G. NON TECHNICAL SUMMARY (NTS)

Project title: Chemistry and Biology of Novel Bone Graft Substitute Materials
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: YES
   (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: NO

Keywords:
Osteoporosis, Bone graft substitute, Ovariectomy

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
The main objective of this study is to use a rat model to study the effectiveness of a new bone graft substitute (BGS). BGS are natural or man-made materials that are used to replace bone in patients with diseased or damaged bone. The project license will focus on osteoporosis, a disease that is characterised by bone loss in women due to oestrogen deficiency following the menopause. The bone loss that is seen in osteoporosis substantially increases the risk of fracture in these women. Using a rat model of bone loss following surgical removal of the ovaries (ovariectomy), we will determine whether new BGS materials can be used to replace the bone that is lost in osteoporosis.

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 data from this study will support an application for regulatory approval for human clinical trials of a new bone graft substitute for use in women with bone loss and skeletal fragility caused by post-menopausal osteoporosis. Although the BGS materials to be tested have been shown to be effective in patients with normal bone, their effectiveness when used in diseased bone is untested. One of the key questions that regulators will need to see answered is how the material behaves in osteoporotic bone. It would be extremely difficult to secure ethics approval to undertake a clinical trial to inject AGN1 (or other BGS) in patients with osteoporosis because the injection procedure in humans will require a
surgical procedure; without some evidence of efficacy in osteoporotic bone, it is very unlikely that ethical review board approval could be secured. This rat study will form the foundation of the preclinical data that will be needed to confirm efficacy in osteoporotic bone, paving the way for subsequent human clinical trials. Additionally, since most women with osteoporosis are now treated with drugs called bisphosphonates that partially block bone loss, it is important that our rat work includes a comparison between the effects of the BGS materials in untreated osteoporotic bone and in bone that has been treated with bisphosphonates.

What types and approximate numbers of animals do you expect to use and over what period of time?:
The first study under this license will evaluate a calcium sulphate bone graft material, and this work will require 108 skeletally mature female rats over the next two years. Additional studies, using other bone graft substitutes alone or in combination with cells, are anticipated in the future. Some of these experiments may focus on combining bone-forming cells with the BGS materials; others will make use of stem cells that will be collected from the patient, grown in the laboratory (to increase the number of cells available for transplantation) and then mixed with BGS and implanted in the bone defect in the rat. These additional studies are under planning and can only be initiated when additional grant funding has been secured. Each subsequent study on a BGS material is expected to require approximately 100 rats and we anticipate testing at least three additional variants of BGS (or GBS plus cells) during the 5-year lifetime of this project license. In total, we would expect to use a total of up to 400 rats under this license.

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?:
This study will use surgically prepared female rats that have undergone surgical removal of both ovaries (ovariectomy, or OVX), performed by the commercial supplier of the animals. 4 to 6 weeks later, when animals have fully recovered from surgery, the rats will be delivered to our institution, where they will begin weekly treatment with subcutaneous doses of either alendronate (a drug used to treat osteoporosis in women) or a placebo (saline). 12 weeks after OVX, when the bone loss has been fully established, rats will undergo surgery to create drill hole defects in the bottom end of the left and right femurs. The defects will be filled with the new BGS material and the animals followed for times ranging from 2 hours up to 6 weeks. At each time point, groups of animals from each treatment group will be killed and tissue samples collected for analysis of (a) the chemical changes in the BGS over time, and (b) the bone response to the BGS over time. In some animals, bone markers (fluorescent labels that bind to bone, providing a way of quantifying new bone formation in the living animal) will be injected into the animal in order to allow quantitative assessment of bone turnover through histological evaluation of bone specimens collected at the time of euthanasia. These labels are well tolerated by animals and will allow us to determine how much new bone forms in and around the BGS materials. The most significant potential adverse effects are expected to be post-operative pain, lameness and an increased risk of fracture due to the creation of bone defects. Post-operative pain and lameness would be considered moderate severity so drugs that will provide pain relief will be administered. Fractures are a potential complication of any surgery involving bone, but the risk will be minimised through the use of careful technique. If a fracture occurs during surgery when the animal is anaesthetised, the animal will be killed without being allowed to recover consciousness.

Application of the 3Rs
Replacement:
We make use of non-animal alternatives wherever possible, including cell culture models for studying the effects of bone graft substitutes on isolated bone cells, as well as for evaluating the effects of simulated body fluids on the chemistry of the biomaterial. However, none of these laboratory systems recapitulates the interactions between the implant, bone, bone marrow and immune system that

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develop in the living animal.

**Reduction:**
This is a pilot study to determine the time course of changes in implant chemistry and bone microstructure following implantation of bone graft substitute materials. Sample numbers are based on best available data from the literature. Within each experiment, we maximise data collection from individual animals by using serial non-invasive imaging and blood tests, allowing us to obtain a number of data readings from the same animal rather than using one animal per measurement, thus reducing the number of animals overall.

**Refinement:**
The rat is recommended by the Food and Drug Administration, the governmental agency that regulates the approval of new drugs and treatments in people in the United States as the preferred preclinical model for studying therapies for osteoporosis. The focus of this study is to better define early changes in implant chemistry and relate them to the bone response around the implant. The surgery involves the lower part of the thigh bone and from previous work in rats we expect that animals will tolerate the procedure well. Pain relief will be provided to all animals during and after surgery and thus any pain effects are minimised as far as possible. The study is also limited to 6 weeks since this is the time period during which we anticipate seeing the most scientifically informative changes in chemistry and biology in and around the implanted material.