Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 9

Projects with a primary purpose of: Translational and applied research – Human musculoskeletal disorders

Project Titles and keywords

1. **Use of sex hormones to treat muscle wasting due to old age**
    - Skeletal muscle, Sex hormones, sarcopenia, age

2. **Gene therapy, muscle loss, metabolic diseases**

3. **Articular cartilage repair using mobilised circulating**
    - Cartilage, progenitor

4. **Role of swimming pools gases on spinal development**
    - Scoliosis, Spine, Growth, Deformity, Chlorine

5. **Mechanisms of Bone Growth and Development**
    - Bone, growth, biomineralisation, remodelling, repair

6. **Finding the causes of cartilage cell death during joint infections**
    - Infection, arthritis, prevention

7. **Novel experimental therapies for muscle disorders**
    - Muscle disorders, stem cells, therapy

8. **Novel tendon attachment and repair strategy**
    - Tendon, Biomaterial device, Achilles, rabbit

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

Project 1	**Use of sex hormones to treat muscle wasting due to old age**	
Key Words (max. 5 words)	Skeletal muscle, Sex hormones, sarcopenia, age	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	As humans and rodents age, they suffer a gradual loss •in skeletal muscle mass, strength and the slowing of movement commonly referred to as sarcopenia. Sarcopenia is mainly seen in people >80yrs and mice >2yrs old. It leads to reduced activity, increased susceptibility to falls and eventually to the loss of independent living. In the United Kingdom the number of people in this age group is expected to double from 2.7 million in 2006 to 5.4 million by 2031. Therefore, the percentage of the UK population suffering from sarcopenia and the demands this imposes on the NHS and society in general is expected to rise accordingly. Despite its physical and socioeconomic importance, the causes and mechanisms underlying sarcopenia are still poorly understood. In this study, a mouse model of sarcopenia will be used to investigate the cause(s) of sarcopenia and whether treating old mice with two male sex hormones, testosterone (T) and its active metabolite dihydrotestosterone (DHT), can reverse/arrest the development of sarcopenia.	

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)?	1. The findings from this study will increase our understanding of the effects of dihydrotestosterone (DHT) and testosterone (T), on skeletal muscle mass and function. 2. They will provide insight into the role of these hormones in the transport of the building blocks of muscle (amino acids) into and out of muscle cells as well as in the synthesis of proteins. 3. They will tell us whether the prolonged administration of these hormones and the solution used to dissolve them has any side effects and what these side effects are. 4. They will also tell us whether these hormones can be used to prevent or treat muscle wasting due to old age (sarcopenia). 5. Finding ways of delaying or reversing sarcopenia will enable people and ageing animals to remain mobile and have a better quality of life. It also has the potential of saving the NHS money and bed space. 6. Better understanding of the processes leading to sarcopenia will pave way for other /further studies /human studies that may translate into better care and management of muscle wasting diseases in man and domesticated animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this study. We expect to use approximately 68 mice a 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?	In this study the main procedure will be the treatment of mice with physiological doses of one of two hormones, testosterone and dihydrotestosterone. Small volumes (<2Omllkg)wiI be injected weekly or pellets implanted subcutaneously ,each for up to a maximum of 8 weeks. Therefore the only adverse effect we anticipate is minor irritation at the site of injection. Animals are expected to make a rapid and uneventful recovery from the injection/implantation. Aseptic technique will be used in order to minimise infection and painkillers will be administered as required. Additionally, the mice will be carefully monitored throughout the treatment period. In the unlikely event that an animal experiences unexpected adverse effects or infection occurs, minor

