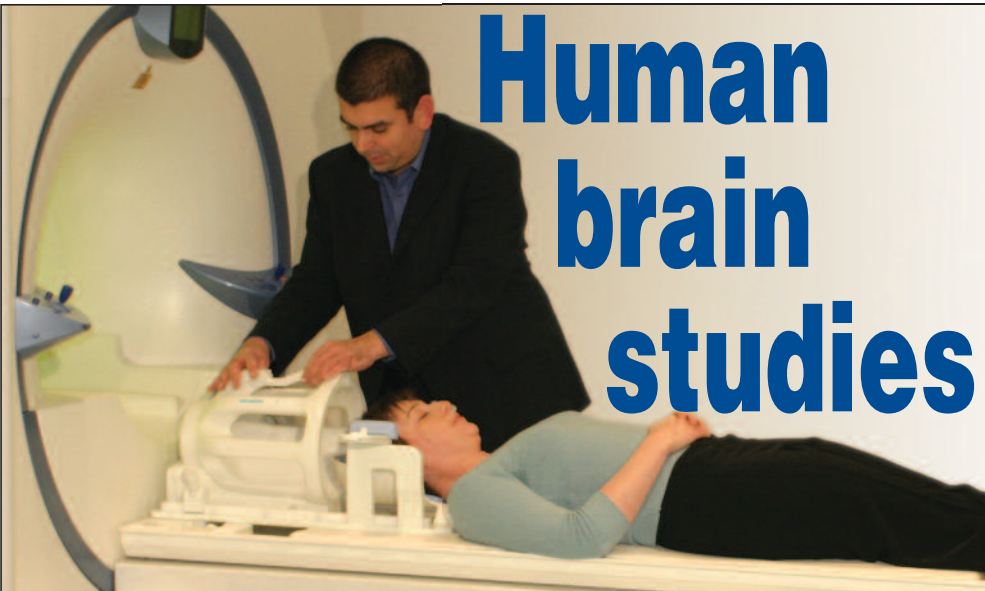


NEW SCIENCE

ADVANCED TECHNIQUES: News from the world of research without animals



Human brain studies

Primates are frequently used in brain research and they suffer enormously. There are also fundamental differences between species, so adoption of new techniques in neuroscience is vital.

For a decade, we have been funding the neuroimaging group at the Aston Brain Centre (ABC), headed by Professor Paul Furlong, which engages in a wide range of research activities studying human behaviour and brain function. The group works to establish how new techniques may be used to replace animal experiments. Projects include vision, neurodevelopment and clinical research, cognition and pharmacokinetics (drug characteristics). This has put us at the forefront of the drive away from horrific animal experiments in neuroscience.

The team examined the effect of a drug, Zolpidem, on a stroke patient. This drug is typically used to treat insomnia and belongs to a class of drugs called sedative-hypnotics. It slows brain activity, allowing the patient to sleep¹.

Zolpidem has previously been reported to have an awakening effect in patients in a persistent vegetative state. It was amongst a few treatments that were shown to "improve the level of consciousness in certain cases"². Several studies have

shown that the drug can provide transient improvement in the condition of patients, which was repeated when the drug was readministered.

The work at Aston involved MRI and MRS scans of the patient, designed to characterize both the anatomical and chemical damage caused by the stroke. Changes in brain circulation and activity were identified and administration of the drug showed improved circulation in the affected half of the brain.

In the field of neurodevelopment, studies were carried out involving two children with rare neurological disorders related to epilepsy. One child's brain was studied as she conducted certain tasks requiring her to move her fingers whilst being scanned for a recording period of 1 hour. The data from this girl was compared to results from her twin sister.

In a second study, EEG and fMRI were used simultaneously on a patient with the epilepsy related disorder, to determine whether the areas of the brain that showed EEG spikes in between seizures also had signal changes due to the blood in that area. Results suggest that certain areas of the brain are connected, but that the

connectivity patterns from these areas suggest that the functions leading to the condition extend beyond these particular areas of the brain.

One particularly intriguing study, another that could not possibly be conducted in animals with any reliability in animals, involves the differences in the brain when participants are critical or reassuring towards themselves. Self criticism has been strongly correlated with a range of conditions such as depression, anxiety and eating disorders, and "self-reassurance" is inversely associated with the same conditions. As little is known about the neurophysiology of these internal processes, the group used a new fMRI task to investigate.

