Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 25

Projects with a primary purpose of: Translational and Applied Research – Human Sensory Organ Disorders – skin, eyes and ears

Project Titles and keywords

1. **Small animal models of wound infections**
    - Wound, infection, therapy, intelligent-dressing

2. **Transplantation of epithelial cells for LSCD treatment**
    - Limbal stem cell deficiency (LSCD), rabbit, stem cells, corneal opacity, impression cytology

3. **Healing Mechanisms of Thermal Injury Wounds**
    - Healing, thermal wounds, skin grafts

4. **Regenerative therapies for skin**
    - skin, regeneration, wound healing

5. **Molecular control of skin development, regeneration, ageing and carcinogenesis**
    - Skin, Hair growth, Wound healing, Cancer, Ageing

6. **Cellular, Drug and Gene Based Therapy for Eye Disease**
    - Rodent, AMD, Vision, Retina, Therapeutics

7. **Stem cell therapies for retinal degeneration**
    - Retina, regeneration, stem cells, therapy, blindness

8. **The role of Rab GTPases in disease**
    - Eye, eye disease, gene therapy, aging

9. **Mechanisms of Intraocular Pressure Regulation**
    - Glaucoma, intraocular pressure, blindness

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<tr><td>Project 1</td><td colspan="2">Small animal models of wound infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Wound, infection, therapy, intelligent-dressing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td></td><td>Basic research</td></tr>
<tr><td>✓</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
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<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">The proposed studies aim to explore the processes of wound infection and identify methods that will limit the infection, by identifying pathogenic microorganisms at an earlier stage of infection or by improving treatment of wound pathogens. A number of possible interventions will be evaluated as part of future studies; e.g.

i) intelligent wound dressings which sense the presence of pathogenic microorganisms and/or toxins in the wound and provide a visible indication of the infection without removing the dressing.

ii) wound dressings impregnated with antimicrobial agents and/or agents that will promote re-epithelialisation and wound closure.

iii) novel topical therapies that might be applied to wounds to treat infections either therapeutically or as prophylaxis.

Whilst a considerable amount of work can be performed using biofilms generated in vitro the true</td></tr>
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	efficacy and clinical relevance of a particular dressing or strategy can only be assessed using an animal model where sufficient numbers can be used to generate statistically significant data and where a lesion of standard dimensions and produced under controlled conditions will allow direct comparison between treatment groups in a physiologically relevant situation. Furthermore a controlled system using a defined microbial challenge against a background of natural healing is essential to assess the predictive value of intelligent dressings in detecting potentially life-threatening infection.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In clinical groups, such as burns patients, reducing the number of times a wound dressing is replaced limits the amount of scarring and helps promote wound healing. This may be particularly important in paediatric populations, where infections are often life threatening and clinicians are currently not able to clearly identify infected wounds; hence dressing may be changed on a precautionary basis. There are also major concerns about the treatment of chronic wounds, notably diabetic foot ulcers, where there are limited treatment options and significant mortality and morbidity associated with infections. Current management of chronic wounds depends on debridement of the wound, the use of broad spectrum antibiotics (often over a long term) and dressings which may contain additional antimicrobial agents. There is speculation that this is already a contributor to the emergence of drug resistance in bacteria that colonise these wounds This explosion of antibiotic resistant microorganisms, includes nearly all of the most common pathogens found in chronic wounds, and means that broad spectrum antibiotics are no longer effective in many cases. There is also little evidence that current antimicrobial dressings, notably those containing impregnated silver compounds, are effective. As such there is an urgent need to develop improved treatments and therapeutic/prophylactic dressings to limit bacterial growth and promote healing to improve

	clinical management of chronic wounds.
What species and approximate numbers of animals do you expect to use over what period of time?	The number of animals used will vary according to the type of evaluation required but in every case statistical advice will be taken on the minimum number of animals that will give significant, meaningful results. Typical studies will involve each prototype dressing being tested on groups of 10 mice over a 14 day (up to 28 day) study period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Experience of skin biopsy with other species at this establishment indicates that punch biopsies are well tolerated with no signs of distress, inflammation or infection and that group housing could be maintained. The mice are likely to experience some mild discomfort from the wound which will be minimised by analgesic and anaesthetic regimes. There is small risk (1-2% deaths) of adverse effects associated with the use of general anaesthesia. The generation of diabetes in mice could lead to the development of polydipsia and polyuria, whilst streptozotocin (STZ) is potentially toxic. An established low dose STZ regime will be administered over 5 days to minimise the risks of acute toxicity and adverse effects. The challenge model establishes a local wound infection and there is a low probability that the infection becomes systemic. Well established clinical scoring systems will be employed that allow early intervention to avoid undue suffering and progression to severe disease. At the end of the study all animals will be killed by a Schedule 1 method or by exsanguination under terminal anaesthesia
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst a considerable amount of work can be performed using biofilms generated *in vitro* the true efficacy and clinical relevance of a particular dressing or treatment strategy can only be assessed using an animal model where sufficient numbers can be used to generate statistically significant data and where a lesion of standard dimensions and produced under controlled conditions will allow direct comparison

	between treatment groups in a physiologically relevant situation. Furthermore a controlled system using a defined microbial challenge against a background of natural healing is essential to assess the predictive value of intelligent dressings in detecting potentially life-threatening infection and in the development and evaluation of novel therapies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will vary according to the type of evaluation required but in every case during the planning stage statistical advice will be taken on the minimum number of animals that will give significant, meaningful results. The Establishment has access to bio-statistical advice and extensive experience in establishing new animal models. Initial work on the project will also lays the foundations to be able to move to imaging infections directly in mice, which will allow future studies to significantly reduce group sizes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	There are a number of mouse models that are well characterised and these have featured in an extensive range of publications in the field. The procedures to be used in the study cause self-limiting infections with the efficacy of measures assessed by measuring the time taken to induce a measurable response indicative of infection, assessing numbers of bacteria in a wound or its diameter. The models are specifically chosen to minimise the severity, whilst using systems that are highly relevant to the clinical setting. In all cases robust schedules of clinical monitoring will be used based on extensive experience of animal models over a range of species such that humane end-points are clearly established to minimise suffering. All animals will be housed in social groups with enrichment provided as appropriate.

