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NON-TECHNICAL SUMMARY

Mechanisms influencing mammalian digit tip regeneration.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Regeneration, Digit Tip, Blastema, Stem Cells, Mammalian

Animal types	Life stages
Mice	neonate, adult, embryo, juvenile, pregnant

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 overall aim is to identify the signals in the environment that are important for successful regrowth of the tips of fingers and toes (called digits) that have been amputated in mammals and to understand how cells in the digit respond to those signals. I aim to learn if we can use this information to promote regeneration in tissues where it doesn’t normally occur.

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?

There are over 25,000 people in England currently living with limb amputations mainly as a result of illnesses such as diabetes or traumatic injury. If you cut off a person’s arm, it won’t grow back. Instead, the wound will heal and you will be left with a scar. Surprisingly, however, removing the end portion of a person’s fingertip does not scar. It will completely regrow all of the missing tissue. Understanding why these two different responses occur in a human could help provide insight into how we can regrow other parts of the body.

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

The main aim of this project is to study how cells in the mouse digit tip respond to injury. We are trying to understand how these cells are able to regrow the digit tip perfectly in order to uncover the basic principles that guide successful regeneration. The results of this study will help us understand how some tissues heal without scarring and will help other scientists working in the regenerative medicine field.

This project will likely result in new information and publications about the following:

1. Understanding how cells in the mouse digit tip communicate with each other and the cells that surround them (tissue regeneration). The mouse digit tip, which regrows upon amputation, is a powerful system for investigating how tissue repair and regeneration works. This process is similar to human fingertip regeneration and functions as an excellent substitute. In both mice and people, digit tip regrowth depends on how much of the finger or toe is removed. If you remove more than 60% of the end of the digit, it will fail to regrow. This is because a key structure made up of a clump of cells capable of growth fails to form. This key structure is called a blastema and it contains all the cells that will regrow the missing parts of the finger or toe. This project will aim to understand which growth factors or molecules in the injured digit tip environment are important for telling cells to form the blastema, and where these signals come from.

2. Understanding how the DNA is modified (epigenetic changes) during mouse digit tip regeneration (development & tissue regeneration). Most vertebrate embryos have the ability to regenerate their limbs. After birth, as animals and humans mature, this ability is gradually lost. There is, however, one exception to this rule. In both mature rodents and humans, regeneration occurs when the end of the digit tip is removed. One reason for this remaining regenerative capacity may be due to changes to the DNA, called epigenetic modifications, that allow genes to be turned "on" or "off". This project will aim to understand the epigenetic modifications that occur in response to injury and how these differ from early embryonic development.

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

One challenge for scientists is to take the knowledge that they discover and make it into therapies that benefit people. The first step in this process is to identify which factors promote regeneration in mammals. Then we need to determine if we can add these factors to the injury or help the person to produce them naturally so that their body will regenerate. This study will aim to answer these important questions. *In the short term,* new information that comes from this study will be presented through publications and conference presentations, or shared with organizations such as Diabetes UK. This will mostly benefit the scientific community. *In the long term,* this information could be used to develop therapies to improve repair and regeneration in human tissues. This research is expected to provide targets for use in regenerative medicine. These targets may be important for people who suffer with diabetes mellitus (Type 1 diabetes) who need to have their fingers/toes and/or limbs removed because their illness was not managed well.

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

To gain the most from this work, we will do the following:

- We will share all experiments and results with the scientific community, including both successful approaches and unsuccessful approaches. This information will be shared by publishing it, presenting at scientific conferences and giving workshops.
- We will share the results of this project with the public. This will allow people who are interested to learn about the research that is being carried out on tissue repair and regeneration.
- We will share results from this work before or at the same time that we send them to be published. This will make sure that the work is available to other scientists sooner and will help other laboratories avoid repeating the same work unnecessarily.
- We will work together with other scientists and/or research and development companies. We will share knowledge, skills and techniques that may result in exciting scientific discoveries.

Species and numbers of animals expected to be used

- Mice: 17332

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.