Results indicated self criticism was linked to activity in the areas of the brain which are connected to error processing and resolution, and behavioural inhibition. Self-reassurance was linked to the brain areas responsible for compassion and empathy towards others. This work is useful for showing the neural basis for certain mood disorders.

This is the type of work that cannot be carried out on animals, and shows the elegance and real scientific relevance of these cutting edge technologies.

1. (<http://www.nlm.nih.gov/medlineplus/druginfo/meds/a693025.html> - accessed 27/10/10); 2. Georgiopoulos, M. et al (2010) "Vegetative state and minimally conscious state: A review of the Therapeutic Interventions", *Stereotactic and Functional Neurosurgery*, vol.88, pp.199-207



APPEAL: We need to raise £50,000 to sustain this project in 2012



YOU CAN HELP end animal research

In these hard economic times, it is more important than ever, that we continue the work of the Lord Dowding Fund.

The Fund is saving animals from research while it also provides the world with advanced scientific

techniques – good for both animals and people.

Countless animals have been saved, and more can be done.

By next year, our work with the European and UK parliaments will have produced new rules to ensure that non-animal scientific techniques are given more weight in the research decision-making process.

So it is important to redouble our efforts. The replacement of the use of animals in research with more sophisticated methods is undoubtedly the proposition that has been the most effective in persuading the scientific community to end animal use.

Our steady progress over the past twenty nine years must continue.

YOUR DONATION CAN HELP US

We urgently need donations to continue our current research projects, as well as to expand our work, bringing closer the day when animal research is something of the past.

Please support the work of the Lord Dowding Fund

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Survey on animal use and alternatives in higher education in Europe

For almost thirty years, the LDF has supported and promoted the replacement of the use of animals in life science education. We believe that in order to change the culture of animal use in industry, science graduates need to learn using advanced techniques, rather than animals.

As part of this ongoing work, we commissioned a survey of the use of animals in higher education in Europe, which was carried out by Dr Akiko Hemmi and Professor David Dewhurst, of the University of Edinburgh.

Aims and objectives;

- To establish the level and extent of animal use in higher education in universities in ten European states.
- To ascertain the level of awareness of alternatives to animals and the implementation of these alternatives.
- To identify those courses in which animals are used and to correlate this to the available alternatives, thus identify any gaps in provision.
- To identify any barriers (such as cost

or language) to the implementation of alternative methods, if available.

- To identify, where necessary, the cause of gaps in the provision of alternatives.

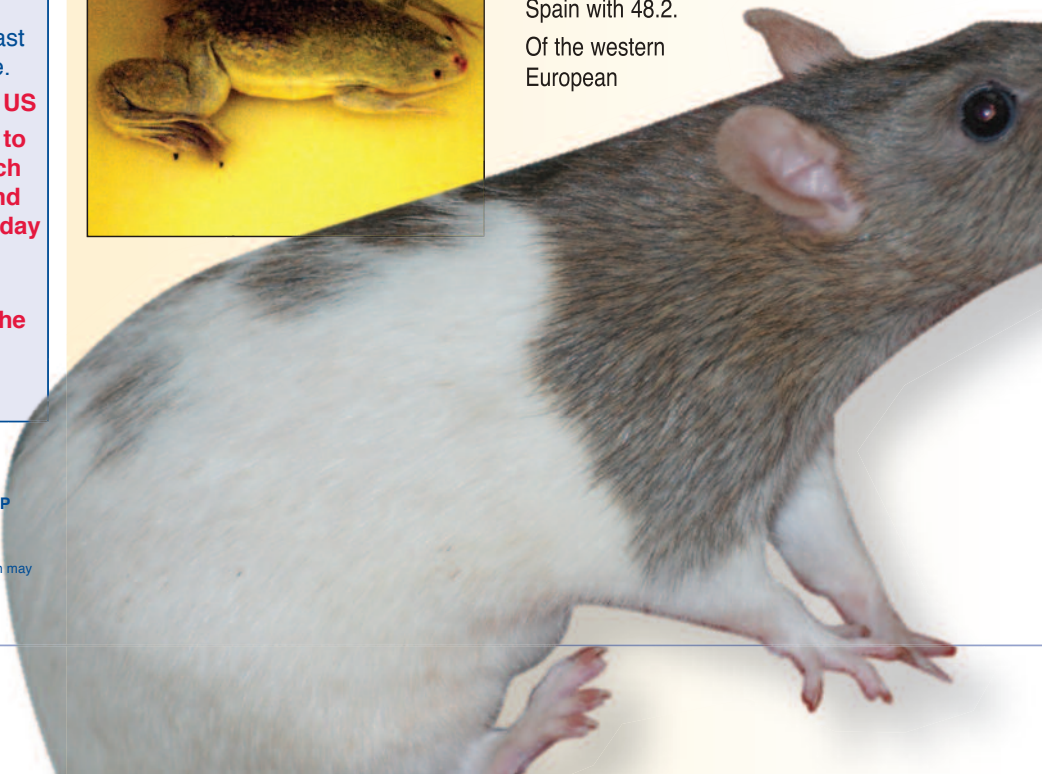
Countries were selected for the survey in order to give a good mix of geography, developing vs. developed countries and languages. Those chosen were the UK, France, Germany, Spain, Italy, Poland, Holland, Slovenia, Czech Republic and the Republic of Macedonia.

