Home Office

NON-TECHNICAL SUMMARY

Repair and resolution of cutaneous wounds and inflammation

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, Inflammation, Psoriasis, Healing, Therapy

Animal types	Life stages
Mice	adult
Rats	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 this project are

- the development of new treatments to improve the healing of wounds;
- the development of new treatments to resolve inflammatory skin conditions;
- to further the understanding of how normal skin function is maintained.

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?

Improved Wound Healing

Chronic wound care is an important area of unmet clinical need and was estimated to cost the NHS £8.3 billion a year in 2018 (Guest et al., 2020), an increase of 48% since 2013 .

Poor cardiovascular health and immunosuppression are the principal underlying reasons for impaired healing. Ageing, dietary insufficiency, infection, immobility, inflammation of blood vessels, diabetes and long-term steroid therapy are other contributory factors. Current therapies are inadequate, focussing on secondary symptoms (such as infection and the die-back of tissue around a wound) rather than the underlying cause, which can contribute to an increased risk of amputation, particularly with diabetic foot ulcers. Standard therapies provide a sterile, moist and anti-microbial environment to improve healing. Only one therapy that is not a dressing or debridement aid (removed dead/dying tissue in the wound) is currently approved by the US and European drug regulatory authorities for the treatment of wounds: Regranex®, which consists of a gel containing recombinant human Platelet-Derived Growth Factor.

A major part of this project will be to characterise novel therapeutic agents that will aid skin repair and combat inflammatory changes.

Treatment of Psoriasis

Around 3% of the UK population suffer from psoriasis, a chronic inflammatory disease of the skin, which bears similarity to and can be associated with other types of chronic inflammatory disease that affect the joints (Rheumatoid Arthritis) and the intestine (Crohn's disease). People who have psoriasis that affects more than 10 % of their skin show an increase in mortality rate. Both genetic and environmental factors contribute to the development of psoriasis. Current therapies, including drugs that suppress the immune system and ultraviolet light, all have some impact on symptoms; however, there is no therapy available that can cure the disease. Therapies can have adverse effects on the skin themselves, such as steroid-induced dermal atrophy (thinning of the skin), and may also be associated with side effects such as diabetes, osteoporosis (loss of bone density) and increased risk of infections and certain types of cancer.

As with cutaneous wound healing, this project seeks to facilitate the development of new therapies for the control and resolution of psoriasis.

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

The project will provide data that will help in the development of new treatments for skin injury, including trauma wounds and inflammatory skin conditions. In addition, studies may produce data to help with better understanding of skin regeneration.

These data may be used to support scientific submissions to regulatory bodies, in order to obtain the necessary approval to progress treatments into clinical trials. Data may also allow the identification of new therapeutic targets or strategies for the treatment of skin injury.

Whenever possible, data will be shared with the wider scientific community, thorough presentation at national/international meetings and publication in peer-reviewed journals, in accordance with ARRIVE guidelines.

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

Short-term, the benefits will be in the pre-clinical decision making process for progressing therapeutic candidates. Any new knowledge that is gained from this project may help and inform other research groups that are working in the same field. Medium-term, this should see more treatments progress to a clinical trial setting. Approval of new clinical therapies is a lengthy and rigorous procedure and so, it may be several years beyond the life of the project for those with clinical need to see any benefits from the outputs of this project.

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

All studies undertaken in relation to this project are performed on a collaborative basis with other, independent researchers.

Whenever possible, data will be shared with the wider scientific community, thorough presentation at national/international meetings and publication in peer-reviewed journals, in accordance with ARRIVE guidelines.

Species and numbers of animals expected to be used

- Mice: 600
- Rats: 200

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.

We are using adult mice and rats in these studies. These represent the animals of lowest sentience in which we can perform these studies. Rats are more intelligent than mice but rat models give superior wound histology. Mouse models can have advantages over rats, particularly for diabetes-impaired wound healing, where use of the diabetic db/db mouse strain avoids the use of a chemical insult to induce diabetes in animals. The species and strain used in each study will be carefully considered, according to its aims.

Adult animals are of sufficient size that any wound sites are not too big relative to the size of the animal.

Use of animals allows us to study the multifactorial nature of the healing response which depends on a range of different types of cells.