Project 2	Transplantation of epithelial cells for LSCD treatment	
Key Words (max. 5 words)	Limbal stem cell deficiency (LSCD), rabbit, stem cells, corneal opacity, impression cytology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Chemical and thermal burns to the eyes are serious emergency that often results in permanent visual impairment and persistent eye irritation/pain. Our work aims to develop clear, corneal epithelial transplants which are patient specific and will not undergo immune rejection. These should be affordable and easily transplantable. The second aim is to ensure that such transplants work well and restore vision in patients with chemical and thermal burns. The epithelial transplants we are developing are of novel nature as they are derived from autologous sources of stem cells that have not been tested previously in humans. It is for this reason that we need to test how they settle and function into an animal model suffering from corneal blindness similar to that observed in humans. This is essential work to ensure that our method has a high chance of success when applied to human patients. The key objectives of this work are:	

	1. To create and validate a suitable model of unilateral LSCD in rabbits 2. To investigate the impacts of immunosuppression on the welfare of rabbit LSCD model 3. To use this chronic model of corneal damage to establish the safety and efficacy of the stem cell derived transplant that is being prepared for human clinical application
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The immediate impact of our project lies in the generation of novel and functional autologous corneal epithelial transplants from stem cells. Stem cells are primitive cells that have the ability to generate various cells found in all of our adult organs. We can test these stem cell transplants using laboratory tests that don't involve animals to select the graft that is most similar to human corneas. However, these tests cannot confirm the ability of our corneal epithelial transplants to reverse corneal opacity, pain and vision incapacity and for this reason we need to perform safety and efficacy studies in animal models such as the rabbit model chosen herein. At the end of our project, we will have found out which stem cell transplants are the safer to transplant and most efficient for restoring vision in patients with chemical and thermal burns. This information will help the scientists and clinicians to develop safe and efficacious procedures for using this graft in humans. The ultimate impact of our project will be to cure a significant proportion of world blindness caused by corneal damage. The provision of human patient specific corneal epithelial transplants will overcome two of the major problems, namely lack of tissue for transplantation and immune rejection of the transplant. Consequently, this will reduce the health care burden by lowering the cost and frequency of transplantation, eliminating the need for drugs that supress the patient’s immune system, and providing a safe, permanent and effective treatment that is readily available to all corneal damage patients If funded, this proposal will change the quality of life of 40,000 people awaiting a corneal transplant every year in Europe, who would otherwise be blind, unable to work and in need of considerable social and

	personal support; with potential to treat many more patients in the future worldwide. A project like this can provide Net Lifetime Benefit (NLB) of €92,657 per patient or a Total NLB of nearly €3,706 Billion in Europe only. Furthermore, it reduces the burden of lost years of life (Disability Adjusted Life Years (DALYs) for severe visual impairment that is estimated at 44%, meaning that one year with visual impairment is equivalent to the loss of 23 weeks of life in good health.
What species and approximate numbers of animals do you expect to use over what period of time?	White rabbits, approximately 90 rabbits over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Rabbits will be used to test the effectiveness of the corneal transplants by inducing corneal injury followed by transplantation of one of the transplants we develop. Rabbits may have an adverse reaction to general anaesthesia; in this case the affected rabbits will not proceed to surgery. Rabbit may also experience moderate to severe pain in the first 24 hours lowering subsequently as the front of the eye becomes covered with a new cell layer. We will use effective and systemic analgesia for severe pain and local analgesics for moderate pain. The pain will be monitored using the rabbit grimace scale. There are also expected effects of the clinical model itself, such as eyelid swelling, redness, inflammation and photophobia; however deeper stromal complications and or ocular surface infections are not expected. These clinically associated effects will be monitored 2-3 times per day in the first 48 hours, daily for the first week and weekly afterwards using a welfare report. Application of immunosuppression can also lead to reduced weight and decreased protein plasma levels which is they key indicator for nephrotoxicity. Reduction of body weight more than 10% and protein plasma levels by more than 20% will be considered humane end points and rabbits will be removed from the study by euthanasia. Eye related post op infections are rare events (< 3%). Risk of infection will also be minimized by using sterile instruments under

	aseptic conditions and topical antibiotics for the first week after the surgery. In view of these adverse effects we have assign the level of severity as potentially severe. We have designed several humane end points for each of the adverse effects outlined above and when these are reached the rabbits will be removed from the experiments using euthanasia. The rest of the rabbits will be sacrificed at 4 months and their corneas will be analysed for engraftment of human cells using methods that are well established in our labs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our current work is focusing on generating corneal epithelial stem cells from a variety of stem cells including human embryonic and induced pluripotent stem cells as well as hair follicle cells. We have tested a large number of protocols over the last four years to enable generation of cells that are as similar as possible to the corneal cells found *in vivo*. Hence we are confident that we can generate corneal like epithelial cells with high efficiency from each of the stem cell types outlined above. Notwithstanding this, we are unable to test how these cells will settle in an injured cornea and how they will function to enable reepithelialisation of cornea and enable restoration of vision in LSCD patients in the absence of an in vivo model. Furthermore, because of the nature of LSCD in humans, we need a model system that replicates key features of human LSCD. These include growth of blood vessels and opaque tissue onto the surface of the cornea from the nearby located tissue known as conjunctiva. Both of these features prevent corneal transparency and transmission of light from cornea to the back of the eye (retina) which is responsible for our vision. Reversal of blood vessel and opaque tissue growth onto the centre of the cornea is a key indicator which we use to evaluate the success of our stem cell transplants. However, none of these features can be achieved with a lab model and for this reason creation of rabbit model which replicates