A search was then conducted to identify courses that contained components of physiology or pharmacology, especially via practical laboratory classes. The number of institutions contacted in each country ranged from 3 to 52, with a total of 294 contacted. Each was invited to complete the questionnaire, which had been translated into their language in order to maximise the response rate and ensure clarity. Follow-up emails and telephone calls were made.

The main findings

The average number of animals used per institution was calculated, although the low number of responses from the Eastern European countries made valid comparisons difficult. However, Romania was found to have the highest average of 110.8 animals, followed by France with 68.6 and Spain with 48.2.

Of the western European



d alternatives rope

universities the UK had the third highest average levels after France and Spain. Underlying these figures is the fact that Spain uses the highest number of mammals, and the UK the highest number of amphibians.

The UK showed a staggeringly high use of guinea pigs relative to other countries, with an estimate of over 400. The next highest figure for guinea pigs was France, with 11. According to Professor Dewhurst "this fits with the guinea pig ileum preparation being the most frequently used in teaching undergraduate pharmacology" in the UK.

The top three reasons why institutions did not use computer-based resources were identified as:

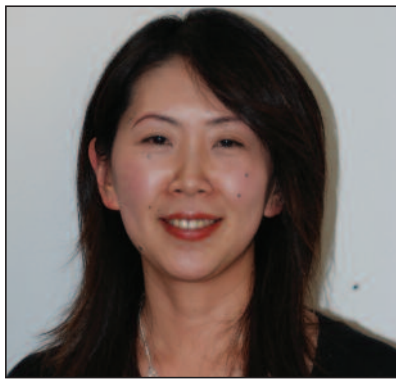
1. Difficulty in finding resources.
2. Lack of money.
3. Available resources did not meet specific learning objectives.

In Germany and Spain, the "lack of capacity to create resources in-house" was reported as an issue and in Italy the barrier was cited as "the lack of support from their institutions and/or colleagues". Three of the four Eastern European countries found that the cost of the programmes was a barrier to use.

Positively, a number of factors that would persuade academic staff to introduce alternatives were identified. These included "published evidence of effectiveness" and "recommendation from a colleague".

Interestingly, in the Western European institutions the students' objections to animal use was "a serious driver", whereas in the Eastern European countries this was a lower priority than saving money. Thus in some countries, moral objections may not be sufficient to reduce animal use if it is perceived that the alternatives are more expensive.

It was noticeable that some institutions chose to remain anonymous, making it difficult to obtain accurate data and calculate response rates. This may have



Above: Dr Akiko Hemmi, of the University of Edinburgh

been because the questionnaire was perceived as being the "thin end of the wedge" to abolishing animal use in education. However, the authors believe that "the study has made a significant first step in this area and possibly represents the most comprehensive survey carried out to date".

As expected, mice and rats were the most common mammalian species used for teaching, although Macedonia and Spain were the only countries to report the use of dogs. Of the non-mammalian species, amphibians were most used; possibly to teach nerve and muscle physiology.

Language was reported by some respondents to be a barrier. This shows the importance of making foreign language versions of these programmes available, especially if they are free or low cost and there is published evidence of their effectiveness.

It is hoped that eventually no animals will be used in teaching. Alternatives are certainly available and were reported as being used to replace animals at high levels in Spain and at low levels in France and Italy. The authors concluded that the "reported use of alternatives in UK universities (35%) is disappointing".

This study shows that despite successes there remains much to do to ensure that alternatives to animals in education are actually implemented. Step one is developing the alternatives but it is vital to maintain the pressure to ensure their use. This study has provided vital information for our drive to end the use of animals in education.



