



Home Office

NON-TECHNICAL SUMMARY

Implantation of seeded scaffolds for tissue/organ development

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

Key words

stem cells, scaffold, organ/tissue, therapy, transplantation

Animal types

Life stages

Sheep

adult, juvenile

Pigs

juvenile, adult

Minipigs

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 create functional tissues/organs using a combination of cells and biomaterials to either repair, replace or regenerate diseased or damaged tissues and/or organs.

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?

To meet the shortage of organs/tissues needed for organ transplantation or tissue repair, both of which are on the increase globally.

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

The outputs from this study will be publishable data which will be disseminated through peer review journals and meetings (we intend to do this after each set of experiments if appropriate).

Additionally, the output may be patentable products (identified once a 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 more than five years (outside the duration of this licence) before being offered clinically.

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

The ability to supply organs or tissues for transplantation, possibly from a xenogeneic source (i.e. non, human), which will not be rejected by the host, will transform the prospects of patients who currently suffer considerable morbidity due to failure of tissue function. This will not only transform the lives and aspirations of the recipients but will also considerably reduce the financial burden on the NHS and Health Care systems.

There are currently 6,079 people waiting for a transplant in the UK and many more who are not on the waiting list would benefit from having a diseased or damaged tissue replaced or repaired.

However, the benefits from our platform technology (cell and biomaterial combination) to create functional organ/tissues are not restricted just to patients and health care providers; there are substantial benefits to the broader scientific and pharmaceutical community. The data generated is vital

for the scientific arena. Both in terms of the bioengineering concepts and in providing basic information on how cells interact with their environment and how their growth and development might be beneficially modified using existing molecular cues present on the extracellular matrix of biologically derived scaffolds.

Additionally, complex perfusion models, whereby the organ's/or tissue's vascular circulatory system are used to repopulate some organ scaffolds prior to implantation, could also be used as miniature validation models. Whilst the 3D culture systems needed to acquire sufficient numbers of different cell populations within a single system may also be used as drug screening platforms.

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

Output (both positive and negative) will be maximised by

- presenting at clinical review meetings to obtain clinically relevant feedback in order to ensure the final approach can be clinically adopted (e.g. is the surgical implantation approach feasible, relevant and appropriate).
- explore collaborative opportunities to better understand how to combine cells and scaffolds ex vivo prior to implantation.
- Present at relevant focused meetings/ international conferences
- Publish in peer reviewed journals appropriate for the topic e.g. F1000Research

Species and numbers of animals expected to be used

- Sheep: 225
- Pigs: 375
- Minipigs: 375

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's anatomy, size, diet, and physiology are similar to humans and is the most appropriate translational transplantation model.

Additionally, as there is potential for xenogeneic implantation for some of the tissues/organs clinically in the future, the donor species needs to be of similar size to humans. Hence pigs and sheep are proposed for most of our studies. Additionally, this is reflected in our de-cellularisation / re-cellularisation technology, which has been directed towards using pig (porcine) tissue/organs to create the initial implantable biologically derived scaffolds.

De-cellularisation is the removal of cells from a tissue or organ, whilst re-cellularisation is the addition of cells to a tissue or organ in which all cells have previously been removed.

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

Animals will fall into two groups; donor or recipient

Donor animals will be used to provide the tissues or organs harvested under **terminal general anaesthesia (GA)** to create the scaffold. Organs (e.g. liver, small bowel/colon, pancreas, trachea/larynx, oesophagus, thymus, or kidneys) will be harvested with their associated vascular supply intact and patent, as this will be used to re-establish a connection to an active blood supply when the tissue scaffold is subsequently re-implanted. Donor animals will also be used to harvest multiple tissue specific cells and tissue biopsies. The harvested organs or tissue will be taken to the lab and undergo a process known as de-cellularisation to create a biological acellular scaffold (ie all the cells will be removed leaving behind a collagen based extra cellular matrix).

