cells**	
Key Words (max. 5 words)	Cartilage progenitor	
Expected duration of the project (yrs)	5y	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Joint surface defects are common problems in human and veterinary medicine. Many of these defects do not successfully repair and lead to osteoarthritis and/or joint replacement. Unlike many other tissues, cartilage (the tissue on the surface of the joint) does not repair itself. There are several surgical treatments for joint surface defects. Whilst useful, none of these treatments is ideal, and often produces scar tissue which itself eventually breaks down and causes diseases such as osteoarthritis. In order to heal, many tissues in the body rely on a population of ‘stem cells’ that can self renew and contribute cells to the healing process. We are interested in manipulating stem cells within the area of the joint and encouraging them to take part in healing the joint surface defect. The cells of primary interest for the repair of bone and cartilage are mesenchymal stem cells that make bone and cartilage cells. However, some recent research	

	evidence has suggested that blood stem cells ('haematopoetic' stem cells (HSCs)) can play a significant role in skeletal repair. In our group, we have experience of working with joint surface defect models in sheep under previous and current project licences. These studies have confirmed the suitability of the ovine knee as a model system for investigating cartilage repair in man. The next step of our experimental 'journey' requires that we begin to investigate the cellular and molecular ***mechanisms*** by which defects in the joint surface heal to provide a better understanding and rational basis for the use of stem cells in joint surface repair. Due to the small number of research tools available to analyse tissues obtained from sheep and the inability to genetically modify these animals, the described experiments are not feasible in the sheep and small animal models are needed. Our objectives are to identify which types of stem cells are involved in cartilage repair in three different models and to assess their ability to produce repair tissue for the skeletal system. In addition we wish to evaluate the effects of cartilage repair therapies, such as biological factors or scaffolds, on the ability of these stem cells to repair cartilage.
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 a genuine unmet partial clinical need for effective long-lasting treatment of joint surface defects in both man and animals. Joint surface defects lead to the development of osteoarthritis, an incurable condition that leads, eventually, to joint replacement in man and often euthanasia in animals. The experiments described in this study are designed to provide key information on the behaviour and interactions of stem cells in disrupted and repairing cartilage. They will also provide information on the effect of cartilage repair therapies on these stem cells. This information will lead to a better understanding of how stem cells repair cartilage and how we can manipulate them to enhance repair.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice 2600 over 5 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?	We anticipate that 100% of animals used in these experiments will experience clinical signs of a moderate severity associated with the surgical procedure. Whilst these animals may experience a transient lameness in the immediate post-operative period, this is anticipated to improve and no clinical signs are expected thereafter. In addition, other adverse effects may possibly occur. These include wound and joint infections. The animals will be very carefully monitored to ensure any animal that has an adverse event will be rapidly checked and treated where appropriate. At the end of the experiments the animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not currently possible to reproduce the complex interactions between cells during the repair process in musculoskeletal tissues outside the living animal. Many of the cells that we are interested in only have access to the damaged tissues via the blood and so we need an intact and normally functioning cardiovascular system to investigate these responses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are ensuring that the minimum number of animals are used in this project by performing calculations based on previous results of similar experiments reported in the literature. We are also conducting ongoing experiments in our laboratory that will allow us to specifically target cells of interest and conduct well focussed experiments, for example, we will not proceed to the 4th named experiment until we have clearly identified which cells are the most interesting.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We are using mice in this project as they will allow us to study the mechanism of how cartilage responds to damage and undergoes repair and regeneration. By using micein our experiments, we can use, advanced research tools to study mouse proteins and molecular

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	events as compared to other species. In addition, the opportunity to use genetically modified strains of mice will allow our research team to isolate individual events in the damage/repair process. We will minimise animal suffering by ensuring that all of our experiments are project managed by the Project Licence holder, a veterinary surgeon experienced in designing and implementing experimental procedures. In order to minimise animal suffering all animals will be provided with systemic analgesia in the peri-operative period.