	medical treatment and/or other minor remedial interventions may be carried out, if in the opinion of a veterinary surgeon, such treatment is likely to ameliorate the adverse effects promptly and successfully, otherwise the animal will be killed humanely. At the end of each experiment, the animals will be killed humanely and tissues will be isolated and used to determine the effects of the hormones on their function.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The long term goal of our research is to find a compound that can be used to reverse/slow the development of sarcopenia in humans. To achieve this, we need first to find suitable compounds and secondly, to test these compounds on animal models of sarcopenia. To provide knowledge and data relatively quickly, the model must also have a short lifespan. Mice are ideal for these studies because they have a short lifespan (-36months) and from 2 years onwards, they suffer from a form of a form of sarcopenia similar to that seen in people over 80 years old. We have considered using muscle cells grown in tissue baths. Currently, there are two muscle cell lines available commercially. However, both these cell lines neither age nor suffer from sarcopenia. Consequently, they are not suitable models for use in these studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To keep the numbers of animals to be used as low as possible, statistics and our previous experience with animal experiments were used. Moreover, we will further minimise animal use by maximising the number of samples per animal by using small muscle fibre bundles rather than whole muscles in the study.
3. Refinement Explain the choice of species and why the animal model(s)	To ensure that our results apply to humans, mice that have not been genetically altered will be used. The genetic makeup of these mice is similar to that of human populations. Also, they have a short lifespan

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.	(—36 months) and from 2 years onwards suffer from severe sarcopenia just like humans. Also, they are widely used in biomedical research as mammalian models of humans. Mouse muscles are also similar to those of humans and consist of basically of the same fibre types This makes it easy for us to compare the results from this study with those from our previouè experiments and experiments from other research groups as well as extrapolate the findings to humans.

Project 2: Gene therapy, muscle loss, metabolic diseases

- Summarise your project (1-2 sentences)

This project studies the use of different vectors and formulations for use in gene/cell/pharmaceutical therapies for neuromuscular and metabolic diseases. This will lead to the development of medicines for conditions with unmet clinical needs.

- 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.

Skeletal muscle undergoes a degenerative process in a number of conditions that at best leads to a diminution in the quality of life and at worst are fatal. A large cohort of human diseases have been described in which skeletal muscle is the primary target tissue. These include Duchenne Muscular Dystrophy which affects 1 in 4000 boys and leads to death at about 25 years of age. Skeletal muscle loss is a key feature of many cancers, metabolic conditions and HIV infection. However we all will experience muscle wasting as it is the key feature of ageing. This process, called sacropaenia leads to a reduced quality of life and has huge costs associated with it to the economy. Therefore advances in developing therapies that reverse muscle wasting are likely to be beneficial to a huge number of people.

- Outline the general project plan.

Work in my laboratory is focused on developing gene therapy approaches for serious neuromuscular and metabolic diseases, particularly Duchenne muscular dystrophy (DMD), oculopharyngeal muscular dystrophy (OPMD) and type II diabetes.

1. Breeding and maintenance of colonies of clinically-relevant mouse models of human disease, including those which are genetically modified, and those with naturally-occurring mutations.
2. Development of dystrophin gene augmentation, RNAi and antisense therapies for DMD, based on viral (rAAV, adenovirus, lentivirus, retrovirus), non-viral (oligonucleotide, plasmid, electrotransfer, hydrodynamic and stem cell) vector systems and pharmaceuticals.
3. Development of gene augmentation, RNAi and antisense therapies for OPMD, based on viral (rAAV, adenovirus, lentivirus, retrovirus), non-viral (oligonucleotide, plasmid, electrotransfer, hydrodynamic and stem cell) vector systems and pharmaceuticals.
4. Development of systems to modulation muscle physiology (bulk, strength, stem cell activity) as an adjunct gene augmentation, RNAi and antisense therapy for muscular dystrophies and atrophies, based on viral (rAAV, adenovirus, lentivirus, retrovirus), non-viral (oligonucleotide, plasmid, electrotransfer, hydrodynamic and stem cell) vector systems and pharmaceuticals.
5. Development of gene augmentation, RNAi and antisense therapies for

metabolic conditions e.g. type II diabetes, based on viral (rAAV, adenovirus, lentivirus, retrovirus), non-viral (plasmid, electrotransfer, hydrodynamic and stem cell) vector systems and pharmaceuticals.

6. Testing of plasmid and viral vectors containing elements for their ability to remain episomal and replicate with the cell during muscle and liver regeneration; providing stable and sustained gene expression in the absence of genomic integration.
7. Development of strategies to induce tolerance to neo-antigens.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

No serious adverse events are expected in this project. However, appropriate monitoring will ensure that if any adverse effects are noted that the animal is treated, with advice from the named veterinary surgeon or undergo a schedule 1 procedure if welfare issues dictate such a course of action.