Human mirror neurons recorded for first time

Researchers at the Ronald Regan UCLA Medical Centre in the US have recorded the neurons in the brain that fire when we perform an action, or see others doing the same. The "mirror neurons" were recorded directly for the first time in humans during research involving 21 consenting patients undergoing treatment for epilepsy¹. Single cell recordings are usually taken in animals, often primates. The current research discovered "two neural systems where mirroring responses at the single-cell level had not been recorded previously, not even in monkeys²".

EU super computer

The Partnership for Advanced Computing in Europe (PRACE) is an international non-profit association, enabling European researchers to access super fast computers located in other countries². The PRACE infrastructure will allow the investigation of the way drugs interact with the human body and protein folding, for example. It could also help to gain knowledge about how to increase blood flow in cardiac disease, potentially predicting heart attacks and preventing deaths³.

Inhalation tests

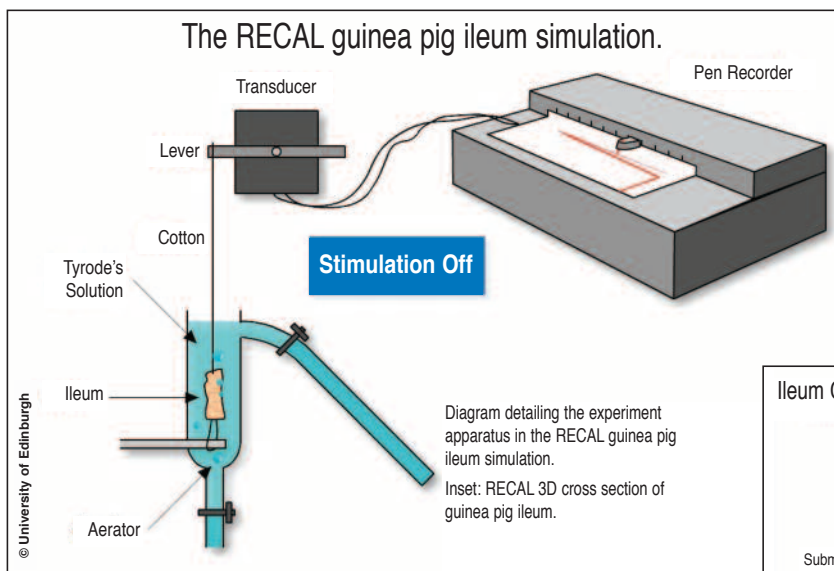
Scientists are working to create technologically advanced alternatives to replace animals in inhalation studies. The results will be useful in drug development for humans.

Biological engineers at Harvard University have created a "microsystem" which mimics activity between the alveoli sacs of the lungs and their connected capillary blood vessels. The scientists have constructed the model in such a way that it is possible to apply a "cyclic mechanical strain" to the cells which mimics breathing in the whole lung⁴.

The researchers believe the new "lung-on-a-chip" model and other micro-devices may "expand the capabilities of cell culture models and provide low-cost alternatives to animal and clinical studies for drug screening and toxicology applications".

1. <http://www.sciencedaily.com/releases/2010/04/100412162112.htm> accessed 5/11/10; 2. <http://www.ehealthnews.eu/research/2110-european-commission-welcomes-launch-of-supercomputing-infrastructure-for-european-researchers> accessed 4/11/10; 3. <http://europa.eu/rapid/pressReleasesAction.do?reference=IP/10/706&format=HTML&aged=0&language=EN&guiLanguage=en> accessed 4/11/10; 4. Huh, D et al (2010) "Reconstituting Organ-Level Lung Functions on a Chip" *Science*, vol. 328, pp: 1662 - 1668;

25 years of replacing animals in education



This is not the case when using animal tissue.

Another programme is the “Langendorff Heart”. This is interactive and simulates experiments that may be performed on the isolated perfused mammalian heart. The program covers the effects of various different classes of drugs and the effect of ions. The simulated data are derived from actual experimental data.

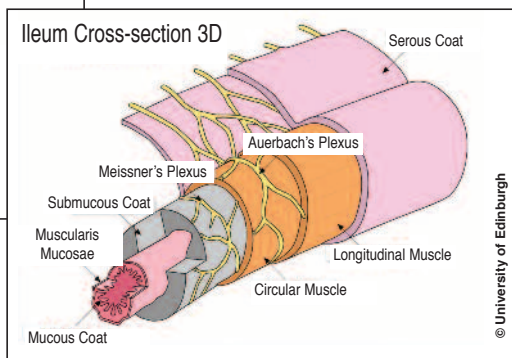
For a quarter of a century, LDF has funded David Dewhurst in his work to develop and refine software packages to replace animal use on physiology and pharmacology courses.