In this study we are using mice to study tissue regeneration. This is because the mouse digit tip and the human fingertip are very similar. Both digit tips are made of skin, nerves, blood vessels and bones. Both naturally regrow the tip when injured as well. The things that they have in common make the mouse digit tip an excellent replacement to studying regeneration in humans as it makes us confident that any results we get will also be useful for people. Additionally, all the genetic tools that we need to help us understand how regeneration works are already available in mice. Our first experiments will use cells of interest to us taken from baby mice (less than 14 days old) that will be grown in the laboratory. This allows us to reduce the number of animals we need because we can increase the number of cells we have by growing them. Most of the animal experiments will use adult mice (more than 8 weeks old) however as we need to understand why many animals have the ability to regenerate tissues when younger but in adults this ability is limited. By studying this process of regeneration in adult tissue we can ensure that all of our findings will have the widest clinical application.

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

In order to study digit tip regeneration, animals will normally have a surgical procedure. During this procedure we will do one of two things. We will remove 1-2 mm of the end of the digit, which will fully grow back. In a human this would be the same as removing the tip of your finger starting from midway through your nail. Otherwise, we will remove 3-4 mm of the end of the digit. This type of injury will not grow back but will heal and the mouse will have a shorter digit. In a human this would be the same as removing the tip of your finger up until the first joint. A small number of animals will also be injected with various substances that will help discover at the cellular level what is happening during regeneration. Animals are subsequently humanely killed and tissue samples taken after death.

To study what certain cells are doing during the process of digit tip regrowth, we will make, breed and use genetically modified animals in some experiments. These animals will receive injection of substances which will either make cells in the digit tip glow, delete a gene in cells affecting their function or make a small number of cells die. In all cases, the mice will be killed humanely and the tissue samples taken after death. We are then able to study what effect this had on digit regrowth under the microscope.

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

Most of the mice used in this project are not expected to display any harmful changes that affect the animal's day to day life. Amputation surgeries will only be performed on the middle three digits of one or both feet in order to make sure the injury does not affect the animal's ability to move and groom itself. Surgeries will only be carried out on hindlimbs (feet). The forelimbs (hands) of the mice will not be touched. All surgeries are carried out under anaesthesia and animals receive pain medication that will remove any discomfort during the surgery and in the recovery period. Mice normally recover from surgeries within 10 - 15 minutes and display normal behaviour. When baby mice receive surgery, immediately afterwards, they are returned with their brothers and sisters to their mother. The mother has always been observed to welcome her babies back. Mice that had 3-4 mm of the tip of the digit removed, in which the digit doesn't regrow, will display a small amount of swelling in the digit. This appears at 7 - 14 days post amputation and will naturally disappear following this period. This swelling has not been observed to cause the mouse distress or impact their normal behaviour. Genetically altered mice that are given the drug, tamoxifen, may lose up to 10% of their weight. This sometimes occurs during the first 1 - 2 days that they are given tamoxifen. These mice normally gain the weight back to normal in the next 5 days and do not show any other harmful side effects.

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)?

This project will use mice. 44% of the protocols are classified as mild and 56% of the protocols are classified as moderate.

What will happen to animals at the end of 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?

The digit tip is a complex structure being comprised of many different cell types. When the digit is injured, it needs to be able to tell all these different cells to regrow a toe over several months. These cells also need to respond to instructions coming from the surrounding cells which tell them where to go and what to do during the regeneration process. It is therefore important to study this process in a whole organism because the response of the different cell types depends on which cells surround them and what instructions they receive from these cells. Today, there are few models that can imitate fingertip or toe regeneration in the laboratory. Those that do, first require the researcher to obtain cells directly from the animal. Additionally, most techniques that work with cells outside of living animals do not fully reflect their normal behaviour over long periods.

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

In recent years scientists have developed a new way of growing (culturing) cells in 3 dimensions. They allow scientists to carry out their research without using animals. This is because they can be made using human or mouse stem cells. They are also very exciting for researchers to use because the cells mimic the structure of a small organ and can be grown for long periods of time. These cultures are called "organoids". So far, scientists have been able to make "organoids" that resemble the brain, kidney, lung, intestine, stomach and liver.

Why were they not suitable?

Despite the excitement around this new way of growing cells in three dimensions, scientists have not yet developed an "organoid" that models the human digit tip. Current "organoid" cultures cannot be grown together with supporting blood vessels and immune cells (cells that keep our body safe from infection) as well. This technology, therefore, cannot be used to model how the environment communicates with and directs the cells of the digit tip to regenerate.

