Home Office

NON-TECHNICAL SUMMARY

Investigating the Use of Fat Derived Stem Cells to Prevent Leakage of Bowel Joins After Surgery

Project duration

3 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

Anastomotic Leak, Stem Cells, Omentum, Alginate Gel

Animal types	Life stages
Mice	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 project’s aim is to harness the healing capabilities of fat derived regenerative cells (which we have identified in lab-based models) and combine this with a rapid setting gel to develop an implant that will be applied around a join between two ends of bowel to promote healing and prevent leakage. This will help us better understand the healing process at the bowel join line following removal of diseased portions of bowel and build on our lab-based characterisation work in humans and preliminary animal study in a pig model.

A retrospective assessment of these aims will be due by 26 November 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Whenever two ends of bowel are joined together during surgery there is a risk that the joint (anastomosis) may leak due to poor healing. Leakage from the bowel (anastomotic leak) can occur in up to one in eight cases, and is the most feared complication of bowel surgery. The patient becomes unwell and often requires further surgery with the formation of a stoma, which is often permanent. Around one in five patients die because of an anastomotic leak. In patients that survive a leak, there can be long term consequences that impact on quality of life. New treatment strategies are required if we are going to reduce the risk of this serious complication.

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

This research has the potential to bring about a step change in clinical management, reducing the risk of bowel join leak and its consequences for patients undergoing bowel surgery. Success with this model will provide early evidence and help to progress the regenerative cell and gel technology towards first-in-man clinical studies. Regenerative medicine scientists will be targeted through open access publication in high impact scientific journals and presentations at national/international scientific conferences. As the research involves mouse models, it will be of general interest to those working in animal research. We will ensure that the animal work complies with the principles of the 3Rs (replacement, reduction and refinement) and the ARRIVE guidelines provided by the Home Office.

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

Short Term (1-2 Years): The methodologies developed will be of use to researchers in the field of regenerative medicine, providing an alternative source of regenerative cells with optimised protocols for harvesting and application. This will be the first time that ODRC/gel implants have been developed and their efficacy tested in animal models of wound healing.

Long Term (3-5 years): This will open up many other avenues for the use of the technology as a promotor of wound healing at other sites of the body.

A) Patients: Up to a third of patients who suffer an anastomotic leak will die. Patients that do recover from an anastomotic leak experience long-term consequences in terms of increased cancer recurrence, high permanent stoma rates, and poor quality of life. If this research is successful, it is hoped that our new intervention will reduce the huge morbidity and mortality associated with anastomotic leak.

B) Clinicians: Many attempts have been made to reduce the incidence and impact of anastomotic leak through advances in surgical technique and perioperative care. Although some strategies have shown promise pre-clinically, they have failed to demonstrate efficacy when translated to clinical practice. As a consequence, the incidence of anastomotic leak remains stubbornly the same as it was 50 years ago. It is hoped that this research will ultimately provide clinicians with a means to reduce anastomotic leak and improve patient outcomes.

C) Healthcare Providers & Policy Makers: In the UK, approximately 100,000 anastomoses are performed each year for gastrointestinal disease, with leak rates varying between 1%-15% depending on the site of the anastomosis. Assuming an average leak rate of 10%, this equates to around 200,000 avoidable deaths and £250m additional healthcare costs per annum. Fewer anastomotic leaks would see a reduction in length of stay following gastrointestinal surgery and a reduction in re-interventions.

D) Industry Partners / Commercial / Private Sector: Preliminary work characterising human regenerative cells was supported in kind by an industry partner. We will continue to develop this relationship and work with a potential commercialisation partner.

E) Society: General Surgery, which includes gastrointestinal surgery, accounts for the highest activity in the UK, with around 1.3M procedures performed per year surgery. The numbers of operations are expected to increase with the aging population and the rise of certain diseases (e.g. increasing cancer incidence). Inevitably, more gastrointestinal operations will result in a greater absolute number of complications, including anastomotic leak, with an increasing socio-economic impact. Anastomotic leak is one of the most serious and most frequent complications of gastrointestinal surgery. Reducing its

incidence will make surgery safer and ensure that more patients are able to return to full postoperative physical function with resumption of normal lives and contributions to society.

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

We will disseminate the findings to relevant surgical forums through presentations to the Association of Coloproctology of Great Britain and Ireland, The American Society of Colon and Rectal Surgeons, and the Society of Academic and Research Surgeons. We will present the findings at relevant scientific meetings, such as TERMIS (Tissue Engineering) and The Gordon Conference of Regenerative Medicine. The group will undertake various outreach activities to ensure that the research, and the role of the funders, is recognised by the general public. To ensure that the relevance of the work is easily understood, we will use modern forms of communication, such as Visual Abstracts. Engagement with public and patients will be central to the design and conduct of the research to ensure that it remains directly relevant to end-users. We will work with the patient and public involvement groups to disseminate the work to its network of stakeholders. The group is part of a national network of academics, clinicians, industry partners, and public and patient members with an interest in facilitating the pull through of novel devices and technology into clinical practice

Species and numbers of animals expected to be used

- Mice: 83 Male and 83 Female Black 8 week old C57BL-6 mice

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.

Our early data supports the idea that ODRCs may promote healing of bowel anastomoses and represent a novel means for preventing bowel join leak. However, to tes this a definitive preclinical small animal model is required. This cannot be undertaken in pigs due to the numbers and costs involved and the fact that there is no good pig model for anastomotic leak due to the high healing rate. We therefore propose to develop the ODRC/ gel technology and test its safety and effectiveness in an established mouse model of bowel join leak. A recent international consensus statement regarding animal models for research on bowel joins supports the use of a mouse model as the closest to the human scenario. Three models have been developed in the C57BL-6 mouse. Our preference is the model described by Pommergaard et al. (2004), with an leak rate of ~ 40%. This is high enough to allow a demonstrable change and keep the number of animals used as low as possible.