As describe, potential adverse effect could include

Anaesthesia: Underdose or overdosing.

Gene transfer: Pain, gastric irritation and/or diarrhoea, bleeding.

Surgical procedure: Pain, wound dehiscence, infection, bleeding.

Exercise: Pain, exhaustion.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The project aims to develop novel medicines for neuromuscular and metabolic conditions in which loss of muscle is a key component of disease establishment or progression. Such developments will lead to clinical translational programs for conditions with unmet clinical needs. Established links with veterinary and clinical colleagues will facilitate clinical translation of novel therapies.

- 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.

Overall it is anticipated that up to 3000 mice will be used over the course of this 5-year programme of work. However, with constant refinement of procedures, it is probable these numbers may be reduced. We require a mammalian model as the studies need to correlate to the neuromuscular, cardiovascular and immune systems. Mice are one of the lowest species commonly used in experiments of this kind and over the last 50 years a great deal is known about their anatomy, physiology, genetics and behaviour. Animals will only be used in our experiments if

they are in excellent health.

- 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 studies using cell culture systems and isolated tissues can generate much helpful information relating to the responses of individual cells to experimental manipulation, the studies proposed investigate the effects of agents on the integrated systems that control muscle function, the complex physiological underlying cardiovascular function, and the interactions with a complex immune system. Some studies require systems that fully model those whole body symptoms found in muscular dystrophy or cardiac patients such as reduced or altered movement and blood vessel occlusion. Such studies can only be carried out with any meaning in animals. However, complementary studies in cell culture and isolated tissues will be used in the first instance to identify potential agents worthy of investigation in animal models. Furthermore, preparation and characterisation of viral proteins is always done using cell culture systems reducing the number of animals required for this work.

The number of animals used will be chosen based on prior experience in experiments of this kind, upon pilot studies and with an aim to reducing this number but retaining effective statistical power in all cases. We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points. For all the experiments proposed we will use a group size which is the smallest compatible with achieving statistically meaningful and robust results. The principles of refinement, reduction and replacement will be adhered to throughout.

- Explain why the protocols and the way they are carried out should involve the least suffering.

All experimental procedure will be carried out by experienced personnel. The protocols are designed to give maximal data outputs with the least number of animals under the least severity of procedure possible. Such conditions produce clinically relevant translational information for the establishment of human clinical trial regimens.

Project 3	**Articular cartilage repair using mobilised circulating**		
Key Words (max.5 words)	Cartilage, progenitor		
Expected duration of the	2		
Purpose of the project (as in Article 5)	Basic research		No
	Translational and applied research	Yes	
	Regulatory use and routine		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals		No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are seeking to investigate how cells harvested from the blood stream can be best used to treat defects of the joint surface. This is of particular importance for the treatment of arthritis and damage to the joint by trauma.		
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)?	Both humans and animals would potentially benefit from this research. If successful, this research will characterise how best to obtain cells from the circulating blood and how to use them to treat damaged joint surfaces. This damage, at the current time, remains very hard to treat and will lead to osteoarthritis and, in severe cases, necessitate joint replacement.		
What species and approximate numbers of animals do you expect to use over what period of time?	48 sheep over 2 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 planned experiments will cause a mild and temporary lameness in the animals which usually lasts for less than 4 days. Lameness will be treated with pain killers. At the end of the experiment the animals are killed humanely.
Application of the 3Rs	
Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of cells from blood to treat cartilage damage is a new and potentially major advance in therapy. However, it has only been reported in 2 small, poorly designed experiments in man by a private, non-UK organisation. , In order to test whether the treatment works and how it works it is necessary to perform the experiment in an animal model.
Reduction Explain how you will assure the use of minimum numbers of animals	We have used a power calculation based on results of previous related studies to calculate the minimum number of animals that we can use whilst still gaining the information required from the experiment.
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 using sheep as they are well recognised to provide a structurally and biomechanically relevant mode of the human knee. The model used i.e. production of a chondral defect in the distal femur, is a good model for cartilage repair in a biomechanically relevant site. To minimise welfare costs to the animals all surgical procedures will be performed by a veterinary surgeon experienced in this specific experimental procedure. Animals are provided with pain relief after surgery and we use specialised equipment to measure how much the sheep weight bear on the operated limb after surgery. Animals with lameness that is not cured by pain killers within12 hours are killed to prevent suffering.

