Home Office

NON-TECHNICAL SUMMARY

Modulation of wound healing and scarring

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
- (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

wounds, burns, pigmentation, chronic, scarring

Animal types	Life stages
Pigs	adult
Mice	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?

To assess treatment(s) that will prevent/reduce wound infection, inflammatory response and/or accelerate wound healing.

To assess treatment(s) that will reduce/prevent scarring following wound healing and investigate the mechanisms of all aspects of scarring including altered pigmentation.

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?

Wounds are a major healthcare burden and in particular chronic wounds and burns/thermal injury are a global problem. Wounds are a leading cause of loss of hours of work and a drain on all healthcare systems around the world. One of the dangers of open wounds such as chronic wounds and burns, is the susceptibility to infection which can delay wound healing, increase risk of gangrene or lead to sepsis with a risk of death. Scarring following injury affects not only the cosmetic and psychological well being of the patient, but more importantly, growth and function, particularly in burn injured children. Current management of wounds relies largely on a large number of dressing products designed for mass therapy and lacks knowledge that can provide personalised care. Wound healing is a complex process and we need to understand the mechanism of why wounds do not heal or heal slowly and scar so that we can develop smart treatments that will speed up healing and prevent/control infection and reduce/prevent scarring. This project aims to address these challenges and advance our knowledge of wound healing and scarring.

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

We will test new substances/treatments designed to speed healing and reduce scarring as well as new treatments that fight wound infection. We will first study how these treatments change wound healing and scarring in animals before we test them in humans. In parallel, we will investigate how and what effects any new treatments of wound healing/scarring may have including side effects. We predict that these studies will provide us with more information into the mechanisms of wound healing and scarring that can be investigated for further development of new targets for personalised care. These studies will provide a safer route into clinical wound and scar management. The studies will specifically focus on the current challenges in wound healing such as improving speed of healing, preventing/reducing infection including targeting multi-resistant organisms and prevention/reduction of scarring.

The new information will be shared widely in the scientific and clinical communities.

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

The ultimate beneficiaries will be humans and will aid the healthcare professionals of various disciplines in managing patients with wounds and scars. Some of the outcomes of these studies may well benefit animals with wounds and veterinarians as well. The scientific community will definitely benefit from the basic science knowledge gained from these investigations.

In the short term, testing of new substances will allow us to identify the potential new treatments from those that need more work or are not suitable for use in human wound healing and scarring. Development of non-invasive technologies to study state of healing/early detection of infection for timely intervention will be of great benefit to children in particular, as it will address the issues of pain and anxiety related to handling of wounds.

In the longer term, detailed scientific studies (molecular and genetic investigations) on tissue samples obtained from these studies will help to better understand the mechanism of action. The knowledge will assist scientists to develop even better target candidate treatments for rapid wound healing, prevent/reduce wound infections, prevent scarring, assist with regaining normal skin pigmentation and texture of the scar as well as prevent deformities/limitations in mobility.

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

The establishment has a large collaborative network in wound healing. We will maximise outputs by peer reviewed publications in the scientific literature and where appropriate, we will consider media engagement. We will collaborate with other researchers in various fields as we have developed expertise in various wound healing models and will have an extensive library of tissues from different types of wounds at various stages of healing/scarring. By wider collaborative working, we will be able to harness a wide range of expertise and be able to investigate different approaches and thereby accelerate the pathway into preclinical testing of such therapies.

Species and numbers of animals expected to be used

- Pigs: 75
- Mice: 210

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.

Rodents have been selected to test the efficacy of the novel therapeutics in accelerating wound healing/reducing inflammation and infection and in particular for mechanistic studies. However, as explained below, pigs will be used for pre-clinical studies and for studies involving wound healing, wound infection, scarring and altered pigmentation.

The pig has been chosen as its skin has similar anatomy and physiology to human skin. Pigmentation in the pig skin, unlike mice, is akin to the human skin in that the pigment cells are present in the hair follicles and in the epidermis (top layer of skin) and both play a role in pigmentation. Mice are loose-skinned with a panniculus carnosus (skin muscle) and heal mainly by contraction that is much more rapid than by re-epithelialisation (cells migrating over the surface of the wounds to form top layer of new skin) seen in 'tight-skinned' mammals such as humans and pigs. Our previous studies on human thermal/burn wound samples have confirmed similarities with the pig thermal/burn wounds.