<table>
<tr><td>Project 9</td><td colspan="2">Mechanisms and treatments of craniofacial birth defects</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">craniosynostosis, cleft palate, FGF, Crouzon, craniofacial</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Collectively, craniofacial abnormalities rank amongst the most common birth defects that may affect a new-born baby. These include cleft palate and premature fusion of the skull bones, which is known as craniosynostosis. Treatment of these disorders is a long term process and typically involves each child being cared for by a dedicated team of consultant specialists including clinical genetics, radiology, dentistry, maxillo-facial surgery, anaesthesia, speech, language, hearing and psychological therapy. The primary treatment for these defects involves complex surgical remodelling of the skull and facial deformities, which is aimed at protecting brain development and facilitating breathing, feeding, visual function, as well as the restoration of a normal craniofacial appearance. Although surgical correction is mostly reliable, there is often a need for repeated surgical interventions from birth to maturity.

Ultimately, the aim is to identify drugs that can be used</td></tr>
</table>

	to prevent or delay the progression of these diseases. Successful drugs can be translated into clinical use as part of a novel non-surgical, non-invasive treatment for craniosynostosis and cleft palate.
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)?	Although the primary surgical treatment of craniosynostosis and cleft palate is often successful, repeated rounds of surgery during childhood are often necessary to either keep up with the continuing growth of the skull or to improve the function of the palate. A non- surgical, pharmacological treatment that will be able to delay or prevent surgery after the initial correction will benefit patients by reducing the clinical burden significantly. Even if pharmacological therapy is used as a supplement to initial surgical correction improving the functional outcome, it could prevent the need for several rounds of repeat surgery during childhood. This will have an enormous impact on the patient's quality of life and the development of the child as a whole, and on the patient's and families' quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice (wild-type and mutant/transgenic) to provide models of the human disease under study. We estimate to use no more than 3000 animals over the course of the five-year study.
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?	This project uses transgenic mouse models for human craniofacial birth defects, Inherently, these animals will be adversely affected by the features of the disease (e.g. cleft palate, craniosynostosis). However, these animals are usually viable and breed normally, indicating a low level of severity. When in use, these animals will be carefully monitored by appropriately trained staff and specific husbandry methods will be applied, when necessary to reduce any adverse effects. Due to the nature of this research any adverse effects as a result of administration of experimental substances or bone loading are unpredictable, but will be managed by the application of anaesthesia, analgesia and regular monitoring of the animals' health post-procedure.

	All animals will be killed at the end of the project by approved (Schedule 1) methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into craniofacial birth defects concerns the mechanisms by which the head of an embryo/fetus develops its specific shape and associated function, and the disturbed mechanisms that underlie abnormal shape and function. Hence, an understanding of normal and abnormal development that leads to birth defects requires analysis of whole animals. While tissue culture experiments can provide useful information on certain specific molecular or cellular phenomena, they cannot mimic the complexity of functioning organs, let alone the entire body.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to reduce the numbers of animals by using the most efficient and state-of-the-art breeding and husbandry methods available. We also include statistical power analyses to identify the minimum number of animals that we need in order to answer the research questions being posed. In addition, preliminary testing of drugs will be done in vitro, so only the most effective drugs will be tested in animals.
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.	Genetically altered mice enable the construction of animal models of human biology and/or disease that can be used in numerous ways to improve our understanding of disease processes and to develop new methods for diagnosis and approaches to therapy. Many studies of embryonic development employ sub-mammalian vertebrate or even invertebrate species. While each of these model systems has its advantages, the over-riding benefit of mouse studies is the relatively straight forward extrapolation of results to humans, and therefore to clinical disease. It is for this reason that genetic modification in mice forms the basis of this research programme. When testing new therapeutic strategies on animals, we will employ the good practice guidelines provided by LASA and the NC3Rs.