	these two key features of LSCD is necessary.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to meet the objectives of this research all attempts have been made to reduce the number of animals and procedures to minimize suffering and yet achieve clinical and statistical significance. For all the new procedures we are going to establish, 1-3 rabbits will be used to enable testing and gaining of skills and experience before moving on with a larger rabbit cohort. We will use expert statistical advice and calculations to ensure we only use the minimum number of animals necessary to achieve the scientific results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rabbits have been chosen for this study because of the similarity in eye size to humans and their wide use in this type of study which enables comparison of our results to published literature. The protocols have been carefully designed to keep suffering to a minimum. Where invasive procedures will be carried out, appropriate anaesthetics and analgesics will be administered. Any loss of condition (weight loss, drowsiness, post-operative inappetance, anaesthetic complications) will indicate removal from the procedure and humane killing by a Schedule 1 method. The use of topical antibiotic medicine after invasive procedures will reduce adventitious infection, and the regular use of analgesics will reduce post-operative inflammation and discomfort/pain and in addition will also reduce the likelihood of transplant rejection.

Project 3	**Healing Mechanisms of Thermal Injury Wounds**	
Key Words (max. 5 words)	Healing, thermal wounds, skin grafts	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Thermal injury is a common cause of injury worldwide, particularly in children, with devastating lifelong consequences. Majority of wound healing research has been done on non-thermal, open and closed wounds to date. Little is known about thermal wound healing. The purpose of this project is to identify key mechanisms that can be altered to reduce scar formation including abnormal pigmentation and/or accelerate healing of a thermal injury (burn).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will discover mechanisms and mediators of healing of thermal wounds and may allow identification of novel drugs/factors that could be used to speed healing, reduce scarring and normalise pigmentation in man and animals. The results of this work will be of interest to both scientists and clinicians.	
What species and approximate numbers of animals do you expect to use over what period of time?	300 pigs over the duration of the project license.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will create thermal and/or open wounds on the backs of the animals to study the rate of wound healing and function of resulting “new skin”. We will investigate effects of novel treatments/dressings on wound healing and ‘new skin” formation including the pigmentation. The procedure can cause post-surgical pain of mild to moderate severity which will be controlled with pain killers. The animals will be humanely euthanized at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Open or thermal wounds require cells from the circulating blood to interact with the local cells of the skin to allow complete healing of the wound and formation of the “new skin”. We can study some aspects of wound healing such as re-epithelialisation (healing of the top layer of the skin) in ex-vivo models which we are currently developing for thermal wounds. However, in order to study the full spectrum of wound healing i.e. development of the “new skin” after open/thermal wounds requires animal studies with blood circulation and there are no alternative models available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The block design of the experiments has been agreed with the statistician to ensure that minimum numbers of animals are used. Furthermore, we will use the same animals undergoing characterisation of wound healing for testing novel bioimaging techniques to gain maximum information from fewer animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The pig has been chosen as its skin has similar anatomy and physiology to human skin. Pigmentation in the pig skin, unlike the rat or mice, is akin to the human skin in that the pigment cells are present in the hair follicles and in the epidermis and both play a role in pigmentation. Mice and rats are loose-skinned with a panniculus carnosus and heal mainly by contraction that is much more rapid than by reepithelialisation seen in ‘tight-skinned’ mammals such as humans and pigs. Our previous studies on human thermal wound

	samples have confirmed similarities with the pig thermal wounds. The animals will be given pain relief during the procedure to ensure minimal or no pain is felt after recovering from the anaesthetic. They will be observed closely thereafter and further pain relief administered as necessary. From our previous studies, we have now refined the technique of surgical procedure and application of dressings and refined the dressings used, thereby reducing the frequency and the duration of general anaesthesia for the animals.

Project 4	Regenerative therapies for skin
Key Words	skin, regeneration, wound healing
Expected duration of the project	0 year(s) 8 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(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 aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

We aim to develop new treatments that increase tissue regeneration after a skin injury, or in injuries that often don't heal properly. Specifically aim to:

1. Find out if molecules that stimulate something called the 'Wnt signalling pathway' work when injected in the skin of mice
2. Find out if these molecules help speed up wound healing rate, the quality of skin repair. and hair follicle regeneration following skin wounding
3. Work out the correct dosage and method most suitable for doing this in mice, and whether wound healing can be promoted by other drugs or molecules in combination
4. See whether biomaterials, like gel carriers, can be used to deliver these drugs
5. Find out if there are any adverse effects (eg, cancer)
6. Try and develop the chicken egg as an alternative way of measuring these effects (for example by growing human skin in fertilised chicken eggs).

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Skin disease and injury often results in wounds that heal very slowly, or which heal through formation of scar tissue, which may be unsightly and which can in some cases can cause pain and restrict movement. We are trying to develop new therapies that improve wound healing in two ways: by promoting the regeneration of tissue rather than the formation of scar tissue; and by promoting the repair of wounds that are very difficult to make heal, like those that occur in diabetes.

