Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 4

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

Project Titles and Keywords

1. **Analysis and therapy of neuromuscular disease**
    - DMD, Muscular Dystrophy, Muscle, *mdx*

2. **Biomaterials for Regenerative / Oral and Maxillofacial Surgery**
    - Biomaterials, Surgery, Tissue Engineering, Orthopaedic

3. **Strategies for orthopaedic translational research**
    - Orthopaedics, bone, cartilage, tendon

4. **Antibiotic Treatment for Chronic Back Pain**
    - Antibiotic, back, pain, bacteria, infection

5. **Muscle loss in chronic disease**
    - Muscle wasting, muscle regeneration

6. **Rabbit cartilage defect model**
    - Cartilage, defects, rabbit, cells, scaffolds

7. **Developing improved understanding and better treatments for bone and joint disease**
    - Arthritis, osteoporosis, fractures, cancer

<table>
<tr><td>PROJECT 1</td><td>Analysis and therapy of neuromuscular disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>DMD, Muscular Dystrophy, Muscle, mdx</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</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 aim of the work carried out under this licence is to develop an effective therapy for Duchenne muscular dystrophy and to find the genes responsible for other neuromuscular diseases so that treatments can be found.</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>DMD is the most common of all the neuromuscular disorders with a prevalence of 1 in 3000 male births. Current treatment is restricted to palliative care which has only modest benefits and is associated with a number of severe side effects. An effective treatment developed through this programme of work will improve the quality of life for DMD patients and their families. Additionally, the clinical development of effective therapies for DMD is paving the way for the development of experimental therapies for other relevant muscle-</td></tr>
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	related disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 17,950 mice over the course of the 5 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?	Animal models used in this programme of work are mostly mildly affected (~95%). The most likely adverse effect comes from the genetic modification found in a small percentage (~5%) of moderately affected animals. These animals show signs of weakness, difficulty breathing and reduced mobility. Mice are monitored and scored to assess welfare and are treated with nutritional support where appropriate. Animals showing signs of deterioration are euthanized. Animals may undergo procedures such as injection, imaging and motor function tests which are minimally invasive and no significant adverse effects are expected. Denervation and nerve crush should not result in pain and reduced mobility, and the animals are closely monitored for any possible, rare adverse effects. If an adverse effect were to occur, animals would be euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to mimic the action of a drug on muscle from cell line work alone because muscle in the body receives signals from the nerve in order to work properly and in general, the drugs for treatment have to be delivered by the blood stream. The regulatory authorities also demand that we have preclinical data in mice before going on to human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of results but also minimising the number of animals used in each study. Wide selections of tissue samples are taken from each animal used in a study to try to cover every eventuality and prevent having to use more mice in a repeat experiment. This ensures maximum use of every animal that we work with. Samples are put in long term storage enabling us to access them if

	and when required at any time in the future.
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 best model to use as mouse muscle is very similar to human muscle. The *mdx* mouse has a mutation in dystrophin and is a good model for DMD and for use in the development of therapies. It is only very mildly affected because mice can regenerate their muscles after damage much more easily than man can. The double knockout (*dKO*) mouse lacks dystrophin and utrophin and is a moderately affected model of DMD that better mimics the disease progression in man. However, due to this increased severity we minimise the use of this model, using the *mdx* mouse in preference where possible.

<table>
<tr><td>PROJECT 2</td><td colspan="3">Biomaterials for Regenerative / Oral and Maxillofacial Surgery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Biomaterials Surgery Tissue Engineering Orthopaedic</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</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>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>~~Yes~~</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">To identify and develop materials that will be useful for promoting the healing of, and replacement of bone in patients who have missing bone as a result of disease, degeneration, trauma of congenital deformity.

To identify surface coatings or treatments, that when applied to implantable materials and devices will encourage healing and minimise complications such as infection of failure of the material or device.