Project 10	**Cell-extracellular matrix interactions and diseases**	
Key Words (max. 5 words)	Liver, Tendon, Extracellular matrix, Fibrosis	
Expected duration of the project (yrs)	5	
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)	The different tissues of the body are composed of defined combinations of specialised cell types and a mixture of proteins outside the cells, termed the extracellular matrix (ECM). The ECM induces many important signals and keeps homeostasis. The disruption of signals from the ECM causes chronic degenerative and fibrotic disorders. However, their molecular mechanisms still remains largely unknown. Over the past 5 years, we have focused on how tissue/organ regeneration and remodelling are regulated by local ECM. We have been particularly interested how we can change the clinical outcome of the debilitating disease, liver fibrosis and tendon injury by manipulating ECM. In this proposal, we will address: How the progression of liver fibrosis occurs; and How adult tendon homeostasis is maintained, and what the slow healing responses to adult tendon injury are.	
What are the potential benefits	Our approach is to use mouse models as a bridge to	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	the development of translational strategies for humans. We envisage that the benefits from this project will be as follows: 1. If the mechanisms of progression of liver fibrosis to cirrhosis will be clarified, it will have a potential to develop novel anti-fibrotic therapies. 2. If novel biomarkers to predict the progression of liver fibrosis to cirrhosis will be developed, it will have a tremendous benefit not only to liver diseases but also in many other human chronic fibrotic diseases. 3. If we will find out a significant role in adult hepatic progenitor cells in response to liver injury, this information is expected to be the base for the future design and rationale used in clinical practice for the novel cell-based treatment of liver fibrosis/cirrhosis. 4. The development of a novel hepatic myofibroblast-based system from transgenic mice for the screening of potentially therapeutic compounds for the treatment of liver fibrosis would vastly enhance our capacity to screen a large number of compounds and reduce the use of animals (and patients). 5. If the novel mechanisms underlying adult tendon homeostasis will be defined, it will have significant economic impacts and beneficiaries in humans to develop new strategies for strengthening the properties of tendon tissues and for improving the quality of life (QOL). 6. If we will find out novel mechanisms underlying adult tendon healing following injury, this will have significant beneficiaries in humans to explore the effects on new therapeutic strategies.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse Total 3,950 in 5 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?	Expected adverse effects are: the development of pyrexia and weakness without local pain due to the hepatotoxic reagents; a mild jaundice due to the cholestasis; pain associated with surgery; infection due to the operation. All proposed experiments will be moderate severity limit. In all treatment experiments, the following clinical signs will be used as humane end-points (using Schedule 1 procedure): perineum; hunched posture; piloerection and lack of social contact; respiratory distress; development of ascites which interferes with mobility; weight loss exceeds 20% All animals will be culled by Schedule 1 procedures or terminal anaesthesia at the end of all proposed experiments
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Extracellular matrix is everywhere within the body and cannot be reproduced using tissue culture. The complex interaction between molecules, in the majority of cases, cannot be replicated in culture. Indeed, no *in vitro* model can faithfully recreate the natural environment of adult tissues/organs and the regeneration process following injury. We thus have to utilise an animal model to reliably test our hypothesis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be minimised by performing multiple assessments on the same animal. Whenever possible import of genetically altered animals will be avoided by searching cryobanks and using frozen embryos instead. We have consulted with an expert who is able to give advice on improved experimental design and implementation of maximising information gained from each animal experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	*In vitro* experiments using cell lines or isolated organs cannot address adult physiology and pathology. To address these phenomena, it is essential to use a mammalian species as an experimental model. Mice are one of the lowest mammals and colonies are easy to expand in a short period of time.

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Furthermore, the mouse has now become the most understood mammal, allowing for detail analyses of various biological and physiological processes. In addition, the availability of reagents such as antibodies and probes is much larger than those of other species. Finally, among the mammals, genetically modified technologies are well established only for mice. The present experimental system that I propose makes it possible to investigate *in vivo* functions of target molecules during tissue repair/remodelling, and fibrosis. Refinement of our research strategies with respect to animal experiments include that the tissues/organs from one animal will be used for more than one experiment. In all experimental procedures, we have carried out operations for a considerable time where we gained experience and have a feel to the mouse behaviour and we try to minimise the length of the procedure and therefore anaesthesia as well as giving analgesic to minimise pain.