<table>
<tr><td>Project 4</td><td colspan="3">Role of swimming pools gases on spinal development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Scoliosis – Spine – Growth – Deformity - Chlorine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 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></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></td></tr>
<tr><td>Preservation of species</td><td></td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td></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 investigate whether early exposure to certain agents found in the environment such as that encountered in public swimming pools causes skeletal growth disturbance of the spine</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">Prevention of scoliosis (spinal curvature) in children. If abnormalities are detected, the research could also provide additional insights into the mechanisms involved in growth.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Mice (200 over 5 years)</td></tr>
</table>

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 propose to expose mice to low doses of chemicals found in swimming pools (By reproducing the levels in the ambient air of an indoor heated swimming pool). The doses used will be in line with typical levels found in pools and other than the potential for development of scoliosis no other adverse effects are expected. If a comparable spinal deformity results no pain is expected as spinal curvature in children is not painful**.** Furthermore the spinal curvature will not be expected to impair mobility as it does not do so in humans. However, the animals will be closely monitored for any signs of distress. The severity is expected to be mild. At the end of the procedures the animals will be killed with a Schedule 1 technique (overdose of anaesthesia).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As the development of scoliosis occurs during growth we cannot use cadaver specimens to investigate this aspect of spinal deformity in children. It would not be possible to control the levels of exposure to the different agents to look at the effect on growth in children. Mathematical modeling can be used for certain spinal problems but it is not suitable for this growth study. Cell culture is not suitable for studies of longitudinal growth.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Human epidemiological studies suggest that there is a three fold increased risk of developing scoliosis in children that went swimming in the first year of life. In wild type mice there is an exceedingly low rate of scoliosis. In order to minimize the number we will carry out the study in batches of 30 in case a high rate of scoliosis is found. Scoliosis has previously been produced in wild type mice and rats after surgical procedures. Initially, we will use wild type mice, however, if no effect is seen we will consider using an additional predisposing factor such as genetically modified strains of mice (e.g. C57B16,

	TgPWS).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are vulnerable to scoliosis, as shown by several previous studies. Pups are needed because scoliosis is a disorder of growth, however to avoid distress, the young animals will be kept with their mothers until normal weaning age. In addition, in order to minimise distress the pups will not be placed into water nor removed from their normal housing cages, but the whole cages will be placed into the controlled environment. AIS/lateral spinal asymmetry in children does not cause pain therefore, if scoliosis occurs, we do not expect any mice developing spinal asymmetry to suffer pain.

<table>
<tr><td>Project 5</td><td>Mechanisms of Bone Growth and Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>bone, growth, biomineralisation, remodelling, repair</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</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>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</td><td>Yes ✓</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>This research programme focuses on studying the basic biological control of bone and cartilage development in both health and disease. In particular, it aims to provide an understanding of how genetics regulates bone growth which is significant for both human and animal development.

These fundamental studies have significant implications for the treatment and prevention of human disease, including some increasingly common and significant diseases such as osteoarthritis, osteoporosis, and arterial calcification (hardening of the arteries). In addition there is a less known problem with bone development in children who are either on long term steroid treatments, for example for asthma, or experience</td></tr>
</table>

	inflammatory disease such as Crohn's disease. In these situations their bone growth can be severely affected and this work will investigate the processes involved with the goal of improving growth in these children. Bone disorders also occur naturally in a range of animal species, such as osteoarthritis in horses, cats and dog; bone degeneration can also occur in livestock, such as osteopenia in egg laying hens. Further, companion species, particularly those involved in competitive events (e.g. horse racing), are also affected by trauma and skeletal degeneration, which have financial and more importantly welfare implications. This research programme will increase our understanding of the genes that control bone formation and also help to identify ways to prevent abnormal skeletal formation in animals and man. Our program of work will investigate: • How switching specific genes on or off affect bone growth and development. • The effects of chronic inflammation on bone growth and bone mass • The regulation of energy homeostasis (energy balance) by bone
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 aim of our project is to determine if the genes/proteins that are responsible for healthy skeletal development and controlled energy homeostasis represent unique therapeutic targets for the treatment of diseases of great public health concern, e.g., short stature, osteoarthritis, osteoporosis, and diabetes. Overall, our research aims to improve the quality of life and mobility of people with bone and joint problems, and associated pathologies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 11,000 mice. **Sample sizes** to be used are based on previous work which was used to estimate the minimum number of rodents required for establishing significant differences between groups.
In the context of what you	All procedures detailed here are to be performed in