Not only does this ensure that the students’ learning experience is humane,

work and the unnecessary suffering caused by the experiments which this software seeks to replace.

The guinea pig ileum program simulates an isolated preparation of the guinea pig ileum. In the animal model, this is tissue that has been removed from the guinea pig prior to its death. The aim of this practical is to explore the effect of drugs and electrical stimulation on the release of, and response to, neurotransmitters in the nervous system in the intestine.

The computer simulation enables a range of drugs to be used, alone or in combination and over a range of doses. In addition the effects of electrical stimulation can be determined. A “magic” wash facility allows the model to be instantly cleaned of all prior traces of drugs, and further experiments to be conducted, thereby speeding up the learning process.

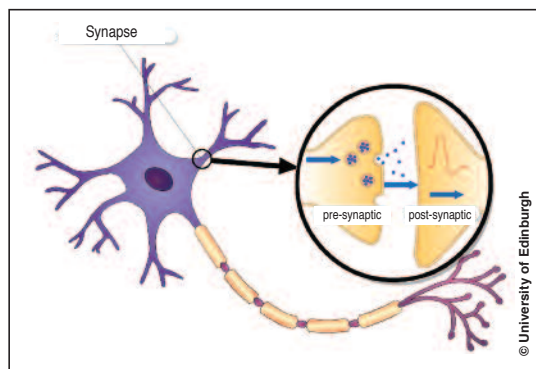


An independent review of the Langendorff heart program stated that “The handling of the program is easy and its appearance attractive... This resource is suitable for the total replacement of an animal lab, thus offering a solution to the difficulties involved in running the animal lab.”

In order to make these exciting alternatives to animal use available to students across the world many topics have been translated into languages including Chinese, Spanish, Ukrainian, Romanian and Lithuanian.



teaching them to use advanced techniques rather than animals, but it also allows the students more flexibility. If a mistake is made when using a humane method, or the student wishes to change a variable, they can simply start the procedure again. Professor Dewhurst’s work replaces several experiments and procedures in teaching. It is useful to look at these in order to appreciate the magnitude of this



Alternatives have been implemented in about a third of UK Universities. But, despite a quarter of century of effective use, two thirds of universities in the UK continue to use animals such as guinea pigs. It is vital that pressure be increased to ensure that alternatives are used.

Right: Diagram of a neuron showing a close-up of the synapse.



Paul Furlong, Professor of Clinical Neuroimaging at Aston University, in conversation with *New Science*.

Professor Paul Furlong, MPhil., PhD. and his group have published 36 high impact international peer reviewed journal articles and presented work at over 25 International neuroscience conferences during their preceding 5 year funding period. LDF has now granted Professor Furlong funding until 2015.

***New Science (NS):* Describe a “typical day” at the Aston Neuroimaging suite.**

The work is varied but is roughly divided between fundamental research, (exploring brain responses in healthy volunteers) and our clinical research work (studying brain function in patients referred to us by NHS consultants). Two different brain scans are performed for both fundamental and clinical research; MRI or MEG based scans.

At the Aston MRI Centre we scan firstly to obtain a high resolution brain image which we can reconstruct in three dimensions, but also to then obtain vital information about how the brain processes information. This is often achieved by projecting information or stimuli to human volunteers whilst they are in the scanner.

Another type of scan we perform is MEG, where we detect brain waves, generated by brain cells. We can create ‘virtual electrode’ data, which can replace some types of invasively implanted brain electrodes.

By merging MRI and MEG scans, we gather a tremendous amount of valuable information about how the brain is working, helping us understand brain function in healthy volunteers as they process information.

***NS:* Outline one use of animals that could be replaced today with non-animal methods.**

In the field of behavioural neuroscience, primates are trained to respond to a stimulus in order to study brain cell changes associated with their response and behaviour. In many cases I believe that the questions could more effectively be studied non-invasively using neuroimaging techniques, especially with pain research, where human cognition is a vital factor in understanding the response to pain and analgesia.

***NS:* What are the advantages of using non-animal methods in neuroimaging?**

Most importantly, human studies in health and disease are directly translatable to the human condition. This is vital if we are to truly understand the underlying causes of mental illness and many conditions that do not naturally occur in animals and are often artificially induced to mimic symptoms and signs.

