Date of second strand review: 26 March 2014

Project title	**Regulation of inflammation in wound repair**		
Key words	Chronic wounds, inflammation, diabetes		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine production		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	Yes	
Objectives of the project	Tissue repair and regeneration require dramatic and coordinated changes in cell behaviour in both wound-resident cells at the site of injury and in distant cells that respond to and are recruited to the injured tissue. In the last decade, the influence of inflammatory cells on wound healing has been shown to be highly significant, as they can function to promote or inhibit wound healing. Discovering the underlying mechanisms controlling the behaviour of inflammatory cells is key to controlling these cells for therapeutic benefit. It is often very instructive to compare normal processes with diseased processes in order to understand how that process is regulated. Diabetic patients and animal models have severely impaired wound healing and often develop chronic wounds. By comparing factors in diabetic wounds with normal wounds, we can begin to understand what is important for efficient wound healing and how to promote impaired wound healing. Inflammatory cells from diabetic patients and animal models are dysfunctional and inhibit wound healing. However, this process is poorly understood and the key mediators that control these cells are not known. Many pro-inflammatory factors are over-expressed in diabetic chronic wounds compared to normal wounds, but whether they are causative or a consequence of the dysfunctional inflammatory cells is not known.		

	Our objectives are to (1) determine differences in how genes are controlled between diabetic and healthy inflammatory cells, (2) identify the key factors controlling these genes (3) test whether we can alter these factors and reverse the dysregulation of inflammatory cells in diabetic wounds. The results of this study will be important in future therapeutic development.
Potential benefits likely to derive from this project	The results of this project are intended to: 1. Identify the underlying molecular mechanisms that contribute to dysregulated inflammation. 2. Contribute to scientific knowledge related to chronic wounds. 3. Identify potential new therapeutic strategies to promote healing in diabetic humans and animals. The potential benefits of this study include the development of potential gene and cell based therapies to aid patients with chronic wounds and reduce the need for limb amputation. In addition, this study would benefit animals with diabetes, particularly pet dogs and cats, which like human patients, develop complications associated with this condition.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Over a 5 year period: 4,500 mice (approximately 2,500 for breeding purposes and 2,000 for experimental procedures) 800 rats (approximately 400 for breeding purposes and 400 for experimental procedures)
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	This study is designed to understand how inflammation is controlled in a normal wound environment and what might be different in a diabetic wound environment. Anaesthetised mice (non-diabetic and diabetic) will receive small (10 mm diameter or less) wounds on their skin so that we can compare the processes involved in wound healing in diabetic mice with the normal situation. We will apply different factors to the wounds that we believe will enhance wound healing, including growth factors and stem cells, in order to find the best treatment for diabetic wounds. After surgery, mice will be provided with pain relief and monitored closely for any signs of distress. Distress in mice after this type of surgery is very rare, however, if there is any indication of suffering we will seek veterinary advice. In some studies we may need to exchange bone marrow from one mouse/rat to another mouse/rat in order to determine the effects of the diabetic

	environment on how bone marrow cells develop and behave. To do this we condition a recipient with a dose of radiation that will allow for the donor's bone marrow to replace the original. The animals do not feel anything during the radiation treatment and they are given the replacement bone marrow right away following their treatment so they should not feel any ill effects. Four to six weeks later we will be able to track their bone marrow cells using blood sampling and biopsies. In addition, we are developing methods of live imaging in order to reduce the number of animals needed for each study. Strategies to minimise adverse effects due to our treatment, as well as minimise the number of animals needed for these studies include testing the effects of the factors we are putting on the wounds in cell culture first. In this way we will be able to identify the most promising candidate factors without using animals. This will reduce the chances of inducing an adverse effect, and reduce the number of animals needed to accomplish the objective. Animals will be humanely killed at the end of each study.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	We have to use animals in this study because understanding how inflammatory cells interact with wound healing in a diabetic environment must be studied in the complete physiological setting in order to get an accurate picture of this process. Mice and rats are the least sensitive animals that accurately mimic the disease in humans. We use mouse and rat models of Type I and Type II diabetes combined with transgenic animals that expresses green fluorescent protein in all of their cells. We can put these cells into wounds and find them again because of the green fluorescent protein marker. This allows us to keep track of how they might be interacting with the wound to enhance healing. We cannot use humans for these experiments because we would not be able to modify their genes nor track the cells from the bone marrow.
2. Reduction How the use of minimum numbers of animals will be assured	By reading the scientific literature, we will avoid repeating anything that has already been done. By consulting with colleagues that have expertise in our area, we will refine our experimental design. By conducting experiments in cell culture (in vitro) first, we will identify many of the factors that may regulate inflammatory cells. We will also test potential therapeutic treatments in cell culture models of wounds first. To plan for our animal work, we have consulted a statistician to establish the minimum number

	of animals required for each study. Also, where possible, we will use two wounds per animal to reduce the number of animals required.
3. Refinement Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	The species and models we have chosen are based on how well they mimic diabetes in humans, their sensitivity (they are the least sensitive models we can use for our study), how well-characterised they are, and our expertise. The animals will be given anaesthesia so they will not feel anything when they undergo wounding. They will also be given pain killers so when they wake up they will not have any discomfort. They will be watched closely to make sure they do not show any signs of being in pain or becoming ill. If they appear to be in pain or appear unwell, veterinary advice will be sought.