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NON-TECHNICAL SUMMARY

Creation and repair of Ano-Rectal Fistulae in a porcine model

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Fistula, ano-rectum, therapy, stem cells, scaffold

Animal types	Life stages
Pigs	juvenile, 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 determine what combination of stem cells and biomaterials has the greatest impact on promoting new tissue growth in an ano-rectal fistula track.

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?

Currently there is no gold standard treatment for treating ano-rectal fistulae and achieving long term healing. Patients with active fistulae experience considerable pain and reduced quality of life.

The expected benefits for patients with ano-rectal fistulae include:

The potential for different therapies to be applied as less invasive surgical procedures. This approach would preserve the anatomy of the surrounding tissue and thereby prevent incontinence (inability to control bowel movement).

If successful, it may also be used for fistulae in other parts of the body (e.g. head and neck; this area is currently not under investigation).

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

One output from this study will be publishable data which will be disseminated through peer reviewed journals and national/ international scientific conferences/meetings.

An Additional output may be patentable product(s) (identified once detailed analysis of the competing market has been undertaken) which can be commercialised and offered clinically to patients (once regulated studies have been completed). If a successful product is identified, it is likely to take longer than 5 years (outside the duration of this licence) before being offered clinically.

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

Long term the additional benefits will be to the patients, approx. 2 per 10,000 of the population in the UK experience chronic and recurrent fistulae, a successful treatment outcome would significantly improve patient's quality of life, reduce hospital visits and in-patient time thus reducing NHS cost.

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

Output will be maximised by

- presenting at clinical meetings to obtain relevant feedback in order to ensure the final approach can be clinically adopted
- explore collaborative opportunities both with regards to cell type and source, biomaterials and mode of application/delivery

- Present at relevant focused meetings/conferences

Species and numbers of animals expected to be used

- Pigs: 50

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.

The pig is an established model for ano-rectal fistulae. The model was developed and published by our group. The pigs anatomy, size, diet and physiology is similar to humans for this model and is the most appropriate translational model available. The model allows fistulae to be created in pigs which mimic human ano-rectal fistulae and have similar pathophysiology. Smaller animals do not provide appropriate clinically translatable data.

Female pigs are used in preference to males as their pattern of glandular distribution (groups of specialised cells that produce secretion to aid lubrication) within the internal anal sphincter (muscles that help the anus to open and close) is similar to humans.

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

For each animal under the first general anaesthesia (GA), 3 ano rectal fistulae will be created using an indwelling seton (a surgical thread), a blood sample and any tissue biopsies may also be taken at this stage, (these will be used to harvest cells and used as part of the therapy). The animal will be allowed to recover.

Approx. 30 days later, under a second GA the fistula will be treated. The seton within the fistula track will be removed, the fistula curetted (i.e. cleaned out) to remove granulation tissue (old scar tissue). A different treatment will be applied to each fistula (based around a combination of different biomaterial and cells). An MRI/CT image may be taken and the animals allowed to recover. Additional MRI/CT images (under GA) may be taken at monthly intervals to assess the healing of the fistula track.

The duration of the treatment stage may vary between 2 -6 months depending upon the healing outcome under investigation. All Animals will be killed at the end of the treatment stage and tissue removed for histological and molecular analysis (i.e. the tissue will be analysed under the microscope and using different laboratory techniques)

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

In our experience of this model, all animals tolerated the procedure well showing little discomfort over the duration of the study. Any pain was transient and was alleviated by pain relief following each

surgical procedure. They did not experience any weight loss or abnormal tissue growth.

Animal suffering will be kept to a minimum by regular monitoring by experienced husbandry staff. Prior to, and following the creation of the fistula and treatment application, animals will placed on a softer mash diet for a approx. 3 days to ensure the production of soft stool and prevent straining. Pigs are normally fed a pelleted diet which is harder.

The use of immuno-suppressive drugs (helps to prevent rejection when cells from one individual are placed into a different individual) may be considered when it is not possible to obtain sufficient number of autologous cells (i.e. cells from the same animal) without impacting on the animal's physiological function.

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 this model is 100% moderate.

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?