***NS:* Can you envisage a time when animals will not be used in medical research? What, if anything, is currently preventing this happening?**

I believe that eventually animals will not be used in medical research because

alternatives will offer the most effective and productive data for treatment of human illness and disease. There are already compelling alternatives which will be refined and improved over time.

Seeking alternatives needs to be a strategic priority, with appropriate resources in place. Establishment of the NC3R in the UK was a step in the right direction, although resourcing and scope of action are relatively modest.

Government strategy and policy needs to put pressure on our commercial partners in the pharmaceutical and cosmetics industries to make animal alternative strategies viable and ultimately financially attractive. Financial considerations remain the greatest obstacle to progress in my view.

***NS:* What do you think neuro-imaging will be like in 25 years time?**

It would be wonderful to think that our techniques will be in the forefront of screening for developmental disorders and brain disease. Together with gene screening and gene therapy, that infants and young children could be scanned to aid profiling for the likelihood of diseases in later life, and preventative therapies used to eradicate many of the brain disorders and mental health issues that affect so many people today.

Human model for cancer research

In 2010, we awarded a grant to Dr Debbie Holliday and her team at the Leeds Institute of Molecular Medicine to validate and test therapeutics in a novel, all human, model of breast cancer.

Breast cancer is a complex disease with several different molecular alterations involved in its development and progression. A comprehensive approach, taking this into account, is required to improve the efficacy of target-based therapy in breast cancer. None of the above can be easily or accurately replicated in animal models.

The primary aim of the project is to validate two novel *in vitro* models of breast cancer; a 3D

cell culture model and a tissue slice model.

The 3D cell culture breast cancer model is the first to contain the 3 major epithelial (lining) and stromal (connective and supporting) components of the breast. The group have now extended their model, to include human ductal breast epithelial tumour cells, which form spheroid structures in culture similar to structures seen in breast tissue. Data produced so far is encouraging, and confirms that the 3D model is a good representation of living breast tissue.

The tissue slice model allows the validation of the 3D culture model, ensuring that it retains characteristics

representative of the original tumour. The team have obtained samples of both normal and tumour containing breast tissue, and successfully cultured the samples for up to 7 days. These have been treated with different doses of two common breast cancer drugs.

The models will be validated against published data, to show that they provide data comparable with those observed in animal experiments, and thus are viable replacements.

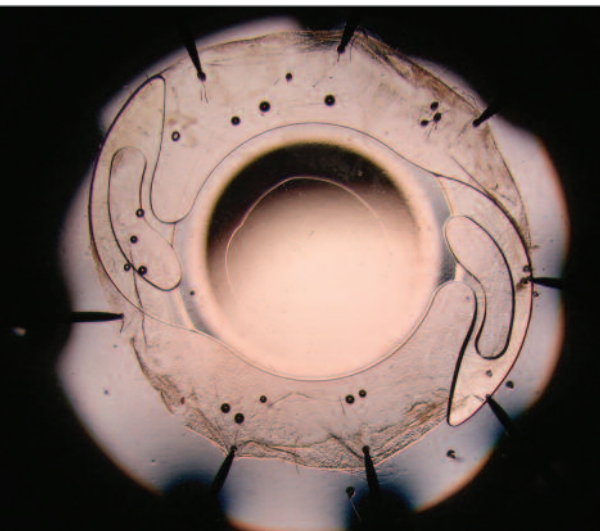


APPEAL: We need to raise £ 20,250 in 2012 to continue this important cancer research

Tissue banks and ethical c

One of the best ways to research human disease is to use human tissue and cells. This can be either in living subjects or donated tissue, which might be given after removal during surgery, if surplus to requirements. In legal terms human tissue is defined as “material that has come from a human body and consists of, or includes, human cells”.

The metastasis of a cancer cell (green) into a donated human brain cell (red).



Above: A capsular ocular bag, from a human donor, with an intraocular lens implanted as used in project carried out by Dr. Michael Wormstone at the University of East Anglia.

The LDF is funding Professor Geoff Pilkington at the University of Portsmouth for his brain tumour research. We recently asked Prof Pilkington about the difficulties he has faced in obtaining tissue for study.

Prof Pilkington has been researching brain tumours for over 35 years and in his view, in order to progress science and research at a sufficient pace to save lives, the system for tissue donation needs to change.

