Home Office

NON-TECHNICAL SUMMARY

# Using RNAs to investigate the mechanisms of skin repair

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research

**Key words**

skin, repair, wound, matrix, epidermolysis

| Animal types | Life stages |
|---|---|
| Mice | adult, embryo, neonate, juvenile, pregnant, aged |

## 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?**

Skin can be injured by a range of actions such as surgical intervention or unintentional trauma. Studies in mice have already shown that small wounds on their backs heal very efficiently when filled with wound matrix: the temporary tissue, which naturally replaces blood clots. The aim of this project is to find out how the wound matrix changes and test if small RNAs can be used to change the process of wound repair. Furthermore, the identification of adhesive molecules that improve the formation of wound matrix could help wound healing and alleviate devastating disorders such as epidermolysis bullosa (EB), which is characterised by fragile skin that blisters and tears easily.

**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?**

As a major barrier protecting the body, skin is often damaged. While healthy skin repairs well, many underlying health conditions such as diabetes may result in persistent ulceration or large-area wounds with impaired healing. More efficient healing of ulcerated wounds can be achieved if we learn more about the adhesion mechanisms controlling wound matrix formation. Wound matrix consists of many different cells that produce adhesive molecules responsible for efficient skin repair. Much of these functions rely on adhesive properties of the molecules by which cells are held together in the wound gap. In addition, the cells themselves have adhesive molecules that allow them to migrate to close the wound gap. These molecules are also crucial for keeping the skin layers together and when they lack functionality, it can lead to dissociation of skin layers, known as blistering. Thus, the identification of new adhesive molecules that improve the formation of wound matrix could help wound closure and alleviate blistering diseases like EB, a life-threatening genetic disorder with no cure.

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

1. Publication in scientific journals

2. Identification of new molecular mechanisms controlling adhesion in skin.

3. Testing new potential novel therapies for wound repair.

4. Development of wound assays in vitro with potential application for the replacement animals.

5. Proof of principle that the inhibition of small RNAs - tiny switches that help control which genes are turned on or off - can restore adhesion in EB.

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

1. The scientific community in academia and industry by knowing how adhesion works in mice and cultured human skin cells.

2. Patients will eventually benefit from the development of improved wound treatments.

3. A new avenue for epidermolysis bullosa (EB) treatments may open up for further clinical research, and patients may eventually benefit.

4. Animal welfare will be enhanced through the development of non-animal methods to evaluate the effectiveness of tissue regeneration treatments using wound matrices.

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

To disseminate the output of this project, I will participate in professional meetings, collaborate with other researchers in wound repair field. I will communicate with other mouse-based project leaders in the field about the use of the experimental methods to evaluate the effectiveness of wound treatments using wound matrices. Publications, social media and press releases will also be used.

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

- Mice: 880

# 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.**

Mice remain the main model of impaired (or improved) cutaneous wound healing in vivo for three major reasons:

1. Genetically modifiable and genetically controlled breeding lines make it possible to test specific molecular mechanisms by turning different genes on and off.

2. High resemblance and conservation of mechanisms of skin repair between mice and other mammalian species, including domesticated animals and humans.

3. Short reproductive cycle of mice makes the project aims feasible to achieve within the terms of typical funding and the license.

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

1. Mice will be bred to achieve the genetic type needed for experiments by deletion of specific genes.

2. After pain killers and anaesthetics are given to mice, they will be shaved to expose skin on the back. Then, each mouse will receive two small (6mm) wounds on the back skin.

3. As an option, wounds will be treated with a simple dressing pre-soaked with oligonucleotides. These are short, custom-made strands of nucleotides, the basic building block of nucleic acids which all living beings need. If the dressing doesn't stay on, the oligonucleotides will be injected near the wound site once, after wounding has been administered.

4. All animals are expected to make a rapid recovery from the anaesthetic within two hours. Animals that fail to do so or exhibit signs of pain, distress or of significant ill health, which is rare, will be humanely killed.

5. EB blistering mouse models will be genetically modified so that the blistering disease will eventually heal.

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

Though the incidence of adverse effects during surgery is very rare, the animals will be monitored regularly for any signs of infection or abnormal behaviour. If any of these are indicated, the animal will be humanely killed.

The treatments to be applied or injected in to the wounds are not anticipated to cause any adverse effects to the animals. They may accelerate wound healing, or they may impair wound healing, however this should not produce any additional discomfort for the mice.

EB mice will only be observed for blister development on their ears, footpads, and tails, and may experience discomfort associated with redness and irritation of the skin covering these parts of the body. As soon as the symptoms appear, they will be scored as described in the protocol, and the animals will be humanely killed. We expect to alleviate the blistering of the EB mice by crossing them to mice with genetically deleted microRNA-29, a small, non-coding RNA molecule that plays a crucial role in regulating gene expression.

