Home Office

NON-TECHNICAL SUMMARY

# Evaluation of Novel Strategies for Bone Repair

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
    - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

**Key words**

*No answer provided*

| Animal types | Life stages |
| --- | --- |
| Rats | adult |
| Rabbits | adult |

# Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

# Objectives and benefits

**Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.**

**What's the aim of this project?**

This project will determine the safety and effectiveness of different natural and synthetic materials, alone and in combination with cells and/or adjunct treatments, that might be used to enhance the healing of bone.

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

Millions of patients each year develop problems secondary to incomplete healing of fractures caused by trauma or disease (e.g. cancer, osteoporosis). Fracture complications negatively affect the patient's quality of life, with prolonged hospitalisation, potentially crippling disability and, in extreme cases, infection and a risk that the limb may be lost. The average healthcare cost for managing a non-healing fracture has been estimated to range from £17,000 to £79,000 per patient.

There are a number of reasons why fractures fail to heal normally. First, the amount of bone lost as a result of the original trauma or disease may be too great, exceeding the ability of the skeleton to repair itself. The second major reason is that the patient's bone may not be healthy – this is common in elderly patients and in those taking certain medications. Another important reason for fractures failing to heal is that the bone may be infected, either at the time of the original injury or at the time of surgery.

Whatever the underlying cause, there are currently two main ways to replace bone and stimulate healing in patients with these problematic fractures. The first relies on bone that is removed from the patient him/herself, then placed into the site that is not healing. The use of the patient's own bone – so called "autograft" – is relatively easy from the surgeon's perspective – the bone is immediately available (it is usually harvested from the pelvis of the patient) and since it comes from the same patient, there is no risk of disease transmission or of adverse immune responses to the graft ("rejection"). However from the patient perspective autograft bone suffers from some important limitations. For instance collection of the bone graft requires a second incision, which causes additional discomfort for the patient following surgery. Additionally, there is a physical limit to how much bone can be harvested from the pelvis, especially in children and in smaller adults. Finally, the quality of the bone that is collected can be quite variable, especially if the patients are being treated with chronic doses of medications such as steroids, which are known to reduce the biological properties of autograft bone.

As an alternative to using the patient's own bone, the surgeon can elect to use bone that is harvested from cadavers. This bone – known as allograft – is typically obtained through commercial tissue banks. While quite plentiful, allograft can be very expensive to buy. Additionally, because it comes from a different person, there is potential for transmission of infectious diseases such as HIV, Hepatitis B and Hepatitis C. Donors are screened for these diseases, but a small risk is always present.

There is tremendous interest in the development of safe, effective and reasonably-priced alternatives to human tissue for enhancing bone repair following bone loss secondary to trauma or disease. Given the concerns over both availability and potential disease transmission from natural human bone products, there is a necessary drive to develop alternatives to human bone, including the use of non-human (xenograft) bone or bone derivatives, synthetic biomaterials, or combinations of natural and synthetic materials.

We and other groups are actively working to develop these so-called bone graft replacements. In most cases, these are based around either chemically processed forms of native bone, synthetic forms of bone mineral (typically derivatives of calcium phosphate), or some form of natural or man-made polymer (common examples include collagen, poly-lactic acid (PLA) or poly-caprolactone (PCL)).