Recipient animals under general anaesthesia (GA) will receive either

- a seeded matrix/scaffold - Biological matrices will be developed using the tissue or organs harvested from the donor animals. Each matrix will be seeded with tissue specific cells or progenitors. Cells used to seed the scaffold may be autologous (from the same animal) or allogenic (different animal but same species). If Autologous, animals will undergo GA one month prior to receiving a scaffold at which point the appropriate cells/ tissue biopsies will be harvested (as not to physically compromise the health and wellbeing of the animals). These seeded matrices will be implanted (up to 6 months) without comprising the tissue or organ and will provide information on how the matrices integrates with the surrounding tissue and cellular fate on a smaller scale.
- a seeded organ/tissue scaffold (i.e. intact organ scaffolds) which will be implanted in situ (i.e. tissue and site specific with connected vasculature). Cells used to seed the scaffold may be autologous or allogenic. If Autologous, animals will undergo GA one month prior to receiving a scaffold at which point the appropriate cells/ tissue biopsies will be harvested (as not to physically compromise the health and wellbeing of the animals). Implanted scaffolds will be left in situ for up to 2 years and assessed for tissue/organ regeneration and integration. During this period they will be monitored in terms of potential functionality using both imaging (e.g. endoscopy, bronchoscopy, MRI/CT/PET) and non imaging techniques (e.g. blood tests and functional assays).

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

Adverse effects will be different for each organ or tissue under investigation.

For **donor animals**, we expect no adverse effects as tissues/ organs will be harvested under a non-recovery terminal GA.

For **recipient animals receiving a seeded matrices**, we expect no clinical adverse effects other than the surgical procedure for which they will be given analgesia. Animal suffering will be kept to a

minimum by regular monitoring by experienced husbandry staff.

For **recipient animals receiving a seeded scaffold**, we expect the adverse effects to be influenced by the organ/tissue being replaced. For example, animals receiving a tracheal tissue scaffold would require greater monitoring and post-operative care than animals receiving a bowel segment in a two-step procedure. In each case, and for each organ, we will have a clinical post-operative care plan based on human clinical care criteria (e.g. blood analysis) and parameters to monitor and maintain overall animal health.

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)?

Pig Moderate 100%

Sheep Moderate 100%

Mini pig Moderate 100%

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 focus of this research is geared toward using animal tissues or organs to create scaffolds that are identical to the tissue/organ that needs to be replaced in human patients. By using animal tissue/organs to produce the scaffolds, we will not compete with human tissue destined for transplantation which is already in short supply.

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.

Substantial initial work using rodents for biocompatibility, degradation and immune response to the biological scaffolds has already been undertaken. In vitro cell culture work to assess cell-scaffold interaction has been completed. Additionally, in some cases, complex ex vivo perfusion systems and

bioreactors have been used to determine how best to deliver cells to scaffold using its existing associated vasculature before in life implantation studies.

Why were they not suitable?

We require a large animal model for direct clinical translation, especially as the experiments have now developed to the point where we need to show function and efficacy in a full physiological model, and for that, there is no substitute for the whole animal.

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?

As our in vivo (in life) studies will aim to establish function of the seeded scaffolds and tissues, they are observational rather than statistically driven which means we will be able to use very few animals to prove function (for example, typically no more than 6 per tissue/organ per experiment- based on previously published and peer reviewed studies and following discussion with the Regulatory Bodies (e.g. MHRA) before progressing to regulated studies.

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

By assessing cell-scaffold interaction in vitro, using perfusion and bioreactor systems and conducting as much of the pre-implantation logistics on the bench by conducting detailed pilot studies, we have reduced the number of animals used. By sampling/ harvesting seeded scaffolds at multiple time point over the course of the study fewer animals will be required.

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. Additionally, by conducting initial studies with seeded matrices before progressing to seeded organs, we can make iterative changes to ensure the successful outcome of the implanted seeded organs. Thus helping to limit the number of animals used.

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 organ transplantation and tissue repair.

Sheep may, under certain circumstances be a better anatomical model for tracheal/laryngeal implantation.

Minipigs provide an opportunity for implanting seeded organs when assessing for long term paediatric development.

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

These experiments have now developed to the point where we need to show function and efficacy in a physiological translational model.

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 (including increased handling and interaction, accompanied by enriched environment), any adverse effects or injury to the wound site from pen mates can be minimised. Post surgery, if necessary animals will continue to be single housed but in sight of litter mates. Animals will be monitored regularly, pain relief administered (if appropriate). Detailed post-operative clinical care plans will be developed to ensure the highest standard of animal welfare.

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

The use of

- best surgical and human clinical care practice associated with organ/tissue repair and transplantation and subsequent monitoring practices
- 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, PREPARE guidelines, 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, (e.g. transplantation 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.
- LARN webinars