Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 22

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

Project Titles and keywords

1. **Inflammation and cancer-induced bone disease therapy**
    - Bone, inflammation, ankylosing spondylitis, osteoporosis, cancer

2. **Accelerating Fracture and Cutaneous Wound Healing**
    - Fracture, Bone, Wound, Skin, Healing

3. **Nutrition, insulin resistance and grazing in ponies**
    - Nutrition, insulin resistance, grazing

4. **Microvesicles and the prevention of tissue wasting**
    - Microvesicles, Stem Cells, Aging, Tissue wasting

5. **Understanding and treatment of neuromuscular conditions**
    - Neuromuscular disease; muscular dystrophy; skeletal muscle; stem cells

6. **The Mechanics and Energetics of Locomotion**
    - Flight, running, bird, muscle, energetic

7. **Homeostatic calcium regulation in fish**
    - Ca^{2+}, scales, blood, freshwater, seawater

8. **The role of muscle stem cells in health and disease**
    - Stem cell, skeletal muscle, regeneration

9. **The Safety and Efficacy of Cell Therapies**
    - Stem cells, Pre-clinical testing, Medicinal products

10. **Pre-clinical analysis of therapies for neuromuscular diseases**
    - Muscular dystrophy; Therapy development

11. ***In vivo* models for tissue engineering**
    - Stem cells, growth factors, scaffold, tissue engineering, regenerative medicine

12. **New biomaterials for healing of bone defects**
    - Synthetic material, bioactivity, bone, regeneration

13. **Purinergic signalling, bone cell function and tissue calcification**
    - Vascular calcification, osteoporosis, loading, bone

14. **Angiogenesis in health and disease**
    - Capillaries, blood flow, skeletal muscle, ischaemia, hypoxia

15. **Models of tissue repair and reconstruction in the limb**
    - Injury, limb, repair, reconstruction, regeneration

16. **Understanding muscle maintenance, regeneration and ageing**
    - Muscle, regeneration, inflammation, muscular dystrophy, stem cells, Duchenne muscular dystrophy

17. **Manipulation of signalling pathways in zebrafish**
    - Zebrafish, HSPG, regeneration

18. **Genetic and hormonal regulation of the skeleton**
    - Osteoporosis, osteoarthritis, fracture, genetics, hormones

19. **Musculoskeletal Tissue Regeneration**
    - Musculoskeletal, regeneration, repair, bone, cartilage

Project 1	**Inflammation and cancer-induced bone disease therapy**	
Key Words (max. 5 words)	Bone, inflammation, ankylosing spondylitis, osteoporosis, cancer	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project centres on ways to prevent bone loss and/or replace bone once it has been lost. This is an unmet clinical need as ways to replace bone once it is lost are limited. We will be investigating bone loss in three clinical contexts; osteoporosis, arthritis, and bone cancers like multiple myeloma. In these disorders, bone loss occurs faster than it can be accrued leading to complications of a weakened immune system, bone pain and deformity. The notable exception is that in ankylosing spondylitis there is inappropriate new bone growth that occurs in addition to the arthritic bone loss in the joints and it is this new bone growth that is one of the hardest aspects of disease to treat. We aim to understand how bone loss and growth is triggered with a view to applying this not only to the disease being studied, but also to apply that knowledge to treat some of the biggest issues for musculoskeletal health such as osteoporotic bone loss. To do this we need to know how bone cells are behaving in these diseases (arthritis, osteoporosis and cancer-induced bone disease) and how other cells of the body are directing bone cell activity due to the factors and stimulants that they produce. Armed with this information, we will be able to develop new ways to prevent bone loss and thus increase quality of life and longevity.	

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)?	Autoimmune disorders affect up to 1% of the population and present an ongoing burden on clinical resources with joint destruction presenting the need for costly revision surgery in advanced cases. Anyklosing spondylitis has the added complication of developing inappropriate new bone formation as well as joint destruction and gut inflammation. Of even greater prevalence is osteoporosis and whilst therapies such as bisphosphonates have revolutionised its treatment, there remains the need to find ways to replace bone once it has been lost and to treat the complications such as osteonecrosis of the jaw. Furthermore, cancer-induced bone diseases such as multiple myeloma and metastasis to bone have a very poor clinical prognosis and ways to increase lifespan and therapeutic interventions are vital. This project has the potential to address a number of unmet clinical needs that despite recent advances continue to elude therapeutic intervention. Additionally the discovery of novel bone forming agents and how they body normally produces them, has the ability to provide the next generation of treatment for osteoporosis – one of the largest bone disorders affecting our ever ageing population.
What species and approximate numbers of animals do you expect to use over what period of time?	The project license application involves the use of mice only (wild-type and genetically modified). Breeding of genetically modified mice is estimated at 4000 over 5 years. Many of our disease models are only run using one sex of mice, due to the prevalence of disease in one sex as is also seen in the human population. Wild-type mice will be purchased as needed and it is estimated that 1000 will be used over the 5 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?	The severity limit is moderate. Possible adverse effects could include pain from surgery, irritation from local injection, local inflammation as shown by redness, weight loss or mobility problems. Any animal showing deviation from normal health as judged by daily monitoring of food and water intake, body weight, general and coat appearance, gait or behaviour will be treated with pain relief and food supplements. Any animal that fails to respond and remains unwell will be culled. All animals will be sacrificed at the end of the protocol with the exception of protocol 1 (Breeding and maintenance of genetically modified and mutant mice).

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will investigate the causes and ways to reduced bone loss diseases in murine models of ankylosing spondylitis, osteoporosis and bone cancers. Due to the complexities of these diseases it is not possible to address the factors that cause disease initiation without using a whole body system as multiple organs/cell types are required. Where possible we will be using patient samples and lab based assays to inform us and to address the effectiveness of any treatment before resorting to animal models of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used statistical calculations to determine that we will need to use 8-10 mice per group. Mouse numbers will be kept to a minimum by sharing control groups where possible and using bones from other places to confirm findings (and statistical significance) instead of increasing mouse numbers.
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 are interested in reducing disease burden in these models and investigating the first steps in the development of disease. Therefore mouse models of disease will be stopped once the mouse begins to show early signs of disease so we can determine the causative factors. We will develop a new, less invasive model of osteonecrosis of the jaw, a complication of treating bone cancers. Each experimental model will be monitored daily following intervention and mice will be assessed for any signs of distress such as pain and inability to feed. Surgical interventions will be undertaken using the most appropriate anaesthetic and analgesia will be given. Humane endpoints will be strictly adhered to at all times.

<table>
<tr><td>Project 2</td><td colspan="2">Accelerating Fracture and Cutaneous Wound Healing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Fracture, Bone, Wound, Skin, Healing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">Five</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>
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<tr><td></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">The aims are to elucidate the pathways which lead to:
1. Bone regeneration in order to develop strategies to accelerate fracture healing in both normal and osteoporotic fractures, and to improve orthopaedic implant fixation in osteoporotic bone.
2. Cutaneous wound healing in order to develop strategies to accelerate wound healing in the clinical setting especially in diabetic wounds.</td></tr>
<tr><td>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)?</td><td colspan="2">Acceleration of Bone Repair

Each year in the UK 3.6% of the population suffer a fracture, and more than a third of the population will do so during their lifetime, one in ten of these being left with a significant permanent disability. A study of the figures for the UK in 2000 showed that the health and social care of hip fractures alone was £726 million a year. Thus there is a substantial cost both in terms of disability to the individual and of the socioeconomic burden of treatment and rehabilitation to the State.

Acceleration of bone repair in normal and osteoporotic bone, and reduction in implant failures will drastically reduce costs to the NHS as well as positively impact the quality of life for patients, and reduce the death rate in older people.

Advancing knowledge of bone repair and formation</td></tr>
</table>

	will greatly benefit clinicians for therapeutic and management strategies for the large numbers of patients who suffer fractures and undergo orthopaedic implant operations. A large proportion of these patients also suffer from osteoporosis, which leads to poor bone healing. **Cutaneous Wound Healing** Patients with any of the following risk factors may suffer impaired cutaneous wound healing: diabetes, age, smoking, obesity, radiotherapy, chronic kidney disease, immunosuppression and long-term steroids. Common conditions include diabetic feet, pressure sores and surgical wound dehiscence. Strategies that can stimulate and accelerate cutaneous wound healing will reduce infection rates, long-term debilitation and hospital stay. Fibrosis represents a key component in cutaneous wound healing, and the cell responsible is the myofibroblast. Hence factors that regulate myofibroblast development are also likely to affect cutaneous healing in adult tissues and be involved in pathological conditions. We aim to reach a better understanding of the molecular biology and cellular mechanisms in a skin fibrosis model, and thereby to identify novel therapeutic targets to accelerate and promote optimal cutaneous wound healing. Furthermore, these studies will likely benefit the treatment of connective tissue disorders. Our study has been designed to have direct clinical relevance and we expect findings to have an impact in the clinical setting.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 2000 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?	In depth study of the processes of bone repair and cutaneous wound healing requires the availability of fractures and cutaneous wounds, and we create these surgically. The typical experience (80%) is of moderate severity, with the remainder being of mild severity.