To do this, we're investigating molecular pathways and biomaterials. One of the pathways we're investigating is called the Wnt signalling pathway. This pathway is involved in shaping the embryo at the very beginning of life, and in some animals, such as newts, can control the regeneration of whole limbs. We have found in preliminary studies that when we artificially increase the level of Wnt signalling in wounded mouse skin tissue, we can promote the formation of new hair follicles and alter the amount of collagen that forms in the healing wounds. This leads to the exciting implication that we may be able to exploit this signalling pathway to promote better wound healing in people. In parallel we're investigating biomaterials, including clay gels. Clays have been used to help wounds heal since prehistoric times, but very little science has been done on whether this helps and, if so, how.

Unfortunately, it is very difficult to simulate wound healing in experiments in culture dishes. There are often lots of tissues, cells and molecules involved, many of which might arrive at the wound from very remote locations, like the bone marrow or distant blood vessels. In addition, many tissues are involved, for example, nerves, blood vessels, immune cells and stem cells, in addition to the cells present in the skin. In short it is a very complex process. For this reason we believe animal experiments are necessary and we have proposed to use mice. Mice are a well-understood 'model' of skin injury, wounds can be made of a reproducible size, and heal in a predicable way, by a mechanism not dissimilar to that which occurs in people. Im this study we will test the effect of our drugs and biomaterials by making small wounds and by measuring (using a number of molecular techniques) the rate and quality of wound healing. We aim ultimately to develop new therapies that accelerate and improve the quality and degree of wound healing in people, helping them live better lives.

What types and approximate numbers of animals do you expect to use and over what period of time?

We plan to use mice and chicken eggs. Over five years we have planned to use a maximum of 700 mice and 200 eggs.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

This protocol involves making wounds in the back of mice that are not bigger than 2.25 cm2. We always give pain killers to mice before, during and after surgery and of

course the mice are given general anaesthesia during surgery. This is defined as a procedure of 'moderate' severity. Mice tolerate skin wounding well, and a wound of around 1cm2 in size heals in around 7 days. There is a small scar but otherwise the animal remains healthy. T We do not expect to see anything more than small changes in the condition or the behaviour of the animal - if more obvious changes in clinical condition (for example, hunching, lack of grooming, hairs standing on end, or behaviour indicating distress such as subdued and self-isolation) occur then the animal will be killed humanely. We also separate the mice after surgery while a dressing is in place, so that they cannot hurt each other or affect each other's wounds. When we do this, we also provide enrichment in cages - this consists of cage furniture (a mouse house) and extra bedding.

For around 10 days post-surgery, the wound dressings may have to be changed and treatments added. We do this under inhaled general anaesthesia, similar to 'gas' that humans are given at the dentist. This makes the animals less distressed and so they do not experience discomfort in this procedure. We expect no ill effects from these procedures, though we will humanely kill immediately any suffering or distressed animal humanely

Application of the 3Rs

Replacement

Wound healing is a complex physiological process involving a variety of biological systems and cell types. For example, the systemic immune system is involved in regulating healing, and often immune cells are recruited from remote anatomical locations to take part in the wound healing process. For these reasons, wound healing cannot be modelled effectively presently in cell culture or ex vivo conditions.

As it is not possible to test the therapeutic efficacy of novel molecular modulators of intracellular signalling in humans at this stage because of the ethical and regulatory concerns, we have chosen to use mice as our experimental organism. Domesticated mice have been used for more than a century for scientific research and in many cases have been bred especially for this purpose. We believe that the potential therapeutic advances that might be made as a result of this project warrant the use of our small group of experimental animals. Despite this, we are making thorough and serious attempts to validate our proposed methodology in vitro before any in vivo study is attempted.

We will **replace** animals in our research by conducting extensive in vitro experiments to troubleshoot the composition of nanoparticles, the method of delivery, and the concentration of incorporated growth factor and/or compound. To test this, we will obtain primary human keratinocytes and fibroblasts from patients undergoing cosmetic surgery and apply our therapeutic molecules and materials to these cells. We will test the activity of any molecule under investigation by measuring the cellular

corollary of molecular stimulation by, for example, measuring target phosphorylation or activation. In particular, the emphasis of our current research is on Wnt signalling, and we will use a stable cell line that produces an enzyme in response to Wnt signalling, the activity of which can subsequently be detected by luminescence using an added enzyme substrate. These strategies will enable us to determine the efficacy of our technology prior to in vivo work. In parallel, we have proposed to develop a new technology (CAM culture) to refine the use of animals in our experiments in later studies (See '**Refinement**').

Reduction

We aim to **reduce** the numbers of animals used in our research by conducting extensive in vitro work, as described in '**Replacement**'. We will reduce animal use by using in vivo live whole-animal imaging to assess the rate of wound healing. In short, following surgery and treatment with nanoparticles, the rate of wound healing will be measured using x-ray µCT (micro-scale computed tomography)at 3 timepoints during a 14 day post-surgical experimental time-course, where otherwise we would have sacrificed the animal, extracted the skin and measured healing rate by histomorphometry. We calculate that this will lead to a reduction in animal use of 18 mice per 2-factor experiment, and a total reduction in our animal use by 54 animals.

We have also conducted a power analysis and will conduct a statistical evalution of our proposed methods in collaboration with experts in statistics

Refinement

We aim to **refine** the use of murine models in our research in parallel with our murine injury models by devising an ex vivo model of skin wound healing on the chorioallantoic membrane (CAM) of the chick egg. The CAM is an extraembryonic tissue found in the eggs of birds which can provide a surrogate blood supply to tissue cultured on it. This allows the culture and medium term survival of tissue explants which would otherwise undergo core necrosis due to lack of nutrient transport. Furthermore, the CAM has no neural infiltration and therefore these kinds of studies inflict no pain on the experimental organism in question. Briefly, we will isolate human skin tissue obtained under local ethics committee approval following cosmetic surgery. We will trim skin before making small circular full-thickness defects of 3-8 mm in diameter before transfer to the surface of the CAM from embryonic day 10 to day 19.