Firstly, there are strict regulations on the use of human tissue. The Human Tissue Authority (HTA) is an independent statutory regulator, sponsored by the Department of Health. The HTA licences organisations that store and use human tissue for research, teaching, post mortems or treating patients and approves the use of organs and bone marrow for transplants¹.

The Human Tissue Act 2004 (HT Act), which covers England, Wales and Northern Ireland, is the regulation that governs the removal, use and storage of human tissue. Scotland is covered by the Human Tissue (Scotland) Act 2006.

The HT Act also makes it unlawful to have human tissue with the intention of analysing the DNA without the donor's consent.

The red tape

The HTA code of practice relates directly and solely to research, and licences organisations that store human tissue for research.

There are different rules regarding tissue from the living and the deceased. Tissue taken from a patient for diagnosis, and then stored in a diagnostic archive can be valuable in research, but can only be released when the following conditions have been met:

- The patient has given consent for use of their tissue in research; or
- The tissue will be released to the researcher in a non-identifiable form; and
- The tissue will be used in a project that has been approved by a recognised ethics committee (REC).

The guidance also states that “Ethical approval can only be given by a recognised research ethics committee”. This being either an REC operating to standards issued by the UK Health department, or an ethics committee recognised by the United Kingdom Ethics Committee Authority (UKECA) to review clinical trials of certain products.

If the researcher intending to use the tissue is a university researcher, the situation is slightly different in that “A university ethics committee is not, for the purpose of consent exception considered to be a recognised research ethics committee. Therefore consent is still required for tissue to be used in a research project approved by a university ethics committee, even if it uses tissue from the living and the researcher is not in possession, and is not likely to come into possession, of information identifying the participant”².

Professor Pilkington comments that the system of applying for ethical clearance to use tissue is cumbersome and, rather than increasing the availability of tissue and thereby the amount of research that could be conducted with it, it actually hampers such research. This leads to frustration, not only to researchers but also to those who wish to donate tissue for research, and their clinicians.

Professor Pilkington explained to *New Science* the complexity of the on-line forms and the periodic reviews of ethical clearance which take up time. He is regularly contacted by patients who would like their tissue to be used in research to save lives, but he has to inform them that this is unlikely - he must have separate ethics clearance for each hospital from which he is to receive tissue. If that ethics clearance is not in place, even if the patient wishes to donate their tissue, Professor Pilkington would be unable to accept it.

In his words, this is leading to “around 99% of all brain tumour tissue, which could be used in research, being discarded”. He also points out that “This system is not helping patients, clinicians or researchers, and it is ultimately wasting time and hampering our research”. He would like to see “the system being replaced with an opt-out, where tissue, which is removed during surgery and will be discarded, would automatically go to researchers who could use it in their research. If patients were unhappy with this, they could opt out of the system”.

Professor Pilkington would also like to see the anonymisation of samples removed, giving researchers



Right: Brain cells, from a human donor, used in Prof. Geoff Pilkington's project at the University of Portsmouth.

APPEAL: We need to raise £37,739 to sustain our brain tumour research project in 2012

clearance

access to patient information that may help with research. This would also allow patients to follow the progress of their tissue in the laboratory.

How to donate tissue

The HTA advise that "If you want your tissue to be used for any medical research, or you may want it to be used only for specific types of research, it is important that you make these wishes clear to the healthcare professional who seeks your consent, and that they are stated in writing on a consent form. However, you should be aware that some tissue establishments may not be able to use your tissue at all if you impose very specific requirements for its use"³.

Tissue and Brain Banks

The HTA also note that people who are on the NHS organ donor register have not consented to their tissue being used after their death for research, although it may be possible for someone to do both, should they wish to³.

1. <http://www.hta.gov.uk/aboutus.cfm> - accessed 12/11/10;
2. HTA Code of Practice 9 – Research <http://www.hta.gov.uk/legislationpoliciesandcodesofpractice/codesofpractice/code9research.cfm> - accessed 02/11/10; 3. <http://www.hta.gov.uk/donations/howtodonateyourtissueforresearch.cfm> - accessed 11/11/10



In the UK, around 10,000 people a year suffer with cartilage damage significant enough to require treatment. The treatment for cartilage damage currently includes supportive braces, physiotherapy, painkillers and possibly surgical intervention. However, due to a lack of blood supply to cartilage, it can take a considerable amount of time to heal and regain normal function¹.

Recent studies have examined the use of therapeutic ultrasound, involving high frequency mechanical vibrations, as a potential tool for repairing cartilage damage. The healing properties of this method have been beneficial in treating bone fractures, repairing them significantly faster than normal.