	Animals will be subject to all of the risks of surgery and will have careful post-operative observation. Meticulous aseptic surgical technique using sterilized instruments and materials will be used. The fur will be shaved and suitable aseptic preparation of the animal will be carried out. The animals will be kept warm and continuously monitored until recovered from anaesthesia. The mice recover within minutes from the anaesthesia and are free to ambulate. To minimise operative related pain, analgesia will be administered at induction.. The animals will be examined at 6-8 hours following administration of analgesia to ensure that they are pain-free. Clinical signs of pain include withdrawn behaviour, reduced mobility, dehydration, hypothermia and incontinence or diarrhoea. If they still continue to exhibit signs of pain, a repeat dose of analgesia will be administered, and if pain signs continue they will be killed immediately Animals will be inspected, examined, and weighed daily, until the removal of non-absorbable sutures under anaesthesia, with ongoing inspections and examinations. We will examine the animals to ensure that mice are able to feed and groom without problems, and for signs of local infection or foreign body infection including local swelling, seroma, lameness, systemic signs including hunched back, piloerection, weight loss of >20%, and respiratory distress. Animals showing any of the indications listed above will be killed immediately. Occasionally animals may have some residual lameness beyond 24 hours, which has been attributed to minor nerve contusion and resolves within a few days. The animals will be rehydrated subcutaneously post-operatively. LASA guidelines of administration of substances will be adhered to. At the end of the experiments all animals will be killed, and relevant tissues will be collected post mortem for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Bone and skin healing involve complex interactions between numerous cell types and cytokines, many of which have not yet been identified, so this system is not reproducible in vitro. The mouse genome is well-studied and more reagents e.g. antibodies and genetic knock-outs are available for biological

	investigation than for any other laboratory animal, including rats. Therefore to study fracture healing, we create stabilised fractures in mice, and to study skin healing, we create skin wounds in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot studies will be performed before experiments are conducted. This will allow us to determine the total number of animals that will be required. The number of animals is further reduced by non-invasive analysis tools that allow the same animal to be followed during the course of the experiment without the need to kill at each time point. These tools include in-vivo microCT imaging, and gait analysis.
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 mouse is a relevant species for trauma-based translational research. The genomic response in murine trauma models are similar to those seen in humans. Our fracture models have been well-established. The animals receive adequate post-operative analgesia and appropriate monitoring and care and tolerate the procedures very well due to the careful use of aseptic technique. Our cutaneous wound model and dermal fibrosis model are also well described and we have had experience with the techniques. It incurs minimal discomfort to the animals, which will all receive adequate post-operative analgesia and appropriate monitoring and care.

Project 3	**Nutrition, insulin resistance and grazing in ponies**	
Key Words (max. 5 words)	Nutrition, insulin resistance, grazing	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to investigate the relationship between two diets, grazing, bodily condition, insulin resistance, laminitis and Cushing's Disease in ponies. By keeping ponies in two groups, one fed hay, the other fed haylage, both groups being allowed limited grazing during the summer months; we aim to mimic common husbandry conditions of ponies in the UK. In order to try and identify significant differences between the two groups over a 12 month period we will sample faeces, urine, blood and sub-cutaneous fat samples. All done atraumatically using aseptic techniques under local anaesthesia. We intend, by DNA sequencing and culture to identify the microbial populations in the hind gut (via faeces). Blood and urine samples will be processed using advanced laboratory techniques in order to identify specific toxin, molecules and bioactive entities. Small fat samples will be examined using similar techniques. Forage analyses both for nutritional parameters and biological contaminants will be examined. The ponies are to be weighed and condition scored monthly and faeces samples obtained in order to measure pH. In addition blood samples are to be taken in order to measure eleven analytes which are likely to be correlated with insulin resistance and Cushing's Disease.	

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)?	Correlations of the information obtained from this study may help to elucidate the puzzles surrounding pony diets and conditions scores and alleviate the suffering of equids afflicted by laminitis.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use twenty ponies 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?	All procedures used during the study are mild. Adverse effects of localised inflammation or infection at blood sampling or fat biopsy sites are minimized by the use of aseptic techniques. Should ponies show increased strength to their digital pulses, a sign of laminitis, they will be temporarily withdrawn from the study until they recover. Should ponies develop Cushing's Disease they will be treated using recognized therapies. All ponies will remain on site, probably to continue the study but will never leave the site. Provision has been made for their retirement in comfort within their companions with whom they have lived for the last five years.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is postulated that substances from the feeds undergo changes within the equine hind gut leading to the production of metabolites which are responsible for the development of insulin resistance. Faecal sampling after differential feeding is necessary to identify the species found in greatest numbers in faeces, enabling identification of significant organisms. One of the main objectives of this study is to correlate insulin resistance with changes in the pony gut microbial populations. Currently, identification of insulin resistance cannot be achieved using in vitro techniques. In vitro techniques are being used widely and throughout this study.
2. Reduction Explain how you will assure	Statistical methods have indicated that twenty ponies is the minimum number likely to produce results

the use of minimum numbers of animals	which will survive peer review prior to publication of results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This study mirrors the typical management conditions under which many UK ponies are kept. Ponies are being used as they are a type of equid which commonly suffers from insulin resistance, laminitis and Cushing's Disease. The methods used are the least traumatic, only involving procedures under local anaesthesia, whilst being able to provide the data upon which significant conclusions can be based. Laminitis induction is not included in this study. There are no substantial severity protocols involved. The ponies' diets will not be nutritionally deficient in macronutrients.

Project 4	**Microvesicles and the prevention of tissue wasting**	
Key Words (max. 5 words)	Microvesicles, Stem Cells, Aging, Tissue wasting	
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)	The objective of the project is to explore the clinical benefit of stem cell secretory factors on age related degenerative diseases and those associated with cancer. To facilitate the safe translation of pre-clinical testing to human clinical testing, animal testing is necessary in this project.	
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)?	Previously we have established clinically accepted and approved methods for the generation of the patient's own stem cell conditioned media for therapeutic uses. However our previous work had concentrated on degenerative illnesses including multiple sclerosis and injury conditions like joint damage. This project will serve to explore and expand the range of diseases and conditions that our therapy could help. Specifically we will now focus on age related tissue loss and cancer. This will provide pre-clinical data on safety and efficacy to facilitate early clinical application of therapy in diseases which currently have few treatment options. The clinical use of differentiated or undifferentiated stem cell conditioned media provides an attractive, less invasive, safer alternative (minimising cancer, reducing immunological risks) to stem cell transplantation.	
What species and	The project will use both rats and mice (wild-type and	

approximate numbers of animals do you expect to use over what period of time?	transgenic). The project will last 5 years and use approximately 6,500 animals per year
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 project will use an accelerated ageing model to study the effects of stem cell secretions on age related degenerative disease. As a consequence, animals will show some symptoms of age related decline. At the end of the studies animals will be euthanized. A further model will involve cancer cachexia where animals will show some muscle wastage, at the end of the studies animals will be euthanised at humane end points.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Stem cell secretory factors ameliorate disease by a variety of mechanisms thus experimental treatments are not suited to mathematical or computer simulations to replace the use of experimental animals. Appropriate animal testing for optimization, safety and efficacy are needed before any form of clinical trial on human subjects. With some aspects of the project, experimental animals can be replaced by in-vitro testing, and as outlined in the programme of work such in-vitro testing will replace intact animal experimentation where ever possible in the project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of in-vitro methods limits the number of animals required for the in-vivo investigation phases of the project. The use of progeric models will greatly reduce the number of animals bred for the work due the issues related to maintaining wild-type animals to geriatric ages. To further reduce the number of animals used in the project, the proposed methods of experimental designs and methods of analysis of the results have been discussed with our Consultant Statistician, reviewed by our scientific advisory board and reviewed by the University local ethical advisory board.
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 mouse is the species of choice due to availability of appropriate genetically modified lines that phenotypically display progeria (accelerated ageing). Transgenic Ercc mice (Delta) are an excellent model of normal age related pathologies enabling analysis of key features of aging namely decline of endogenous stem cell function, build up of inter and intra cellular debris, tissue atrophy, cognitive decline. There shortened lifespan gives less variability on onset of such changes than in natural aged animals.

	Rats are the species of choice since their metabolism and other clinical features, including sarcopenia resemble the pathological presentations found in humans.

Project 5	**Understanding and treatment of neuromuscular conditions**	
Key Words (max. 5 words)	Neuromuscular disease; muscular dystrophy; skeletal muscle; stem cells	
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 anim als
		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 purpose of this project is to understand the cell populations and mechanisms responsible for the loss, repair, maintenance and regeneration of skeletal muscle and to determine if the application of stem cells, or changing the expression of either genes that are modified in dystrophic muscle, or related genes with similar function, can improve or worsen the structure and function of dystrophic or aged muscles.	
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)?	Findings from this project will provide new knowledge on the regeneration, repair and maintenance of skeletal muscle and how these processes are affected by old age and/or neuromuscular conditions. These findings will pave the way for new treatments for muscular dystrophies and muscle atrophy that occurs as a consequence of aging.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice – approximately 7,000 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?	Some of our mouse models of neuromuscular disease develop symptoms of the disease, particularly as they get older. In addition, we study normal mice into old age and some of these mice lose weight and may develop tumours that are more common in old age. We carefully monitor our mice for these symptoms.	

	We study muscle regeneration, either derived from transplanted cells, or from cells already present in the mouse's muscle. To induce regeneration, we injure the muscle, which may cause pain that is alleviated by analgesics. We perform surgical techniques under general anaesthesia and carefully monitor the mice post-operatively, being particularly careful to keep them warm and well-hydrated. We transplant muscle stem cells into host mice that are immunodeficient, so that they do not immunologically reject the transplanted cells. Immunodeficient mice are prone to infections, so are kept in individually-ventilated cages in barrier conditions, to minimize risk of infection. The majority of the procedures are of moderate severity. At the end of the experiment, mice will be killed by a schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tissue culture experiments give us information about the early stages of muscle stem cell activation, proliferation, stress, death and self-renewal. However, complete degeneration/regeneration of skeletal muscle is possible only in a living animal. Agents that may be either beneficial or detrimental to skeletal muscle are first tested on cells in culture, but to optimize systemic delivery and to determine downstream effects we need to test agents in dystrophic mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimum number of mice per experiment to achieve statistically-significant results and minimize the size of breeding colonies to produce the required numbers of mice.
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 use mice, as normal, immunodeficient and genetically-altered strains and models of neuromuscular conditions are available for us to perform the proposed experiments. We minimize harm to the animals by performing surgery under general anaesthetic, using analgesia where appropriate and keeping immunodeficient mice under sterile conditions.