Concurrently, we will treat wounds with our putative therapies as above. We will observe the rate and quality of skin healing at various time points post-'injury' with histochemical and molecular assays. These innovative models represent a refinement of animal experiments by replacing often painful and invasive surgical procedures in a sentient animal with an ex vivo model that causes no or little pain to the chick fetus. Furthermore, we will advance knowledge in the 3Rs by devising and proving the utility of a wholly novel skin repair /development model in *human* tissue.

We will also refine our work in in vivo murine models of skin injury by providing pre- and post-operative analgesia (carprofen) as well as during dressing changes (as described in protocol 2). During dressing changes, to minimise distress to the animal we will provide inhalation anaesthesia (isoflurane). Prior to any procedures being carried out in a restraining jig, we will train the animals to become accustomed to this, by introducing them to the restraining jig several times without any procedu

Project 5	Molecular control of skin development, regeneration, ageing and carcinogenesis
Key Words	Skin, Hair growth, Wound healing, Cancer, Ageing
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Here, we will address the mechanisms underlying a role of distinct signalling/transcription and epigenetic regulators in skin development, regeneration, ageing and carcinogenesis. Specifically, we will address the following objectives:

1. To define the mechanisms underlying the cross-talk between signalling/transcription and epigenetic regulators in the control of skin morphogenesis, hair growth, postnatal homeostasis and ageing.
2. To delineate how the cross-talk between distinct signalling/transcription and epigenetic regulators contribute to the control of skin regeneration and wound healing.
3. To explore how the cross-talk between signalling/transcription and epigenetic regulators is altered during skin carcinogenesis.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The generated outputs from this project will provide new important insights into fundamental mechanisms that regulate skin regeneration, ageing and carcinogenesis, and will serve as an important platform for the development of novel epigenetic drugs for the needs of regenerative medicine and clinical oncology.

What types and approximate numbers of animals do you expect to use and over what period of time?

This 5-year project will be performed using mice and will involve about 5,000 animals.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The expected levels of severity on this project are moderate. We don't expect any severe adverse effects on this project. At the end of the project, animals will be processed for Schedule 1 termination.

Application of the 3Rs

Replacement

Available in vitro skin models (primary skin cell and tissue cultures) as well as computer models cannot recapitulate the complex processes of skin development, homeostasis, wound healing and carcinogenesis at this time.

Thus, without properly conducted and responsibly performed animal experiments, it is impossible to understand the mechanisms of normal skin development and define why alterations in these mechanisms lead to skin cancer and wound healing impairment.

Reduction

To minimize a number of animals used for experiments, several approaches will be used:

1. Only genetically engineered mice that show high probability to develop distinct alterations in skin development and regeneration will be used for the experimental programme;
2. Homogeneous populations of genetically engineered mice will be selected for pharmacological studies based on their cutaneous phenotypes and distinct alterations of gene expression in skin;
3. Pilot studies will be performed prior to each large-scale pharmacological experiment;

Statistical methods designed for repeated observations, or non-parametric tests on summary measures will be used for calculation of the number of animals in the experimental and control groups.

Refinement

Mice are extremely useful for these studies, because of establishing within last decade a large number of models for studying human skin disorders, including skin

carcinogenesis, distinct hair loss conditions, etc. Because of these unique features, phylogenetically lower species cannot be used.

Several approaches will be applied to minimize animal suffering during and after the experiments: i) Local or general anaesthesia will be used prior and during the experimental procedures; ii) Animals will be monitored while under anaesthesia and during recovery; iii) Animals will be regularly monitored for pain, distress and general health status; iv) The NVS advice will be used to monitor the animal health status and to determine the presence of pain or distress when necessary.

Project 6	Cellular, Drug and Gene Based Therapy for Eye Disease
Key Words	Rodent, AMD, Vision, Retina, Therapeutics
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Age-related macular degeneration (AMD) is a major cause of blindness in the developed world. Currently there are no curative treatments for this very complex disease. Our aim is to increase our understanding of the disease which will allow us to create more competent models of AMD which will then facilitate the development of new treatments and cures.

There are two forms of AMD, wet and dry. We have a treatment for the wet form already in use in the clinic, which reduces the degeneration of the retina by multiple injections into the eye. Our hope is to develop a gene therapy technique which will halt the degeneration with only one injection.

The dry form of AMD currently has no clinical treatments. By investigating the pathways of the disease we hope to open up treatment options to be developed.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

We are developing a laser model of dry AMD in our lab which will aid in the research into treatments of this disease. Current models are either genetic models or require the injection of damaging substances into the eye, while the laser model will be non-invasive and reproducible. The development of new models provides platforms for the discovery of novel therapeutic targets.

What types and approximate numbers of animals do you expect to use and over what period of time?

We estimate a total of 1680 animals used in the five years of this project. Exact numbers will depend on results of breeding and required numbers for statistical relevance which shall be assessed with each experiment. We have chosen to use small rodents, in particular mice, which have a large and ever growing range of models available. Although there are some species differences the basic biology of retinal degeneration in mice is comparable to that of humans. The models of retinal degeneration chosen will be well characterised and representative of human disease states.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The expected level of severity for most animals used is mild. However due to the risk of several adverse effects the licence is proposed to have a moderate severity. Many of the techniques proposed require the animal to remain completely still. Rather than restrain (and potentially stress) the animals they will be put under general anaesthesia. These techniques are used in humans with only topical anaesthetic and they appear to be similarly non-painful in rodents. However they will be closely monitored following treatment, and appropriate action will be taken where an animal is thought to be distressed at any time. Potential surgical trauma such as cataract may occur (the lens becomes opaque), however these effects are not expected to cause pain or distress to the animals, and these animals are simply kept until the end of the procedure. If however they do show signs of distress they will be culled. When using immune deficient mice the protocols will be carried out under HEPA filtered conditions to minimise the likelihood of infection. If animals appear to be unwell/ show signs of infection they will be culled immediately by a Schedule 1 method. There can be a dose dependent adverse effect of the fluorescein during fluorescein angiography, particularly in older mice and mice that have been on a high fat diet. This is believed to be due to the kidneys being unable to completely remove the fluorescein from the system. To prevent this becoming an issue we have instigated a low maximum dose and the need to titrate this dose on new rodent models.

