JOURNAL OF OROFACIAL INVESTIGATION

Ponto de vista

Animal-free research: Where are we currently?

Cleo White B. Med. Sc

Postgraduate researcher
Biomaterials Department,
School of Dentistry, University of Birmingham, UK
CLW058@bham.ac.uk

The use of animals has been long documented throughout history however, over the last 50 years, there has been a significant increase in the number of humane and animal-free research undertaken within science. This can be attributed to numerous factors including the development of the 3R's framework, difficulties in translating results and experimental variability, an increased negative public response towards animal testing and the constant improvement and development of new technology [1].

The 3R's

The 3 R's which stands for reduce, refine and replace was a term first described by Russell and Burch (1959) [2] in their book titled 'The Principles of Human Experimental Technique'. Research heavily influenced by the 3 R's either involves experiments that actively avoid the use of animals (replace), experiments that uses the minimum number of animals required (reduce), involves methods that minimises actual or potential pain, distress or any other adverse effects during the animals lifetime, whilst being involved in experimental work (refine) or a combination of the three [2,3].

When Russell and Burch devised the humane experimentation framework, they couldn't have imagined the impact that this framework would have on future research. However as guidelines and regulations become stricter concerning the use of animals and animal welfare coupled with an increased incorporation of the 3 R's in funding guidelines, what alternatives are available to researchers [4]? The simple answer is more than you think.

Alternatives to animal testing

As science and technology has been developed and adapted, the number of alternative methods to animal testing has never been greater. The alternatives range from human volunteers and *in vitro* testing, to computer models, imaging studies and patient simulators. Selecting the most appropriate alternative method is undoubtedly de-

pendant on the nature of the research and what information is hoping to be gained.

In vitro testing

In vitro testing is the most commonly used and most familiar alternative to animal testing. This can range from developing 3D tissues such a skin, which can be used to understand topical effects of a drug, to organ on chip, which can be used to understand drug toxicity on an organ system [5]. The clear benefit for using these systems is that it completely eliminates the need for animals and high throughput testing can occur. However there are disadvantages to these methods. *In vitro* testing commonly requires supplementation of animal derived products such as foetal calf/ bovine serum (FCS /FBS) which in itself can cause problems such as lack of control due to high inter-batch variability. Nevertheless, scientists are still working with this technology and some are even looking at developing total animal-free environments, where no animal supplementation is required [6].

Using human volunteers

Human volunteers can be used in experiments known as 'microdosing' which is where participants are given a very small one – time dosage of a drug and are closely monitored to see how the drug behaves within the body as well as imaging studies which is where powerful imaging equipment such as CT, MRI and ultrasound are used to scan the body, which again, can be used to look at the systemic toxicological effect of a drug and psychological disorders [7]. Similarly to the in vitro testing, using human volunteers totally eradicates the need for animals to be tested. However, using volunteers for imaging studies will not unveil all the effects of the drug apart from those that can be picked up by the imaging equipment. Additionally microdosing experiments are classed as Phase 0 trials and in order for the drug to be approved, full clinical testing will need to occur. Unfortunately, this still involves animal testing with the full drug dose to identify if the drug is safe and effective.

Computer Modelling

The sophistication of computers has developed exponentially over the last 20 years. An increasing number of studies have used computers to model disease progression as well as being able to simulate human biology. These models can be used to study, plan and predict various outcomes such as how a nanomaterial will behave in a biological environment or studying how an implant will behave within a patient [8]. However with all methods, there are limitations with use.

Computer models rely on data, which can only be obtained from experimental work. Furthermore, the outcomes are typically specific to the organism that is used to generate the initial model. Humans are extremely complex to model, so this makes human modelling a long and arduous task especially if basing the results on human studies (which can take months if not years to be approved) [9]. Additionally models rely on assumptions; therefore you commonly end up with a model that is not representative in terms of organism complexity and can render the model invalid [9].

Animal experiments – no decline

Although it can be seen that there are many suitable alternatives to using animals in experimental work, research conducted by Cruelty Free International and the Dr Hadwen Trust, the UK's leading non-animal medical research charity, has revealed that the number of animal experiments worldwide has not declined and in some areas of the world, this number has actually increased [10]. Why has this happened; why are we slow to adopt the 3 R's and embrace animal-free research? There are three main reasons as to why.

Firstly, although science and technology has progressed significantly, it has not progressed enough for example, some of the more complex tissues found within the body such as the brain are yet to be engineered within the lab. Secondly, the scientific community calls into question the validity of results from alternative methods as well as those who do entirely animal-free research. This stems down to the tradition of using animals in research which extends back to Ancient Greece and Rome. Many of the scientific community have a mentality that to truly test a hypothesis, this must be tested within a living animal or use tests that involve animal derived products 4. Thirdly, the alternative methods that have been developed, have their own limitations which have been mentioned above. So where does that leave us?

The future for animal free science

Animal experiments are not going anywhere based on the statistics and the current paradigm mentality we have towards them. However, as scientific knowledge and technology advances, so too will the alternative methods develop. As they develop, and increasingly become used as scientists look to transition away from current experimental methods, maybe one day we could see the majority of all research being animal free. The question is, as a community are we doing enough to facilitate the necessary developments and are we providing enough support to those paving the way?

References:

1. Flecknell, P. Replacement, reduction and refinement. ALTEX. 2002;19(2):73-8.

2. Russell WMS, Burch RL. The Principles of Human Experimental Technique. London. Methuen. 1959.

3. Balls M. The principles of humane experimental technique: timeless insights and unheeded warnings. ALTEX. 2010;27 (1):19-23.

4. Broadhead CL, Bottrill K. Strategies for replacing animals in biomedical research. Molecular Medicine Today. 1997;3 (1): 483-7.

5. Mathes SH, Ruffner H, Graf-Hausner U. The use of skin models in drug development. Advanced Drug Delivery Reviews.2014; 1:81-102.

6. Richards S, Leavesley D, Topping G, et al. Development of defined media for the serum-free expansion of primary keratinocytes and human embryonic stem cells. Tissue Engineering. Part C Methods. 2008;14 (3): 221-32.

7. Tewari T, Mukerjee S. Microdosing: Concept, application and relevance. Perspectives in Clinical Research. 2010;1 (2): 61-3.

8. Makarucha AJ, Todorova N, Yarovsky I. Nanomaterials in biological environment: a review of computer modelling studies. European Biophysics Journal. 2011;40 (2): 103 -15.

9. Brodland GW. How computational models can help unlock biological systems. Seminars in Cell & Developmental Biology. 2015; (1): 62-73.

10. Cruetly Free International. Facts and figures on animal testing. (2015). Available at: https://www.crueltyfreeinternational.org/why--we-do-it/facts-and-figures-animal-testing (Accessed 27th January 2017).