<table>
<tr><td>Project 6</td><td>The Mechanics and Energetics of Locomotion</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Flight, running, bird, muscle, energetics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of this research is to improve our understanding of the mechanics and energetics of terrestrial locomotion.

The mechanics and energetics of locomotion are linked because the skeletal muscles that propel the animal consume energy as they interact with the animal’s morphology and the environment to determine the locomotor mechanics. However, forging the links between mechanics and energetics in a detailed and deterministic way has proved to be difficult in many systems. The difficult nature of the problem relates to the complex, multiway interactions among the intrinsic properties of muscle, the often deformable morphological links to environmental loads, and the complex nature of these loads. What is needed is an integrated approach that quantifies mechanical performance and metabolic energy expenditure at the level of the tissues and the organism.

In these experiments we will quantify metabolic and mechanical energy expenditure:

(1) Organismal and tissue level metabolic energy expenditure. Whole animal energy</td></tr>
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	expenditure will be determined using respirometry and the energy used by all of the individual muscles and other tissues will be determined using regional blood flow. This will allow us to quantify the energy expenditure of all of the muscles used during locomotion (flight or running) over a range of speeds and to separate the factors contributing to overall locomotor energy expenditure by quantifying the energy used by all of the muscles and by other, non-muscular physiological systems (e.g. respiratory, circulatory, osmoregulatory systems) in relation to speed. **(2) Muscle mechanical performance**. We will quantify muscle mechanical performance by using a combination of *in vivo* and *in vitro* measurements. Together with measurements of muscle metabolic energy expenditure this will allow us to understand the link between the mechanics and energetics of locomotion
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)?	Improving understanding of the mechanics and energetics of locomotion is important to understanding the ecology and evolution of various animal groups, and is of great practical importance for understanding a variety of locomotor impairments in humans. The results from this research will assist engineers adopting a bio-inspired approach to design.
What species and approximate numbers of animals do you expect to use over what period of time?	Birds will be used in this project [e.g. jackdaw (*Corvus*); budgerigar (*Melopsittacus)*; zebra finch (*Taeniopygia)*; canary (*Serinus*); cockatiel (*Nymphicus)*; pheasant (*Phasianus);* jackdaw/crow (*Corvus*); chicken (*Gallus*)] It is estimated that an average of 40-50 animals will be used annually.
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?	Some of the animals will undergo surgical procedures with moderate severity. It is possible that some of the animals could experience discomfort from the procedure. All animals will be humanely killed at the end of the experiments. Other animals will undergo procedures with mild severity. It is possible that some animals could experience transient discomfort. All animals will be humanely killed at the end of the experiments.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The overall aims of our research are to improve our understanding of how muscles function *in vivo*. This work can only be done on live, freely moving animals and could not be replaced by a non-animal method.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 number of animals is 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.	The criteria that have been used to select potential species are: (i) regional blood flow: animals that utilise either flight or running as the primary mode of locomotion and exceed 400g for determining metabolic energy expenditure by regional blood flow (flying animals must also be of a suitable size to fit within the working section of the wind tunnel; (ii) formation flight: animals that are of suitable size to fit in the wind tunnel in small groups and that are suitably sized to perform in vivo/ in vitro muscle physiology. Post-surgery we will observe the animals for appearance and potential behaviour indicators of pain. In event of observing indicators of pain, analgesia will be used. Any animal exhibiting excessive swelling at the implantation/cannulation site will be killed humanely using a schedule 1 method.

Project 7	**Homeostatic calcium regulation in fish**	
Key Words (max. 5 words)	Ca^{2+}, scales, blood, freshwater, seawater.	
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
		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 primary objective is to investigate fish scale metabolism under different calcemic stresses and to assess whether fish scales might be used as a model to study human bone metabolism, disease, repair and regeneration. Samples of fish scales and blood will also contribute to an understanding of the growth and genetics of wild populations.	
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)?	If it is found that fish scales can be used as a model to study human bone metabolism, disease, repair and regeneration then the potential future benefits in terms of medical and pharmaceutical research are huge. If fish scales can be used as a model there is the potential for significant reduction, replacement and refinement of mammalian models currently used in this research area.	
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 100 individual fish will be used over the course of the 5 year licenced project. These fish will be salmonid species.	

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 level of severity will be mild. Fish will receive a single blood sample and removal of a small number of scales under general anaesthesia. There are no expected adverse effects on the individual fish. The fish will either be released at their source after sampling.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to investigate the role of fish scales in maintaining blood homeostasis under different calcemic stresses scales taken from live fish are required and these need to be maintained in a metabolically viable state. Samples will also contribute to understanding the growth and genetics of the species. There are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The equipment and techniques to be used have recently been developed elsewhere and under a collaborative project are to be used here. By working in close collaboration with the developers of the techniques the numbers of samples required for successful implementation of the techniques will be minimised.
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.	Sea trout have been chosen for this research because they can migrate from sea- to freshwater and when they do this they are able to maintain blood homeostasis under significant environmental stress. This makes them the best model to use to investigate the role of fish scales in maintaining blood homeostasis under different calcemic stresses. The procedure is mild. Individual fish capture, anaesthesia, sampling and recovery will be undertaken using well established best practice methodologies.

<table>
<tr><td>Project 8</td><td>The role of muscle stem cells in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cell, skeletal muscle, regeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>X</td></tr>
<tr><td>Translational and applied research</td><td>X</td></tr>
<tr><td>Regulatory use and routine production</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td></tr>
<tr><td>Preservation of species</td><td></td></tr>
<tr><td>Higher education or training</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Skeletal muscle is able to repair and regenerate because of resident stem cell. However, as people get older, this repair becomes less efficient, leading to muscle weakness and wasting. Repair also fails in some diseases, again leading to weakness and wasting. Muscle stem cells can also become deregulated, which can lead them to forming cancerous tumours. We investigate the regulation of muscle stem cells to understand how they are controlled in healthy muscle, and what goes wrong in disease. This research will inform the development of therapies to treat such muscle disease and possibly ameliorate some of the effects of aging on muscle function.</td></tr>
<tr><td>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)?</td><td>Understanding how muscle stem cells are regulated in healthy muscle, and what goes wrong in disease and cancer, will lead to potential therapies to ameliorate this deterioration in muscle stem cell function.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We use mouse, and estimate that we will use approximately 5615 over the five year duration of this licence.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>To understand how muscle repairs itself after injury, we need to damage specific muscles while the mouse is anaesthetised and then examine the repair process. Since we only injury one muscle,</td></tr>
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level of severity? What will happen to the animals at the end?	the vast majority of the limb musculature is unaffected, and so gross limb function remains overtly normal. After the experiments, the mice will be humanely killed and their tissues removed for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Obtaining muscle cells directly from patients for every experimental procedure is impractical. We will use human cell lines derived from healthy and patients, together with mouse cell lines where available. The most effective model for routinely obtaining stem cells however, is mouse, and mice have been generated that model many human diseases. These cells form muscle fibres in tissue culture, but do not mature without nerves, blood vessels and connective tissue. This is why we have to examine muscle stem cells directly from the mouse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our extensive use of cell lines limits the numbers of animals required. Where possible we initiate regeneration in one leg and use the other as the control. Mice will be breed so that we get the maximum progeny with the correct genotype that we need. Pilot studies will be performed to determine the lowest effective dose with new reagents and estimate sample size. The aim is to use the minimum number of animals to obtain statistically significant results and thus be able to determine any difference between experimental and control group.
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 standard non-human model is the mouse, a mammal with a short gestation period and the ability to be relatively easily genetically altered, thus there are many mouse models available. Mouse is also a good model to study regulation of muscle regeneration since there are many useful reagents available. Furthermore, human and mouse cells will fuse together to form mosaic human/mouse muscle fibres in culture, showing that many of the signalling molecules are able to interact. Importantly, many of the key genes involved are conserved in man. We would therefore expect findings on mouse satellite cell regulation, muscle regeneration, disease progression and therapeutic intervention to be broadly applicable to human. Good animal husbandry practice is used, and

	advice sought where necessary. There is a regular use of pain-killers after surgery, and steps like providing moistened food on the floor on the cage used where needed. For transgenic and genetically altered mice, inducible constructs will be used whenever possible, so there should be no phenotype until candidate gene expression or deletion is induced. By targeting the induction to muscle for example, we should avoid any potentially damaging effects of systemic expression. We do not undertake any procedures that are classified as severe.