Application of the 3Rs

Replacement

This work is at a pre-clinical stage and it is unethical to conduct experiments on humans which involve delivery of substances wherein those experiments require the removal of parts of the nervous system for ex-vivo investigations. Therefore, there is no feasible alternative that would entirely replace the use of living animals.

We are using ex-vivo models alongside our planned animal work. While there is no ex-vivo cell culture system which can completely mimic the complex physiological environment found in a living eye, we have developed an ex-vivo culture model of the outer retina which has some advantages over using live animals. Live confocal and ultrastructural imaging can provide data at single molecule resolution which is not feasible in-vivo. We will reduce the number of experiments required in animals, and will produce supplementary data that cannot be achieved from animal experimentation.

We are always looking into ex-vivo methods to give results we currently require from animal work. For example while “gold standard” for testing the pluripotency of iPS cells is the teratoma assay, other methods are being developed and increasing in popularity. We plan to assess the viability of a PCR based “scorecard” method, and if suitable we plan to focus on using that method.

Reduction

The design of individual experiments will be optimized to ensure that the maximum amount of data is obtained from the minimum amount of resources.

We will use the ARRIVE guidelines to inform our experimental design and to help with that we have a member of staff attending the NCR3s summer school.

The numbers of animals needed will be determined by accurate statistical calculations allowing us to use the minimum number of animals possible for each experiment.

Refinement

The laser model for wet AMD is well established and produces a reliable and reproducible model. This reduces the need for potentially harmful genetic mutant models to be used, and since the procedure is known to be painless in humans and does not appear to cause distress in mice, it is a very useful non-invasive technique.

The laser model of dry AMD using a long wavelength laser developed under our previous licence replicates retinal cell loss. Other models of dry AMD include genetic mutants and the use of sodium iodate to selectively kill photoreceptors. But the laser model is a non-invasive model.

The procedures described for this project can be successfully performed in humans with topical anaesthetic eye drops. The same anaesthetic will be used for animals. However potential adverse effects include surgical trauma at the time of injection, transplantation or laser application. If this occurs it will be easily visualised via an indirect ophthalmoscope (vitreous haemorrhage, cataract or retinal detachment). Animals in this situation will be immediately euthanized by an appropriate Schedule 1 method.

Project 7	Stem cell therapies for retinal degeneration
Key Words	Retina, regeneration, stem cells, therapy, blindness
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The aims and objectives of the research are:

1. To investigate whether a type of stem cells, known as Müller glia, which can be isolated from human embryonic and adult stem cells can be potentially used for therapies to treat retinal diseases such as glaucoma and retinitis pigmentosa.
2. To identify drugs or biological factors that could be injected into the eye to induce self-repair of the diseased retina, in order to restore vision without the need for stem cell transplantation.
3. To design and improve methods of cell transplantation that can be applied to the human eye, using biological materials as cell carriers to promote survival and integration of transplanted cells.

We expect that the result of our studies will pave the way for the formulation of a pre-clinical plan to translate our research into human treatments.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

It is expected that this study will lead to the formulation of a plan to undertake pre-clinical studies to develop cell based therapies to treat patients with advanced glaucoma and other conditions that affect the light sensitive cells of the retina, such as retinitis pigmentosa and age related macular degeneration. Stem cells constitute the only hope for restoration of visual function in many patients for whom other therapies are not available or are non-effective. In addition, we expect that our

studies to investigate the effect of small molecules on the self-regeneration of the rat retina can identify targets to treat retinal diseases in humans without the need for stem cell transplantation.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use normal and dystrophic rats and expect to use approximately 2200 rats over the course of the 5 year study. We will also use rabbits and expect that the maximum number of animals of this species to be used over the course of the study is 50.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The animals will receive stem cell transplants into the eye. We expect minimum adverse effects to the immune –suppressant drugs to be administered, as well as minor risk of infection as a result of the ocular injections. We will use the minimum doses of immuno-suppressants that are effective and animals will be sacrificed at the end of the experiments. Some of the rat species we will use, such as the P23H rat, have genetic defects that makes the animals become blind at 6-8 weeks of age. Similarly, when we induce glaucoma in normal animals, the animal may become blind in a single eye. However, animals with glaucoma can still see. Animals that become blind do not suffer distress as they adapt well to their own environment. The overall level of severity is Moderate. All animals will be sacrificed at the end of the experiments.

Application of the 3Rs

Replacement

Transplantation experiments in the laboratory can be undertaken using human retinas transplanted with stem cells (the only laboratory-based model available) . These studies only provide evidence that stem cells are able to attach and survive onto the retina under laboratory conditions. However, these studies do not provide knowledge on the ability of these cells to restore vision. These answers can only be obtained by transplanting the cells into living animals, which will allow us to determine the nerve connectivity of the cells and the transmission of nerve signals amongst them and onto the host using electrophysiological techniques. Where possible, *in vitro* experiments will be undertaken before proceeding to *in vivo* work.

Reduction

We have performed extensive studies in the laboratory and will continue to assess methods for cell transplantation using human donor retina and rat retina . These studies minimize the number of animals to be used for *in vivo* studies. We have sought Statistician’s advice and power calculations have been made in order to

minimize the number of control and treated animals needed to obtain meaningful data.