Project 11	**Connective Tissue fibrosis**	
Key Words (max. 5 words)		
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)	vital tissues such as lung, kidney muscle and skin are affected by scarring which impairs the organ function. the objectives of this proposal is to investigate the cells that are involved, determine the function of specific genes in this process and the time of day we can intervene in scarring.	
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 main benefit would be to understand the reof certain genes in scarring and how they work in the cells that produce scarring. We can potentially block their effect if they are detrimental and potentiate their role if they reduce scarring. The other benefit is to learn when is best to administer drugs during day or night that may target scarring better.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used, genetically altered compared to wild type. We estimate the number to be: 12240 in five years Most experiments lasts no more than three months after inducing scarring.	
In the context of what you	The injury model may involve surgery where the	

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?	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 tissues are examined for the extent of fibrosis compared with controls. In our experiments, we are trying to reduce the scarring and therefore the adverse effect should be lower with the treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Scarring depends of several cells in the tissue and the way it takes hold of the tissue is variable in different organs. Hence we need to examine this in different tissues and cannot be tested in cell cultures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Imaging of mouse lines throughout the progression of the disease would reduce the amount of mice by following the same mouse rather than killing a number of them at each stage of the disease. We have worked out the minimum number that we are required to get meaningful results and we will not breed mice that will not be used in experiments.
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 have introduced genes in mouse lines that can be visualised and can be tracked throughout the experiment. We are looking for levels of proteins that indicate to us the severity of the disease and therefore able to reduce the severity by stopping the experiment before it becomes detrimental to the animal welfare.

Project 12	Chorioallantoic membrane model for tissue engineering
Key Words	Chorioallantoic, skeletal, angiogenesis, tissue, engineering.
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):

The chick chorioallantoic membrane model provides a unique functioning vascular network system to undertake and investigate the complex interactions of skeletal and vascular biology. Therefore, our rationale for undertaking this project is centred on understanding human skeletal developmental biology, specifically the developmental niches present in bone, cartilage and the vasculature, to underpin and inform our approaches to skeletal 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)?

The potential benefits likely to derive from this project is understanding how the blood vessels interact with tissue to help regenerate and repair organs. This is critical in skeletal repair because if no blood supply develops during the regenerative process the bone fracture will not heal. If we can harness the coordinated generation of blood vessels and tissue regeneration then this science could advance our therapeutic approaches to regenerate and repair tissue damage. Both humans and animals can benefit from the success of this project. Tissue engineering techniques can transcend across a number of species, so successfully developing regenerative medicine therapies for humans could well benefit animals in the future.

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

The species to be used are Gallus domesticus (chicken), or Struthio camelus (Ostrich) and we expect to use 2,500-3000 eggs over the 5 years

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?

We intend to implant tissue samples onto the vascular chorioallantoic membrane and assess the blood vessel integration and the bone regeneration capacity of these samples. The adverse effects are expected to be minimal and the likely/expected severity be sub-threshold to mild. The animals will be culled at the end of the experiments.

Application of the 3Rs

Replacement

Clinically, tissue repair particularly bone regeneration cannot successfully be achieved without the development and interaction of a blood supply. Therefore, regenerative clinical approaches can only to a certain degree be tested *in vitro*, subsequently, the quality and quantity of tissue repair can only be assessed by *in vivo* studies.

Reduction

In vitro and organotypic culture testing of cells, tissues and bio-engineered constructs will be rigorously tested before any suitable candidates will be considered for experimental testing in the CAM angiogenesis model.

In addition, more sensitive analysing techniques, real time microscopy and micro CT (X-ray) scanning will reduce the numbers of animals required at varying time points of assessment, therefore reducing the numbers of eggs required.