Our lab has focused in particular on the development of a chemically processed form of bovine bone that might be suitable as a building block for making a bone graft replacement. We elected to focus on bovine bone because of its widespread availability (via the food chain) and established history as an implantable biomaterial (including both soft tissues such as heart valves, and as bone and tendon grafts). Bovine bone has similar macrostructure and microstructure to human bone (more comparable than is seen in sheep and goats, for example) and displays Haversian (osteonal) remodeling. It also has excellent material/mechanical properties that are well aligned with those of native human bone. By combining the processed bone with a resorbable polymer, we will be able to create a bioresorbable patient-specific implant with mechanical properties that are specifically tuned to meet the demands of the site in which it is implanted. The material is amenable to being fabricated into complex structures through the use of 3D printing techniques. Laboratory testing of the processed bone has shown that it is well tolerated by bone cells, and that it its mechanical properties are similar to those of native bone. In order to explore its potential effectiveness in healing bone, we now propose a series of animal studies that will establish whether (a) there is any evidence of toxicity or adverse tissue response when the material is implanted in animals, and (b) whether 3D printed cylinders of the material can serve as a framework, or scaffold, for bone repair. It is critically important that any new candidate material performs at least as well as currently approved products in vivo - otherwise there is no value to the new material.

This project license describes a series of rodent and rabbit studies that will allow for robust, systematic screening of a range of candidate materials (biomaterials) as scaffolds for bone repair. In addition to testing the safety and effectiveness of these biomaterials as stand-alone scaffolds, we will also establish whether it is possible to further enhance bone healing by adding stem cells to the material prior to implantation, or by using post-operative treatment with biophysical stimuli such as shockwave therapy to see if this further enhances the reparative capacity of the scaffold-cell combination.

**What outputs do you think you will see at the end of this project?**

We expect to publish a minimum of 2-3 scientific papers reporting the results from the work under this license. These papers will describe the development of a new type of bone repair material. At the same time, positive results from these animal studies would likely lead to interest in the development of a commercial product for use in the veterinary clinic (for repairing fractures in cats, dogs and horses). With time, there may then be interest from the human market for a product that could be used in orthopaedics, dental surgery and possibly spine surgery.

**Who or what will benefit from these outputs, and how?**

We anticipate direct benefit to veterinary and human clinical patients who require surgical management of bone defects. As part of the work, we will validate both the rat and rabbit models for bone defect studies and our findings (and subsequent publications) should be helpful to other researchers who might be considering similar work in the future.

**How will you look to maximise the outputs of this work?**

We are working to establish collaborations within our institution, as well as with collaborators at other scientific centres around the world. These collaborations will benefit from the data developed under this project license. The animal models described in this project license will be published and we would expect this to lead to more general adoption of these models in the future.

**Species and numbers of animals expected to be used**

- Rats: 120
- Rabbits: 324

# Predicted harms

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**Explain why you are using these types of animals and your choice of life stages.**

Rats and rabbits are widely recognised as appropriate preclinical screening models for determining the tissue response to orthopaedic, dental and spinal implants. Regulatory bodies that oversee the testing and approval of medical devices recommend the rat and rabbit as appropriate models. The rat model involves a relatively small bone defect and is therefore ideal for screening a lot of different possible materials. The rabbit model involves a much larger and more clinically relevant bone defect that is better suited to providing more detailed information about the materials that perform best in the rat model. We use skeletally mature animals because most of the patients that we see with complex fractures or tumours are adults.

**Typically, what will be done to an animal used in your project?**

For the surgical models, the common procedures will be anaesthesia, surgical implantation of one or more biomaterials, then follow-up for periods of up to 6 months. For the subcutaneous model in rats, small cylinders of the test material will be placed under the skin. For the rabbit model, a bone defect will first be created by removing a 15-mm length of bone from the femur; this defect will be replaced with a cylinder made from one of the test materials, and the bone will be stabilised with a metal orthopaedic plate and screws. At intervals after the surgery, chemical dyes that mark sites of active bone formation will be injected into the animals so that we can measure bone healing. In some animals, additional treatments (including the use of shockwave treatment to stimulate bone repair) will also be evaluated. None of the animals will have more than one surgery to implant the materials, and for the bone defect models only one leg will be operated.