Project 9	**The Safety and Efficacy of Cell Therapies**	
Key Words (max. 5 words)	Stem cells, Pre-clinical testing, Medicinal products	
Expected duration of the project (yrs)	5 Years	
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)	Injuries and diseases which affect tissues within the musculoskeletal system are common and cause pain and discomfort to the affected person and cost a lot of money to treat. Stem cells are a population of cells that are capable of generating large numbers of themselves before maturing into functional cells which repair damaged tissue. The processes involved in this can be mimicked in the laboratory using stem cells taken from patient's blood or bone marrow. Our research group is using stem cells to develop therapies that repair, replace or regenerate damaged musculoskeletal tissues. Specifically, we are developing cell therapies to improve healing following surgery to the knee, the shoulder and the foot. The aim of this project application is to investigate the safety and efficacy of the cell therapies before they are used in humans. Understanding the behaviour of cells in the body is difficult due to the complexity of the body and relevant data can only be obtained using animals. Indeed the experiments that we will perform within the project are required by European law. The questions that we need to address include: • Does the cell therapy form tumours or cysts? • Is there an adverse s toxicity or immune response	

	to the cell therapy? • Do the cells survive transplantation? • Do the cells stay at the site of transplantation? • Does the cell therapy improve 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)?	Our primary objective is to demonstrate safety and efficacy of our cell therapies. This will permit us to move into first-in-human clinical trials and the evaluation of our therapies on diseases that have a significant global impact both on people and the economy. Our research will also increase the scientific understanding of how stem cells can be used to treat diseases. We anticipate that the knowledge gained will be relevant to other conditions inclusive of those treating the brain, heart, eye and nervous system. We note that the musculoskeletal disorders treated by our cell therapies are also prevalent in animal species and the work performed within this application will be useful for veterinary practice and the improvement of animal welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	The design of our experiments has been approved by an expert in statistics to make sure that the correct number of animals are used to meet our goals. It is calculated that 240 Severe Combined Immune Deficient (SCID) mice will be used over the five years of the project. It is calculated that 40 rabbits will be used over the five year project. It is calculated that 40 rats will be used over the five year 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?	Adult stem cells will be transplanted into mice to investigate the behaviour of the cells. In some of these experiments, cancer cells will be transplanted into the animals to confirm that the technique has worked. It is inevitable therefore that some mice within these experiments will go on to form metastatic tumours. Because these cells are used to confirm that the technique has worked, any animal that shows signs of pain or distress will be removed from the study by humane killing before they reach any level of severe suffering. For the rabbit and rat models of ligament and tendon repair, the procedures require invasive surgery to cut the ligaments/ tendons. Analgesics will be administered to alleviate pain. The most affected will be the negative control groups in which the ligament/ tendon is not repaired. We do not anticipate any adverse effects relating to pain and distress after the immediate post-operative period. Other ligaments/

	tendons will remain intact so the knee/ shoulder will not be completely destabilised. We will only operate on one limb allowing for the other limb to compensate. Additionally, both animals and humans are capable of functioning with ligament/ tendon deficient joints without pain or discomfort. At the end of the experiments animals will be killed humanely and tissues and organs removed for further analysis to maximise the amount of data generated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Evaluation of safety and efficacy is required by law because adverse side effects following human transplantation may have life-ending/ life-changing consequences. However, some experiments investigating the development of surgical techniques will be performed on human cadaveric tissues in accordance with the Human Tissue Act.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary experiments are carried out using laboratory models to develop and optimise the techniques and methods of analysis.Surgical techniques will be practiced on cadaveric animals. Experiments have been designed so that multiple outcome measures can be obtained from one experiment. Statisticians have been consulted over experimental design so that the numbers of animals used are appropriate to meet our 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.	Severe Combined Immune Deficient (SCID) mice are a breed of mice lacking a functional immune system. Human stem cells can be transplanted without the risk of rejection. Experiments performed using SCID mice include: investigating the formation tumours/ cysts, cell survival following transplantation and the migration of transplanted cells around the body. Rabbits will be used for investigating the effect of cell therapies on the ability to repair ligaments within the knee. The rabbit model is the most well-known and characterised for evaluating knee ligament repair allowing us to interpret the results of our studies. The larger size of the rabbit knee joint will facilitate technical delivery and increase the likelihood of surgical success, thus minimising the number of animals required. Rats will be used for investigating the effect of cell therapies on the ability to repair tendons within the shoulder. The rat model is the most well known and

	characterised for evaluating shoulder tendon repair which will enable us to interpret the results of our studies.

Project 10	**Pre-clinical analysis of therapies for neuromuscular diseases**	
Key Words (max. 5 words)	Muscular dystrophy; Therapy development;	
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)	Neuromuscular diseases are generally severe debilitating inherited conditions, often affecting children, which cause major disability, with associated consequences for sufferers, their families, the NHS and wider society. Unfortunately, the overwhelming majority of these diseases remain incurable, with only supportive and palliative treatments available. The aim of this project is to participate in the development and testing of existing and novel therapeutics for neuromuscular diseases. Our overriding goal is to deliver therapies to neuromuscular disease patients in the shortest possible time frame.	
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 are to advance the development of both existing and novel therapeutics for neuromuscular diseases. As an example, drugs which restore dystrophin expression for sufferers of Duchenne muscular dystrophy are now entering Phase III clinical trials and are likely to become clinically available shortly. However, there is a lack of understanding of how these drugs are metabolised once they enter the body. This lack of knowledge hampers refinement of the drugs, and slows the progression towards safer, more effective treatments. Our project aims to address this deficiency in this class of compounds and others by measuring how these drugs are taken up, utilised and excreted in mouse models of the human diseases, and how this correlates with the effect of the drug on slowing or	

	reversing the consequences of these diseases. The same approach will also be applied to novel therapeutic approaches such as cell grafts and gene therapy using viral vectors.
What species and approximate numbers of animals do you expect to use over what period of time?	The models used under this license are mice, many of which have been genetically altered to express mutations in neuromuscular disease genes. We anticipate that we will utilise a maximum of 11,500 animals over the 5 year duration of the license.
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 mice that we use are afflicted by the same genetic diseases that we are trying to address in patients. As such, this may cause them distress and shorten their lifespan purely due to the disease state that we are trying to model. Mice do not generally get as unwell as the patients with neuromuscular disease, probably due to their smaller size and shorter lifespan, but they may suffer similar consequences to the patients. In the more severe models we use (e.g. Sgcd-/- mice) this may significantly shorten the animals' lifespan and mice may experience muscle weakness, heart problems, shortness of breath and difficulties moving around. This is likely to be a maximum of moderate severity, but will often be mild or sub-threshold as we will aim to kill animals humanely before they reach an age at which they become seriously ill. The therapeutic approaches that we will test in the animals have been extensively tested for toxicology in other laboratories and will be used at what we expect to be non-toxic dosages. We anticipate that the effect of these therapeutics on the animals will either be neutral or beneficial in terms of their genetic condition. Therapeutics may however be administered on repeated occasions and where appropriate, for more chronic treatments, they may be administered via surgical approaches such as mini pumps (to minimise stress of repeated handling and administration) . Application of therapeutics is therefore expected to reach a maximum of moderate severity. Non-invasive imaging of mice is subject to the adverse effects associated with general anaesthesia and where mice are imaged longitudinally the expected adverse effects are expected to be of mild severity. Where animals are subjected to invasive procedures such as heart monitoring or stimulated muscle strength measurements, these techniques are done under

	anaesthesia and animals are not allowed to recover, in order to prevent any suffering. These procedures therefore have a maximum severity of non-recovery. At the end of each protocol the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The neuromuscular system is an interrelated complex of multiple cell types which depends on both nervous input and regulation by cells of the immune system for appropriate function. Whilst any single aspect of this can be effectively modelled using simpler *in vitro* systems such as cell based models, as a whole it can only be effectively modelled in living organisms. Furthermore, regulatory authorities require specific proofs to have been undertaken in animal (usually rodent) models before therapeutics can progress towards clinical deployment. Since our overriding aim is to facilitate and accelerate the development of therapies from pre-clinical to clinical testing (usually referred to as translational research) use of rodent models enables us to maximise the impact of our research by satisfying some regulatory requirements as well as furthering research. In addition to *in vivo* studies such as whole animal imaging or functional tests, a significant component our work is *ex-vivo* based. Studying the tissues harvested from animals helps us to understand the disease pathology and the effectiveness of a therapeutic intervention. Both *in vivo* and *ex vivo* analysis enables us to conduct more robust investigations which are essential for facilitating the development of better and safer therapies for humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prior to any animal experiments we undertake a statistical analysis based on published or our previous data to establish the minimum number of animals required to demonstrate an effect of a stated magnitude (usually 90% power to detect an effect with a statistical significance of $p<0.05$). This requires an estimate of the likely variability and proposed effect size. Where such information is unavailable we undertake small scale pilot experiments to define these parameters before engaging in the research.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The mouse is the model system of choice in this field due to the high degree of homology in muscle structure and function between rodents and humans. Many aspects of muscle function, such as the role of individual proteins and the structure of the

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	neuromuscular junction, can be effectively modelled in lower vertebrates (such as the zebrafish), invertebrates or even in cell culture. However, when it comes to drug metabolism and efficacy, rodent models are required to be confident that effects can be translated back to patients. The mouse is the most amenable rodent available, with a large repository of existing control data and well established standard procedures for the measurement of relevant parameters. As such, the mouse is the only model system with both the necessary and sufficient characteristics for the project to meet its aims. Mice are checked daily by trained personnel and any concerns referred to the relevant veterinary staff who provide 24/7 cover. Particular care is taken in the early phases of any project where the potential outcomes are not known in detail. Where any unexpected consequences arise, the experiment is stopped and the procedures reviewed in consultation with veterinary staff. Any animal exhibiting signs of stress or unwarranted discomfort is humanely killed.

<table>
<tr><td>Project 11</td><td colspan="2">In vivo models for tissue engineering</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Stem cells, growth factors, scaffold, tissue engineering, regenerative medicine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">Five years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)
(Mark all boxes that apply)</td><td>yes</td><td>Basic research</td></tr>
<tr><td>yes</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></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">The ability to repair/regenerate damaged tissue/organs in human body is a major clinical need. In case of bone lose, doctors would normally repair damaged/lost bone by autograft (e.g. bone taken from patent’s own body – which is called the donor site) or allograft (e.g. bone obtained from a donor – i.e. from other people). Similar to the use of spare parts to repair an old car. However, these available technologies have many limitations including 1) lack of stock; 2) need multiple surgeries; 3) risk of infection at the donor site; 4) transmission of diseases from donor to patient.