Refinement

The Chorioallantoic vascular membrane is unique to the egg and develops very rapidly. It can be manipulated so that test samples can be investigated similar to that of larger animal *in vivo* models but without harming the developing chick embryo.

This model is well characterised and developed in our laboratory. Rigorous *in vitro* testing will ensure biocompatibility for use in the CAM and very aseptic culturing techniques will minimise any infection rates.

Project 13	Non-coding genes in osteoarthritis and musculoskeletal ageing
Key Words	snoRNAs, osteoarthritis, ageing, murine, cartilage
Expected duration of the project	4 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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Arthritis is a common disease that causes joint pain and results in failure of the joint cartilage. Age is an important factor in the development of arthritis. We think non-coding RNAs (such as snoRNAs and microRNAs), which are novel and important molecules that control other RNAs and processes in cells, have a role in how cartilage alters in arthritis and why there is an increased risk of arthritis as we age. We also think that the genes that contain snoRNAs may also have a role in arthritis. We will identify which non-coding RNAs change in cartilage ageing and arthritis. For some specific non coding RNAs we will find out what their role is in arthritis. This is because we believe in the long term development of ways to alter non-coding RNAs will allow techniques for early diagnose and new treatments for arthritis.

The musculoskeletal system is severely affected by the ageing process. Articular cartilage is susceptible to age-related diseases, such as OA. OA is the most common degenerative joint disorder worldwide, affecting 8.75 million people in the UK, and presents with degradation of articular cartilage, leading to loss of joint mobility and function, accompanied by chronic pain. There are few treatments available beside total joint replacement and pain relief at present.

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 will develop an understanding of the role of non-coding RNAs in cartilage ageing and OA. This is important as OA is a major burden on millions of individuals in the UK as well as society and the NHS. Therefore as an ever increasing ageing population, in which OA is predominant, patients (for which there is no treatment for OA apart from pain relief or a new joint), society (through less sickness leave) and the NHS will benefit. Animals will also benefit. In particular dogs and horse, also with an ageing population suffer substantially from OA. Potential new targets for diagnostic markers and treatment options are a likely outcome.

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

The project will use mice that have been genetically altered so that they are not able to regulate their genes properly. Over 3 years we will be using1550 mice.

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?

In humans the effects of osteoarthritis include a significant reduction in mobility, altered gait and pain. In the mice, the procedures do not exceed moderate severity because we set a humane end point that does not impede their mobility. The animals will undergo joint surgery under general anaesthetic. Potential adverse effects would be wound breakdown at the surgery site. At the end of the experiment mice will be sacrificed and tissue collected for analysis. At the end of the experiment mice will be sacrificed and tissue collected for analysis.

Application of the 3Rs

Replacement

The DMM model is an in vivo OA model. It is used to assess changes in the joint in a surgically induced arthritis. Mice in which targeted snoRNAs have been removed from cartilage will be used in order to determine the effect on normal cartilage ageing and OA. The only other models available are in larger animals such as sheep.

Reduction

The project licence applicant and surgeon for this project is a trained veterinary surgeon. Surgical techniques will be practiced on cadaveric mice and specific training given by experts in the field of DMM surgery.

Experiments have been designed so that multiple outcome measures can be obtained from one experiment.

We have undertaken power analysis based on previous similar studies and following expert advice in order to define the minimum number of mice required in order to determine an affect.

Refinement

Transgenic mice will be used in the study. Some of these mice will be aged prior to surgery in order to assess the effect of age. The DMM mouse model is the most well-known and characterised for evaluating OA allowing us to interpret the results of our studies. We anticipate that the DMM model will only be required for approximately six months within the three year project.

Project 14	Controlling osteoarthritis progression
Key Words	Osteoarthritis, ageing, trauma, mouse
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Osteoarthritis (OA) is the commonest chronic disease, affecting almost 9 million people in the UK. The World Health Organisation estimates that 9.6% of men and 18.0% of women aged over 60 already have symptomatic OA, making this disease one of the ten most disabling disease in humans. OA affects joints and include various pathological changes in all tissues of the joint that contribute to pain and restriction in range of movements of the joints. Despite the prevalence of OA, there is currently no treatment to slow the progress of the disease. As the aged population is increasing, new treatments for OA will only become more needed.