Refinement

The vast majority of the work will be undertaken in rats because of their low neurological sensitivity and because there is increased success rate of inducing experimental glaucoma in rats compared to mice. Although we have gained much knowledge from our previous transplantation studies, which showed partial restoration of sight in the rat eye. We also need to establish delivery protocols in an eye that is closer to the human eye in size. The rabbit has a vitreous cavity proportionally much larger compared to the lens, and it is the reason for using the rabbit eye in our studies.

We will undertake all the procedures under general anaesthesia and will limit the number of times in which anaesthesia will be administered. All protocols will be performed under aseptic conditions to minimize risks of infection. Animals will be carefully monitored for signs of suffering and will be sacrificed by schedule 1 method if there is any evidence of suffering.

Project 8	**The role of Rab GTPases in disease**	
Key Words (max. 5 words)	Eye, eye disease, gene therapy, aging	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Transport of material within and in-between human cells isimportant for the well-being of human cells. Small proteins, called Rabs, regulate the transport. We study three human diseases that are associated with abnormal transport of material in human cells caused by Rab defects: 1. Choroideremia (CHM) is a hereditary blindness with no cure that affects males; CHM is caused by a faulty CHM gene. Healthy CHM gene is necessary for the correct function of Rabs. Previously we had generated a unique mouse model for choroideremia. The aim of the project is to deliver a healthy choroideremia gene to ocular cells in choroideremia mouse. This approach is known as gene therapy. The work is needed to develop gene therapy treatment for humans. 2. Age-related macular degeneration (AMD) is a debilitating loss of vision in older persons. Cells of the eye (ocular cells) have no ability to divide, and like neuronal cells stay with a person for life. Due to	

	stress in general and aging in particular, there is an accumulation of waste material in the cells, which is toxic and can kill or seriously damage cells. We had found a mouse model of AMD (Rab38 mutant, known as chocolate mouse) where we see changes in ocular cells that are similar to changes in AMD patients. We plan to study aging process in ocular cells with the purpose of developing treatment for AMD patients. 3. Despite huge scientific effort and significant advances cancer remains a life threatening disease. Cancer cells are efficient in self organisation and avoidance of immune surveillance. Recent study had shown that communication between cells occurs through release of specific messages (exosomes) which are small fatty envelops packed with important instructions. We had created a mouse model (Rab27 knock-out) where we disrupted cell communication between immune cells. We plan to use this model to advance this study of cell communication further and aim to develop new treatment for cancer and immune diseases.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The study of choroideremia is important for human gene therapy trial in the UK and worldwide. Choroideremia is caused by defect in a single gene and therefore is suited for gene therapy. Delivery of healthy choroideremia gene to retinal cells is safe due to majority of retinal cells being terminally differentiated and non-dividing, reducing potential risk of tumour formation. Since disease is diagnosed before major histological changes this leave a time period when the disease can be treated. Gene therapy approach was shown to be successful in Phase I clinical trial in principle.. The study of chocolate model will help to understand changes in age related macular degeneration (AMD), thus leading to a potential development of new AMD treatment. The proposed Rab27 study is likely to provide a range of scientific, health and wealth benefits to different interest groups. Firstly, this study will provide information useful to the researchers investigating

	Rab27 function in inflammation and cancer. Secondly, improved insight into mechanisms will result in new opportunities for development of novel anti-inflammatory drugs (e.g. drugs that impose a short-term blockade of Rab27 function) that will provide health benefits for patients. Development of new treatment will reduce health care cost associated with the diseases and so will provide economic benefits to taxpayers.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 5,000 animals over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For Protocol I (Breeding and maintenance) expected level of severity is mild as genetically altered mouse strains do not suffer adverse harmful effects. (PT: tamoxifen and ageing adverse effects). Injections were moved onto protocol 3 and aging was moved onto Protocol 2. (PT: Briefly describe the adverse effects associated with protocol 2 steps.) For protocol 2 expected severity is moderate which is due to normal age-related possible outcomes. For protocol 3 expected severity is moderate due to possible effects of injections including gene therapy treatment. At the end of protocols animals will be humanely killed and their organs harvested for tissues.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CHM and AMD are diseases of the eye which is a highly specialized multilayered organ. Mouse, as a small mammal has a very similar to human organisation of the eye and thus is ideal as a model organism. Majority of cells in the eye are terminally — differentiated non-dividing cells, and thus could not be kept in culture. When possible, experiments will be performed in culture using established cell lines. For example, efficiency of gene therapy vector carrying a healthy CHM gene wilt be tested in tissue culture using standard cell lines and primary skin fibroblasts from CHM patients. We will use primary retinal pigment epithelial (RPE) cultures from CHM mice to assess effect of a healthy CHM gene in CHM cells.

	We will study cells isolated from harvested organs and tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce number of animals that we keep majority of animals will be kept up to age of 6 months. For aging study standard 'old age' point is 12-14 months which is a midlife point, only minimal numbers of animals will be kept beyond this time point. In cases, where a specific time point is not important, organs will be harvested at the earliest possibility to reduce the number of the animals in the colony. (PT: Briefly present how experimental design and statistics will minimise numbers appropriate to meet objectives). To reduce number of animals we will use primary RPE cultures that are established from 3-week old animals. Use of cultures will allow us to generate sufficient sample size to achieve 80-90% power and statistical significance (less than 0.05).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	CHM and AMD are diseases of the eye, which is a highly specialized multilayered organ. Mouse, as a small mammal has a very similar to human organisation of the eye and, thus, is ideal as a model organism. Previously, to obtain animals with CHM we used animals that were injected with tamoxifen to induce CHM disease in all tissues, which caused moderate sickness 4-6 months post injection. We refined the CHM model by inducing CHM only in the eye tissues; these animals have no general health implication. We also refined the ubiquitous CHM model by using female carriers of CHM"" allele (CHM nu1rr) where 50% of the cells in all organs are normal and 50% are CHM cells due to the CHM gene being positioned on the Xchromosome, which undergoes random X-inactivation in female cells. Choroideremia carriers exhibit typical CHM phenotype in all ocular cell layers, and at the same time do not suffer adverse harmful effects. We had also refined a breeding scheme that we used to produce CHM carriers. Previously, we generated CHM carriers through breeding animals injected with

	tamoxifen to induce CHM. Our current strategy is to cross healthy CHMF!OX male and PGK-Cre transgenic female; majority of female offspring have CHM null's genotype (identified by genotyping).

