Home Office

NON-TECHNICAL SUMMARY

Skeletal Tissue Engineering

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Skeletal, Tissue engineering, Biomaterials, Stem cells, Repair

Animal types	Life stages
Mice	adult
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?

The aims of the project are to use tissue engineering to develop the best conditions for cell and tissue growth on biomaterials to repair a broken or diseased skeleton. Our rationale is centred on the urgent need to develop materials capable of activating and growing both blood vessels and bone to help patients repair their damaged and diseased skeletal tissue.

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?

With an increasing ageing population, skeletal tissue loss due to injury or disease is rapidly growing. This significantly impacts the quality of life for the patient over time and has socio-economic costs for healthcare providers. For example, each year in the UK there are over 50,000 primary hip replacement operations at a cost in excess of £350million. Hence, for reconstructive bone surgery and fracture repair, the need to develop better techniques and alternative bone therapies is vitally important.

From a patient's perspective, the ultimate goal is to repair and replace their damaged skeleton with bone material, harvested with minimal complications from their own skeleton. However, the current clinical approach for the treatment of bone defects and non-unions is bone grafting or metal implants, which both have significant drawbacks. The combination of a bone stimulating material combined with bone tissue generating cells to produce a therapy would be an invaluable surgical option, reducing both complication rates occurring in patients undergoing bone replacement surgery. Therefore, generating materials that can help bones repair themselves will be invaluable for patients and animals suffering from diseases and fractures affecting the skeleton. These include non-healing bone fractures and weakened bones due to diseases like osteoporosis and bone cancers.

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

The proposed models being used in these studies will help us to gain new insights in the development of tissue regenerating biomaterials to rebuild broken bones

Specifically.

- We want to use cutting-edge technology to figure out how biomaterials stimulate bone cells and tissue to grow and repair, and then use these imaging and stimulating devices to direct biomaterial formulations to the exact location of the bone fracture.

- We want to create new biomaterial constructs that can stimulate bone regeneration so that we can provide new and better treatments for people who have fractured bones or bone lost due to other diseases such as cancer.

- We will present our findings at scientific and medical conferences and we will publish these outcomes in peer-reviewed scientific journals.
- Finally, any new procedures/methodologies improved welfare settings developed throughout the period of the project license we will aim to publish and share among the scientific community to benefit both human and animal patients.

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

As with all our research goals the aim is to develop improved therapeutic strategies and products for patients who suffer from skeletal diseases and bone trauma injuries. We envisage that these project protocols and novel biomaterials have the potential to provide new treatments to help rebuild bones and skeletal tissues. In addition, the findings from musculoskeletal research can be applied to the veterinary field due to similarities in orthopaedic conditions between people and animals, leading to novel applications in a ‘one health’ approach.

Beneficiaries will include:

- Patients and animals suffering from broken bones and skeletal diseases.
- Healthcare providers.
- UK, EU and worldwide tissue engineering/biotechnology companies involved in tissue regeneration, stem cell biology or developing innovative tissue scaffold technologies,
- the academic community in the generation of new protocols and avenues for skeletal tissue regenerative research.

Many materials we use in our studies are biocompatible and currently used in clinical practice for other applications. Modifying them to enhance their properties can result in new therapies in a relatively short time frame, benefitting patients due to the known track record of efficacy and safety. However, with the complex materials proposed the final outcomes for patients may require months to years of investigation due to thorough testing to ensure safety and efficacy prior to use in the clinic.

When these new treatments become available, we envisage growing the tissue constructs in the laboratory and transferring the regenerated samples to the patient in theatre. Ultimately, we believe this work will be translated to the clinic and benefit patients within the National Health Service and the wider medical community in the area of musculoskeletal repair. Alternatively, the development of materials that exploit the regenerative potential of the patient's own repairing cells would minimise costs and hasten the material's therapeutic implementation.

In addition, the information from this project, including the study protocols and techniques will be made freely available via publication in peer-reviewed journals, in order to benefit patients, other researchers, doctors, vets, and pharmaceutical companies who are involved in the development and assessment of novel therapeutics which target the repair and regeneration of skeletal tissues due to disease and injury.