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

Under general anaesthesia, thermal and/or open wounds on the backs of the animals will be created (maximum 12 wounds measuring 2.5cmx2.5cm on the backs of pigs and maximum two wounds no larger than 1cmx1cm, on backs of mice). The size of the wounds are well tolerated by the animals and are known to heal spontaneously. Some of the wounds will be infected with bacteria to produce local wound infection. The wounds will be treated with novel treatments/dressings which will be applied either topically or by injection into the wound margins. The wounds will be dressed and animals allowed to recover. Wound healing, reduction/prevention of wound infection and "new skin" formation including the pigmentation and scarring will be monitored by imaging and measurements over various time points up to 10 weeks post-injury. At varying time points, wounds will be removed with the animal under terminal anaesthesia. After taking out the wounds, the animal will be humanely killed. The wounds will be processed and subjected to in depth analyses to study the mechanisms and pathways of wound healing/scarring using cellular and molecular techniques as well as effect of infection on wound healing.

In addition, novel non-invasive methods for checking progression of wound healing/wound infection through the dressings will be investigated at regular intervals. These methods have previously been tested in non-living animal experiments and shown to capture images through the dressings. We will perform tests to ensure it is safe and represents the stages of wound healing before the methods are used in humans.

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

The procedure can cause post-surgical pain of mild to moderate severity which will be controlled with pain killers. These effects are well controlled once the wounds have been dressed and the pain relieving drugs are administered in a timely manner before the animal wakes up. The animals will be monitored closely for signs of pain and distress and treated promptly with further pain killers as required. The welfare of the animals will be closely monitored to check on any side effects of the novel therapies. With smaller animals weight loss can be a measure of animal distress and will be carefully monitored.

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 severity of pain is estimated to be between mild and moderate and animals will be monitored very closely and appropriate pain relieving drugs administered as required. From experience, once the wounds are dressed and animals have had their pain medicine before waking from anaesthesia, they are comfortable and do not show any signs of pain and distress. Wounds can also become colonised or rarely infected with microbes but will be managed or treated within the moderate severity range.

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?

Open or thermal wounds require cells from the circulating blood to interact with the local cells of the skin to allow complete healing of the wound and formation of the “new skin”. We can study some aspects of wound healing such as re-epithelialisation (healing of the top layer of the skin) in ex-vivo models (culturing whole skin in petri dishes and wounding the skin) which we are currently developing for thermal and open wounds. However, in order to study the full science of wound healing i.e. development of the “new skin” after open/thermal wounds, requires animal studies with blood circulation and there are no alternative models available to us at present. Furthermore for testing new substances to improve healing and scarring, we need wounds on animals with circulating blood as ex-vivo studies are not suitable.

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

1.We have considered collaborating with colleagues who can keep pig body parts from the abattoir "alive" by perfusing with artificial blood.

2. We are optimising an ex-vivo burn wound model of human and pig skin (surgical/abattoir waste) to study the effect on re-epithelialising (healing of the top layer of skin).

Why were they not suitable?

1.Use of perfused body parts for wounding and studying mechanisms are not suitable because the perfusion can only be maintained for 24 hours maximum to date and we need to study the long term effects of wound healing and scarring.

2. With ex-vivo experimental wounds or burn model, there is no circulating blood which contributes to the healing process. Ex-vivo burn wound model can only be kept alive for short period of time. However, we are currently working on optimising culture conditions in order to study some elements of

ex-vivo thermal/open wound healing and intend to compare the in vivo thermal/open wounds with ex-vivo thermal wounds.

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?

Data from previous studies have informed the estimated number of animals to be used. However, we will continually review the data and update the validity of the sample size calculations and number of animals required.

In this project, we aim to test up to 5 treatments. The generic design of the experiments is step wise where the pilot experiments will inform the design of the experiment i.e. comparison of treatment versus standard of care in same animal or between different animals dependant on whether the test treatment diffuses into the surrounding skin and gets absorbed or remains within the wound bed to avoid confounding the results. The pilot studies will also inform the optimum route of administration and doses to be tested for efficacy studies. Therefore the efficacy studies will have the benefit of data from which to recalculate the exact numbers required as mentioned above.