**What are the expected impacts and/or adverse effects for the animals during your project?**

We expect to see some weight loss as a consequence of interruptions to normal feeding patterns. For animals undergoing orthopaedic surgical procedures, it is very likely that there will be some degree of lameness secondary to the bone surgery – this is what we see in clinical patients undergoing orthopaedic surgery. We will use clinically approved pain-relieving medication to minimise pain and discomfort after surgery. The risk of infection - also ever present in surgical patients - will be reduced by the use of aseptic technique, sterile instrumentation and appropriate wound monitoring and topical wound care. It is not our routine clinical practice to use antibiotics for elective orthopaedic surgery, so antibiotics will not be used routinely in rats and rabbits on this project.

**Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per animal type)?**

All of the rats on this license are expected to fall under the moderate category as they will all undergo surgery. The rabbit procedures are also considered moderate severity. That said, we anticipate that the overall impact of surgery will be less for rats than for rabbits - the implants in rats are placed in the tissues immediately under the skin, while those for the rabbits are placed directly into bone. We do not expect to see lameness in rats where we will see this in the rabbits.

**What will happen to animals at the end of this project?**

- Killed

# Replacement

**State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.**

**Why do you need to use animals to achieve the aim of your project?**

Animals are needed to explore the specifics of the tissue responses that happen following implantation of a biomaterial. Biomaterials are natural or synthetic materials that are designed to replace or augment an organ, or a bodily function, or to stimulate natural tissue repair. For this project, we are focusing on biomaterials that will improve the body's ability to make new bone. All biomaterials that are intended for clinical use in human or veterinary patients must first undergo animal testing to ensure that they are safe and effective. The material that we have developed is novel and has not been tested previously. As such, there is no way to secure approval for clinical use without screening the material in an animal model.

**Which non-animal alternatives did you consider for use in this project?**

The preliminary work underpinning this project has been performed exclusively using cells growing in the lab. The biomaterials that we have identified as being potentially useful must first be shown to be safe for normal cells. We then go on to test the mechanical properties of the different biomaterials to ensure that they are strong enough to survive in a bone defect environment. None of this preliminary work involves the use of animals, and only biomaterials that pass all of these early tests are considered for animal testing under this project license.

**Why were they not suitable?**

Although cell culture and lab-based models can be very helpful in exploring the effects of different compositions on biological and material properties, they do not and cannot address fundamental questions regarding the interactions between the material and the tissues in which it is implanted. For example, bleeding at the surgical site may interfere with the integration of the material into bone, while immune reactions stimulated by foreign cells or proteins may lead to inflammation that could compromise new bone formation and integration. Additionally, the rate at which the polymeric (plastic) component of a biomaterial is degraded cannot be determined without testing in animals – if the material disappears too soon the mechanical integrity of the structure may be compromised, while if it too slow it may adversely affect new bone formation. The use of clinically relevant animal models allows us to perform temporal studies that can address these interactions and compare the performance of the new material (in its different compositions) against that of materials that are already in clinical use. Current options for bone replacement in animals and humans exist but all have limitations in terms of cost, availability and flexibility when used in different applications. The materials that we are exploring offer substantial advantages in terms of being cost-effective, safe and adaptable to the patient's unique anatomy (through the use of 3D printing based off CT or MRI scans).

# Reduction

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

**How have you estimated the numbers of animals you will use?**

We initially estimated the numbers from a review of previously published data from the rat and rabbit models, which indicate sample sizes of anywhere from N=8 specimens per group (for standard rat subcutaneous models for assessing tissue response to biomaterials) to 10-15 specimens per group (for rabbit bone defect models in which biomaterials are placed into long bones).

**What steps did you take during the experimental design phase to reduce the number of animals being used in this project?**

We undertook a number of steps ahead of submitting this project application, including:

1. Detailed literature review to look for alternatives and best practices to reduce overall animal numbers

2. Consultation with NC3Rs Experimental Design Assistant to ensure that we are maximising data collection from every animal

3. Sequential design of experiments so that treatments that are found not to be successful in rats are eliminated from further consideration and do not go into rabbits.