This project is going to investgate the possibility of using patient’s own cells (such as the ‘stem cells’ from the bone marrow, fat tissue and dental pulp/peridontal ligament) to regenerate tissue/organ in the laboratory or inside an animal’s body. To achieve this goal, we will need to develop/optimise new materials and chemicals to support/simulate the growth and function of these cells. The long term aims of this project are to develop new methods to regenerate the patient’s own tissues/organ to reduce suffering and improve their quality of life.

The specific aim of this work is to develop new methods for the repair of damaged tissues and/or to regenerate living tissue substitutes in regenerative medicine and dentistry, This is similar to providing a new ‘part’ for repairing an old car in the garage To get this ‘new part’, we will need three basic elements: 1) the cells (like the</td></tr>
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	engineer in a factory); 2) growth factors (chemical/mechanical stimulations); and 3) biomaterials (similar to the scaffolds or frameworks in the factory, which can support the cells to grow on). The objectives are: 1. to test if the new cells, growth chemicals or materials can be used in human without being toxic to our body or rejected by our immune system as well as if they could still function properly. 2. to design some new animal models which are similar to real clinical case in the patient and use these to test the possibility of regenerating tissues/organs. 3. to investigate the ability of growing human **cells**, or cell lines to repair damaged tissues and/or to regenerate living tissue substitutes using various skin, muscular, bone, cartilage and complex tissue models: 4. To undertake dynamic monitoring of **tissue healing/repair/regeneration/ remodelling** after the application of stem cell therapy, growth factors and biomaterials using X-ray, ultrasound, microCT, MRI and histology.
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 work should provide innovative information for isolation (e.g. how to get the cells from patient), characterisation (how we know if these are the right cells), optimisation and identification of the better cell sources (e.g. which cells are the best for which treatment) for stem cell therapy and tissue engineering. It should also provide information to guide the use of proper growth factors (e.g. which factors? When to use? How to use? How many? How long?) to regulate human cell growth and differentiation leading to clinical treatment for patient. It should be possible to discover the ideal innovative biomimetic materials (e.g. which material is good for which disease) for use as scaffolds for tissue regeneration, which may lead to industrial standard products (e.g. the food or tools we can easily buy from supermarket) to meet the clinical need.
What species and approximate numbers of animals do you expect to use over what period of time?	180 mice or 110 rats per year (including immunodeficient animals); 70 rabbits per year for the next five years.
In the context of what you propose to do to the animals, what are the expected adverse effects	My clinical orthopaedic background and expertise on *in vitro/in vivo* models (since 2000) made me in the excellent position for high quality surgery and to reduce the adverse effect. Our previous experience suggests

and the likely/expected level of severity? What will happen to the animals at the end?	that animals tolerate the drugs, implanted cells, growth factors and biomaterials scaffolds well. For all the drugs/therapies that we propose, we do not expect any adverse effects but animals will be monitored for signs of pain/distress should unexpected outcomes occur. For experiments when surgery will be required (e.g. subcutaneous implantation, peritoneal implantation and defect models), the animals at a surgical plane of anaesthesia. Following this anaesthesia, animals will be brought around but pain levels controlled by the use of painkillers as required. Whilst we make every effort to use sterile techniques to minimise the risk of infection following surgery, this minimal risk remains. If this occurs, and the animal found to be in pain/distress, we will humanely euthanase the animal. In each case where it would not be possible to alleviate pain or implement effective treatment to an animal, it will be humanely killed. Following all end procedures, animals will not be re-used for any other procedure and will be euthanased humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tissue engineering involves the application of scientific principles to design, construction, modification, growth and maintenance of living tissues. This is an extremely complex process involving the growth of stem cells (proliferation, differentiation), growth factor regulation (locally and systemically), extra cellular matrix deposition/remodelling and other physiological/physical or mechanical stimuli, which are essential for the regeneration of functional living tissue substitute. Because of the requirement to assess the processes within a living physiological model none of these objectives could be achieved without *in vivo* testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	*in vitro* screening will be used for the selection of cells/growth factors/scaffolds to be tested in vivo. Also non-invasive methods will be applied to monitor stem cells and tissue formed during the experiment, which will reduce of the numbers of animal to be used in the project (e.g. replace the killing of animals at set time points). The PI is an orthopaedic surgeon and has the expertise to perform high quality experimental surgical procedures resulting in high quality data and thus reducing total number of animals used in in vivo experiments.
3. Refinement Explain the choice of	We have been working with animal models since 2000 and have expertise to choose the appropriate species

species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	(mice, rats and rabbits), models and methods according to the aims and objectives of each individual study. Many of the methods have been refined from our previous in vivo studies. Many new procedural and sample assessment methods have been employed and will be continually optimised to improve the quantity and quality of outcomes from in vivo study. For example: continual use of imaging modalities like microCT, MRI, spectroscopy, Caliper life sciences *in vivo* imaging system during experimental period. These will not only reduce the number of animals used (the animals do not have to be sacrificed at different time points) but also improve the quality of the outcomes (e.g. high resolution images and quantitative assays of repair mechanism).

Project 12	**New biomaterials for healing of bone defects**	
Key Words (max. 5 words)	Synthetic material, bioactivity, bone, regeneration	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will develop new materials that improve bone healing in difficult fractures. These synthetic materials can enhance the effects of proteins and other molecules important in healing processes. We will test prototypes in experiments with animals, an important step for the translation to bedside of this new technology.	
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)?	Non-union bone fractures are a serious failure of healing in a bone fracture with a current prevalence of around 20 cases per 100,000 population per year. We believe our proposal will help to develop a product that will improve current treatments. A higher number of cases could be successfully treated with a lower cost and lower number of adverse responses, thus reducing the number of cases with permanent failure of healing. Because of the advantages of our simplified concept, a cell-free scaffold manufactured using proven technologies and materials, we expect our proposal to overcome key barriers to translation to a clinical application.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use male B6-129 mice, a very common line of mouse used in lab research, during up to 12 week long experiments. We expect to use a minimum number of implant compositions, depending on the final results from the previous cellular experiments, therefore the overall total number of animals will not exceed 1000 animals.	
In the context of what you propose to do to the animals, what are the	We expect the animals to experience post-operative pain related to the surgery performed in their front paw for the bone defect model and implantation which will be relieved	

expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	as far as possible with analgesia. Based in our collaborators experience with this model we expect the animals to recover quickly after surgery, displaying normal behaviour. We also expect all the animals to experience minor discomfort and stress during and after the anaesthesia for CT scans. Protocols are designed to minimise these effects. We expect a very low number of animals to experience wound infection, splitting, or implant displacement. Treatments and corrective surgeries will be provided in these cases. Because of the need to collect all the implant samples for the planned testing, at the end the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In this project we propose a new approach for the healing of bone fractures. It is related to the repair of damaged tissue and the creation of new blood vessels by our own cells and body. The responses of cells and proteins properties to the materials used are not possible to fully reproduce in the lab, and often different in an animal or in a cell culture. Therefore, animal experiments are a critical step of this project, to validate the efficacy of the platform in an environment similar to a human body, and are an essential validation of the new technology before a clinical application. Before starting the animal experiments we have planned extensive research on cell cultures of the most promising systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	During the animal trials we will use non-invasive techniques (CT scans) to get periodical images of the healing process, without severely hurting the animals and making it possible monitor bone growth using the same imaging test in the same animal over time. This will drastically reduce the number of animals to be 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.	We have chosen a model where a defect of a critical size is performed in one of the front limb bones. It is a defect that does not heal spontaneously. This is close to the clinical situation of non-healing bone fractures in long bones such as the human leg and arm bones. In this model, the ulna and the radius are exposed and a defect is created in the radius with a surgical tool. The ulna provides sufficient stabilization of the defect and no fixation plates/hardware is required, thereby considerably simplifying the surgical procedure, reducing the risk of infection, and at the same time reducing animal pain, compared to other bone defect models in rats. At the same time we consider the model the least invasive to evaluate

	how our new technology enhances the healing process. We strongly believe that non-healing models are necessary as they are rigorous test-beds for bone repair strategies. Also, this model has been already successfully used by collaborators.

Project 13	**Purinergic signalling, bone cell function and tissue calcification**	
Key Words (max. 5 words)	Vascular calcification, osteoporosis, loading, bone	
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 objective of this project is to determine how extracellular nucleotides and purinergic signalling regulate bone cell function and tissue calcification (in particular vascular calcification). Adenosine triphosphate (ATP) is best known as the 'currency' of energy within living cells. ATP belongs to a large family of molecules essential in cellular function called nucleotides. However, ATP and related compounds are also released from cells, acting as important 'messengers' for cell-to-cell communication. Cells respond to ATP (and other nucleotides) using special recognition sites, known as P2 receptors; the classic analogy is a 'key and lock' mechanism. Research over the past few years has shown that ATP and P2 receptors play important and complex roles in controlling the activity of cells responsible for bone turnover. ATP stimulates the bone destroying cells (osteoclasts), whilst powerfully blocking bone calcification by the cells that form bone (osteoblasts). There is now increasing interest in the possibility that ATP and P2 receptors could also be involved in the development of common bone disorders such as osteoporosis (which results from an imbalance in the actions of osteoclasts and osteoblasts). Although vascular calcification shares many similarities to the process of bone mineralisation, the mechanisms leading to its development are still not	