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

Training & Collaborations

The team undertaking the research will acquire skills and expertise in bone repair, blood vessel and skeletal stem cell biology, biomaterial development, imaging techniques in real-time such as 3D x-ray scanning and 3D optical imaging. Training will be achieved through an extensive local network of multidisciplinary collaborations within the University, including: Clinical Orthopaedics, Biomedical Research Facility, Biomedical Imaging Unit and the Histochemistry Research Unit, and established national and international collaborations in regenerative medicine.

In addition, the protocols and research work will be communicated to our Student & Postdoctoral Interactive Network to provide new students and postdoctoral researchers information regarding the models available for research projects in our University and to foster collaborative projects. We are part of the national and international Regenerative Medicine collaborative group networks where we share our methods and results from acellular materials that will be available to be tested in skeletal and other tissue/organ regenerative models.

Education & Public engagement

The information and findings generated from this project will be presented through the scientific community by presentations at national and international scientific research meetings. We will also communicate findings to the public through outreach activities such as the University Science Day, and laboratory open days for GCSE and A-level students in tissue regenerative medicine.

Progress of the study and results will be regularly presented at ongoing teaching events and public lectures. The press office of the University will also publicise the results.

Species and numbers of animals expected to be used

- Mice: 660
- Rats: 120
- Rabbits: 60

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.

Whilst all attempts are made to reduce the use of animals by using in vitro methodology (in vitro experiments on human and mouse skeletal stem cells, explant studies and organotypic models), it is inevitable in work of this nature that in vivo investigations are required to be undertaken. The animal models detailed are critical to facilitate in vivo proof of concept and efficacy in an relevant biological system, not possible in humans. Adult mice and rats will be used in these investigations because they are the most reproducible and well characterised animals for (1) skeletal tissue regenerative models and (2) bone fracture models.

Bone disease and fractures may occur across the lifespan, ranging from young to old, however, it is predominantly in the adult and aged populations that the repair mechanisms in bone start to fail. Therefore, it is expected that the vast majority of experiments will be performed in adult animals.

One of the biggest hurdles to overcome in clinical orthopaedic repair is the regeneration of large segments of bone that have been lost due to disease or trauma. We will therefore use rabbits at the final stages of these studies as this provides a larger bone defect model to test the potential of our repair materials to regenerate large amounts of bone tissue.

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

Scenario 1 (50%)

- Various biomaterials, cells or substances will be either injected and assessed for localisation to the bone or will be injected/implanted under the skin of mice to assess that the agent is retained at the injection/implant site and is able to stimulate new bone formation followed by recovery. As it takes some time to make new bone the experiments will last between 6-12 weeks. Because we are seeking to localise biomaterials, cells, substances to bone locations and in particular where there is a bone deficiency, the injection studies may be shorter (1-7 days). We will assess the rate of bone formation by 3D X-ray scanning at various timepoints over the course of the study under anaesthesia. Optical imaging will also help us to pinpoint the exact location of the injected biomaterial in the bone.

Scenario 2 (30%)

- Mice and rats under anaesthesia will have a portion of bone (limb-long bone) removed and biomaterials, or cells are implanted in its place during surgery or delivered by injection locally or systemically, followed by recovery. The overall limb stability will be maintained with fixation pins. This experiment will be run for 8-12 weeks as the biomaterial implant has to build the bone and then it needs to be shaped by the rodent cells to fit exactly into the broken bone site. We will assess how well this repair is going by 3D X-ray scanning, optical imaging, under anaesthesia at vary timepoints over the course of the study.

Scenario 3 (15%)

- Mice and rats will have a section of bone (skull) removed and biomaterials and/or cells implanted in its place during surgery, or injected locally or systemically followed by recovery. This model is used as the skull bone develops and repairs differently from long bone fractures. This experiment will be run for 8-12 weeks as the biomaterial implant has to build the bone and then it needs to be shaped by the rodent cells to fit exactly into the broken bone. We will assess how well this repair is going by 3D X-ray scanning at varying timepoints over the course of the study.