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?	rodents and do not exceed 'moderate' in severity. The animal models and protocols to be used here have been developed by us and our colleagues over numerous years to investigate how the skeleton interacts with factors in specific areas of the body and across all tissues as well as environmental factors to form a fully functional organ. All experiments will be performed by appropriately trained technicians 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	Fundamental *in vitro* ("test-tube") studies that have used cell and organ culture approaches have been invaluable but are in themselves limiting. As the overall aims of these studies are to mirror what occurs in animals and humans during normal physiological development and chronic disease (e.g. inflammation and osteoarthritis) it is essential to extend these *in vitro* observations to the whole animal. *In vitro* approaches have a number of recognised limitations: • *In vitro* cell cultures do not represent all *in vivo* tissues. • There is a loss of organ interactions between organs; thus indirect effects of agents on bone physiology are not readily detectable *in vitro*. • Many tissues *in vivo* do not change, whereas cell lines are continuously proliferating. • Cell lines often do not provide a reliable model of *in vivo* responses to any kind of biological change. This is often because a cell type in culture loses the characteristics it would have in a live animal. Transgenic Mice Removing or increasing the activity of a gene provides information about what that gene normally does in a physiological (whole body) context. This information cannot be obtained from *in vitro* models where, for example, cell-cell interactions and systemic regulatory pathways are lost. However once information has been obtained from these *in*

	vivo models all efforts will be made to minimise the number of mice used by carrying out well designed *in vitro* experiments using cells from the wild-type and transgenic mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim to reduce the numbers of animals we use. The principals of our experimental design have already been established through our previous work, and that of our close colleagues and collaborators. This therefore allows us to simply undertake the key experiments that will answer our specific questions. Statistical calculations are always used to identify the minimum number of animals that we can use to provide meaningful results. These calculations are based upon our extensive experience in the studies detailed here – for example, we have established in our DSS inflammation model that a minimum of 6 mice per group is required to secure statistically significant differences. In these studies we will also take repeated measures on the same mice. This and other *in vivo* imaging methods will enable serial data acquisition and remove the need for culling multiple groups of mice at set time-points. We have many collaborations with other scientists so maximum use is made of animal tissues across many related projects.
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 on mice. This decision has been made to provide us with the potential to explore the role of specific genes in any biological response we observe, through the use of mutant and transgenic mouse models. We have chosen mice because their basic skeletal biology is very similar to humans and the models to be used here *e.g.* joint loading model, have been developed for, and are widely used in, mice. Furthermore, there is the advantage of available transgenic mouse modes and reagents (antibodies, probes etc). Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. Drugs will be administered at non-toxic

	dosages and if unknown, this will be tested in a carefully graded dose-finding protocol. The alternative strategies which others have used to attain similar end-points frequently involve surgery and our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia.

Project 6	**Finding the causes of cartilage cell death during joint infections**	
Key Words (max. 5 words)	**Infection, arthritis, prevention**	
Expected duration of the project (yrs)	**1 year**	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Throughout our lives, damaging bacteria are trying to get access to the inside of our bodies. The lubricating fluid inside our joints is an ideal environment for bacteria to flourish because of a plentiful supply of food. Bacteria multiply rapidly once they gain entry to a joint and cause a devastating infection called 'septic arthritis,' Approximately 10000 people in the UK develop septic arthritis each year. This is a destructive disease resulting in rapid breakdown of the smooth cartilage surface leading to the development of crippling arthritis in up to half of the affected patients. The most common bacterium causing septic arthritis is *Staphylococcus aureus.* This bacterium releases a range of toxins and some of these can kill cartilage cells. Cells of the body's immune system respond to the infection by unleashing powerful substances that are toxic to the bacteria. However, some of the chemicals released by the body's immune cells are also thought to kill cartilage cells as a side effect. It is not clear whether it is the toxic substances released	

