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NON-TECHNICAL SUMMARY

Biocompatibility and pharmacology of novel nanotechnologies

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Nanomaterials, Nanotechnology, Biocompatability, Neural Interface, Nanomedicine

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Nanomaterials are very small materials with at least one dimension (eg. width, length, height) that is less than 100 nanometres (one billionth of a metre) in size. The overall goal of this project is to design and test novel nanomaterials or nanomaterial enabled devices. We aim to design and identify the nanotechnologies that have the biocompatibility (compatibility with living tissue or a living system by not being toxic, injurious, or physiologically reactive) and functionality to be able to translate into clinical use (therapeutic, diagnostic and monitoring) for a wide range of diseases.

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?

The use of novel technologies based on nanomaterials (nanotechnologies) seeks to design smarter solutions with potential to overcome key clinical problems. These could have applications in various diseases including cancer or brain disorders. However, in order to design nanotechnologies for these applications, it is essential to first understand how these nanomaterials interact within living organisms. We aim to test the safety/biocompatibility and biological interactions of nanomaterials or devices incorporating nanomaterials, first in cells grown outside the animal, and then in healthy animals. In addition to biocompatibility, by studying where the nanomaterials go in the body and how the body reacts, we can learn about their safety or identify potential medical uses, which will inform other researchers interested in using the same tools. It will also inform health and safety protocols for those who may be exposed to nanomaterials during their jobs (occupational) and be relevant for the wider public who may be exposed to nanomaterials present in the environment.

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

The new information gained from this project will be shared in the form of scientific publications, conference communications and through public engagement activities throughout this project. While this work is in the early preclinical stages in the development of medical nanotechnologies, the information gained will allow us to further design and develop nanomaterial and nanotechnology approaches for various *in vivo* and clinical applications.

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

In the short to medium term, many of the nanomaterials being investigated are being produced and used commercially for medicine or other applications. The biocompatibility profiles of these materials are very important to study to be able to provide safety information both to researchers who will further develop the technologies and members of our society exposed daily to engineered nanomaterials present in the environment (natural, occupational, or at home).

Eventually, in the long term, patients will benefit from any effective new technologies developed for the diagnosis, monitoring or treatment of diseases.

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

Publication and communication of our findings will always be the primary aim of this work. The goal of every experiment conducted under this licence will be to generate valid, high quality and therefore publishable results and we will endeavour to ensure that all findings meeting these criteria will be published (majority in open access journals) to inform the wider scientific community.

Species and numbers of animals expected to be used

- Mice: 2500
- Rats: 700

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.

Mice are chosen for many of these studies for a variety of reasons, primarily as they are the least sentient mammalian species that will provide data applicable to humans. The similarities between mice and humans in terms of physiology, genetics, immunology and other systems allow us to make reliable predictions about the likely interaction of nanomaterials and nanomaterial devices in humans. Rats are used as an alternative rodent model for some of this work primarily where the larger size provides more anatomically relevant information for nanomaterial device development and biocompatibility testing.

The majority of research will use adult animals as these will have the most closely related biology to adult humans, where these nanomaterials are intended to be used.

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

For the initial testing of nanomaterials, healthy animals without an existing disease or clinical characteristic will be administered (by injection) or implanted (by a surgical procedure) with these nanomaterials via an appropriate route (usually a single administration at the start of the experiment but occasionally repeat administrations throughout). These routes will vary depending on how the nanomaterials are intended to be used as medical technologies, or the likely route of exposure when considering environmental nanomaterials. In the case of nanomaterial interactions with the brain (either as materials or as devices) this initial administration will require a surgical procedure.

Following dosing or device implantation, animals will be monitored by a range of approaches. Outcome measures in the majority of these studies would include one or more of the following: live imaging,

blood sampling either to recover nanoparticles, or for assessment of systemic markers, urine or faeces collection and post-mortem tissue histology to assess local and systemic inflammatory reactions, as well as distribution patterns. For many animals, the only procedure performed would be administration of materials, with the assessments done after humanely killing, or from samples collected under terminal anaesthesia.

For nanotechnology based devices, once initial biocompatibility of the devices is carried out (as described above) and we are confident that they are not having any effects beyond standard devices already in clinical use, we can begin recording and/or stimulating from the devices to assess how this may impact the biocompatibility or functionality of the device. These assessments will utilise a number of techniques to assess the state of both the tissue and the animal as a whole. This will include blood sampling to check systemic effects, and behavioural tests. This will allow baseline behaviour of the animal before implantation of the device, assessment after implantation with and without recording for a full comparison. These behavioural tests will be minor and non-stressful for the animal, utilising widely used techniques such as rotarod for motor function. The effects of any neuroprotective or other agents embedded in a material surrounding the electrodes can also be assessed to determine whether their presence has beneficial effects on the tissue. At the end of the experiment, animals will be humanly killed and tissues collected (particularly those in contact with the device) for detailed assessment.