To evaluate new barrier membranes in guiding tissue growth and healing; these are placed over bone defects and work by preventing the in-growth of other tissues into bone defects thereby allowing</td></tr>
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	the body time to grow new bone. To identify tissue engineered constructs that will restore lost hard and soft tissues in and around the mouth but also, potentially, at other sites in the body. These constructs comprise a scaffold (framework) into which cells are allowed to grow in culture in a laboratory before being grafted back into a patient.
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 include the development of products to improve clinical outcomes in surgical practice, to reduce the complexity of surgery by avoiding having to take bone grafts from another part of a patient's body and to reduce the length of stay in hospital in patients who have lost body tissues and who require surgical reconstruction for functional and aesthetic rehabilitation.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats, probably less than 250 over the 5 year period of the licence.
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?	Animals will have test materials placed under the skin of the back or into a surgically created bone defect in one leg, one side of the lower jaw or skull. Surgery will be carried out under a general anaesthetic and post-operative pain relief will be provided; antibiotics will also be given if appropriate and the animals will be monitored regularly for any signs of adverse effects. The overall level of severity for this project is moderate, therefore it is anticipated that the animals will suffer a degree of pain and the after effects of an anaesthetic; in our experience the animals make a rapid recovery and resume normal activity in a short time. We have never had to kill an animal as the result of undue suffering or adverse effects following surgery. The expected adverse effects include wound infection; difficulty eating or mobility problems (less than 0.5% incidence in our previous studies) persistent or worsening adverse effects would be

	an endpoint and any animal suffering unduly would be humanely killed. At the end of the study animals will be killed using a schedule 1 method of by perfusion of fixatives under a terminal general anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Testing in cell or organ culture cannot, unfortunately, fully simulate in life evaluation of biomaterials. We need, therefore, to undertake studies using animals to determine the response of various tissues to agents and materials that may be worthy of further evaluation. Prior to animal studies we test materials extensively in the laboratory and use cell, tissue and organ cultures with cell lines or using human tissue. Animal tests are only done on materials with the potential for future clinical use. . We use rats as these are the smallest animal that can reasonably be used for our type of study; some of the materials that perform well and that can be expected to enter the market place for use in humans and animals may subsequently require further testing in larger animals, but this is not part of our work. Initial testing of potentially useful materials in rodents can reduce the need for testing in larger animal models by selecting out the best performing material(s) under investigation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Materials will not be tested on animals until we are sure that appropriate results have been obtained from non-animal based laboratory testing. Small scale studies will be carried out to assess the response to materials at a single time point – only if results are promising will further testing be considered. Appropriate statistical evaluation will ensure best use of animals whilst minimising the numbers required to achieve meaningful 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.	The work we undertake is basic science and the animal study is the end point of our main investigation using non animal based evaluations. The final evaluation we do informs and determines if further in-depth regulatory studies before clinical application but this is not part of our precompetitive work. We aim to minimise suffering of the animals by careful husbandry and the use of analgesics and antibiotics as appropriate. The animals will be housed in groups and regularly monitored for signs of distress or complications arising from surgery and appropriate steps will be taken to minimise any suffering in line with current guidelines.

<table>
<tr><td>PROJECT 3</td><td colspan="3">Strategies for orthopaedic translational research</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Orthopaedics, bone, cartilage, tendon</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[5]</td><td colspan="3"><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[6]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The work proposed in this project will continue to advance the basic knowledge on the remodelling, repair and regeneration of the musculoskeletal system in order to translate understanding of mechanisms to new clinical strategies and devices in prevention and management of skeletal disease.
This project licence incorporates a number of procedures designed to elucidate the mechanisms that control remodelling, repair and regeneration of tissues and structures in the musculoskeletal system.</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</td><td colspan="3">1. Determine the response of specific skeletal tissues and composites of skeletal tissues to defined mechanical and biological stimulation.