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

First of all, animals used in this project will be anaesthetised and an area on the back of each animal will be shaved and any remaining hair removed using wax strips or cream.

Whilst still anaesthetised, animals may undergo procedures to the skin (the shaved area) that will result in injury. The injury may be an incisional wound (i.e. a cut made by a scalpel) or an excisional wound (i.e. a hole made by removing a plug of skin using a surgical biopsy punch). Animals will receive pain relief (usually by injection).

Various factors can affect the speed at which a wound heals, including infection, aging and diabetes. In order to study these, we will vary the procedure that we follow. For some studies, we may inject substances into the skin near the wound in order to mimic infection. In other studies, we may use aged animals or we may use female animals that have had the ovaries removed, in order to study the effects of aging. Finally, we may use animals that have been genetically-modified so that they develop diabetes.

For some studies, instead of a physical wound, a chemical may be applied to the skin that will result in inflammation. This is relevant for studying treatments for allergic dermatitis and psoriasis, for example, according to the type of chemical that is applied.

We may apply treatments to the animals in order to speed up the healing of the wound, or reduce the inflammation (if appropriate, this will be performed under anaesthesia); treatments may include

dressings, gels, injections or substances given in the food or drinking water (or by a feeding tube directly into the stomach). Placebo treatments will be included in any study, together with any appropriate reference treatment (i.e. a treatment known to have a beneficial effect).

Studies will typically last one or two weeks. Animals will be weighed daily and welfare checks performed at least once per day. Experiment treatments will be administered/applied as appropriate for each study. Blood samples may be taken from animals during the study. Animals will be humanely killed at the end of each study and skin and other samples taken for analysis.

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

It is expected that there will be some discomfort from wounds and any chemically induced inflammation of the skin.

We expect there to be a temporary drop in body temperature (a couple of hours) and body weight associated with anaesthesia (one or more days).

During a study, the administration/application of substances may be associated with discomfort or localised irritation at a site of injection (a few seconds). The restraint of animals for the purpose of blood sampling may cause transient distress and minor pain/discomfort may be experienced when blood is sampled (a few minutes).

Diabetic animals may drink more and urinate more than healthy animals.

Application of a dressing to a wound may cause some discomfort and local irritation depending on its mechanical properties (i.e. how soft and flexible it might be) and how it is fastened to the animal (i.e. the adhesive used).

Unless stated, we expect to see some evidence of one or more of the effects for the duration of any study (up to two weeks).

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)?

We expect the majority of animals to be classified as moderate (90 %), with some control animals being classified as mild or non-recovery (< 10 %).

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 skin can be regarded as the largest organ of the body. It has many cellular and structural components and performs a range of roles including a barrier to infection and harmful chemicals, sensory perception (touch, pain, heat/cold) and temperature regulation. The skin also possesses a community of microorganisms on the surface (microbiome) which serves to modulate skin function in health and disease.

Animal models are necessary as we need to be able to study the integrated response of all the different cells in the skin, and those cells that migrate into the skin, following injury (whether caused by trauma or inflammation).

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

Our skin research projects to make use of a range of animal alternatives. We can culture small pieces of skin (called explants) for short periods, which can allow us to study the effects of different treatments on specific processes that occur in the two major skin cell types, the epidermal cells (or keratinocytes), which form the top layer of skin (epidermis) and fibroblasts, which form the dermal layer, on which the epidermal cells sit. These models also allow us to study how molecules penetrate the skin.

We can make three-dimensional cell cultures, by making a synthetic dermal layer on top of which we can culture epidermal cells. This actually looks and grows a bit like skin, and the cultures are made from cells we can isolate from normal skin and keep frozen in the laboratory until we wish to use them. These cultures can be used in a similar way to the small pieces of skin, and can be used for looking at how substances protect skin cells from ultraviolet radiation (i.e. sun damage).

We have a cell culture system that allows us to monitor in real-time how epidermal cells or fibroblasts respond when a single layer of cells grown in culture is "damaged". This damage is caused by a metal pin which scratches through the layer of cells, making a gap. The cells migrate to fill the gap and we can look at how different chemicals can slow down or speed up this process. We can also perform experiments to study the formation and branching of blood vessels in culture dishes.

Why were they not suitable?