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

From our previous work, we don’t expect the nanomaterials or devices we use to have any particularly strong adverse effects. Usually the nanomaterials would not be associated with any clinical signs and the effects of these on cells or tissues are only observable when conducting detailed analysis post-mortem (microscopically/histologically/molecularly). Where unexpected substantial reactions occur, animals will be humanely killed as these effects would likely interfere with the aims of the studies. The procedures used to administer the nanomaterials are usually the least invasive possible. Where surgical administration is needed, this will be done under anaesthesia and analgesia/additional support is provided to minimise the effects associated with this.

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)?

Mice: 50% mild, 50% moderate

Rats: 100% moderate

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

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Before materials or devices are tested in animals, they will first be tested in cell cultures, or in relevant *in vitro* systems to check the safety and to help determine the doses that would be safe, or provide a particular effect *in vivo*. Many responses to nanomaterials, including the pharmacology and biodistribution, are driven by complex interactions with multiple cell types as part of whole systems (eg. the immune system, the cardiovascular system etc). These cannot be effectively modelled and integrated in an *in vitro* setting and therefore require the use of live animals.

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

In vitro cell cultures including 3D models and co-culture systems (up to and including organoids which are 3D *in vitro* models that contain multiple cell types and mimic organs more closely).

Why were they not suitable?

Non-animal alternatives such as those listed above can provide important information and are always used in the first instance for all new technologies and nanomaterials. This includes testing in organoid systems which is an ongoing effort by our group. However, none of these systems (including organoids) can effectively recapitulate the complex multi-system interactions of a whole organism, as is required for our objectives.

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?

Numbers of animals have been estimated in consultation with statisticians using historical data from our own experiments with the same models and approaches with similar nanomaterials or nanomaterial devices.

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

We aim to use the minimum numbers of animals required to adequately and robustly address the research question. This has been determined with support from statisticians and use of rigorous

experimental design considerations (as guided by the NC3Rs Experimental Design Assistant). Use of adequate numbers of animals will reduce variability, improve experimental consistency and confidence in outcomes. All assumptions on which sample size estimates are based will be re-evaluated once additional or new data is available from these studies and if necessary numbers of animals required will be revised for subsequent studies.

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

The main way we will reduce the number of animals we use will be to use longitudinal monitoring and live imaging techniques. This will allow us to obtain data from the same animal over time instead of the more traditional method of killing a different animal at every timepoint. Where nanomaterials or nanomaterial devices are being tested for the first time in animals, pilot studies will be run with smaller numbers to ensure safety and provide an initial assessment of effect or functionality that will be used to statistically determine the correct number of animals to use for further investigations. Where possible (eg. device implantation to a hemisphere of the brain) we will use animals as their own control (eg. non-implanted hemisphere). Finally, at the end of each experiment we will collect as many tissues as possible in order to maximise the potential output from each experiment.

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.

For initial assessment of nanomaterials, or nanomaterial devices, healthy animals without any disease or clinical phenotype will be used. This will minimise the suffering and harm to animals while giving the most clear picture of the effect of the nanomaterial, or nanomaterial device without any confounding factors.

For administration of nanomaterials, the least invasive route that is relevant for the particular application will always be used. Where more invasive routes are necessary (eg surgical administration) this will be scientifically justified and through proper aseptic technique, pain management and careful monitoring is not expected to cause any additional distress or prolonged suffering.

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

The animals proposed are the least sentient mammalian species. The use of non-mammalian species (eg. Xenopus, Danio) would not be appropriate for the clinical translation of the nanotechnologies under development. Mice and rats are the most appropriate for the work being carried out as they have circulatory, nervous and excretory systems very similar to humans, which allows us to model where the

materials go, how the body breaks them down and how they are removed from the body in a system similar to humans.

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

Animals undergoing surgical procedures will receive appropriate analgesia to prevent any post-operative pain, will be carefully maintained at a suitable depth of anaesthesia and may also receive additional fluid support to prevent dehydration associated with longer procedures. These animals will be provided with additional husbandry such as mash/wet food, heated housing and careful monitoring in the immediate hours following surgery until normal activity is resumed.

Animals will be group housed and where animals have been individually housed for a particular purpose (post-surgical recovery) these will be grouped as soon as is appropriate.

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

All experiments will be planned and executed with reference to the PREPARE and ARRIVE 2.0 guidelines to ensure effective experimental planning and proper reporting of experiments respectively. We will follow guidance from BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinements and LASA guiding principles for Administration of Substances and Aseptic surgery.

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

The researchers working under this licence will be regularly encouraged to actively stay informed on advances in the 3Rs as is required by the conditions of their PIL. We will regularly check information on NC3Rs website and newsletters and we will attend institutional and regional 3Rs symposia. Any relevant advances, for example refinement of techniques or approaches, will be readily implemented into this project.