2. Elucidate the influence of mechanical, biological and material factors on the remodelling and repair</td></tr>
</table>

project)?	processes of skeletal tissues and structures. 3. Define the influence of mechanical, biological and material characteristics on the integration of prosthetic implants with skeletal tissues 4. Develop strategies to regenerate skeletal tissues and structures through manipulation of the mechanical and biological environment to attract, cue and selectively differentiate multi-potent cell populations.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep 1300 Goat 300 Rat 550 Mouse 250 Rabbit 300 All over the 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?	The models used are predominantly surgical and as such fall in the overall "moderate" severity category. However, the staged approach of the work is consistent with minimising the severity within this category wherever possible. At the end, animals will be killed under Schedule 1 of ASPA.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many aspects of our work are being achieved through the use of laboratory based in vitro cell culture, tissue culture, computer modelling and bioreactor studies using novel materials and chemicals. These techniques allow identification of the most likely treatments to be validated in vivo. This replacement reduces the numbers of animals used. The integrated physiological environment of the living animal is still essential to elucidate the patho-physiological mechanisms prior to application in advancing clinical management of musculoskeletal conditions in both veterinary and

	human patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have ascertained in our study designs that in most experiments minimum group sizes in the order of six animals are required to evaluate levels of effect that have both statistical and translational significance. Standard models have been developed and will be used where appropriate for comparative data to avoid replication of some control groups.
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 shall adopt a hierarchical approach using simple models before using complex implants. The strategy used would minimise the animal suffering and this would be further minimised by use of appropriate analgesia protocols. Our previous experience and refinement of models used indicates that NO procedures would be required at the substantial severity level. The complex surgical models would be at the moderate severity level, with appropriate protocols and end points to manage pain and infection.

<table>
<tr><td>PROJECT 4</td><td colspan="3">Antibiotic Treatment for Chronic Back Pain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Antibiotic, back, pain, bacteria, infection</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td colspan="3"><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[8]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">To define the pharmacology and efficacy of intravertebral disc injectable slow release antibiotic formulations to treat bacterial infection of the spinal disc</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="3">In the UK 1.5 – 2 million people suffer from chronic back pain at any one time. It is a significant burden on society, estimated to cost the UK economy annually >£12bn in treatment, lost productivity and disability costs. This project is to develop a novel treatment for chronic back pain that could provide a more effective alternative to long-term use of systemic antibiotic therapy.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td colspan="3">The current plan is to use up to maximum of 150 pigs and 150 sheep to complete this project,</td></tr>
</table>

over what period of time?	
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?	There are two experimental protocols. Both have been categorised as being of moderate severity. Animals may show symptoms of back or neck pain, reluctance to walk/lameness and decreased appetite. Whenever possible, all appropriate means of pain relief will be used to ensure the animals feel no pain, or at least it is kept to the minimum achievable. Changes to the diet may help to stimulate the appetite if reduced food intake is seen. At the end of a study the animals will be killed by schedule 1 and authorised non-schedule 1 methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is a point in biological research when in vitro experiments cannot provide all the necessary conditions to further research. In vitro models can mimic aspects of the pathophysiology of disease. They cannot, however, reproduce the complex interactions between different cells and mediators or reproduce the functional changes that occur as part of the ongoing disease process. Our strategy is, where appropriate, to both replace the use of animals with *in vitro* tests and reduce the numbers of animals used in efficacy testing. The use of ex-vivo spinal disc preparations as the first step in the cascade is an example of where we will reduce the number of animals used, by pre-screening formulations and only selecting the most promising for *in vivo* testing
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prior any *in vivo* studies all formulations will be investigated using *in vitro* and *ex vivo* studies to guide dose and formulation selection. Only the most promising candidates will be selected for *in vivo* testing initially for PK profiling. The formulations with the best PK profile will then be studied for efficacy in the infection model. The availability of in-life imaging techniques allows

	the progression and resolution of the infection to be tracked in each animal, removing the need to kill an animal to quantify the response at each time-point. This reduces the number of animals needed to complete this project.
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 intravertebral disc injection of formulated antibiotics requires a surgical procedure guided by imaging techniques. The small size of rodent spines and their discs stops this species being suitable for the models. Larger animal, e.g. pig and sheep, spines and discs have anatomical and biological similarities to humans and for practical reasons (overall size) are therefore the species of choice for this project to demonstrate the pharmacokinetics, efficacy and pharmacodynamics of the therapy. Clinical observations and imaging of the animals (infection model) will be designed to ensure that harmful effects resulting from the procedure will be detected early. Studies run within the Biological Services Unit are under regular veterinary supervision by the Named Veterinary Surgeon. Any incidental illness or injury will be treated, where possible staying within the constraints of the study protocol, or the animal euthanised.

