Home Office

NON-TECHNICAL SUMMARY

Modulation of Wound Healing in Small Animals

Project duration

5 years 0 months

Project purpose

- (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
- (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 following aims mentioned in paragraph (b)

Key words

Wound Healing, Acute, Chronic, Therapies, Efficacy

Animal types	Life stages
Mice	adult, aged
Rats	adult, aged
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 aim of this project is to assist in the development of new therapies to: - minimise blood loss and disfigurement after traumatic injury, and promote or otherwise improve the healing of acute and chronic wounds.

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?

Acute traumatic injury to the skin and underlying tissues (due to accident or elective surgery) is extremely common, effecting millions of people each year. The control of blood loss immediately after injury and the subsequent development of disfiguring scars are key issues following such acute trauma. The NHS spends ~£8.3billion annually on treating wounds, ~£5.6billion of which is spent on chronic wounds (venous leg ulcers, pressure areas and diabetic foot ulcers). These debilitating and slow healing wounds preferentially afflict (and severely impact) the elderly. Reflecting the progressively aging population their prevalence is rapidly increasing, year on year. Slow-healing (or otherwise defective) wounds are a significant burden to patients and their Quality of Life, their families, the NHS, and the economy. Existing treatments are largely ineffective – new, effective therapies are urgently required.

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

Based on past experience (gained from work undertaken under the authority of previous project licences), it is anticipated that work undertaken on this project will assist in the development and clinical uptake of new, more effective, therapies able to: - prevent excessive blood loss and scarring after acute traumatic injury, and accelerate or otherwise improve the healing of acute and chronic wounds.

While our work is invariably commercially sensitive and undertaken under confidentiality agreements, we have and will publish our findings in respected peer-reviewed journals - wherever possible.

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

The process of new product development, testing and receipt of approval for clinical use is very time consuming - and as such, it is unlikely that any of the therapies tested in this project will achieve approval for clinical use within the lifetime of this licence.

That being said, three developmental therapies tested over the past 5 years (under our previous Project Licence) are now nearing approval for clinical use.

In the long-term, it is hoped and expected that patients with problem wounds or individuals that display abnormal responses to injury will be the principal beneficiaries of our work under this licence.

The development of new more effective wound healing therapies, that accelerate or otherwise improve wound healing, would also be expected to reduce costs incurred by the NHS, and increase the profitability of those companies engaged in the development of these therapies.

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

We will maximise the value of our outputs by dissemination through a variety of means. Subject to approval from study sponsors, we will endeavour to present our findings at scientific wound healing meetings and publish as much information as possible from studies conducted under this Project License.

Species and numbers of animals expected to be used

- Mice: 2500
- Rabbits: 150
- Rats: 500

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.

Adult (and aged) mice, and adult rats and rabbits will be used in this project as they have repeatedly been shown to be effective screens to assess the impact of a wide range of therapies on the wound healing process.

As it would be unreasonable and unacceptable to fully recreate the extreme conditions and complex pathologies of human chronic wounds in an animal, models have been established that exhibit delayed healing due to one or other of the pathologies associated with chronic wounds in man. Studies involving the use of adult and aged diabetic animals, specifically the spontaneously diabetic 'db/db' mouse (which display characteristics similar to those of Type II diabetics - including delayed wound healing) are particularly widespread and considered clinically relevant. Similarly, the surgically-induced rabbit ischaemic ear wound healing model is widely accepted as a clinically-relevant impaired healing model.

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

Prior to any procedures, animals will be acclimatised to their new environment for a period of between 7 and 10 days - during which they will be largely left undisturbed other than to replenish their food and water provisions and to refresh their bedding materials.

Under general anaesthesia, the fur at the planned wound/implantation site will then be removed by clipping and/or depilatory cream and the skin cleaned and disinfected.

The subsequent 'typical experience' of animals will depend on the species of animal and the protocol being followed.