**What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?**

Steps to optimise animal use include:

1. Use of shared control groups across the different phases of testing.

2. Use of non-invasive imaging (such as X-ray and CT scans) and non-destructive tests that allow specimens to undergo mechanical testing and still remain sufficiently intact to be useful for biochemical or histological studies. By doing more than one set of measurements on the same specimen, we are better able to make direct comparisons between outcome measures.

3. Use of injectable chemical dyes (fluorochromes) to measure bone formation at different time points in the same animal. These dyes stain the bone and provide a time stamp - by looking at the pattern of this staining (with a microscope) after the animal is dead, we can retrace the history of bone healing in the animal...rather like examining tree rings and establishing periods of relative drought and relative plenty. Multiple labels can be injected in each animal, allowing us to compare healing patterns at different time points, without the need to kill animals at individual time points.

4. Compliance with PREPARE and ARRIVE guidelines related to the design, conduct and reporting of animal studies.

# Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

We will first start by establishing that the combination materials (composites) are safe (non-toxic) when implanted in subcutaneous sites in rats. The subcutaneous model, with implantation on the dorsum of the rat, is well tolerated, with transient pain/distress that is similar to that seen with repair of a simple surgical incision. Material and material-cell combinations that demonstrate acceptable safety will then go on to testing in a clinically relevant bone defect model in rabbits.

The rabbit bone defect model provides a clinically challenging environment for bone repair. The instrumentation used to stabilise the femur is strong enough to protect the bone even if the candidate

material is ineffective at supporting new bone growth, so we do not expect to see a significantly higher rate of complications in animals that are implanted with biomaterials that prove not to be effective.The alternative to the rabbit would have been the sheep - this would have offered the opportunity for an even larger bone defect, but we felt that this fact alone was insufficient to justify moving up to this species at this time. If the candidate materials are especially promising and we are able to commercialise the material, regulatory bodies may subsequently ask for sheep data, at which time we would submit an amendment to this license.

**Why can't you use animals that are less sentient?**

We cannot assess acute or chronic tissue reactions to implanted biomaterials in anything other than a live- animal model. Cadavers and terminal surgery are helpful in optimising surgical technique but can never replace the need for live animals in work of this type.

**How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?**

We leverage the skills of our technical and animal care teams to ensure effective monitoring of animals from both a behavioural and a welfare perspective. Pain-relieving medications are given routinely after invasive or potentially painful procedures, and validated pain scoring/behavioural scoring is used to confirm that pain relief is sufficient. Daily direct observations of the animal, and routine monitoring of body weight and food intake are used to ensure the welfare of animals in our care. When we can, we will train animals to allow for oral (or in-food) administration of pain-relieving medication. We have used shockwave therapy in clinical veterinary patients (dogs and cats) for over 2 years without any need for sedation, and we anticipate that the procedure will be well tolerated in rabbits without sedation. However, to ensure minimal stress we will use sedation or short-acting inhalation anaesthesia in this preclinical study. We use non-invasive and non-painful techniques for imaging, and minimise the number of interventions needed to secure a scientifically valid answer.

All animals will be group housed in order to ensure social interaction and normal behaviours (e.g. play and social grooming). Species-appropriate environmental enrichment will be provided (e.g. Cheerios and plastic shelters for rats; plastic balls for rabbits to play with).

**What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?**

All of our experimental work is designed and performed in accordance with established best practices. These vary from the use of the LASA Guide, NC3Rs guidance, development of surgical standard operating procedures based on publications, use of validated pain scoring schemes, and the design and reporting of studies using PREPARE and ARRIVE guidelines, respectively.

**How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?**

I receive regular updates on developments in the application of the 3Rs through institutional communications and mailings from international animal care organisations such as the Association for

Assessment and Accreditation of Laboratory Care International (AAALAC International). My lab is committed to using best practices in our work and would certainly institute advances in, for example, pain scoring and rabbit analgesia if demonstrated during the course of this project.