PROJECT 5	**Muscle loss in chronic disease**	
Key Words (max. 5 words)	Muscle wasting, muscle 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
	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[9]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to identify important molecular pathways which result in muscle wasting and muscle weakness in chronic diseases. This is with a view to developing drugs that can target these pathways to treat muscle wasting and muscle weakness in patients in the future. This will be achieved through the manipulation of genetic material in the muscle of healthy mice, mice undergoing muscle wasting and mice undergoing 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)?	Muscle wasting leads to a decrease in exercise tolerance and a reduction in quality of life for many of these patients. Improving muscle mass and muscle strength in these patients can positively impact on exercise tolerance and quality of life breaking the vicious cycle of disability, inactivity and depression. Increase in muscle mass may also lead to increased survival in patients, since reduced muscle mass predicts reduced survival. The potential benefit is that drugs can be developed to treat muscle wasting and muscle weakness in patients based on targeting the molecular pathways that maintain and regenerate muscle mass. Many chronic diseases result in loss in	

	muscle bulk and structure.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used as they closely mimic the genetic makeup of humans and we have a well-established procedure for all the processes used in our protocols in mice. Electroporation - 5 mice per gene in control and treatment groups, atrophy and regeneration models — 8 mice in control and treatment 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?	Care has been taken to ensure the minimal number of procedures required to generate the results and that all procedures are undertaken under appropriate anaesthesia and pain relief. The expected level of severity is moderate. We have set humane end points for any animal involved in this research project and all animals will be closely monitored for signs of suffering which are: Dexamethasone model of muscle atrophy — SC injection of dexamethasone for 6 consecutive days (AA) — Humane end points: Local injection site infection or bleeding, loss of weight. Regeneration model of muscle injury — IM injection of myotoxin (AB) — Humane end points: Local injection site infection or bleeding, post procedure pain, lameness / hind limb paralysis, complications of anaesthesia Both protocols involve injecting genetic material into the muscle followed by application of an electrical current to the surface of the muscle to allow the genetic material to penetrate into the muscle cells - Humane end points: Local injection site infection or bleeding, pain, lameness / hindlimb paralysis, complications of anaesthesia (e.g. not waking up). At the end of the study, the mice will be killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The most mature muscle cell in vitro is the myotube. The myotube is still a developing cell and is not a myofibre as is seen in adult muscle. Therefore, it is not possible to be certain of the effect of a genetic manipulation on adult muscle by using muscle cells in culture. Furthermore, many of the mechanisms we are testing are not specifically targeted by drugs that can be used in man, and generally it is considered inappropriate to test effect in man before testing the effect in animals. Therefore animal research is necessary for our goals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will test molecular pathways using muscle cells in culture and only test the most promising pathways in animals, to reduce the animal numbers used to the minimum. Also the electroporation model allows control and treatment to be applied to the two legs of one mouse which more than halves the number of mice required if control and treatment were given to separate groups 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.	Mice will be used as they closely mimic the genetic makeup of humans and have similar muscles to humans so we anticipate that any mechanisms that regulate muscle mass in mice can be translated to humans. The atrophy and regeneration models are refined because they produce a consistent and significant, but not devastating, loss of muscle mass and therefore are commonly used in our research field (and by our collaborators who we learnt the procedures from). The electroporation model allows allows control and treatment to be applied to the two legs of one mouse — having an internal control is more accurate than using a control value from a separate mouse that will have different genetic makeup/sliqhtly different environmental factors