For mice and rats undergoing wound healing protocols

Standardised (incisional or excisional) wounds will be created under general anaesthesia and aseptic conditions. Animals will be given appropriate pain-relieving drugs and antibiotics to minimise post-surgical discomfort prior to recovery. Wounds will usually be dressed to prevent contamination with soiled bedding. Animals will then be allowed to recover from anaesthesia under warmed conditions. Animals will be re-anaesthetised at various time points after injury (typically every 2 to 3 days for non-diabetic mice and rats, and every 3 to 7 days for diabetic mice) to permit photography, wound site assessments and re-application of topical treatments. Pain-relieving drugs and antibiotics will usually be given by injection under the skin on each occasion. These studies will typically run for up to 14 days (20 days in diabetic mice as they heal slower) after injury - though longer-term studies examining the effect of treatments on long-term parameters (such as scarring) may be performed. On conclusion of the study, animals will be humanely killed and tissues harvested for histological investigation (or other analysis).

Test or control treatments may be applied topically to the surface of wounds or to the animal as a whole (systemically). Topical treatments will usually be applied immediately after injury and subsequently re-applied at each dressing change (i.e., every 2 to 3 days for non-diabetic mice and rats, and every 3 to 7 days for diabetic mice). Systemic treatments will usually be applied to restrained fully-conscious animals by oral dosing or injection under the skin on a daily (or less frequent) basis for the duration of the study.

For rabbits undergoing wound healing protocols

Under anaesthesia and aseptic conditions, the vessels supplying blood to one of the rabbits ears will be tied- off to reduce blood flow and thereby delay wound healing. Biopsy wounds will then be created in both ears. Test or control treatments will then be applied to the biopsy wounds, and dressings applied to prevent contamination with soiled bedding. Animals will be given appropriate pain-relieving drugs and antibiotics by injection under the skin prior to recovery. Animals will then be allowed to recover from anaesthesia under warmed conditions. Animals will be re-anaesthetised at various time points after injury (typically every 3 to 5 days) to permit photography, wound site assessments and re-application of treatments and protective dressings. Pain-relieving drugs and antibiotics will typically be given on each occasion. These studies will typically run for 15 to 20 days after injury. Test or control treatments will usually be applied topically, immediately after injury and subsequently re-applied at each dressing change (i.e. every 3 to 5 days). On conclusion of the study, animals will be humanely killed and tissues harvested for histological investigation (or other analysis).

For mice and rats undergoing the sub-cutaneous (under the skin) implantation protocol

Depending on how test products present (liquid, gel or solid), they will be inserted under the skin by hypodermic injection (liquid or gel) or surgical insertion (gel or solid) under general anaesthetic. Surgical insertion will typically involve the creation of small scalpel wounds (typically 1cm in length) followed by the creation of pockets under the skin by blunt dissection (separation of the skin from underlying muscle involving minimal damage). Test substances will then be inserted into the pockets. The insertion (scalpel) wounds will be sutured closed and animals allowed to recover under warmed conditions. Animals will be given appropriate pain-relieving drugs and antibiotics by injection under the skin prior to recovery from anaesthesia. Sutures will be removed under general anaesthesia 5 to 7 days later. Animals will be killed and implantation sites harvested (for histological, or other analyses) at varying time points up to 12 months.

All animals following these wound healing and subcutaneous (under the skin) implantation protocols will typically receive a marker of cellular proliferation (or other tracer) by injection into the abdomen whilst restrained and fully conscious - one hour prior culling.

All animals following the wound healing protocols will be singularly housed for the duration of the study - as when housed in groups they have a tendency to groom one another which invariably results in dressing damage. This can result in loss of test materials and contamination of wounds with soiled bedding - which can impact on the progression of wound healing and thereby invalidate the study being undertaken.

All animals following the 'under the skin' surgical implantation protocol will usually be singularly housed for 7 days after implantation (to allow incisional wounds to heal) and group-housed thereafter; whereas, animals following the 'under the skin' hypodermic injection implantation protocol will be singularly housed for 2 days after implantation and group-housed thereafter.

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

Based on over 20 years of conducting studies using similar protocols, we believe the protocols to be followed in this project to be very well tolerated.