	by the bacteria, substances released by the immune system or both that result in the cartilage destruction.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The goal of our study is to determine each of the factors that cause destruction of the cartilage in septic arthritis caused by *Staphylococcus aureus* including the specific bacterial toxins and any damaging components of the affected individual's immune response. Understanding what causes the damage will allow us to develop methods to prevent the joint, destruction that occurs in septic arthritis and save people who are affected from a lifetime of pain and disability.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice. A maximum of 150 mice will be required for the whole experiment that will be conducted over the course of 1 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 potential adverse effects include the development of severe infection and animals will be monitored and will be humanely killed if there are signs that this is developing. The severity of procedures is moderate. At the end of the experiment the animals will be humanely killed and then their tissues will be harvested for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Owing to the complex interactions of bacteria and the host immune system it is not possible to study the full effects of joint infection without using an animal model. Where possible we have used models using tissues from animals culled for other reasons to identify the bacterial toxins of interest but these models do not take into account the effects of the immune response. It is not possible to study this in human patients for a number of reasons. Firstly the patient population is highly varied with a range of other health problems such as immune deficiency that are likely to affect results. Secondly the time from the infection onset to the study of the joint must be known and this is almost impossible to quantify in human patients. Lastly, this investigation requires samples of cartilage from the joint and taking these from patients would cause arthritis.

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.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are capable of developing spontaneous episodes of joint infection and they also harbour the same types of bacteria as humans on their skin. The response to infection in mice has many similarities to the observed situation in human patients. There is a ready availability of commercial re-agents for the analysis of the immune response in mice. We have refined the anaesthetic and surgical procedures to minimise animal suffering. In conjunction with the animal house technicians and the named veterinary staff we have validated our pain relief protocol by using sensitive techniques. We expect to see very few problems using this model, but still we will closely monitor the animals for general indicators of ill health. Any serious adverse effects such as severe systemic infection would result in immediate humane killing of the animals.

Project 7	**Novel experimental therapies for muscle disorders**	
Key Words (max. 5 words)	Muscle disorders, stem cells, therapy	
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)	Muscle disorders and specifically muscular dystrophies are among the most severe human disorders and no cure is currently available. One of the most severe forms of muscular dystrophy is Duchenne muscular dystrophy (DMD), which is also one of the most common genetic diseases of childhood, resulting in long term disability and ultimately death, usually in the 3rd decade of life. There are approximately ¼ million patients in the world with DMD (not considering all other forms of muscular dystrophies) and 26,000 new cases are discovered every year, each one costing to the society up to $75,000 per year and to the household and family about £40,000 per year. The development of therapies is particularly complex since skeletal muscle is the most abundant human tissue. Therefore the main aim of this project is to develop therapeutic strategies that may enter clinical experimentation and improve life expectancy and quality of life for this large group of patients affected by chronic or acute	

	muscle disorders. Overall, muscle diseases pose major medical, ethical and socioeconomic issues that justify the need for biomedical research.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project is likely to enhance the understanding of muscle disorders. Demonstration of safety and efficacy in animal models of muscle disorders is expected to lead to the development of a possible therapy in patients. Notably, the most severe forms of muscular dystrophies affect children, whose compromised quality of life and need for continuous supportive care carry a severe burden also for their parents, families and for the NHS. Although cell-based therapies are initially expensive, on the long run they would be cheaper than the total costs described above. An example is bone marrow transplantation for blood disorders (e.g., leukaemias and lymphomas), a cell-based procedure routinely done every day in hundreds of hospitals around the world.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rodents will be used. Great care will be taken in order to use the minimal number of mice to obtain statistically significant results. When necessary, consultation with a statistician will be also taken into consideration to optimize experimental design. Strict in vitro validation of the experimental model will be pursued before in vivo testing, such as testing cell differentiation in 2D or 3D cultures. Approximately 1,000 mice will be needed over a 5 years period. A few rats (approximately 50) could also be used.
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 vast majority of the planned experimental procedures described in this project licence are routinely performed in clinical settings and the level of severity will range from mild up to moderate. Animals will be monitored for beneficial effect (e.g., amelioration of dystrophic signs). In the presence of unforeseen complications, adverse events or side effects mice will be promptly humanely culled. In case of successful therapeutic outcome (e.g., amelioration of the muscular dystrophy) the animals will be monitored up to one year of aqe and afterwards they