PROJECT 6	**Rabbit cartilage defect model**	
Key Words (max. 5 words)	Cartilage, defects, rabbit, cells, scaffolds	
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[10]
• Summarise your project (1-2 sentences)	The project aims to analyse the potential of cells, biomaterial scaffolds and tissue grafts of cells and scaffolds for repair of defects created in the articular cartilage of knee joints of rabbits that are analogous to the defects in osteoarthritic cartilage.	
• Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.	Around 8.5 million individuals in the UK suffer from Osteoarthritis (OA), which is a progressive debilitating joint disease that mostly affects articular cartilage. Articular cartilage is a specialised tissue that covers the ends of bones in joints and, by acting as a shock absorber, enables joints to move freely. Adult articular cartilage has limited self- repair capacity and can be easily damaged due to falls, traumatic sport accidents, previous untreated knee injuries or wear and tear over time as a result of ageing. Currently there are no effective pharmacological agents that promote comprehensive healing of articular cartilage defects. Although a number of surgical interventions are used for functional	

	restoration of articular cartilage defects, no technique has yet been completely successful. It is therefore crucial to repair these defects in the early stages of OA because, if left untreated, the defects grow larger and deeper over time, contribute to progressive articular cartilage degeneration, joint immobilisation, increased pain and ultimately require expensive joint replacement surgery. Tissue engineering is a promising alternative strategy that encompasses the application of cells, scaffold biomaterials, grafts of cells ÷ scaffolds for the repair of tissues lost through trauma or disease. Although initial screening studies to assess the potential of cells! scaffolds! tissue grafts to promote tissue repair can be performed using ex vivo (i.e. in the tissue culture dish in the lab) cartilage defect models, these models cannot faithfully reproduce the complex biological and mechanical environment of the joint that plays an important role in tissue repair in vivo (i.e. inside the body). The consensus opinion in the field of skeletal regenerative medicine is that the rabbit cartilage defect model is an ideal system to analyse the ability of cells, biomaterials, 3-Dtissue grafts for stimulation of repair of articular cartilage defects in the load-bearing environment of the knee joint.
• Outline the general project plan.	A partial (approximately 5 mm diameter, 1-1.5 mm deep) or full thickness (approximately 5 mm diameter, 3-5 mm deep) defect will be created in the articular cartilage covering the lower extremity of the thigh bone in the knee joint of the anaesthetized rabbit, for implantation of cells! scaffolds! grafts of cells ÷ scaffolds into the defects. The defect will then be covered by a flap of tissue referred to as periosteum harvested from the shinbone. Following successful surgery, the rabbits will be allowed free movement and sacrificed at 48 weeks post-surgery for examination of defect repair using established techniques.
• Predicted harms: Give a brief description of the procedures to be	Adverse effects can be expected due to, 1. Creation of the bone defects.

applied to the animals used in this project and describe the expected adverse effects.	2. Implantation of cells! scaffolds! cells + scaffolds into defects — lack of bioconipatibility (though, extremely rare a,s materials will be tested initially in the ex vivo model). 3. Periosteal flap harvest. 4. Wound dehiscence 5. Immunosuppressive agents.
• Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.	It is a clear aspiration that this programme of work will provide a real opportunity to derive a new strategy for cartilage repair involving the application of cells! novel biomaterials! 3- D tissue grafts that can be subsequently used in the clinic for repair of cartilage defects in patients with CA.
• Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.	36 adult rabbits will be used. The rabbit cartilage defect model has been chosen due to consensus opinion in the field that it is an ideal load-bearing large animal model for the realistic assessment of the potential of cells, scaffolds and cell + scaffold grafts to promote cartilage repair. The ability of cells! scaffolds! tissue grafts for repair of cartilage defects will be screened initially using a widely applied ex vivo cartilage defect model. Only those cells! scaffolds! tissue grafts that promote robust repair of the defect in the ex vivo model will be used for the animal work, thereby significantly reducing the number of animals in the study. Further reduction in the use of animals will be achieved by using the in vivo micro CT (computed tomography) machine, which will allow us to monitor repair tissue formation without having to sacrifice the animals at various time points leading up to 48 weeks.
• Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non- animal studies in parallel with the project.	Although the ex vivo cartilage defect model is a good screening tool to select candidates for the animal studies, it cannot faithfully reproduce the complex biological and mechanical environment of the joint, which plays an important role in cartilage repair. Cells, scaffolds, cell + scaffold grafts therefore demonstrate a certain threshold of function in the ex vivo model. The application of the rabbit knee cartilage defect model is crucial to carry out realistic assessment of the potential of cells,