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)?

By better understanding the mechanisms controlling OA progression, we expect to find new targets for therapy, as well as new ways of detecting severity of the disease. These would be of great benefit for the animal and human population which suffer greatly from osteoarthritis, by providing potential new treatment as well as make sure the right patients are treated appropriately.

What types and approximate numbers of animals do you expect to use and 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 10,000 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 levels of severity? What will happen to the animals at the end?

In the mice, the procedures do not exceed moderate severity because we set a humane end point that does not impede their mobility that may occur with severe osteoarthritis. At the end of the experiments the animals are killed and their joints are examined for the extent of osteoarthritis compared with controls.

Application of the 3Rs

Replacement

This project aims to define what controls osteoarthritis progression (hence severity), which is a slow complex disease, with interactions from many tissues in the joint and in the whole animal (for which there currently no good alternative).

Reduction

Careful design of the experiments must be implemented to avoid unnecessary use of animals. Calculations of the number of animals needed is based on previous identical experiments. For testing new agents and new protocols, in vitro methods will first be used when possible, followed by experiments on small groups of animals. Results from these can then be used to properly calculate the number of animals needed.

Refinement

Mice are the most appropriate species due to techniques available to modify their genome. In addition, osteoarthritis development in our models is well described and resembles closely human pathology, and is non-invasive.

The system used allows to “activate” the gene deletion or activation in specific tissues and at specified timepoints; thus we can avoid unnecessary complications linked to effects in other organs that may be fatal as well as avoid complications due to effects on the embryo stages.

Project 15	Assessing biomaterial and cell transplant strategies for bone formation
Key Words	Biomaterials, Scaffold degradation, Bone formation, Stem cells
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Tissue engineering is a newly developed scientific field which aims to develop new therapies for the treatment of diseases which cannot currently be adequately treated with existing therapies, for example, osteoporosis or fracture non-union. Tissue engineering techniques use patients' own cells, which are grown within a scaffold made from biomaterials to enable the cells to grow and develop into functional tissues. These biomaterials for scaffolds play an important role in guiding the cells' growth and also initially provide mechanical, structural support. However, the scaffolds have to be degradable. In other words, as the new tissues become established, the scaffolds should break down naturally as the new tissue is formed. It is therefore essential that the breakdown profile of the biomaterials utilised within a tissue engineering therapy, are well defined. The objective of this project is to assess our newly developed biomaterials which enable real-time, non-invasive, non-destructive monitoring of the scaffold degradation rate as well as assessment of bone formation rates. This project will also address whether the incorporation of stem cells functions to enhance bone formation and therefore promotes better healing.

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 ultimate goal of this project is to develop tissue engineering based therapies for patients with bone disease. The successful prediction of the scaffold degradation rate and stem cells' incorporation on bone formation will help to speed up the new therapy development. Our project could accelerate the development of new biomaterials and establish a new technique to monitor the implants' stability and degradation in real time, non-invasively and non-destructively.

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

We will use mice and rats for the experiments. We estimate use up to total 2,000 animals over 5 years.

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?

Both mice and rats will undergo procedures that involve incision in skin or creation of a defect in the non load bearing, non-articular surfaces of the skull. Scaffolds will be placed into small pockets created in the flank of the animals to assess biocompatibility. Small holes will be made in the skull of rats and repaired with newly designed and developed scaffolds, and the healing rates will be monitored and characterised. These animals may experience moderate discomfort due to the surgery and the introduction of foreign materials, but anaesthesia, and pre and post operative analgesia will be provided to minimise this. In addition, the animal may experience local inflammation at the site of implantation. This is rare but could cause signs of distress to the animals. Overall the level of severity for procedures in these implantations is Moderate. At the end of studies animals will be humanely killed so that further analysis can be performed to assess the performance of the grafts and the level of graft integration into the host, and ultimately the potential for the grafts to facilitate and promote bone healing.