Scenario 4 (5%)

- Rabbits through a surgical procedure will have section of bone (forearm) removed and biomaterials implanted in its place followed by recovery. This experiment will be run for 8-12 weeks as the biomaterial implant has to build the bone and then it needs to be shaped by the rabbit cells to fit exactly into the broken bone. We will assess how well this repair is going by 3D

X-ray scanning/optical imaging, under anaesthesia at vary timepoints over the course of the study.

In addition to the above scenarios

1. the animals may receive an injection of bone inducing factors to localise to the bone defects to enhance the repair.

2. The animals may be subjected to a scanning device, such as ultrasound, in order to improve the implanted biomaterials' ability to promote bone healing under anaesthesia. We will use ultrasound because it is routinely used in the clinic and can activate certain biomaterials by pressure waves to release bone repairing factors (locally at a bone fracture site) without any surgical interventions for the patient.

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

In 2 of the protocols the animals will exhibit transient mild pain. In the other 3 protocols (the bone fracture models) animals will experience moderate short term pain due to the procedure. With the limb defects there will be a 12-24 hour period where the mice will limit load bearing on that limb. Normal movement and locomotion resumes thereafter. Mortality rate through these procedures is very rare and will be less than 1%.

Although the animals will be regularly monitored, weight loss has not been an issue with these procedures.

X-ray scanning. There is the risk that radiation doses during a micro-CT X-ray examination may produce adverse effects such as delayed growth or repair. However, doses of x-rays and duration of scans are way below any threshold that will cause problems to the animal. These scans are usually in the duration of 2- 5 minutes.

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)?

The expected severities are moderate as the animals will be undergoing a surgical procedure.

In the skin implant model, the severity can be mild as only an injection of biomaterial is added under the skin. For larger biomaterial implants the animals will be anesthetised and a skin incision made, implant inserted under the skin and sutured. Animals will be given pain relief post-surgery. Therefore, this procedure will have a moderate severity. We envisage about 60% will be moderate and about 40% will be mild.

For the bone defect models in the other protocols, we estimate the severity to be moderate and that 100% of the animal will experience this due to the surgical procedure. Again, these animals will be given pain relief post-surgery.

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?

Initial bone tissue engineering research work and biocompatibility will utilise all relevant in vitro methods at our disposal. However, the clinical relevance of the approaches proposed, and the quality and quantity of bone tissue formed can only be assessed by in vivo studies in relevant animal models. In vitro approaches cannot, to date, mimic the complex physiological in vivo microenvironment involved in tissue repair. Thus, this proposal necessitates the use, in part, of animals to test the ability to generate new bone using the bone tissue engineering principles outlined. It is important to stress that initial in vitro studies are undertaken to evaluate and optimise the growth of human bone cells and skeletal stem cell populations on the scaffolds under examination. Where possible we will utilise our organotypic/organ culture in vitro models as a replacement to in vivo models to examine the ability of our regenerative strategies to repair skeletal defects. However, to address the efficacy of biomaterials in skeletal repair that require an understanding of its effects on integration, inflammation and blood vessel formation, then in vivo skeletal models will need to be used.

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

Using laboratory cell culture experiments we are gaining as much information as possible to understand the functions and toxicities of new biomaterials being developed. In addition, we are using 3 dimensional bone tissue known as organoids to study the interaction of the many cell types that create fully functional bone tissue. We are using this data to create biomaterials with functioning coatings which can mimic the bone growth processes.

In other studies conducted in our group Artificial Intelligence (AI) modelling is being developed to test biomaterials implanted into the developing chick membrane, so that this technique can be used to predict and determine how a new material will act in producing blood vessels and new bone tissue. However, there are limitations at the moment as this AI modelling is based on data provided from real studies. Once enough data has been accumulated for AI, this can, in principle, be applied to the animal models used in these protocols when researching new biomaterials. As AI modelling obtains a better understanding of the outcomes and mechanisms of these materials in the aforementioned bone models, the number of animals required will eventually decrease. With continual learning of the models and how new therapies interact in repairing and rebuilding bone AI in the future will be one avenue to replace the use of animal models.

Previous human studies. There is a wealth of clinical literature providing data on the efficacy of biomaterials on implants in hip replacements and bone fracture repair and in bone fracture that will

provide us with guidelines of what works in the patient. In addition, there are number of clinical trials which have data on the dosing of growth factors and of skeletal stem cells which give insights into the positive outcomes and the negative outcomes. We'll use this information to improve our biomaterials and avoid the negative consequences that have already been discovered in these clinical trials.