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

Animals will be put to sleep (anaesthetised) and abdomens opened. The omentum will be removed and taken to the laboratory for further tests and will be incorporated into the gel. The bowel join will be made with the animal asleep and the gel conatining cells applied around the join. The whole procedure will

last no longer than 1 hour. The mice will receive analgeisa and fluid through a vein during the procedure and pain relief after. The animal will be allowed to recovery and monitored closely for 7 days before Schedule 1 sacrifice to excise the bowel join for analysis.

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

Recovery from openieng of the abdomen: Pain for 7 days which will be mitigated through regular administration of pain relief.

Abdominal pain caused by large amount of bowel leakage (peritonitis): Pain mitigated through regular administration of pain relief and schediele 1 at the first sign.

Abdominal pain caused by a small amount of bowel leakage (Abscess):Pain for 7 days which will be mitigated through regular administration of pain relief.

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 following severties are expected in the animal model desribed:

Mild: Group Receiving Bowel Joing + Cells + Gel (60-100%) Group Receiving bowel join +/- gel (60%)

Moderate: Group Receiving Bowel Join + Cells + Gel (0-20%) Group Receiving bowel join +/- gel (20%)

Severe: Group Receiving Bowel Join + Cells + Gel (0-20%) Group Receiving bowel join +/- gel (20%)

Non-recovery : Group Receiving Bowel Join + Cells + Gel (0%) Group Receiving bowel join +/- gel (0%)

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 26 November 2024

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

It is only possible to test the safety and efficacy of our ODRC/gel implant in a living biological model. We have previously undertaken simulated experiments using wound healing models in the laboratory and the results suggest that the ODRC/gel implant increases the rate of wound healing. These results now need to be verified in a biological model.

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

We have already tested our ODRC/gel implant in laboratory models of wound healing including wound scratch assays which demonstrated positive results. Following a small pig study (n=4) the next step is to trial the safety and efficacy of the implant in a larger animal study.

Why were they not suitable?

Verification experiments in a living biological model are now required to demonstrate preclinical safety and efficacy prior to first in man clinical trials.

A retrospective assessment of replacement will be due by 26 November 2024

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

Model Establishment: 2 Male and 2 Female Black C57BL-6 mice

Main Study: 81 Male and 81 Female Black C57BL-6 mice. Total = 162 Mice

Assuming a 20% attrition Rate and including model establishement, maximum number = 198

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

We utilised the NC3Rs experimental design assistant to guide the numbers needed to demonstrate safety and effectiveness of the cell / gel technology in a mouse model of bowel join leakage. The study

has been designed online with the up to date ARRIVE guidelines and adhering to the principles of the 3Rs. The ideal study design to evaluate effectiveness is a randomised controlled trial where subjects are randomised to receive one of two treatment options.

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

The model of bowel leakage will be performed on 10 synthetic tube models the same diameter as mouse bowel. This will also be completed on 10 schedule 1 mice. A 4 mouse pilot study will be used to establish the model of anastomotic leak. Tissues will be processed such that analysis can be made in replicate within each mouse. We will also perform an interim analysis after 24 animals to verify the animal model of AL and check the sample size assumptions allowing for adjustment if necessary. The ideal study design to evaluate efficacy is a randomised controlled trial. In line with the 3Rs of animal research the control group will contain two sub groups, gel alone and no treatment, to keep number of mice to a minimum. Randomisation & Blinding: Mice will be randomised using block randomisation through SealedEnvelopeTM at a 1:1 allocation ratio. All mice and tissue samples will be labelled such that researchers assessing the effects of the treatment and analysing the results will be blinded. Control: It is not anticipated that there will be any difference in leak rate between the animals treated in the combined control group. The purpose of including gel alone in the control group is to allow assessment of its integrity analysis. An exploratory subgroup analysis will be performed to ensure no difference exists between these groups.

A retrospective assessment of reduction will be due by 26 November 2024

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

Using the model described by Pommergaard et al. in 2014 we expect 40% of animals in the control groups (no ODRC/gel) to experience bowel leak. Of those that leak we expect half to experience abdominal pain due to a large anastomotic leak (faecal peritotnitis) and undergo schedule 1 within the first 24 hours and the other half to become unwell and undergo schedule one between day 4 and 7 post op. It is believed this model provides the least pain, suffering, distress and lasting harm to the fewest number of animals in order to demonstrate a minimum level of efficacy to move on to first in man clincal trials.

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

The anatomy of less sentient animals does not allow for the formation of a bowel anastomosis. If the mice were terminally anaesthetised we would not be able to measure the healing effects of the intervention at the required post-operative stage.

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

Animals will be reviewed regularly by the research team and staff from the Animal House with the aforementioned pain relief administered to ensure animals are in as little pain as possible. At the first sign of bowel leakage the animals will undergo schedule 1 killing as soon as possible.

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

NC3Rs, ARRIVE and local guidelines will be followed. In addiiton the LASA guidelines on aseptic techniue will be adhered to.

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

We have reviewed the NC3Rs website regularly to ensure that our practice is up to date, for example the recent change to the ARRIVE guideline recommendations. We are also of the local animal house mailing lists which distributes urgent updates which are reviewed on a regular basis.

A retrospective assessment of refinement will be due by 26 November 2024

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?