Animals will be kept warm and carefully monitored during the procedure and in the recovery period afterward.

**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 mice which are genetically modified will experience mild severity, and mice which experience wounding moderate severity. We expect to alleviate the blistering phenotype of the EB mice, which has a moderate level of severity, by crossing them to microRNA-29 deletion mice which has a mild level of severity mild.

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**What will happen to animals used in this project?**

- Killed
- Used in other projects

# 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?**

We must use animals in this study because testing how different types of skin cells interact during wound healing, as well as detachment of epidermis from the dermis in the EB model, must be studied in the complete physiological setting. That way, we get an accurate picture of this process. We cannot fully use human cells for these experiments because we would not be able to monitor the physiological whole-body response to wounding.

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

Human skin cells isolated from patients' skin biopsies could be used to partially answer the aims of the project. Using the information from mouse wound matrix, we will test the conditions to reconstitute the matrix in vitro using human cells to develop novel replacement models. While it is not possible to fully replace the mouse model, the human in vitro model will help to reduce the number of animals in future research not only for our projects but also for other researchers in the field.

**Why were they not suitable?**

There is not enough known about the adhesion molecules regulated by the microRNA-29 oligonucleotides to make the full use of an in vitro system. We need to study wound matrix adhesion in the microRNA-29 deletion mouse first. Based on this, we will develop the human in vitro model of wounds modifiable by the oligonucleotides.

While the EB model exists in human skin equivalents, we cannot test the genetic rescue of the disease without using genetically altered mice.

# 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?**

By consulting with expert colleagues, I have refined the experimental design. I will also test potential therapeutic treatments in cell culture models of wounds before we work with animals which will both reduce the risk of adverse effects and reduce the number of animals needed. To plan for our animal work, I have consulted a statistician to establish the minimum number of animals required for each study. Also, where possible, I will use two wounds per animal to reduce the number of animals required, using wounds collected from both male and female mice.

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

1. I used the Experimental Design Assistant (EDA), a free online tool from the NC3Rs to accurately reduce the number of animals being used in this project.

2. Control mice will be the wild type littermates (in microRNA-29 knock-out experiments) or non-specific control oligos applied to one wound vs the other on the same animal. This will maximise the data output from the mice I use and will also minimize the variability.

3. I have data from my previous work to help to determine the minimal number required per experimental group.

4. I developed parts of the protocol for the replacement of mouse wounds with human in vitro assays.

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

I will use a commercial genotyping service to cut the time for genotyping PCR to ensure efficient breeding. My group uses computer modelling to analyse wounds and we also receive and share wound samples for sectioning and imaging with other groups.

# 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 are the least sensitive mammalian species that can mimic a systemic wound healing response in humans. In addition, the only genetically modified models we are working with are available in mice. We have expertise in experimental and surgical work with these models and always use the most refined experimental design and employ sharing of tissue from each animal used whenever possible.

The use of anaesthesia and analgesics are used where appropriate to minimise discomfort and stress to the animals. All team members will be aware of the need to minimize the impact on each animal and the necessity within each experiment to define humane endpoints.

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

Unlike mammals, lower organisms commonly used in tissue regeneration studies such as the fruit fly lack all microRNA-29 molecules. Thus, only mammalian, namely, mouse, skin regeneration model can be employed to study microRNA-29 function in wound healing.

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

Skin inflammation is an integral part of healing, though skin irritation is minimal and the inflammatory symptoms are transient. We shall use analgesia and the aseptic technics to minimise the inflammation.

We are using the least severe blistering model to test the possibility of genetic rescue of the blistering symptoms. The Junctional Epidermolysis Bullosa mouse model (JEB) we're using, where outer and inner layers of skin are separated, has less severe symptoms compared to both other mouse models and the actual disease in humans. It is the most suitable model for the specific purpose of the study which allows us to focus on the specific genetic mechanisms involved in the condition and potential treatments, despite the mouse model not fully replicating the severity seen in human cases of JEB.

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

I will be keeping up to date with wound healing literature and have designed studies using appropriate controls. I also used the smallest group size per experiment to effectively test effects on wound matrix formation. Wounds can be treated as ‘independent’ because previous studies have shown greater variability in wound healing between wounds within a mouse than between litter mates.

The breeding of mice developing a mild blistering has been performed and published by academic labs and commercial breeders.

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

The animal facility regularly conducts 3Rs workshops and send me the most updated 3Rs information. This information is also updated on the Establishment SharePoint, which I check regularly.

I will also keep up with the current literature reporting the development of alternative models of wounds and consult the local 3Rs managers and will be supported by the NVS advice at every stap of the project.