30 July 2015

Project title	Models of tissue repair, reconstruction and regeneration of the limb		
Key words	Injury, limb, repair, reconstruction, regeneration		
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	We aim to study the biology of inflammation in different types of injury to the soft tissues in the limbs of animals. This will allow us to understand how the soft tissues heal and whether we can make them heal better. The injury types will be very similar to injuries seen in the clinical setting. The repair methods will simulate the surgical procedures used. Other methods will also be used based on reconstructive surgery techniques to develop vascularized tissues that can be used to heal major trauma wounds. All the procedures have been optimized to be of minimal disturbance to the animals studied. The findings of the studies will be used to develop better therapies and surgeries to these common clinical situations resulting from trauma.		
Potential benefits likely to derive from this project	We see these injuries and perform elements of these repairs and reconstructions on a day to day basis in the clinical setting. We lack a true understanding of the healing process that occurs between all the tissues which means that humans rarely get a perfect outcome. We have chosen to recreate the simplest component of each injury we see. This allows us to assess the biological interactions between the different tissues and study the different cells that are involved. We have used this approach in previous projects which have gone from basic lab research all the way		

	through to clinical trials. We will use our well-defined transgenic models to simulate injury, repair and reconstruction of damaged tissue in the limb. This will aim to discover new novel therapies, which ultimately could be beneficial to man. Our main focus is to develop our reconstruction model that uses a new method to vascularize and promote new tissue formation. This is used as a vehicle to support cell therapies or biomaterials as a realistic platform to heal big wounds like pressure sores, cancer wounds or trauma wounds. We will have a portfolio of clinically relevant models to help us tackle the problems that arise from minor injury and severe injury to the limbs.
Species and approximate numbers of animals expected to be used, and anticipated period of time	We plan to use mice and rats as our model systems to study the effect of injury. We will use mice for their genetic alterations which will allow us to look at specific cell types and molecular pathways of interest. The use of rats provides us with a larger model that can be used to study different biomaterials, which may be too large to study in the mouse. We have a limit of operating on 1200 rats, 1200 mice and 1200 genetically modified animals on the licence. We also have the aim of breeding genetically modified mice of around 2000 for this project. From previous licence activity this is a realistic limit over 5 years.
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	Breeding: Animals bred on the license will be expected to have either none, or mildly abnormal characteristics which do not affect animal survival. If animals have a visible disability they will not be used further for the surgical component of the study. Bone marrow transplant in mice: Mice will be used that have had bone marrow transplants to study the contribution of bone marrow cells to tissue formation or wound healing. These mice may have diarrhoea or failure to thrive in a small percentage of animals (from experience 5-10%). If this occurs animals will be humanely killed. The generation of these mice will be of mild or moderate severity. Only after full recovery from reconstitution will mice be used for surgery. Having conducted these surgeries over a number of years we have had very few adverse problems because we have ensured the surgery is of the highest standard. The severity rating for the procedures is moderate although some procedures have minimal physiological insult. Tissue for specific assessment is all acquired by schedule 1

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