The adverse effects we expect to observe in animals following protocols in this project are: localised wound pain, wound infection and weight loss.

Post-surgical pain

Animals may experience transient discomfort after surgery which is usually mild and self-limiting. Animals will be wounded under general anaesthesia, and will be given appropriate levels of pain-relieving drugs at the time of surgery (and thereafter) in order to reduce post-surgical discomfort. Animals will be monitored regularly for the development of adverse effects and any animal found to be displaying signs of distress or discomfort, that does not respond to remedial actions (as advised by the NVS), will be killed by a schedule 1 method.

Wound infection

The loss of the skin barrier as a result of experimental wounding in rodents and rabbits can occasionally result in localised wound infection (occurrence under previous Project Licences <0.5% approx.). In order to minimise the likelihood of infection, surgical procedures will be carried out in

accordance with Laboratory Science Animals Association Guiding Principles for Preparing for and Undertaking Aseptic Surgery. All animals will also be given appropriate antibiotics (as advised by the NVS) prophylactically (unless there is a potential for interference with scientific data).

Wound site infection is associated with increased (cloudy) exudation and wound malodour together with elevated peri-wound inflammation (heat, pain, redness & oedema). These indicators will be monitored, and if evident appropriate systemic antibiotics will be given. Wounds on rodents and rabbits will be monitored for infection on a daily basis for the first week after injury and subsequently on a twice weekly basis. Where systemic antibiotics are ineffective, and the infection is considered to be compromising well-being, animals will be killed by a schedule 1 method. With frequent monitoring for wound infection, the duration of this adverse effect will be limited to 1-3 days, which we believe is insufficient time for significant clinical manifestation of adverse signs.

Weight loss

Repeated anaesthesia, initially for the purpose of wounding and subsequently for follow-up assessments and re-application of substances, and the repetition of other 'mild' activities (such as dosing) can result in some weight loss. While this is typically small and limited in wild-type (normal) animals, it is more common and more extensive in obese animals. Animals will be monitored regularly for weight loss, and where greater than 5% loss is observed in wild-type (normal) animals, or greater than 10% in observed in obese animals (relative to their starting weight) they will be provided with an enriched more palatable softened diet. The provision of such enriched diets normally results in weight stabilisation and often in gain. Any animals that display greater than 20% loss in body weight (25% for diabetic animals) will be humanely killed.

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)?

Mice (including diabetic mice) - wound healing protocols- expected severity **MODERATE** - proportion of animals 100%

Rats - wound healing protocol - expected severity **MODERATE** - proportion of animals 100%

Rabbits - ear wound healing protocol - expected severity **MODERATE** - proportion of animals 100%

Mice - sub-cutaneous implantation protocol - expected severity **MILD** - proportion of animals 40%

Mice - sub-cutaneous implantation protocol - expected severity **MODERATE** - proportion of animals 60%

Rats - sub-cutaneous implantation protocol - expected severity **MILD** - proportion of animals 40%

Rats - sub-cutaneous implantation protocol - expected severity **MODERATE** - proportion of animals 60%

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?

The mammalian response to injury is multifaceted and complex - involving actions, interactions and responses of numerous cell types present at the site of injury and delivered to the site from other parts of the body via the blood stream.

Wound healing research in animals is necessary as, while laboratory investigations on cells or pieces of skin (from animals and humans) can generate important preliminary data (such as toxicity data, and some indication of 'likely' efficacy); they are unable to fully replicate the multiplicity of physical and biochemical reactions, cell types and cell interactions, that occur in and around wounds as they heal.

In vivo wound healing studies in animals also offer the possibility of investigating mechanisms of action of, and/or, the development of unexpected adverse interactions to wound healing substances/therapies at the tissue/cell/molecular level, which, as this invariably requires wound tissue excision, would be largely ethically unacceptable in the clinical setting.

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

We considered, and use (together with academic collaborators), a variety of non-animal approaches to achieve our research aims.

