NON-TECHNICAL SUMMARY (NTS)

Project Title	Tendon homeostasis, injury and repair
Key Words	tendon, repair, progenitor cell, inflammation
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Tendon injury is common in both athletes and the general populations. Injured tendon heals poorly and is prone to re-injury. Currently, there are no effective treatments for tendon injury; this is largely because we do not understand what happens within the tendon during the early stages of injury or how the cells resident within the tendon respond to injury. We know that there are several different cell populations within tendon; in the

project we will establish how these different cells maintain healthy tendon structure and respond to injury. We will also determine the role of microorganisms (e.g. bacteria) on tendon inflammation and healing.

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

Understanding the roles of specific cells within tendon in health and with injury will provide information that will allow the development of more effective treatments for tendon injury, targeted at developing methods to stimulate the resident tendon cells to fully repair the tendon after injury and resolve chronic inflammation. Not only with this benefit humans, but findings will also be of benefit to animals that suffer from naturally occurring tendon injuries, such as horses and dogs.

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

We will use rats and mice in all the planned experiments. We anticipate we will use approximately 600 rats and 100 mice over 5 years. In preliminary work to develop and refine techniques, we will use rat cadavers obtained as waste material from other unrelated experiments, which will reduce the number of rats used. All experiments will be carefully planned and we will perform calculations to ensure that we use the minimum number of animals required to obtain statistical significance. Animal numbers will be further reduced by using one hind limb as a control, and performing experiments on the other hind limb.

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

We will use a minimally invasive procedure to label proteins within the tendon by injecting heavy labelled water into the abdominal cavity and mixing into the rats' drinking water. This substance is non-toxic and has no side effects, and therefore this is classed as a mild procedure. After varying periods of time, we will cull the rats and analyse the tendons to determine the rate at which proteins within the tendon are being synthesised and degraded. We will develop 2 models of tendon injury – one will be induced by creating a small wound in the tendon with a needle, and the other will be induced by loading the hindlimb while the rat is anaesthetised. Both these procedures are moderate – they involve anaesthesia and may result in short term pain which will be reduced by giving the animals painkillers before and after surgery. All animals will be culled at the end of the experiments so we can analyse their tendons within the laboratory to assess how the cells within the tendon respond to loading and where within the tendon injury initiates. We also intend to suppress the immune system in some of the rats so we can perform a bone marrow transplant prior to tendon injury to assess how bone marrow stem cells respond to tendon injury. For the majority of rats, this is classed as a moderate procedure, however if the bone marrow transplant fails there is a high risk that the rats will die. This is expected to occur in less than 10% of rats, such that the overall severity limit for this procedure is classed as severe. We will also perform experiments using germ-free mice to establish the effect of microorganisms on the development and progression of needle-induced tendon injury. This is classed as a moderate procedure, as germ-free conditions can affect the intestines, which may result in dehydration and diarrhoea, therefore these mice will be monitored closely and will be culled if showing signs of distress.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Developing new treatments for tendon injury is currently limited by a very poor understanding of the roles of the cell populations within tendon during health and disease.

Little is known about the initiation of tendon injury as samples from naturally occurring injuries in humans and other species can only be obtained in the late stages of injury.

In our previous experiments, performed without the use of live animals, we have started to understand what happens when tendon injury starts. However, these studies have raised important questions that need to be answered before we can develop effect treatments for tendon injuries. These questions can only be answered by performing experiments on animals, as current models of tendon injury in the laboratory are not able to replicate the complex structure and loading environment within tendon, and are not suitable for longer term experiments. The experiments we have planned will allow us to identify which cells are responsible for repairing the tendon. We can then isolate these cells and perform experiments on them in the laboratory which will allow us to develop methods to activate these cells and promote tendon repair.

Moreover, it is not possible to determine the role of the microbiome in tendinopathy in other models or other animals, as currently only mice are available as animals raised in a germ-free environment.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We always aim to reduce the number of animals we use. We have performed calculations to ensure that we use the minimum number of animals required to obtain statistical significance in each experiment, and will use randomisation and blinding approaches to reduce any bias. Animal numbers will be further reduced by using one hind limb as a control, and performing experiments on the other hind limb. We will source rodent cadavers from other unrelated experiments to use in pilot experiments to refine techniques, reducing the number of animals that will be culled for this project.

Refinement

Explain the choice of animals 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.

Refinement

The mouse and rat are well-established models for investigating tendon injuries and the effect of mechanical loading. We have chosen a needle-injury model, as this creates a small, highly reproducible injury which will not result in excessive pain or lameness. We will also induce tendon damage by loading the hind limb under anaesthesia. This allows us to apply well defined and controlled loading protocols, leading to similar levels of damage between animals, decreasing the variability and increasing the sensitivity of the experiments.

Animal suffering will be limited in our studies by our strict monitoring of actual severity and severity limits. Our protocols are also designed not to produce excessive trauma or suffering, and painkillers will be administered before, during and after any procedure that is expected to caused pain. Animals will be killed if they approach the limit of severity.