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 predicted number of animals needed for this project has been based on the following:

1. Animal numbers have been worked out by talking with a trained statistician. This is to make sure experiments are designed properly and have also been based on the number of studies that we expect to carry out each year. It is important to make sure that experiments use the proper number of mice so that the results of the experiments are accurate.

2. We have asked for advice from experienced researchers to help us work out the number of genetically altered mice needed. We have also predicted the number of animals needed from our own past experience. We have carefully considered the best way to make sure we keep the lowest number of different types (strains) of mice for breeding. At the same time, we have also made sure that we have enough mice to use for experiments.

3. We have considered the type (strain) of mice that we are working with to estimate the number of young animals that we can expect each time they give birth. For example, mice that are brown normally give birth to 6 - 8 animals. However, white mice give birth to 10 - 15 mice normally. Animal numbers will also be reduced because we will be buying in a large number of them. By doing this, we can control how many mice we have and will not waste any extra that are born and cannot be used.

4. Test experiments, called pilot experiments, will be used to calculate the amount of a substance that we can safely inject into the animal. It is important to find the smallest amount of the substance that has an effect but that won't harm the animal in anyway. This will then allow us to perform the intended experiment using enough mice to be sure that the results we obtain are right.

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

To work out the number of animals required for each experiment we used the NC3R's Experimental Design Assistant. This makes sure that the number of animals used in this study will produce data that is reliable and accurate. We have also taken the advice received from a local, qualified statistician. This will make sure that each experiment includes methods of reducing the chance that we unfairly interpret our data. We will also use a type of mathematics, called statistics, to help us calculate the chance that our ideas, called a hypothesis, are correct. Where possible, all experiments are designed to get the most information using the least number of animals possible. For example, we can give the digits on one side of the mouse the treatment and digits on the other side of the mouse can be given the control. Additionally, all experiments were designed taking into account the PREPARE guidelines (a document that gives scientists advice on how to plan animal research and experiments).

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

In order to use the smallest number of animals that will give us useful results, we will do the following:

- We will try and breed our genetically modified mice in a way so that none are wasted. Any types (strains) of genetically modified mice that are not being used for scientific study will be frozen as embryos.
- Where possible, we will do test (pilot) experiments. For example, when working with substances that stop cell function, we want to use an amount that will have an effect. These test experiments will help us to work out how much this amount is but will only use a small number of animals. We will only work with substances that we know. These substances will also have been used in the past for experiments and will have been shown to have an effect before when other scientists used them.
- We will try to work with other scientists and share tissue from the mice that we have removed the digits from. This will reduce the need to breed more mice.
- We will keep up to date with any new techniques that allow us to replace the experiments where we use animals with different experiments that do not use these.

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.

This project will use mice to study the naturally occurring process of digit tip regrowth. The procedure of digit tip amputation will be carried out in two different ways. Amputations that only remove 1-2 mm of the tip of the toe will result in regrowth of the digit to its original shape after approximately 30 days. Alternatively, amputations that remove 3 – 4 mm of the tip of the toe will result in wound healing and scar formation. Additionally, digit tip amputations in mice only affect the skin, the very tip of the bone, minor nerves and nail. This injury does not damage muscles, tendons, glands or joints. Because these amputations only affect a small amount of tissue, the digit is able to heal quickly. It does not affect the ability of the animal to move or it's day to day activities. This project will also use genetically altered mice. Using these animals, we are able to follow cells or kill cells that we are interested in. These genetically altered mice have been used by other researchers before. They are not expected to show any harmful clinical signs.

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

The mouse is a good system for studying naturally occurring regeneration. The process of mouse digit tip regrowth mimics human fingertip regeneration. In this project, most of the time we will be studying adult mice. The reason for this is that many animals have some ability to regenerate when they are young. When the animals reach maturity however, this ability is lost. In mice, the end of the digit tip is able to regenerate throughout the mouse's life. This also occurs in humans and we would like to understand why this is so. Mice were also chosen for this study as this is the only type of animal in which all of the genetic tools needed are available. Regeneration in classes of animals that are less aware (sentient) such as amphibians,, fish fish or worms have been shown to act through different mechanisms to mammals. These animals can produce and use specialised proteins which mammals can't make.