	well understood. Evidence now suggests that purinergic signalling could also play an important role in preventing the development of this condition. The aim of this project is to study in much closer detail the ways in which P2 receptors and related proteins control bone turnover and regulate tissue calcification.
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 primary potential benefit relates to new knowledge about the regulation of bone cell function and tissue calcification by ATP and related compounds. There is also the possibility that the *in vivo* studies will provide molecular targets for which pharmaceutical products could be developed to treat vascular calcification, Clinically vascular calcification is accepted as a significant risk factor for the development of adverse cardiovascular effects such as heart attack and stroke. Currently, there are no treatments available to prevent or treat vascular calcification.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will utilise rodents to address its research questions. It is anticipated that ≤4500 mice and ≤200 rats will be used over the 5 year duration of the licence. The rodent species to be used are appropriate because their fundamental skeletal biology is very similar to humans in many regards and there is the advantage that genetic models, probes and antibodies are available. The most appropriate models of vascular calcification are in rodents. Sample sizes to be used are based on previous work and a calculation to estimate the minimum number of rodents required for establishing significant 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?	We have developed over the years appropriate animal models of for studying bone and vascular cell function *in vitro, ex vivo* and *in vivo.* Most of the transgenic mouse models to be studied are not expected to display any adverse phenotype and therefore are classified as mild. NPP1 knockout mice have a phenotype which progressively worsens with age and leads to reduced mobility, grooming and weight loss. These animals are classified as a moderate severity. Mechanical loading and *in vivo* scanning and the associated general anaesthesia are unlikely to cause adverse effects but if they do occur they are likely to be moderate in severity. Rodent

	models of vascular calcification are also well validated and widely used. Adverse effects (such as weight loss) will be due the induction of renal failure and are expected to occur in all animals. These effects will be moderate in severity. All experiments will be performed by appropriately trained experimenters and are essential for the success of this project. Animals will be sacrificed by Schedule 1 at the end of experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Purinergic signalling is a complex system comprising of multiple receptors and ecto-nucleotidases. Due to a high degree of sequence homology, the number of selective agonists and antagonists for these is limited. Furthermore, the long duration of our cell culture protocols means that it has not been possible to obtain stable long term knockdown of gene expression in our primary cell culture systems. Consequently, culture of cells derived from knockout animals is required *to* establish the role of these proteins in bone turnover and vascular calcification. *In vivo* the skeleton is a highly metabolic organ with numerous interactions between different cell types and systems (e.g. bone cells, the vasculature and the nervous system). These interactions cannot be reliably reproduced *in vitro* and thus ecto-nucleotidase/receptor removal may exert some skeletal effects that would not be evident from *in vitro* experiments. Longitudinal *in vivo* µCT analysis and *ex-vivo* analysis of isolated bones will determine whether there are any changes in bone phenotype and if these effects are influenced by age. The 3D structure of blood vessels and the interactions between vascular cells can also not be fully reproduced by cells in culture. The analysis of isolated aortas, culture of *ex vivo* aortic rings and the use of *in vivo* models of vascular calcification allows us to determine if the effects seen *in vitro* are reproducible *in vivo.*
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim to reduce the number of animals we use. For example, our *in vitro* protocols have been designed and optimised so that we can isolate all three bone cell types (osteoblast, osteoclasts and osteocytes) as well as vascular smooth muscle cells from a single animal. For *in vivo* and *ex vivo* work, power analyses are always applied in order to identify the minimum number of animals required to answer

	the specific question being posed. For instance, we have established that our tibial bone loading studies require group sizes of no more than eight to secure statistical significance. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce the numbers of animals required still further.
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 chosen to focus our work on rodents. Genetically altered mouse models have been widely employed to investigate the effect of numerous factors on bone turnover and vascular calcification. Using a combination of *in vitro, in vivo* and *ex vivo* techniques, this study aims to understand how purinergic signalling influences bone cell function and tissue calcification. The receptors to be investigated are identical in rodents and humans and thus any findings may be of potential therapeutic benefit. Animal suffering will be limited in our studies by our strict monitoring of actual severity and severity limits. Our protocols are also designed not to produce excessive trauma or suffering. Animals will be killed if they approach the limit of severity.

Project 14	**Angiogenesis in health and disease**	
Key Words (max. 5 words)	Capillaries, blood flow, skeletal muscle, ischaemia, hypoxia	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Adequate control over the extent of the microcirculation is critical in health and disease, with numerous pathologies characterised by either uncontrolled expansion or failure to elicit adequate growth. We have identified a number of new avenues that require further work to mature the topic, mainly based around the ability of the cells making up the capillaries (or nearby perivascular cells) to sense changes in the local mechanic environment. We aim to exploit these models further in two ways: a) better understand the cellular and molecular regulation involved and b) refine the animal models of exercise to provide a more targetted intervention.	
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 may be able to avoid ‘off-target’ effects of many current pharmacology-based angiotherapies, optimise the efficacy of other angiotherapies, and provide guidance about specific forms of exercise that would most benefit individual patient groups.	
What species and approximate numbers of animals do you expect to use over what period of time?	In the main we will use rats due to the appropriate size for invasive surgery and challenging terminal experiments (~1500/5 years), with mice used where genetic modification or expensive molecular interventions are required (~500/5 years), and rabbits where we wish to more closely mirror surgical interventions conducted in patients (~150/5 years).	
In the context of what you propose to do to the animals,	The adverse effects are likely to be mainly in response to surgery, as identified in each of the	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	protocols. From past experience of animal welfare monitoring the maximum level of severity will be moderate. The animals will be humanely killed after experimentation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternatives to animals cannot be used to meet the aims and objectives of this work because *in vitro* models of angiogenesis differ fundamentally from the *in vivo* process. Many of the characteristics of endothelial and smooth muscle cells are altered in tissue culture systems, the contextual significance of extracellular matrix molecules and interstitial cell types is lacking, mechanical stimuli are usually absent, and the time course of adaptations cannot be mimicked realistically. The different factors initiating or controlling angiogenesis *in vivo* have also been proved to be different from those *in vitro*. The integrative nature of *in situ* tissue function is thus of paramount importance to the problems being addressed and the scientific rationale of the work is best served by use of animal models. Although it is encouraging that such data may be translated into the clinical setting, fundamental research is still needed in order to understand the mechanisms involved. Such investigations are impossible without the use of animals, all alternatives having been shown to be of limited value. Where parallel studies have been made (e.g. muscle ischaemia, hypoxia/COPD, elevated shear stress), qualitatively similar processes have been observed in animals and humans, giving confidence to continue to develop the animals models to inform clinical interventions. This work has progressed slowly in part because there are very few, specific inhibitors that can be used to probe the role of these signals *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Appropriate experimental design and data analysis are routinely employed to minimise sample size without compromising the validity of outcomes. Sample sizes may be set using statistical insight ('power analysis', where the least practical difference between groups and the likely variability of the data is estimated to determine how many animals/group are required). Exact numbers of animals required will vary depending on the nature of the experimental intervention; in practice group sizes of 6-10 have been required in past experiments, with typically a 1-2% failure rate. Given genetic drift in commercial

	stocks, it has not been possible to reduce animal numbers by repeat analysis of control groups, though we continue to try by means of loop experimental design.
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.	Experiments will be carried out mainly using rodents as appropriate mammalian models to develop fundamental knowledge that may act as a prelude to veterinary application and human intervention. Previous experience has allowed refinement of the procedures in order to obtain reproducible angiogenesis in a relatively short duration, with reduced trauma. This facilitates the study and interpretation of mechanisms in a minimum number of animals. Mice will be one target because the use of transgenic and knockout technology can be used to investigate further the molecular basis of observations made in the rat. Sometimes the use of genetically-altered animals may be the only way to establish the *functional* role of a e.g. protein in supporting angiogenesis. In general, we aim to minimise harm by reducing: the frequency and/or duration of the procedure, the likelihood of known adverse effects and the proportion of animals likely to be affected, and the severity level by range-sighting to optimise efficiency. In addition, we adopt a monitoring regime whereby animals are closely monitored following invasive surgery, assisted by approved welfare assessment protocols, and subject to humane end-points. Triggers for interventions are stipulated in each protocol SOP. Genetically-altered animals may have an increase in susceptibility to infection, although this risk is considered to be minor in consideration of the high standards of husbandry in the CEU and health status of the mice. They may have increase in susceptibility to haemorrhage due to weak blood vessel structure, but this is considered unlikely to be a problem because mice are unlikely to experience excessive trauma in the BMSU, and there exists compensatory pathways in vessel maturity that would help to prevent an excessive loss of blood. A register will be maintained all genotypes/phenotypes and associated adverse effects, to be available for inspection by all personal licencees. Adverse effects that have been noted prior to transfer of the animals from another project licence will also be recorded on the register.

	Any newly identified adverse effects in wildtype and genetically modified animals will be recorded along with the appropriate route of action to be taken. The register will guide control measures and humane endpoints, and will be used to record the phenotype of new strains of mice for which we do not have specific information.

<table>
<tr><td>Project 15</td><td>Models of tissue repair and reconstruction in the limb</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Injury, limb, repair, reconstruction, regeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to study the biology of inflammation in different types of injury to the soft tissues in the limbs of animals. This will allow us to understand how the soft tissues heal and whether we can make them heal better. The injury types will be very similar to injuries seen in the clinical setting. The repair methods will simulate the surgical procedures used. Other methods will also be used based on reconstructive surgery techniques to develop vascularized tissues that can be used to heal major trauma wounds. All the procedures have been optimized to be of minimal disturbance to the animals studied. The findings of the studies will be used to develop better therapies and surgeries to these common clinical situations resulting from trauma.</td></tr>
<tr><td>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)?</td><td>We see these injuries and perform elements of these repairs and reconstructions on a day to day basis in the clinical setting. We lack a true understanding of the healing process that occurs between all the tissues which means that humans rarely get a perfect outcome.

