July 2016

Project title	**Extracellular matrix in development and disease**	
Key words (max. 5 words)	Extracellular matrix; body clock, connective tissue; cartilage; osteoarthritis	
Expected duration of the project (years)	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[1]
Objectives of the project	i) To understand the function of various components of the extracellular matrix (ECM, the supporting scaffold material for cells), and their daily rhythmic regulation, in its early development; ii) To determine the mechanisms by which mutations in these matrix and body clock (circadian rhythm) genes result in specific diseases, including osteoarthritis; iii) To test potential novel therapies using the mouse models generated in our programme.	

[1] At least one additional purpose must be selected with this option.

Potential benefits likely to derive from this project	Advancement of biological knowledge This work will help understand how this molecular supporting scaffold is formed and maintained on a daily basis. This information is of relevance and interest to the whole field of extracellular matrix research. In addition, the knowledge gained underpins our understanding of changes in the connective tissue in disease. Defining disease mechanism and identifying novel treatment opportunities Osteoarthritis is common, affecting 60% of people over the age of 65. Chondrodysplasias (the malformation of the cartilage cell) is caused by mutations in ECM genes. These are relatively rare. There are currently no effective treatments for chondrodysplasias and osteoarthritis and the only options for patients with these conditions are joint replacement and analgesia. There is an association between these two conditions which affect cartilage cells (chondrocytes) and various forms of abnormalities, such as the endoplasmic reticulum stress (ER stress) and the body clock system. The importance of these findings is that they highlight new potential avenues for the treatment of these conditions.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Mice. In total, 10,000 mice are expected to be generated and used over 5 years.

Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	Protocol 1: *Breeding and maintenance of genetically altered mice-* Severity level: Mild *Chondrodysplastic strains have a mild dwarfism and animals do not exhibit obvious pain or discomfort.* *We do not anticipate any adverse effects as a consequence of removing relevant genes.* *A small number of animals will be observed up to 18 months. We do not anticipate any significant age-related side effects in mice up to that age.* Protocol 2: *Treatment of chondrodysplasia or osteoarthritis-Severity level: Moderate* *Those animals that undergo anaesthesia on several occasions will only be re-anaesthetised when they have fully recovered from the previous anaesthetic.* *Protocol 3: Induction and treatment of surgically induced osteoarthritis-Severity level: Moderate* *Mice will be humanly killed for detailed studies.*

Application of the 3Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used	We are studying the complex biological process by which long bones grow and by which the articular cartilage subsequently can become degraded in osteoarthritis. We make extensive use of cell culture models. However, the holistic process of bone growth and osteoarthritis cannot be modelled using in vitro techniques. We are now at the stage of directly determining whether endoplasmic reticulum (ER) stress and body clock disruption are pathogenic factors in the causation of osteoarthritis and for these studies, there is no alternative but the in vivo model.
2. Reduction How the use of minimum numbers of animals will be assured	Some of the measures are essentially qualitative. Others are quantitative, requiring statistical analysis. For qualitative experiments, defined breedings (where all the progeny have the desired genotype) are employed to reduce numbers of animals produced with unwanted genotypes. The number of observations will be the minimum necessary to provide an adequate description. For quantitative aspects of the programme, age and sex-matched animals are used. For quantitative assessments of disease severity, a blinded assessment of outcome will be performed. Where feasible, the animals are littermates to reduce variation. Clearly, the exact numbers of animals required will vary with the particular experimental design, and the estimate of the variation, and so on.
3. Refinement Reasons for the choice of species and why the animal model(s) to be uses are the most refined, having regard to the objectives. General measures that will be taken to minimise welfare costs (harms) to the animals.	Mice are the ideal model for these studies. Mice have a skeletal system that develops in a similar fashion to humans; they develop osteoarthritis similar to that seen in humans in response to joint destabilisation in a relatively short (8 week) time frame. Thus we have been able to produce genetic mouse models of specific human chondrodysplasias and osteoarthritis.