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

All methods in this project will use techniques that reduce animal stress and make sure the animal does not suffer as detailed below:

- We will make sure that animals suffer as little as possible during surgical procedures by giving the animals medication to manage their pain. All digit tip amputation surgeries and injections into the digit tip will use anaesthesia. Pain medication will be given before the surgery and where necessary after the surgery. We will talk with the Named Veterinary Surgeon to make sure we do this correctly.

- We will make sure to watch the animals before and after surgery to make sure they are healthy and not distressed. We will record what we see on a chart. If the animal needs anything such as more pain medication, we can give this to them. We will always talk with a Named Veterinary Surgeon and get advice if the animal shows any signs that are outside its normal behaviour.

- Surgery will be carried out in a clean manner (using aseptic technique). We will make sure to meet the level set out in the Home Office Minimum Standards for Aseptic Surgery and the LASTA Guidance on Preparing for and Undertaking Aseptic Surgery (2017).

- We will make sure to use the best care methods to improve the quality of life for the animals. Mice will be placed in cages with other mice that they get along with. The mice will be given enough bedding material and at least one shelter (e.g. cardboard tube or plastic house). This will encourage the mice to explore and give them a refuge so they can escape the other mice when they want to.

- We will make sure to kill any animals before they suffer too much. This is called a Humane Endpoint. We are not expecting animals that undergo any procedure in this licence to suffer very much or for very long. If an animal does begin to look unhealthy, we will look at it and decide if it needs to be killed. If the problems cannot be relieved with pain medication or other methods, the animal will be killed.

- Some substances have to be given to animals by specific routes. We have therefore asked to use several different routes (e.g. intraperitoneal [injection into the abdomen], oral and subcutaneous [injection under the skin]) in this project. We will always use the least harmful route possible to give an animal a substance, which is normally under the skin (subcutaneous). This is to make sure we cause the smallest amount of discomfort or pain to the animal.

- All animals that are brought into the animal facility will be allowed at least 7 days to get used to their surroundings. This process is called acclimatisation. We will also allow them to get used to their handlers prior to use. This will reduce the amount of stress the animal experiences and will improve their well-being.

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

To make sure experiments are done in the best way, we will follow the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. For surgical procedures we will follow the Laboratory Animal Science Association (LASA) Guidance on Preparing for and Undertaking Aseptic Surgery (2017) and the Home Office Minimum Standards of Aseptic Surgery. For the breeding of genetically altered mice, we will follow the guidelines provided by the Home Office and the NC3Rs Resources on 'Genetically altered mice' detailed in:

- https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf

- https://www.nc3rs.org.uk/GAmice

Additionally, for making sure that chemical inhibitors or ligands are given in the best way, we will refer to the following resources:

- *"Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare". Morton DB et al. Lab Anim. 2001 Jan;35(1):1-41*

- *"Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider". Turner PV et al. J Am Assoc Lab Anim Sci. 2011 50(5): 600–613*

- *http://www.procedureswithcare.org.uk/lasa_administration.pdf*

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

In order to stay up to date about advances in the 3Rs, I will use the following methods:

- I will make sure that I have regular talks with the Named Persons and animal technicians at my Institute. This will help me to go over current approaches and remain informed if there are any new 3R opportunities.
- Sign up for the NC3Rs e-newsletter. This is where information on the latest NC3R publications is promoted. I will also attend NC3Rs events or workshops.
- I will regularly use our Institutional 3Rs search tool. This contains a database of information that is frequently updated about ways to reduce or replace the number of animals we use in experiments. It also contains information about the best ways to perform experiments that will reduce stress on the animal.
- I will regularly search published literature to stay informed about the latest techniques and approaches that may be used that will allow me to replace, reduce or refine my experiments that use animals.

To make sure that I can use any new techniques properly, I will collaborate with other scientists or staff members that already know these new techniques. This will make sure that all scientists working under this licence receive proper training and are qualified in the new procedure. Test studies will be performed for any refined methods to make sure these new methods do not cause the animal any more pain, suffering or distress. If any of these new methods occurs in a procedure that requires authorization (regulated procedure), permission will be sought before any experimental work is started.