Currently, it is not possible to recapitulate all aspects of skin structure and function in cell culture models. All these culture systems lack a blood supply and immune cell populations, and a microbiome (the population of resident bacteria and other microorganisms). Our three-dimensional cultures are quite fragile and so do not lend themselves to study the healing of wounds. All cell culture models depend on the availability of surgical skin samples from consenting individuals. This has particular relevance to wound healing, where the three-dimensional aspect of the healing process and the role of immune cells that migrate into the wound space need to be studied. A range of functional immune cell types are also required to study inflammatory conditions of the skin, such as allergic dermatitis and psoriasis; many of these cells are not usually resident in the skin and migrate, in response to

inflammatory signals. Hopefully, as we become more able to add complexity to our cell culture models, we will see some replacement of animals in these types of study.

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?

This estimate is based on running three mouse and one rat study each year for the project, combined with the use of current non-animal alternatives.

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

We have sought independent statistical advice based on data obtained from previous studies. Animal numbers used will be those required to meet the objectives of the project. To minimise animal numbers, we will set realistic limits for the size of effect that we wish to be able to detect in our studies (e.g. the fold improvement in the speed of wound closure). We will choose the most appropriate model for the project and utilise in-bred animals (i.e. animals that are genetically identical), in order to reduce variation in the results that we obtain.

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

We may perform small pilot studies in order to measure the concentration of drug that is achieved in the blood and in the skin following its administration; this will be done with small numbers of healthy animals per group (five or less), with one group per concentration of drug. Such studies can inform us of the most suitable concentrations to use in a bigger study to look at the effect of the drug on wound healing or inflammation. The range of concentrations tested will be within the toxicity limits of drug; this will have been determined by previous work by other laboratories, based on animal studies and/or cell culture experiments.

We always seek to maximise the amount of information that we can obtain from each study. This is achieved by collecting clinical observations during the study and collecting as many tissue samples as required, for analysis at the end of the study. We have available a wide range of analytical platforms to support our studies and give us access to multiple readouts that can inform us about different aspects of a drug's ability to promote healing and/or to suppress inflammation. In collecting as much data as possible from each study, we minimise the risk of having to run another study in order to gain further knowledge.

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 rodent models of wound healing and skin inflammation. These models will include excisional and incisional wounding and chemically induced inflammation (psoriasis and dermatitis). These models are well-characterised in terms of their time-course and severity of harms. Because of this, a suitable frequency of monitoring animals for welfare purposes can be incorporated into study protocols and informed decisions about when it is appropriate to end a study can be made (according to the question we are trying to answer by running the study). Pain will be minimised by the use of pre- and post-operative analgesia for all surgical procedures and throughout the time-course of the study, where it does not frustrate the outcome (i.e. mask the effect of any treatments being tested). Also, humane endpoints can be set to maintain animals within moderate severity limits. All animals will be humanely killed at the end of each protocol.

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

We require models to be robust and well-characterised in order to reproducibly evaluate new therapeutics of varying modalities, ranging from injectables to gels applied directly into wounds and material dressings. The chosen species and models best allow us to perform these tasks. Immature animals have immature immune systems; a fully functional immune system is fundamental to models of inflammation and the immune system is also important for cutaneous wound healing.

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

A study will run only for the length of time required to meet the aims and objectives of that study. Monitoring animals for welfare purposes will be increased in response to any observed increased in signs of discomfort and informed decisions about when it is appropriate to end a study prior to any scheduled endpoint will be made. Pain will be minimised by the use of pre- and post-operative analgesia for all surgical procedures and throughout the time-course of the study, where it does not frustrate the outcome (i.e. mask the effect of any treatments being tested). Also, humane endpoints can be set to maintain animals within moderate severity limits.

A process of continuous review will operate, to ensure that the models we run are fit-for-purpose; this will involve looking at all the data that we collect and seeing how it may change with time and if required, making appropriate changes to models.

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

We will perform procedures according to our own standard operating procedures. We will also follow guidelines issued by the Laboratory Animal Science Association (LASA) and guidance from the NC3Rs, as appropriate.

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

We will stay informed about the 3Rs advances through attendance at lectures and seminars run locally, as part of a continuing professional development programme; through the NC3Rs website; by keeping up with the latest scientific publications in the field.