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

The design of the experiment was discussed with the establishment's statistician who analysed some endpoint data from our previous experiments. We also discussed with the NC3R team.

The experiments are designed such that the treatments and controls are within a block or incomplete block (depending on the number of treatments analysed within an experiment) and this allows to reduce the actual number of animals used per experiment. Furthermore, by using smaller pilot experiments initially, we can optimise the final experiments and recalculate the sample size at each stage.

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

For each treatment to be studied, pilot experiments will be used to optimise the design of the experiment, route of administration and doses to be tested for efficacy studies. Data will inform the validity of the sample sizes used. Previous experimental data has also been used to inform the current sample size. Observations from each of the dose response and efficacy studies will add to the data. Our previous studies on pigs have provided data to combine re-epithelialisation (top layer of new skin)

and early inflammatory infiltrate (blood cells which help with wound healing) studies to day 10; ongoing inflammatory response and myofibroblast phenotypes (cells which lay down the collagen in the new skin)in wound bed (early marker of scarring) to day 21 and long term effects on re-pigmentation, vascularity (blood vessels) and texture of scars to day 70 post-wounding. These aligned time points will reduce the number of animals studied.

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.

Incisional/excisional wounds will be made in mice and incisional/excisional and thermal wounds of varying depths will be made on backs of pigs. These are standard wound models to study wound healing. In addition, we will study antimicrobial treatments on infected wounds in these animals.

The animals will be under the effect of general anaesthesia when the wounding is performed so they will NOT feel any pain or discomfort. The wounds will be well padded and covered. From previous and clinical experience working with patients, once the wounds are dressed and well covered, the pain is minimised.

Wound infection is unlikely as wound management/dressings are applied aseptically after washing with antiseptic solutions and sterile dressings are used.

The animals will be given pain relief at the start of the procedure to ensure minimal or no pain is felt after recovering from the anaesthetic. They will be observed closely thereafter and further pain relief administered as necessary.

From our previous studies, we have now refined the technique of surgical procedure and application of dressings and refined the dressings used, thereby reducing the frequency and the duration of general anaesthesia for the animals. The animals will be closely observed post-wounding for any signs of distress or pain and prompt management initiated as appropriate. After wounding, the animals will have robust dressings to avoid self-interference with the dressings and will be singally housed, but will be adjacent to other animals to allow social interaction.

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

Following initial ex-vivo studies, we will initially check effects of test treatments on mice before we study the effect on porcine wounds (considered akin to human wound healing/scarring/re-pigmentation). The pig has been chosen as its skin has similar anatomy and physiology to human skin. Pigmentation in the pig skin, unlike mice, is akin to the human skin in that the pigment cells are

present in the hair follicles and in the epidermis and both play a role in pigmentation. Our previous studies on human wound healing have confirmed similarities with the pig wound healing.

The pig has been selected as the model of choice for our more developed treatments down the translational pathway that have already been validated in small animal studies. For our early discovery pipeline treatments we will be using less sentient animals such as the mouse. Despite their lack of similarity to human skin, the detailed characterisation, genetic tractability and convenience of experimentation due to most of the literature being based on rodents, make mice ideal for earlier phase discovery studies such as initial trialling of novel compounds and mechanistic studies where specific pathways can be identified for more targeted therapy.

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

The animals will be monitored very closely by the specialist animal care staff who are conversant with handling and welfare of mice and pigs. The animals will have time to acclimatise, for at least 7 days. Only staff competent in animal handling and assessment of animal welfare and surgery will be performing these procedures. They will interact with the animals during their acclimatisation and build up a rapport to minimise distress.

Following the procedure, enrichment will be provided such as bedding and toys.

Animals will be weighed periodically and their food and water intake monitored as well as their bowel movements along with the health of the skin and eyes to ensure they are healthy and not distressed throughout the period of the experiment. Animals will be assessed for pain and pain medication given timely. General appearance, body function, environment, status of the dressings and behaviour of the animals will be closely observed.

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

ARRIVE guidelines and guidelines by joint working group on refinement

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

By attending the NC3R workshops, keeping abreast of the literature and regular communication with the Establishment 3Rs manager. If there are new advances in procedures and welfare of animals, we will seek amendments to refine our techniques and implement changes after discussion with the named veterinary surgeon.