Why were they not suitable?

The above strategies are suitable and we do use them to screen, in part, the many biomaterials that are developed. We only resort to animal studies due to the complex factors that are involved in regeneration and repairing of bones. The generation of new bone requires multiple steps and the interplay of many different bone factors (inflammation, skeletal stem cells, blood vessel cells, collagen structures, calcium and phosphate (for hardening the new tissue), mechanical stimulation and cells that re-shape the new bone in a 3D environment to fit the defect bone exactly (without scarring) in a co-ordinated manner.

This interaction cannot be consistently or correctly reproduced under tissue culture conditions to meet the criteria expected to inform a clinical translation. In addition, in vitro model systems make it difficult to detect unexpected toxicities and observe the integration and degradation of materials over time.

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 have used our current and past experimental data to inform us of an estimation of the number of animals required per group and the number of control and test groups to give statistically valid experiments.

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

In the design phase we have used organ culture and chick eggs to reduce intra-group variability of biomaterials and by using this model and the results obtained has allowed us to reduce progressive experiments in mice models. This screening has reduced approximately 60% the regenerative biomaterials being taken forward for testing in animal studies.

We have designed experiments using the fewest animals consistent with obtaining statistically valid results as determined from our power calculations. For example, multiple small implants, up to six, can be assessed in one animal. This significantly reduces the number of animals per group required in these studies by 50%. Using the NC3R's Experimental Design Assistant we have calculated the

minimum number of animals required for a determined amount of new bone tissue formation in the test implant material comparison to control (no implants) or control (implanted biomaterials without stimulatory factors). With refined techniques and multiple imaging of the same animal over time, we can confidently obtain significant result outcomes with 4 animals per group.

In certain cases, mathematical modelling may be used to predict the release of growth factors from biomaterials under certain biological conditions, allowing us to determine how many animals we can exclude while still achieving a meaningful outcome in the experiments. In addition, multiple scanning and imaging of the animal bone implant or bone defect site at different time points reduces the number of animals required in these type of studies.

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

Rigorous laboratory testing will be performed on the newly constructed biomaterials before any candidates will be put forward for the skin implant experiments. In Stop/go scenario (Fig.1) only positive biomaterials in these protocols will advance to the bone defect models in the mice and rats. Furthermore, the best candidates in the rodent bone repair animals will be scaled up and used in a larger animal model.

Multiple analysis will be undertaken on biomaterial samples implanted into the animals, this will include X-ray and microscope imaging, blood vessel scoring, biochemical and molecular analysis, and histology. The multiple data that can be derived from one sample will optimise and minimise the number of animals to be used in a single study. In addition, real time optical/microscope imaging will significantly reduce the number of time points required to assess bone formation within the implanted biomaterial. This will further reduce the number of animals required for one study involving multiple time point assessments.

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.

Mice will be used in the majority of the investigations in this project license as they are the best characterised and standardised species appropriate for this research. The microinjection model and the subcutaneous implant model will allow for the assessment of the localisation of the biomaterials to the skeletal site and new bone formation of the implanted samples respectively. This procedure will cause the least pain, suffering and lasting harm to the animals compared to the bone defect model protocols. However, this is a non-skeletal site, therefore, to properly establish the efficacy of these

materials, we must implant them into a bone location and see if they can combine and rebuild the bone to its former state.

Technically the methods refined from previous animal licenses are;

- Surgery is performed early in the week allowing for more detailed observations post-surgery.
- Staggered surgery procedures throughout the week so that there is plenty of pre and post-surgical time to assess the welfare of the animals undergoing the surgical procedures and also to have the optimal time for the preparation of the biomaterials to implant.
- Pre & post analgesia given for the bone defect surgery and implantation.
- In the first few days after surgery, tunnels, housing in the cages will be removed to reduce the potential risk of affecting the healing at the surgical site of the animal. Also, non tangling nesting materials will be used in the initial days after surgery.
- Food will be placed on the bedding/floor of the cages initially in the first couple of days after surgery in the limb defect models to minimise increased load bearing and rotation on the operated femur.
- With the calvarial defect model soft food will be administered as eating hard food pellets could cause some pain and discomfort in the cranial defect site

Finally, to address the clinical problem of large areas of bone loss failing to mend, the rabbit model is a well-characterized model for evaluating large biomaterials to repair this bone deficiency, allowing for the possibility, if successful, of transferring these therapies to the clinic.

