Submission No 225

INQUIRY INTO USE OF PRIMATES AND OTHER ANIMALS IN MEDICAL RESEARCH IN NEW SOUTH WALES

Organisation: Codex Research Pty Ltd

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Codex Research Pty Ltd – submission to the NSW Parliamentary Inquiry “Use of primates and other animals in medical research in New South Wales.”

Codex Research Pty Ltd is a NSW-based biotechnology start-up working on a technology that aims to greatly increase the effectiveness of basic and translational bioscience and biomedical research. We feel that the recent explosion in silicon chip technologies has not produced a parallel explosion in knowledge of human biology. Modern medicine is making improvements in its ability to manage symptoms, but the list of diseases that we know how to cure remains stubbornly short.

The reasons behind this problem are many and complex, but we believe that a significant issue is a reluctance to move beyond proven, but ancient, research methods. We have been studying cells in dishes for centuries, and conducting experiments on animals for millennia. Certainly, we have learnt a great deal using these methods, and modern enhancements make such experiments look very different from early efforts. But there are shortcomings to these techniques that no amount of high-tech tweaking can overcome. Human cells in a dish “know” they are in an alien environment, and respond very differently than the cells in a living human body. Animals have genetic similarities to humans, but as we are now trying to unravel the deep complexity of human biology, and particularly hoping to explore personalised medicine, it is increasingly clear that animal experiments are less and less relevant.

Codex Research has been developing a device designed to overcome the problems with existing methods. The device incorporates a smart pump with a tissue-like scaffold within which human cells can grow in a realistic, dynamic environment, experiencing the pulsing pressure, flow, fluid shear and mechanical strain that is normal for the cells in the human body. Our technology is at a very early stage, and a lot of development work needs to be done, but we are already seeing some very interesting results with our laboratory prototypes.

Attempts to approximate a human blood vessel in a box

We can now co-culture multiple vascular cell types together for at least 6 weeks under pressure and flow conditions approximating human vessels. This includes vascular endothelial cells lining the inside of the vessel and both smooth muscle and cardiac fibroblasts in the vessel wall. Grown this way, the cells behave very differently than in traditional 2D (cells in a dish) cell culture, with a greater representation of 3D physical cues as well as physical forces. We have shown *de novo* collagen growth in these structures.

Such a system then provides us with a new tool to study disease conditions and risk factors such as high-glucose to model diabetes, and calcification to model atherosclerosis. Already at this early stage, this has allowed us to study vascular devices and implants (e.g. balloons and stents) – deploying them in our system and studying cell recovery. Moreover, we have been able to add inflammatory cells – macrophages – to the system, a path to modelling the earliest stages of cardiovascular disease in a lab-based system.

Our academic collaborators in this technology have already received some small research grants to further this work using our devices.

Personalised medicine

A very exciting development was the award of a National Heart Foundation Vanguard grant for a study of patient cells in an effort to uncover previously unknown risk factors for cardiovascular disease. Libraries of patient-derived cells are difficult to build and represent an extremely valuable resource. This recent grant success for our collaborators, using the bioreactor as an enabling tool, demonstrates a growing appetite for advancement in this area and speaks to the potential of the bioreactor approach.

Other findings of interest

Further experiments have been conducted using our bioreactors to see if we could observe other interesting outcomes. In experiments with cancer cells we have observed spontaneous clustering, a phenomenon we expect would be of interest in research into secondary tumour formation.

We serendipitously observed the spontaneous formation of structures that might indicate early angiogenesis. An ability to research angiogenesis in depth, in a properly relevant biological environment, is fundamental to progress in many fields of study.

We need to stress that these findings are extremely early and a great deal more work needs to be done by experts in these fields before any claims are made about the value of these results. However, we do feel that it would be a great shame not to pursue these avenues of research using our bioreactor technology.

Possible benefits to NSW

The move to replace animal-based research with better alternatives is an idea whose time has come. More and more people are coming to feel that conducting experiments on animals is ethically wrong, and we are seeing the results of this social movement in new efforts by governments in Europe and the US to move away from animal experimentation. At Codex Research, we believe that alternative technologies have great potential to not merely substitute for animal-based research, but to dramatically improve our research outcomes.

These are still early days in what we see as an inevitable development of a new field of technology, which means NSW has a great opportunity to participate in this process. This has the potential for a huge boost to the NSW economy. Developing this sort “deep tech” is expensive and takes time. Private investment funding these days is focused on the next “killer app,” some piece of software that is quick to market with a predictable growth cycle. The NSW government provides some excellent funding opportunities for technology start ups, such as the Medical Device Fund and the Physical Science Fund grants. A similar grant specifically aimed at alternatives to animal-based research would be a great boost to the development of this technology in NSW.