This project will address a clinical question i.e. is it possible to heal an ano-rectal fistula similar to that seen in humans using the different therapies proposed. As such, a clinically translatable (a treatment plan which is transferable to humans), relevant large animal model is needed. All work leading up to this stage that could be done either in vitro (in a petri dish or in the lab, specifically is the material cell friendly ie cytotxicity) or in smaller animals (potential material degradation and immune response) has been completed.

The next phase is to assess the combinational therapy (combined cells and biologically derived materials) for its intended clinical application and for this a large animal model is required.

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

A non- animal alternative animal was not considered as we require a large animal model for direct clinical translation.

Why were they not suitable?

We require a large animal model for direct clinical translation and all necessary biocompatibility studies (i.e any toxicity relating to the materials, specifically immune response and material degradation) have already been conducted in rodents.

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?

The experimental design is based on our previous studies (resulting in published data) using this model to assess a commercial collagen paste together with fibroblasts (special cells involved in healing). The previous data will also help to determine the group size, cell dosage, and the amount of biomaterial for each experiment.

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

In order to minimise the number of animals, up to 3 fistulae will be created in each animal with no additional cumulative adverse effect thereby reducing the overall number of animals required. Control fistulae created will receive no treatment, however, based on previous experience/data, controls may be created per study and not per animal (i.e. it is not necessary for there to be a control in every animal).

Biological variability within the animal model will be controlled by using the same sex and similar weight animals and is based on our previously published study and experience.

The use of non-invasive assessment (e.g. MRI imaging), whilst potentially increasing the number of anaesthetics an individual animal has over the course of a study, can significantly increase the amount of information gained per animal and therefore reduce the overall number of animals used.

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

Post-mortem tissue sharing between different projects and collaborators will also ensure maximum usage of each animal.

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.

The pig is the most appropriate animal model with regards to anatomy and physiology when compared to humans for the creation of fistulae of relevant size. In our experience of this experimental design, all animals tolerated the procedure well showing little discomfort over the duration of the study.

Ano-rectal fistulae created in pigs are unlikely to be as debilitating because unlike humans who sit on their “bottom” thereby placing pressure on the ano rectal fistula, pigs predominantly lie on their belly or on their side resulting in less pressure and soreness on the outer surface of the ano-rectal fistula

Additionally, this model allows for the creation of fistulas of comparable size and potentially similar complexity. Whilst this is the best model, we have been able to develop it is recognised that the pig is quadrupedal (stands on 4 legs) while man is bipedal (stands on 2 legs) resulting in different pressure distribution on the fistulae.

Animal suffering will be kept to a minimum by regular monitoring by the Named Animal Care and Welfare Officer (NACWO) and the Named Veterinary Surgeon (NVS) throughout the study. Prior to, and following the creation of the fistula and subsequent treatment, animals will be placed on a mash diet for a minimum of 3 days to ensure the production of soft stool and prevent straining.

All surgical procedures will be performed in line with human clinical practice, whilst the animal is under general anaesthesia (GA), (for animal welfare and practical purposes a GA is necessary). Pain relief will also be given where appropriate to ensure overall animal wellbeing.

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

Less sentient species are not appropriate because the data generated cannot be clinically translated (i.e. studies of fistulae in rabbits or rats would need to be repeated as their physiology is very different).

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

By acclimatising the animals to single housing prior to the initial surgery, any adverse effects or injury to the fistula site from pen mates can be minimised. Animals will be monitored regularly, pain relief administered (if appropriate) and given a mash diet for a minimum of 3 days to ensure the production of soft stool to prevent straining.

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

The use of

- best surgical practice
- adherence to the principles set out in the LASA (Laboratory Animal Science Association) guiding principles document combined with good pre- and intra- operative care and monitoring will minimise unnecessary suffering.
- The Norecopa, NC3Rs and LASA (and similar animal research and welfare) websites

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

We will review

- the current literature (encompassing changes in veterinary research and human surgery) and
- any revisions to the regulatory guidelines along with input from the
    - local Named Information Officer (NIO),
    - Named Animal Care Welfare Officer (NACWO),
    - Named Veterinary Surgeon (NVS) and other local animal care staff.
    - As well as checking the Norecopa, NC3Rs and LASA (and similar animal research and welfare) websites

and implement any changes where appropriate.