Why can’t you use animals that are less sentient?

The development of new bone is incredibly complex involving factors and cells from the blood, the bone marrow, inflammatory cells, minerals and mechanical forces. In addition, bone fractures takes a considerable time to successfully repair.

Unfortunately, we are still not at a stage to incorporate all these components in the laboratory therefore these animal models are required to help us understand and improve the development of materials to aid in bone growth and repair. We can mimic some of components in the laboratory, and this has led us to refine the materials and cells that we will be used in these studies.

There is a chick egg embryo model that we use to determine the efficacy of these biomaterials in generating bone. This model is a less-invasive in vivo model allowing for the assessment and function of these biomaterials in a living organism. However, these are short term models (max. 10 days) whereas bone repair can take months to repair in vivo. In about 90% of our studies we use this model to test the biocompatibility and efficacy of new materials, gels, cells, growth factors before consideration in using in these in mice/rat models. Only clinically approved materials will be considered to be used directly in the mice/rat studies.

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

We shall continue working to minimize welfare costs for the animals in the following ways:

- Reviewing the need for these experiments to be undertaken. Weighing up the balance of potential successful outcome of undertaking the experiment and the level of harm/distress the animal will endure.
- We will ask for feedback from technicians and welfare officers in the Biomedical Research Facility and their valuable day-to-day knowledge to revise protocols to improve the animals' experience.
- Prior to the surgical procedures, researchers will familiarise and handle the animals on a regular basis to reduce stress for both the animal and the user.
- General best practise guidance for injection, blood sampling and aseptic techniques will be followed.
- Animals are closely monitored for several days after implantation under the skin or after the bone defect surgery, with continued pain relief given over 72 hrs in the first instance and further pain relief administered if signs of pain persists in the animal. Wounds will be carefully monitored to ensure that sutures have not loosened or come off or there are any signs of infection.
- Animal weight is frequently monitored for the duration of the studies.

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

We will follow the ARRIVE guidelines which provide a 20-part checklist of the minimum information required to be reported by groups using animals in research. ARRIVE guidelines are essential to help overcome issues in science such as reproducibility, reducing bias and the correct use of statistical methods of analysis.

In addition, we will follow and consult the Norecopa (Norway's National Consensus Platform for the advancement of "the 3 Rs" (Replacement, Reduction, Refinement)) in connection with animal experiments database platform and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for better science experiments using animals to ensure that we are using the best defined models for our work in the investigations of biomaterials for skeletal biology.

All surgeries will follow the LASA (2010) guiding principles for preparing for and undertaking aseptic surgery. The standard operating procedures (SOPs) and risk assessments for these surgical procedures will be evaluated annually and amended in accordance with published updated aseptic surgical practises and guidelines.

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

We will consult the NC3Rs website to identify any changes that will be relevant to the workings of this model. In addition, we will follow the latest findings from the Laboratory Animal Science Association (LASA) and the PREPARE guidelines from Norecopa in better planning for research involving animals to prepare for better science and advance the 3Rs. Any changes will be implemented directly through the experimental design, and if necessary, through a project licence amendment.

In addition, we follow the latest publications on using these models and identify any new methods that reduce, replace or refine the skin implant and bone defect models. If applicable to this model in improving the 3R's we will request amendments from the Home Office to adjust the techniques/methods required and training or notification of relevant staff in updated techniques.

Continual training will be undertaken to ensure that users will be proficient in running these studies particularly on the aftercare post- surgery.

Attendance at University animal users meetings and/or discussion of University animal users meeting slides at research group lab meetings.

We will liaise with other experienced groups in in vivo research both nationally and internationally who use these models to get advice on updates on best practise for anaesthesia/analgesia, husbandry and surgical procedures similar to the ones we will be undertaking in this project.