We have chosen to recreate the simplest component of each injury we see. This allows us to assess the biological interactions between the different tissues and study the different cells that are involved. We have used this approach in previous projects which have gone from basic lab research all the way through to clinical trials. We</td></tr>
</table>

	will use our well-defined transgenic models to simulate injury, repair and reconstruction of damaged tissue in the limb. This will aim to discover new novel therapies, which ultimately could be beneficial to man. Our main focus is to develop our reconstruction model that uses a new method to vascularize and promote new tissue formation. This is used as a vehicle to support cell therapies or biomaterials as a realistic platform to heal big wounds like pressure sores, cancer wounds or trauma wounds. We will have a portfolio of clinically relevant models to help us tackle the problems that arise from minor injury and severe injury to the limbs.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use mice and rats as our model systems to study the effect of injury. We will use mice for their genetic alterations that will allow us to look at specific cell types and molecular pathways of interest. The use of rats provides us with a larger model that can be used to study different biomaterials, which may be too large to study in the mouse. We have a limit of operating on 1000 rats, 1200 mice and 1200 genetically modified animals on the licence. We also have the aim of breeding genetically modified mice of around 2000 for this project. From previous licence activity this is a realistic limit 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?	Breeding: Animals bred on the license will be expected to have either none, or mildly abnormal characteristics which do not affect animal survival. If animals have a visible disability they will not be used further for the surgical component of the study. Bone marrow transplant in mice: Mice will be used that have had bone marrow transplants to study the contribution of bone marrow cells to tissue formation or wound healing. These mice may have diarrhoea or failure to thrive in a small percentage of animals (from experience 5-10%). If this occurs animals will be humanely killed. The generation of these mice will be of mild or moderate severity. Only after full recovery from reconstitution will mice be used for surgery. Having conducted these surgeries over a number of years we have had very few adverse problems because we have ensured the surgery is of the

	highest standard. The severity rating for the procedures is moderate although some procedures have minimal physiological insult. Tissue for specific assessment is all acquired by schedule 1 or terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At this point in time we cannot accurately mimic the injury and repair/healing or inflammatory response in the absence of nutrition provided by blood flow. We are currently in tandem to our animal experiments working on alternatives that do not use living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have conducted experiments before and used this as a basis for power calculations to ensure numbers of animals used are kept to a minimum. The number of animals proposed for use in this license has been dramatically reduced in accordance with our experience in maximizing data acquisition from previous studies.
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 refined our surgical techniques to a higher fidelity than any human operation so that they may be conducted on mouse which is the lowest sensate mammal available for biological study. These operations are conducted to the highest standard with hundreds of hours of training time acquired to ensure reproducibility and standardisation. Each animal receives husbandry, intraoperative care and postoperative care to a clinical standard.

Project 16	**Understanding muscle maintenance, regeneration and ageing**	
Key Words (max. 5 words)	Muscle, regeneration, inflammation, muscular dystrophy, stem cells, Duchenne muscular dystrophy	
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)	In Duchenne muscular dystrophy (DMD) the muscle cells undergo continuous damage. At first the damaged muscle cells are regenerated by the stem cells present in the musculature, but as the disease progresses the muscle tissue is gradually replaced by scar tissue, a process called fibrosis, and infiltrated with inflammatory cells. Fibrosis and chronic inflammation, in turn, establish a very hostile environment for the resident muscle stem cells, which eventually lose their ability to regenerate the damaged muscle cells thus further contributing to muscle loss. Our laboratory focuses on understanding how the environment established by fibrosis and inflammation affects the maintenance and regenerative properties of the resident muscle stem cells. Using dystrophic mice and cell culture as model systems we have discovered that a family of proteins produced by inflammatory cells are altered in dystrophic muscle and play important roles in the regulation of muscle stem cell survival and function. We now plan to continue to use mice and cell culture to investigate in greater detail the underlying molecular mechanisms. Additionally, we will continue to investigate potential therapeutic targets that we have previously identified, such as the proteoglycan syndecan-3, to unravel the molecular mechanisms that	

	are regulated by syndecan-3 and other similar proteoglycans in muscle regeneration.
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)?	DMD and other forms of muscular dystrophy are devastating diseases for which no cure or effective treatment is currently available. This study will greatly advance our understanding of the way in which muscular dystrophy develops and will lead to identification and characterisation of novel therapeutic targets for the treatment and management of DMD and possibly other forms of muscular dystrophy as well. We also hope to identify novel blood biomarkers of DMD to help us monitor the activity of the disease in the muscle without taking a muscle biopsy.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice, approximately 1600 over a 5 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?	No animal used on this project is expected to experience more than moderate severity and many will experience no more than mild. In one set of procedures animal will receive one intramuscular dose of a myotoxic substance or one application of a cool probe to cause a localised muscle injury. Based on previous experience and a wealth of literature in the field of muscle biology, it is well known that induced muscle injury causes only very limited, often unappreciable pain, which is only transient and fades away quickly within hours. Animals that receive an injury will be carefully monitored in the post-operative time and any sign of unexpected adverse effects will be immediately managed by seeking advice from the veterinary surgeon. Should any animal show signs of pain or distress (e.g. piloerection, hunched back, etc) we will administer an analgesic at a dosage advised by the NVS. Another set of procedures that we will perform on the mice will be administration of medicines that are expected to ameliorate muscle regeneration in response to either acute injury or a genetic disease such as muscular dystrophy. We expect no adverse effects from administration of these drugs, which have all been already characterised in the context of other diseases (e.g. lung disease, inflammatory diseases, coagulation disorders, etc) and shown minimal adverse effects. We now want to explore the usefulness of these drugs to treat muscle dysfunction as we have

	evidence that they might improve the overall health of dystrophic and injured mice. At the end of a protocol animals will be humanely killed so that their tissues may be further examined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying the interplay between several cell types and the environment that they establish in the muscle during regeneration induced by either injury or diseases. Although the individual cell types can be isolated and cultured to address certain scientific questions, not all questions can be addressed by using a cell culture system because it is impossible to reproduce in a petri dish the real environment that exists in the organ and is impossible to reproduce exactly the conditions of an injury, especially the inflammatory response. Thus, it is indispensable to use animals. Nonetheless, every time a scientific question can be addressed by using a cell culture instead of an animal (e.g. when addressing questions about the fine details of a molecular pathway) we will use cell culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposal has been designed with the help of statistical advice to minimise the number of mice used. Moreover, we will plan our experiments in order to get the most results possible out of each animal, this will automatically reduce the number of animals needed to obtain all the results that we are after. Additionally, we will breed the transgenic animals in a way that maximises the number of animals obtained from each mating that can be used in the project and minimises the number of animals that cannot be 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.	We have chosen mice because mice have very similar muscle physiology to humans and there are several genetically altered mouse strains available that would be extremely useful for our project. All mice will be hosted in a state-of-the-art facility at the University of Liverpool that applies the highest standards of animal care and welfare. Cages are equipped with environment enrichment devices, such as little houses and raised balconies. The temperature and humidity in the facility are tightly controlled to meet the needs of mice and the food provided ad libitum is of the highest quality.

Project 17	**Manipulation of signalling pathways in zebrafish**	
Key Words (max. 5 words)	Zebrafish, HSPG, regeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This research aims to provide important knowledge to apply to curing bone diseases and aiding in regeneration. The specific focus is on the role that heparan sulphate (HS) plays in cell-to-cell communication (signalling) during normal development and during regeneration. HS is known to be required for signalling in the skeleton, but the precise mechanism by which HS acts is unknown. Here we will analyse the role of HS at the single cell level to determine precisely the role that it has during skeletal formation and how HS interacts with signalling molecules. This project will involve treating larvae with chemicals which disrupt specific molecular targets, raising the temperature to induce gene expression (within the range of temperature experienced by zebrafish in the wild), using recombination to activate signalling and studying mutant fish strains.	
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)?	HS has been implicated in several skeletal diseases, most notably in multiple osteochondromas (a form of childhood bone tumour). Understanding how HS functions in the zebrafish model will help to develop new treatments for human patients. Our studies into regeneration focus on the remarkable ability of zebrafish to heal without scarring. A long term goal of the regenerative biology field is to aid in the development of more effective therapeutic approaches to improve the quality of life of individuals who have suffered severe tissue loss due to injury or disease.	

What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish (Danio rerio) 30,000 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?	The protocols are designed to minimise suffering and if animals are in poor health or visible pain the experiment will be aborted. Although much is known about the mechanism by which these regimens act, there may be unforeseen effects on the health of the fish. Furthermore, because parts of the skeleton develop after fish are ready to begin feeding, it is sometimes necessary to maintain fish that would normally feed (more than 5 days old) even though they are unable to do so. As there is still yolk present, the microscopic larvae derive nutrients from this. Once the yolk is entirely consumed (8 days old) then larvae will be humanely killed or feeding will commence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As the skeleton develops slowly and has known interactions with the surrounding tissue, it is not possible to do these experiments in vitro. Regeneration involves many interactions with different tissues and immune cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals used by careful experimental planning. The vast majority of the animals used in this project are for breeding purposes and will not be used for experimentation. To minimise the number bred and maintained we use a management database.
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.	Zebrafish was chosen for this study because of its low sentience (neuronal complexity), because it has a skeleton which is similar to that of humans and because it has a high regenerative capacity. Animals are anaesthetized during any procedure that may cause pain and if the fish appear to be in pain after recovery the experiment is terminated and the fish are humanely killed.