Ultrasound has therefore recently been investigated as a possible treatment for the enhanced cartilage regeneration.

Unacceptable animal studies have been carried out in order to prove the efficacy of ultrasound therapy as a healing

ultrasound. By combining the expertise of the scientists in biomaterial science, cell biology and ultrasound technology, they optimised the tissue construct component of the project while perfecting the ultrasound equipment for the study.

This project has established an alternative to animal experiments for investigating the effects of ultrasound therapy on cartilage. The model was designed to be simple to manufacture, with good biocompatibility and mechanical integrity. The early work conducted in this project was presented at an International Biomaterials conference in September 2009 (See *New Science* 2009).

Looking to the future, and encouraging young scientists to adopt humane methods of research, Dr Harle is also a Science, Technology, Engineering and Mathematics Network (STEMNET) ambassador for the North West of England. In this role, Dr. Harle discussed the LDF work in the context of the

Cartilage repair

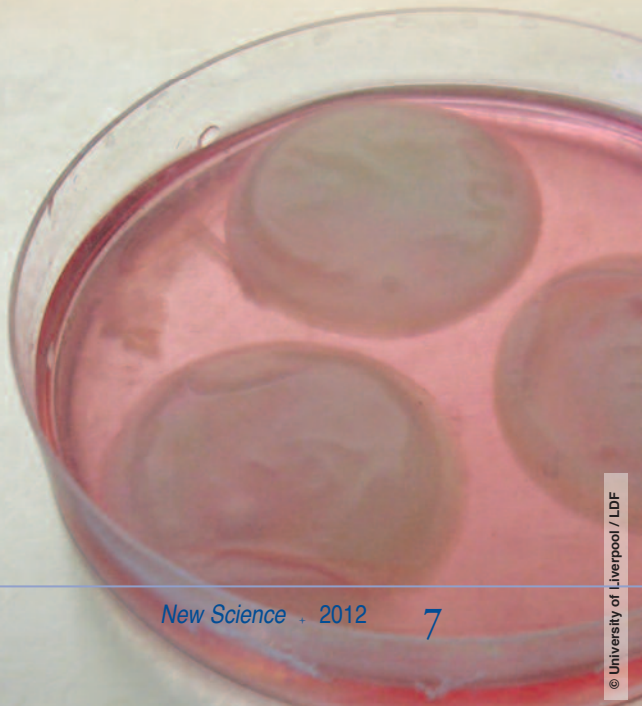
method. These have included dropping a 500g weight, from a height of 35cm, onto the legs of rats, themselves weighing only 300-500g. In another study this time to assess the effects of low-intensity pulsed ultrasound on cartilage repair, 106 rabbits had their legs damaged. The animals underwent surgery involving the opening up of both their hind limbs and the drilling of the femurs, through cartilage and bone, creating a 3.2mm diameter and 5mm deep defect.

Drs Vehid Salih and Jamie Harle, of the Eastman Dental Institute and the University of Liverpool respectively, have completed their LDF funded project, which aimed to advance knowledge regarding the healing effects of ultrasound on damaged cartilage without the unethical and unnecessary use of animals in these painful experiments.

The innovative, multi-disciplinary project established a three dimensional cell culture model for cartilage, free from animal-derived products, in which to investigate the therapeutic effects of

replacement of animals with school and college students interested in careers in science, medicine or related subjects. In addition, the postgraduate students who assisted with the project using non-animal derived products, will go on to carry out PhD and MSc work which it is expected to include these materials and methods, helping to establish a new generation of humane scientists.

Below: Three alginate constructs, seeded with human cells, prior to ultrasound experiments.



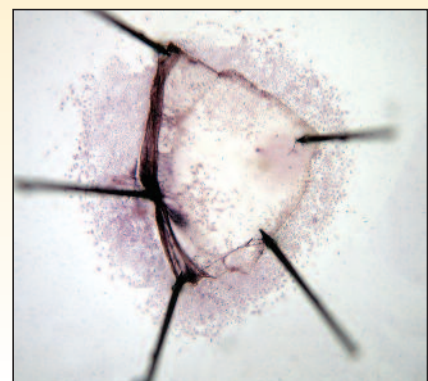
1. <http://www.nhs.uk/Pages/Preview.aspx?site=Cartilage-damage&print=634248319918443869&JScript=1> - accessed 08/11/10



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