Application of the 3Rs

Replacement

Biomaterials degradation can be initiated by multiple mechanisms. The body fluid, enzymes and mechanic force can all trigger the degradation. It is also known that the formation of bone by cells within the biomaterials (scaffolds) depends on the degradation rate of the scaffolds. This complex process cannot be mimicked *in vitro* hence it is necessary to assess the degradation rate and its effects on bone formation in animal models. Although *in vitro* models can give valuable information, they are unable to completely replace *in vivo* models.

We have created ex-vivo models to pre-assess these effects and thus the animal number to be used has been reduced.

Reduction

Our experience with the experimental protocols will be applied to ensure appropriate group sizes are used to identify statistically significant differences between groups, whilst minimising the numbers of animals undergoing the protocol. Group sizes are constantly reviewed and experts in statistics are consulted to ensure the minimum numbers of animals are used.

We will run pilot experiments with a relatively small numbers of animals where necessary, to establish initial biocompatibility, fluorescence tagging intensity and cell-seeding densities, from which the bony healing rate, scaffold degradation rate and imaging quality can be acquired appropriately. This strategy will minimize the chance of an experiment having to be repeated because it was incorrectly designed.

Refinement

To minimise animal suffering, procedures in this application have been designed and selected to be the least invasive and traumatic and as surgically simple as possible. The subcutaneous model will be used to pre-screen scaffolds prior to testing in the cranial model. Only scaffolds showing favourable outcome in the subcutaneous model will progress to the cranial model. The cranial model was chosen as the bone is not jointed or load bearing and has relatively sparse nervous supply, compared to the long bones or the face, for example. This makes it one of the least painful models of bony injury. For most subcutaneous experiments, mice will be used as these are less sentient than rats, but will provide reliable data for assessing the biocompatibility of the scaffolds. For the cranial model, the mouse skull is too small and thin to be used. Such defects are likely to cause significant harm to the animal, and the thin surface is not sufficient for union with the scaffold matrix. For the cranial model, rats have been selected as the most suitable model. Where possible two scaffolds will be used per animal to allow a within animal control/comparison. Non-invasive imaging and analysis of scaffold degradation products in urine will be used rather than a longitudinal study, sacrificing multiple animals at various time points. These measures will minimise the welfare costs to the animals.

Project 16	**Developing drugs to treat Myotonic Dystrophy**	
Key Words (max. 5 words)	Myotonic Dystrophy, drugs screen, animal model	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective is to identify a small molecule (drug) that will provide a treatment for myotonic dystrophy, the most common form of muscular dystrophy in adults. Work in our lab has led to the discovery of a target protein and we are trying to identify an inhibitor that will affect this protein. We have identified compounds, which represent starting points in this process. In effect they are 'hits'. The project is a 'hit to lead' development in which we try to identify better compounds with more drug-like properties for further clinical development. Hundreds of small molecules will be tested in biochemical and cell based assays to identify the most suitable therapeutics. The animal testing part represents the final stage of the process for this phase of drug development. Overall we aim to test 6 to 10 compounds in a myotonic dystrophy mouse model. The most suitable small molecule will be judged in a series of tests and then used in the next stage of the drug development process.	