	scaffolds, cell + scaffold grafts (selected from the initial screening exercise) for cartilage repair in a load-bearing environment.
• Explain why the protocols and the way they are carried out should involve the least suffering.	Animal suffering will be minimised by, 1. Administration of analgesic agent to relieve pain and distress 2. Use of aseptic techniques throughout to minimise any risk of infection, which will be closely monitored. 3. If the wounds open, animals will be anaesthetised and wounds aseptically cleaned and reclipped/resutured. If the wounds reopen again then the animals will be euthanised using humane Home Office-approved methods. 4. An appropriate dose of immunosuppressive agent will be administered until the end of the experiment. Any reaction to the immunosuppressive agent will be assessed and, in consultation with the Named Veterinary Surgeon, a different agent will be administered. 5. If adverse effects due to implantation of materials into the defect site are detected, the principal investigator, licence holder and animal technologists will determine whether or not the animal has reached the humane endpoint determined in the licence and therefore should be killed using humane Home Office-approved method. If there is any doubt the Named Veterinary Surgeon will be consulted.

PROJECT 7	**Developing improved understanding and better treatments for bone and joint disease**	
Key Words (max. 5 words)	Arthritis, osteoporosis, fractures, cancer	
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
	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[11]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Diseases of the bone and joints are a major cause of suffering in modern society which affect as many as 40% of people aged 65 years and above. Once significant damage to the bones and joints has occurred the process is difficult or impossible to reverse. Both genetic and environmental factors contribute to bone and joint diseases and over the past ten years, huge advances have been made in identifying the genetic variants that predispose to bone and joint disease and the possible environmental influences that play a role. Despite this, relatively little is known about the mechanisms by which these genes influence the skeleton or the consequences of an abnormal gene. The objectives of this project will be to use animal models to investigate the function of genes that are thought to cause bone and joint disease and to use these models to test out new treatments.	

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 experiments proposed in the project offer the prospect of gaining better understanding of the causes of bone and joint disease. For most of the diseases under investigation, there is either no effective treatment (such as osteoarthritis) or treatments are incompletely effective (osteoporosis, cancer-associated bone disease, inflammatory arthritis). The experiments proposed offer the prospect of developing better and more effective treatments which may benefit humans with these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	The animals used will mostly be mice but some rats may also be used. It is expected that approximately 5000 animals will be used 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?	Some surgical procedures are being undertaken as part of this project, including surgery to induce arthritis and to remove the ovaries or testes to mimic the effects of postmenopausal osteoporosis and osteoporosis in men with hormone deficiehcy. Adverse effects such as problems with wound healing and post-operative pain may be encountered as the result of these procedures. Other experimental procedures will be employed to induce inflammatory arthritis and to mimic the effects of cancer spreading to bone. The arthritis may be associated with pain or lameness. The procedures designed to mimic bone cancer may be associated with pain or paralysis. Controls are in place to minimise and/or deal with any adverse effects that may arise as the result of the procedures. The overall maximal level of severity in this project is moderate. At the end of the procedures 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	This project forms only one part of an overall programme of research that seeks to gain greater understanding of the genetic and environmental determinants of bone disease. The experiments proposed are restricted to instances where the use of animals is essential and the answers sought cannot be obtained by other experimental models that we use including studies of human disease and studies

	from cells and tissues from humans and animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all animal experiments, statistical modelling and power calculations are carried out to determine the appropriate number of animals required to address the scientific question being posed. Where appropriate, facilities are also available to conduct imaging of the skeleton on more than one occasion at different time points to avoid having to use separate groups of animals for different time points, again with the aim of reducing the number of animals required.
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 vast majority of experiments within this project utilise mice which are a validated model with which to investigate normal and abnormal function of bone and joint disease. The experimental models that are proposed have been carefully chosen to represent the optimal and most refined way of addressing the research questions in hand. Some of the experimental techniques proposed (such as surgical interventions) have adverse effects but procedures are in place to monitor animals for any signs of suffering or distress and to deal with this if it occurs.