	will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Even though a substantial part of the experimental work will be based upon pre-screening in vitro of the therapeutic potential of our strategies (e.g., cell vitality, proliferation and differentiation), there are no validated methods to avoid using cell transplantation in animal models to assess engraftment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant part of this project will be dedicated to the development of a human artificial muscle, which, if successful, will provide a unique way to test cell-based therapies in vitro, therefore significantly reducing the overall number of animals to test a cellular medicinal product.
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.	Several rodent models of muscular dystrophy are available, providing a unique opportunity for scientists to model and test possible therapeutic strategies to counteract disease progression. Specific statistical tests, such as Power Analysis and/or Resource Equation will be utilised to calculate sample size and optimize it in order to use the minimum number of animals possible. Frequent (daily) monitoring, use of national and international guidelines, defined humane endpoints (e.g. culling upon early detection of tumours or other clinical signs of severe distress) are just some examples of the measures that will be taken to further minimise welfare costs to animals.

Project 8	**Novel tendon attachment and repair strategy**	
Key Words (max. 5 words)	Tendon, Biomaterial device, Achilles, rabbit	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to find a better way to improve repair after tendon injuries in man. Specifically we wish to discover how a novel implantable device for tendon repair alters (degrades) with time when when paced into a tendon. Further we wish to identify how safe the implantable device is by determining whether it provokes a greater reaction by the body than stitching material which is currently used to repair tendon injuries in man.	
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)?	Traumatic tendon transections as a consequence of laceration are common in the hand and wrist region. Surgical repair of these lacerations are common, but frequently people end up with some disability due to failure of repair or inappropriate formation of scar tissue. We have developed a novel implantable device which we think will improve outcomes in people with such injuries. Prototypes of this device have been tested in-vitro and in-vivo (mice and rats), with studies showing strong promise for benefit in its use. We now need to test the actual device which will go into human patients prior to first in human use, and the rabbit is the smallest animal which we can use the human prototype device in (rats and mice are too small).	
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbit, up to 50 adults, 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 Rabbits will suffer from some short term pain due to surgical placement of the device or suture which will be of moderate severity and will be minimised by the use of pain controlling medicines. The animals will be subject humanely killed at the end of the experiment
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project is the culmination of 8 years previous work in which the Implantable device has been developed and tested extensively both using non-animal alternative methods and in mice and rats. It is now necessary to test the fully developed implant for human use in an animal model of an appropriate size in which the implant can be placed in the same manner in which it would be used in a human tendon. This necessitates the use of a larger animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed preliminary studies both in the laboratory, and in rats and mice and have data to support our sample size requirements. The type of studies being performed have been performed previously for other devices, and the sample sizes proposed are similar to those used commonly elsewhere. We will review the numbers of animals to be used in consultation with a statistician after the initial experiments to ensure that the minimum number needed to achive the objectives 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 rabbit has to be used, as it is the smallest appropriate model in which the prototype implantable device can be placed into a tendon in the manner in which human tendon repair would be necessary. Previous work has been undertaken with a similar device in both mice and rats; however for regulatory purposes, we need to use the device as it would be used in man. The studies done previously in rats and mice used a smaller device, which was not placed in the tendon in the manner we will have to use in humans. This was because mice/rat tendons are too small. A rabbit Achilles tendon is an appropriate size, hence this work requires the use of this animal. The studies will be undertaken by a highly experienced specialist orthopaedic veterinary surgeon who has performed many tendon repairs. Analgesia will be used peri and post-operatively to decrease surgical pain. Antibiotics and surgical asepsis will decrease risk of surgical site infection.

	We have practiced and developed the surgical technique in cadaver rabbits to ensure we can undertake this procedure appropriately, safely and with the minimum suffering.

Project 9	**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	
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.