Project 18	**Genetic and hormonal regulation of the skeleton**	
Key Words (max. 5 words)	Osteoporosis, osteoarthritis, fracture, genetics, hormones	
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)	The lifetime incidence of fracture for a 50 year old in the UK is 40% for women and 13% for men, and osteoporosis costs the NHS £1.7 billion per annum. The cost of osteoarthritis is estimated at 1 % of gross national product. This financial burden will inevitably increase as the population ages and disease incidence rises. Treatments for osteoporosis generally prevent bone loss but their effectiveness is limited by side effects and patient acceptability, whilst there are no effective drugs to prevent or delay osteoarthritis. Thus, there is urgent need to advance understanding of the mechanisms of bone and cartilage formation and repair in order to facilitate development of new drugs. The basic mechanisms that drive growth and development are also essential for tissue maintenance and responses to injury in later life. Dysregulation of these fundamental processes is thought to underpin the onset and progression of degenerative disease. Recent studies indicate that regulated availability of the biologically active thyroid hormone (T3) in individual cells and tissues is a conserved mechanism that coordinates organ development and function in all vertebrates. We propose that altered T3 availability is a key factor responsible for abnormal tissue repair in response to	

	injury, and the increased susceptibility to chronic degenerative diseases such as osteoporosis and osteoarthritis that is observed during ageing. In addition to obtaining a detailed understanding of the fundamental role of thyroid hormones in tissue repair and the pathogenesis of osteoporosis and osteoarthritis, we also seek to identify new genes that specify bone and joint structure and function, and develop new disease models for skeletal disorders. These studies will provide new understanding of the onset and progression of degenerative skeletal diseases and facilitate identification of novel therapeutic targets for use in osteoporosis, fracture healing and osteoarthritis.
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 lifetime incidence of fracture for a 50 year old in the UK is 40% for women and 13% for men, and osteoporosis costs the NHS £1.7 billion per annum. The cost of osteoarthritis is estimated at 1 % of gross national product. This financial burden will inevitably increase as the population ages and disease incidence rises. Treatments for osteoporosis generally prevent bone loss but their effectiveness is limited by side effects and patient acceptability, whilst there are no effective drugs to prevent or delay osteoarthritis. Thus, there is urgent need to advance understanding of the mechanisms of bone and cartilage formation and repair in order to facilitate development of new drugs. The basic mechanisms that drive growth and development are also essential for tissue maintenance and responses to injury in later life. Dysregulation of these fundamental processes is thought to underpin the onset and progression of degenerative disease. Recent studies indicate that regulated availability of the biologically active thyroid hormone (T3) in individual cells and tissues is a conserved mechanism that coordinates organ development and function in all vertebrates. We propose that altered T3 availability is a key factor responsible for abnormal tissue repair in response to injury, and the increased susceptibility to chronic degenerative diseases such as osteoporosis and osteoarthritis that is observed during ageing. In addition to obtaining a detailed understanding of the fundamental role of thyroid hormones in tissue repair and the pathogenesis of osteoporosis and osteoarthritis, we also seek to identify new genes that

	specify bone and joint structure and function, and develop new disease models for skeletal disorders. These studies will provide new understanding of the onset and progression of degenerative skeletal diseases and facilitate identification of novel therapeutic targets for use in osteoporosis, fracture healing and osteoarthritis.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Average of 1960 animals per year for 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 generated with genetic alterations affecting maintenance of bone and joints. Our prior experience indicates these animals do not experience significant distress. Animals will undergo procedures to manipulate their hormonal status, to provoke mild arthritis or create an internally splinted and stable fracture. The animals will be anaesthetised for these procedures and our considerable experience indicates they do not suffer significant distress. Indeed, following procedures for induction of mild arthritis or generation of a stable fracture, animals typically recover fully within minutes. They only experience a mild degree of lameness for a short period that does not prevent free movement or normal eating and drinking. All animals routinely receive painkillers to prevent any discomfort and they are group housed with appropriate bedding, nestling material and with cage toys for a stimulating cage environment. We monitor animals in accordance with the UK Laboratory Animal Science Association good practice guidelines (LASA guidance). The overall severity of the proposed procedures is considered moderate and at the end of the study animals will be humanely killed or euthanised, or vasectomised males may be kept alive.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Circulating hormones and other factors that act inthe complex multi-cellular microenvironment of the skeleton regulate bone development and maintenance. Skeletal disorders result from abnormalities that may occur during intra-uterine development, post-natal growth or adulthood whilst structural properties of bone depend on movement and weight bearing. Animals are also essential for studies of osteoarthritis, which is a dynamic process involving several tissues and the trafficking of cells from distant sites to the joint via the circulation.

	Similarly, fracture healing progresses over a period of weeks and involves growth factor signalling from surrounding tissues to initiate new blood vessel formation and migration of bone-forming cells in order to repair the injury site. All these intricacies cannot be modelled ex vivo, and investigation of factors that initiate and modify disease progression must therefore involve whole animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Primary bone cell culture will be used in place of animals to investigate molecular mechanisms. Studies will include hormone manipulations to investigate cell maturation and examine gene regulatory responses. Data from cell culture studies will be used to design in vivo experiments; ensuring results are statistically robust using the minimum numbers of animals. We further minimise numbers by employing sequential and complementary analytical techniques on individual tissue samples to gain the most information possible from a single animal. We have also refined an efficient strategy to minimise animal numbers by using an osteoarthritis model only suitable in males, together with a fracture-healing model that is independent of gender and will be performed in females. This ensures all mice will be informative and used for 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.	Key molecules that regulate the skeleton in mice have the same functions in man, and human genetic disorders causing abnormalities of cartilage and bone are recapitulated in mutant mice. Hormonal control of bone and cartilage is faithfully preserved in mice and humans, and the osteoarthritis and fracture models to be used in these studies were established and validated in mice with predictable outcomes, Overall, mice are the only appropriate species for the proposed studies and we will minimise harm by: using the least traumatic and most refined disease models; ensuring best practice by adhering to the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines; and ensuring the best possible animal husbandry is adopted according to LASA guidelines.

<table>
<tr><td>Project 19</td><td colspan="2">Musculoskeletal Tissue Regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Musculoskeletal, regeneration, repair, bone, cartilage</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>✓</td><td>Basic research</td></tr>
<tr><td>✓</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>✓</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">The overall strategy is to –

1) Identify a clinical problem with musculoskeletal patients.
2) Review the literature concerning this problem and asking what (if any) questions remain unanswered.
3) Define the research questions.
4) Determine whether these questions can be answered in (i) a patient study (ii) a cell culture study (iii) a biomechanical measurement study or (iv) a mathematical modelling study.
5) If the research question cannot be answered by any of the above then design an in vivo study to answer this question.

The overall aim of this programme is to increase our understanding of the response of the musculoskeletal system to mechanical stimulation and injury (including failure to heal), and to investigate clinical techniques that may contribute to improved healing after injury.

Specifically:

(i) their ability to regenerate and respond to various injuries and mechanical stimuli</td></tr>
</table>

	(ii) their ability to heal after division with novel cutting techniques.
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)?	Diseases of the musculoskeletal system account for 25% of GP attendances and consume a major proportion of hospital resources. Each year in the UK alone, over 1 million fractures occur and over 100,000 joint replacements are performed, many of these caused by osteoporosis and osteoarthritis, respectively. This major burden of disease will rise further as the age of the population increases. There is therefore a burning need to increase our understanding of these disabling conditions and to investigate regeneration and repair on the musculoskeletal system. One of the major functions of the musculoskeletal system is to exert forces and respond to loads. This interaction between the biological system and the physical world is poorly understood. Yet this mechanobiological interface has a major role in the cause of musculoskeletal diseases as well as regeneration and repair of the musculoskeletal system. In order to reduce suffering, there is also a need to look at enhancing repair where this is impaired, using tissue engineering, therapies using cells and special signalling proteins.
What species and approximate numbers of animals do you expect to use over what period of time?	Estimate of numbers: mice 1410, rats 600, rabbits 130, sheep 190, guinea pigs 20, 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?	The animals will undergo procedures that mimic those happening to patients. In summary (1) musculoskeletal tissues will be divided using novel devices such as ultrasound, (2) studies will be carried out in which bone will be divided under anaesthetic and stabilised with internal or external devices and (3) lesions will be created in cartilage, muscle and nerve and the repair of these defects will be studied to improve the treatment of arthritis and other conditions. These are of moderate or mild severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work outlined in this project license has all been carefully planned in line with the principles of replacements, reduction and refinement. Wherever possible, animals will not be used. For instance, if the answer to the question can be obtained from cell culture, these techniques will be employed. To

	maximise this, we have built a range of equipment which include a four point bending system for lower strains and a hydraulic system for higher strains. In addition, we have purchased and refined a device which enables us to keep whole cores of bone alive for several months. In parallel with this, we have appropriate ethical approval in place to permit us to use (with patient consent), human surgical discard material. Further, in some situations, we are getting suitable data direct from patients either from measurements from external fixators or from CT scans. This has been coupled with mechanical data obtained in the laboratory and fed into complex ("finite element") computer models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be kept to the minimum necessary to enable statistically sound conclusions to be drawn from the studies. The numbers requested reflect this and are, therefore, the minimum number required to achieve the important 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.	All the procedures have been carefully refined in order to minimise discomfort. Most of the techniques described in are similar to those used in patients, in particular, the division of the bone, external fixation and distraction of bone. We know from the feedback from patients that these procedures are well tolerated The majority of the work will be done in rats and mice, occasionally we may use larger species (sheep) in which we will divide a bone under full anaesthesia and study the repair mechanism for the bone. The animals will have the same care as medical or veterinary patients with bone problems. We will also study situations where the cartilage is damaged e.g. arthritis and ways to repair this. The choice of species and strain to be used depends on the study. Some of the procedures were originally developed in rabbits. The bones of this species are quite large and allow precise control of the mechanical environment. In line with the principle of reduction, we have validated these models in the rat and in some cases it has been possible to go down to the mouse. However, internal and external fixation are extremely

	challenging in bones of these dimensions. Mice will be used when animals with a known change in their genes are required. Sheep will only be used when we need to control the amount of movement in the osteotomy or fracture site very accurately or if it is necessary to determine whether or not an effect of an agent observed in a smaller species also occurs in a larger model.