Project 9	**Mechanisms of Intraocular Pressure Regulation**	
Key Words (max. 5 words)	Glaucoma, intraocular pressure, blindness	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall goal of this project is to identify the cellular and molecular factors controlling eye pressure. This is important because eye pressure typically becomes elevated in glaucoma, the second leading cause of blindness affecting 60 million people worldwide. The only way to treat glaucoma and prevent further blindness is to lower eye pressure. While glaucoma medications are available, none are perfect, and many patients continue to experience elevated eye pressure that threatens their vision or requires them to undergo risky eye surgery. In order to develop new drugs that more successfully lower eye pressure and prevent blindness, we must better understand the factors controlling eye pressure. This in turn requires research. The research questions addressed in this project are: 1) What molecular signals or genes control eye pressure? 2) What goes wrong in glaucoma to cause elevated	

	eye pressure? 3) How can these signals be exploited to lower eye pressure?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The direct beneficiaries of this project are other scientists who aim to understand glaucoma, pharmacologists who aim to design better drugs to treat glaucoma and clinicians who aim to provide better care for their patients with glaucoma. Ultimately, our field of science advances by research investigating how eye pressure is controlled, and this leads to improved understanding of how to treat and target the disease. In the long-term, we envisage our research informing the developing of new drugs or technologies that save vision in patients suffering vision loss from glaucoma.
What species and approximate numbers of animals do you expect to use over what period of time?	The project uses mice and rats. Over the 5-year duration of this project we expect to use approximately 6000 mice and 1300 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity is moderate. Our primary experimental readouts are measurement of eye pressure and measurement of aqueous humour outflow resistance. Eye pressure measurement is non-invasive and involves a light-weight probe that briefly contacts the eye. Outflow resistance measurement is invasive and requires that we put a needle into the eye, but these experiments are often performed ex vivo following humane killing. To fully assess the effects of compounds on outflow resistance, it is necessary to perform some in vivo measurements with a needle in the eye, but to minimise suffering these are performed under terminal anaesthesia where the animal is not allowed recovering or experiencing any pain. We include other surgical procedures, such as implantation of mini pumps to deliver drugs over several weeks. These techniques follow standard surgical practise with appropriate use of analgesics, anti-inflammatories and antimicrobials to minimise the risk of infection or distress. On some occasions it is necessary to inject compounds directly into the eye. These injections are

	performed under anaesthesia with recovery with sterilised fine glass pipettes, using analgesics to minimise any ocular pain or distress. Our protocols include methods to induce glaucoma-like symptoms in mice by exposure to a particular class of steroids known as corticosteroids. Corticosteroid-induced glaucoma is a recognised side effect of human patients receiving corticosteroid therapy, and corticosteroid-induced glaucoma allows us to mimic the human disease in rodents to study glaucoma pathogenesis and to screen for new drugs to better treat glaucoma. Prolonged corticosteroid exposure may lead to adverse effects including lethargy, loss of appetite and weight loss. We closely monitor animals receiving corticosteroids to treat animals upon any signs of distress. Affected animals are given a high calorie dietary supplement to help with weight loss and examined by the NVS. We are currently developing new methods using nanoparticles to localise corticosteroid delivery to the eye so to minimise adverse effects including weight loss caused by systemic corticosteroid exposure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mechanisms controlling eye pressure are complex, involving multiple tissues and cell types. There is no model other than a living eye that fully captures this complex system, and we therefore must work with animals if we are to investigate the mechanisms of eye pressure regulation. Whenever possible, we perform experiments using ex vivo tissue specimens to minimise the number of procedures on living animals. We also leverage cell culture studies and mathematical modelling to inform experiments using animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimise animal numbers by performing statistical power calculations that estimate the minimum number of animals required to achieve a predicted effect. These calculations are based on population of measured data acquired over the previous 5 years. Whenever possible, we design our experiments to use paired eyes from individual animals, where one

	eye is treated and the contralateral eye used as an untreated control. This paired design requires fewer animal experiments to achieve a desired scientific aim by controlling for intra-individual variability.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice (and likely rats) mimic the anatomy and physiology of the human eye, and drugs that affect eye pressure in humans tend to have similar effects in mice. All animals will be housed in groups where possible with appropriate environmental enrichment and fed according to current institutional 'best practice'. For our glaucoma research, we have developed specialised equipment to measure the turnover of fluid within the eye; this turnover is the principal factor controlling eye pressure. The measurements are very accurate with minimal noise, and this refinement over previous techniques reduces the overall number of animals used in experimentation. We are also further refining our methods to deliver steroids to the eye; steroid delivery is an established method to induce glaucoma-like symptoms in mice. Because whole body exposure to steroids leads to weight loss, we have developed steroid-eluting nanoparticle that can be injected near the eye to localise steroid delivery to the eye. This method appears to mimic the glaucoma-like features without the weight loss, and represents a further refinement in our methods.