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)?	Myotonic dystrophy is the most common form of muscular dystrophy in adults. It affects around 1 in 8,000 people world-wide and is characterised by progressive muscle weakness and wasting. It is a debilitating condition with an average lifespan of 58 years. There is no treatment for myotonic dystrophy and this project aims to develop a suitable therapy. If we are successful this will benefit the 100,000 patients in countries with developed health care systems. Our aim is to develop a full treatment. However if it were to slow disease progression it would be better than anything currently available.
What species and approximate numbers of animals do you expect to use over what period of time?	We wish to test a small number (6 to 10) of molecules on a mouse model of myotonic dystrophy which is called the HSA^{LR} mouse model. We aim to test 400 mice in total.
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 experiments described in this license application will be performed on the HSA^{LR} mouse model of myotonic dystrophy. This is the best-studied model of myotonic dystrophy available. It has a repeat expansion mutation similar to that found in myotonic dystrophy patients and it shows similar muscle abnormalities to those found in patients. The mice will be injected with compounds, which have been pre-tested by collaborators to determine their properties in animals. If the compounds properties permit they may be given orally, but most likely they will be injected to provide the optimum dose. Typically, the treated HSA^{LR} mouse will, over the period of a week, undergo behavioural testing, on the rotarod, for grip strength and it will spend a set period drinking in the lickometer cages (usually 1 -2 hours). The mouse would then be administered the compound (most likely by daily IP injections for 5 days) and the behavioural testing will be repeated. The animal will experience mild discomfort from the injections, and there is the potential for mild stress during behavioural testing and induction of anaesthesia, although this will be minimised by habituation to handling. This would be the typical experience for 80% of animals. An electromyogram (EMG) will be carried out after which the animals will be subject to schedule 1 killing. An EMG involves inserting tiny electrodes into muscles to measure their electrical activity at rest and when they're being used.

	The HSA^{LR} mouse colony has been maintained in the USA for several years and there are no notable adverse effects from the transgene. The animals have detectable changes in electrical activity in their muscles and some slight weakness but nothing overt. Following testing in the worst case an animal may develop problems and require extra care, including wet mash. The mouse may be required to be left in the lickometer cages for 18 hours, and become stressed during behavioural testing. The compound may have to be given for more than 5 days, and the mouse may have an adverse reaction to the drug or administration method. Or (if the mouse does not have an adverse reaction) behavioural testing and EMG may be repeated up to 3 times after drug administration to get a time course of the drugs action. During the EMG testing the mouse may not be maintained appropriately under anaesthesia, or die due to the anaesthetic. This cumulative scenario is unlikely (less than 1% of cases). The likely level of severity is moderate. After the various tests the animals will be subjected to schedule 1 killing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A series of non-animal alternatives will be used in biochemical and cell based assays to narrow down the number of potential suitable compounds from several hundred to 6 to 10. The HSA^{LR} mouse is the best-used and most suitable model with which to test possible therapeutic small molecules. Other animal models do not have the capacity for us to measure benefit of drug treatment. This study forms part of a project in which potential drug treatments for myotonic dystrophy are tested in biochemical and cell based systems to identify those most likely to have beneficial effect. Thus far 18,000 small molecules have been screened in cell-based assays to identify the target for the present study. Based on this work we are now testing further compounds in a focussed fashion, selecting those most likely to affect the chosen target. Several hundred compounds are being screened through the

	biochemical assay and those showing appropriate selectivity will be tested in cell based assays. Our aim is to identify around half a dozen small molecules for *in vivo* testing. Thus we are already using and will continue to use a range of additional tests to restrict the number of compounds being tested on the mice. A very substantial amount of replacement will have taken place before compounds are tested on the mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Throughout the studies we will strive to use the minimum number of animals required to achieve the scientific objects of this licence. Sample size calculations have been undertaken based on data from the literature.
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.	Throughout the project the most refined procedures will be used to assess the animals. Replicating the complex physiology of myotonic dystrophy requires a mammal. The chosen species for this project as a genetic model of the disease is the mouse. This is a well-characterized model. Alternative possibilities do not allow the assessment of myotonia, nor meaningful measures of weakness that have been developed and quantified previously in the mouse. Prior to conducting the analysis covered on this license application toxicity testing will be performed by our collaborators so we will already know the maximum tolerated dose to test. The specific behavioural tests we will conduct before and after drug treatment will be the most refined for the experimental question as they will be based on empirical data using well established protocols. Repeated testing will only be carried out if a time course of drug effect is required.