These include undertaking wounding assays (called scratch-assays) on sheets of cultured skin cells, and studies of the healing of wounds created on pieces of cultured (live) human skin (taken from patients with excess abdominal skin or following surgical amputation).

Why were they not suitable?

While these non-animal alternatives can provide useful information, that can assist and guide the design of animal studies, such alternatives cannot replace them. The complexity of the wound healing response, particularly the involvement and interaction of numerous cell types from different parts of the body means that wound healing and the effect of developmental therapies on wound healing, can only be studied in intact animals.

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 estimated the number of animals needed for each protocol based on our experience of using these approaches in previous projects and anticipated future requirements.

This includes both our experience of the minimal number of animals required, for each treatment group to provide valid and useful data, alongside our experience in, and understanding of, the wound healing sector, and the likely demand for our research models. We have made assumptions on future requirements based on our best assessment of the science and our previous experience.

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

Group sizes for all of our protocols are based on 20 years previous experience with these animals and protocols, the relevant scientific literature and power calculations. We utilised available online resources such as the NC3Rs experimental design assistant to plan experiments and perform power calculations to determine sample size. These calculations were based on knowledge of the mean values and variability of the primary outputs for each protocol - taken from our prior experience and that of others.

Our group sizes are the lowest possible to allow for the infrequent “in study” loss of animals (due to death or complications); whilst maintaining a high probability that a study will be sufficiently powered for statistical analysis on completion. This reduces the likelihood that animals will undergo unnecessary procedures in statistically underpowered studies.

We design experiments so that multiple experimental readouts can be derived from a single animal. We use imaging when possible so that wound healing can be tracked non-invasively and confirmed by tissue approaches after humane killing.

The creation of multiple wounds sites in individual animals allows for “within animal” controls to be used (where appropriate) which reduces the total number of animals required for a given study.

We standardise our experimental variables in order to minimize variation, such as using animals that are closely matched in terms of age and of a single strain. This also reduces the total number of animals required for a given study.

As we have managed to reduce variation, we have established very reproducible protocols. This means that it is not always necessary to repeat certain positive control groups and that historical control data can be used – thus reducing the number of animals required in a given study.

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

We work closely with an academic group that have extensive experience in the development and use of non-animal alternatives – including wounding assays on sheets of cultured skin cells (scratch-assays) and assays of the healing of wounds on cultured (live) human skin.

Where appropriate, these non-animal alternatives will be used to screen-out inappropriate investigational agents and determine the most effective dosing regimens for subsequent animal studies - thereby reducing the numbers of animals required for effective *in vivo* investigation.

In instances where the pre-existing data (in relation to potential efficacy) is considered insufficient to undertake a fully powered study, or where a large number of formulation variants exist, preliminary pilot studies, involving small numbers of animals/wounds, will be undertaken to determine the need for more extensive investigation or to screen-out less effective variants – and thereby reduce the numbers of animals used.

We regularly share animal tissue with other research groups and have a good communication network within the University to alert other groups to available tissues.

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 use mouse, rat and rabbit models of wound healing, mouse and rat models of *in vivo* implantation, and a rat model of haemostasis.

Our overriding rule, is to use the model and methods with the least likelihood of causing pain, suffering, distress or lasting harm that is necessary to address the scientific question being asked.

The surgical methods used to create wounds or implant materials, the routine use of pain killing drugs and warmed recovery, the size and number of wounds or implants/implant sites per animal, and the frequency, form and duration of follow-up assessments and dosing procedures/dosing regimens that we use, have been progressively refined to minimise harms - during the course of our previous licences.

For a given animal, we create the smallest and fewest wounds or implants under general anaesthesia, provide appropriate levels of pain relief, and undertake the fewest and shortest follow-up assessments and substance administrations by the most refined route, possible.

Wherever possible, we avoid single housing of animals unless it is essential for scientific reasons or animal welfare. Group-housed animals have a tendency to groom one another, which for our protocols invariably results in removal of sutures or damage to dressings - which in turn can lead to rupture or

contamination of wounds - and ultimately invalidation of the study. That being the case, all animals are housed-individually for at least 7 days after wounding, and where necessary for the duration of the study.