A brief summary of recent literature on the value of using animals in medical research

Some general information about mouse vs human

Mouse and human had a common ancestor 80m years ago and share 92% of DNA. About 5% of each species’ genome are protein coding (genes) and out of ~4000 genes that have

been studied, less than 10 are found in only one of the species. On average, the protein-coding regions are 85% identical, however non-coding regions are not as well conserved at 50% or less. Overall they are highly genetically similar but it only takes differences in one gene to affect clinical translation. Further, mouse disease models usually don't reflect the full component of disrupted genes that contribute to human disease, and within a study mice are too genetically and environmentally similar and so results are often not replicable or generalizable even to mice.

Translation of Research Evidence From Animals to Humans

Hackam and Redelmeier (2006) JAMA 296(14):1727-1732

Only ~1/3rd of highly cited animal research (2000 articles published between 1980 and 2000 in leading journals) translated to human randomized trials – expected to be even worse for other published studies.

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

Suntharalingam et al (2006) N Engl J Med 355:1018-1028

In phase 1 clinical trials, six young, healthy men were given 1/500th of the dose of TGN1412 (leukaemia drug) given to monkeys, they all experienced a cytokine storm and multiorgan failure with unknown mechanism, luckily all survived. Turned out to be likely caused by only slight differences in the amino acid sequence of the drug's target that affected the strength of the antibody-antigen interaction.

Genomic responses in mouse models poorly mimic human inflammatory diseases

Seok et al (2013) 110(9):3507-3512

Genes that are significantly altered in human inflammatory diseases are highly similar between humans, but very little correlation with mice - in corresponding mouse models the orthologs are close to random in matching their human counterparts (R-2 between 0.0 and 0.1).

Lost in translation: animal models and clinical trials in cancer treatment

Mak et al (2014) AJTR 6(2):114-118

Less than 8% of cancer clinical trials translate to humans from animal models, largely due to physiological differences (failure to account for the complexity of human carcinogenesis) and imperfect homology of molecular targets between animals and humans.

The flaws and human harms of animal experimentation

Akhtar (2015) Camb Q Healthc Ethics 24(4):407-419

This review makes the case that animal experimentation is net harmful to human health, based on data showing that animal models and research are inadequate to predict human clinical outcomes for the vast majority of biomedical science. The resulting harms to humans are through unreliable drug safety and efficacy data (e.g. TGN1412 leukaemia drug from above) and through opportunity cost of potentially effective abandoned drugs that didn't work in animals as well as by diverting resources from more worthy causes such

as the development of human-specific models. Examples of drugs which may not be on the market today if they were subject to current animal testing regulatory requirements include aspirin and penicillin as well as Gleevec (for chronic myelogenous leukemia) which was discovered through predictive human based testing but had serious adverse effects in at least 5 species tested, and tamoxifen (effective for breast cancer) which caused liver tumours in rats but this was only discovered after being on the market for years.

Conserved cell types with divergent features in human versus mouse cortex

Hodge et al (2019) Nature 573(772):61-68

Used single cell and single nucleus RNA-seq across human and mouse cortex samples to characterise cell types. They found that homologous cell types between species often have highly divergent gene expression profiles. Key genes such as neurotransmitter receptors were amongst those with the highest expression variation between homologous cells – so developed drugs often hit the wrong signalling pathways entirely (i.e. homologous neurons often have different receptors). This key finding helps to explain why there have been so many drugs that have cured mice of brain disorders such as Alzheimers, schizophrenia and autism but none of these have worked in humans.

Organ chips, organoids and the animal testing conundrum

Horejs (2021) Nat Rev Mater 6:372-373

We still use animal models 1) because they are there and we know how to work with them and 2) many *in vitro* models are too simplified to be predictive, but now we are at a point where we can make much more complex *in vitro* models that are as good as or better than animal models. *In vitro* models can be tailor made to the specific question/tissue at hand and have many benefits over animal models (as well as being based on human biology) such as being able to decouple the effects of mechanics and fluid flow, being able to add in relevant components one at a time and control them independently as well as things like being able to culture microbiome with human cells (as animals have a different microbiome). Need to keep developing the technologies and engaging more researchers to achieve critical mass so that people start looking beyond animal models.

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