Wherever possible, environmental enrichments (e.g., forage food, nesting materials and wooden chew-sticks) will be provided to animals following our protocols.

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

The mammalian response to injury is multifaceted and complex – and as yet, these complexities haven’t been fully recapitulated in less sentient species. Recent research undertaken on Zebrafish suggests that whilst their response to injury is similar to mammals in some ways – it is clearly different in others; and it is well known that not all data derived from Zebrafish studies and other less sentient models is relevant to humans.

Because wounds take days or weeks to heal, it is not possible to study the entire wound healing process under terminal anaesthesia. That being said, one of our protocols, in which we determine the impact of agents on the process of haemostasis (halting blood flow after trauma), is undertaken entirely under terminal anaesthesia.

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

As noted earlier, the surgical methods used to create wounds or implant materials, the routine use of pain killing drugs and warmed recovery, the size and number of wounds or implants/implant sites per animal, and the frequency, form and duration of follow-up assessments and dosing procedures/dosing regimens that we use, have been progressively refined to minimise harms - during the course of our previous 3 project licences.

Opportunities for further refinement are are continually sought.

The following refinements were made during our most recent project licence:

1. We moved from oral (gavage) dosing with steel gavage 'needles' to dosing with less rigid and thereby less damaging flexible plastic 'needles'.

2. We routinely use 'black-out' felt head covers (like falcon hoods) when dosing conscious animals. This has an immediate calming effect and appears to make dosing procedures less stressful.

While we do not expect any significant physical, behavioural or physiological deviation from normality in animals following our protocols, we undertake regular monitoring of key well-being parameters which facilitate the early detection of unexpected adverse effects (that may impact on animal pain, discomfort or distress) and thereby allow the rapid deployment of remedial action.

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

We will use the following resources in planning and conducting experiments:

ARRIVE Guidelines 2.0. https://arriveguidelines.org/arrive-guidelines

PREPARE Guidelines. https://norecopa.no/prepare

NC3Rs Experimental Design Assistant. https://eda.nc3rs.org.uk/

NC3Rs guidance on blood sampling in mice. https://www.nc3rs.org.uk/3rs-resources/bloodsampling/blood-sampling-mouse

NC3Rs guidance on blood sampling in rats. https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-rat

NC3Rs guidance on blood sampling in rabbits. https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-rabbit

NC3Rs guidance on microsampling, including the microsampling decision aid. https://www.nc3rs.org.uk/3rs-resources/microsampling

NC3Rs Mouse Grimace Scale. https://www.nc3rs.org.uk/3rs-resources/grimace-scales/grimace-scalemouse

NC3Rs Rat Grimace Scale. https://www.nc3rs.org.uk › 3rs-resources › grimace-scales › grimace-scale-rat

NC3Rs Rabbit Grimace Scale. https://nc3rs.org.uk › 3rs-resources › grimace-scales › grimace-scale-rabbit.

NC3Rs guidance on anaesthesia. https://www.nc3rs.org.uk/3rs-resources/anaesthesia

NC3Rs Guidance on analgesia. https://www.nc3rs.org.uk/3rs-resources/analgesia

NC3Rs Guidance on handling and restraint. https://nc3rs.org.uk/3rs-resources/handling-and-restraint

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. https://www.lasa.co.uk/wpcontent/

uploads/2018/05/Aseptic-Surgery.pdf

EFPIA/ECVAM good practice guide to the administration of substances and removal of blood, including routes and volumes. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jat.727

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

Our group will stay informed through the NC3Rs website. Relevant information, including the NC3Rs newsletter, is circulated within our institution by email to all personal and project licence holders. We will attend local events organised by our Animal Welfare and Ethical Review Committee and

information sessions on NC3Rs funding streams organised by our institution's Research & Innovation Service. We will share best practice within our institution and have well developed interdisciplinary networks to facilitate this. We attend regular local user-group meetings for project licence holders at which the group will receive updates on any changes to best practice or requirements.