Retinal 3D: A Physiologically-Competent Human Retinal 3D Model

Background

Over 60 million people worldwide are blind (Zhang K. *et al.,* 2012). One of the leading causes of blindness in the ageing population is Age Related Macular Degeneration. There is currently no cure for this debilitating condition.

The eye is a complex organ comprising three major structures: the cornea, the lens and the retina. For the cornea and to a minor extent for the lens, *in vitro* models are available that enable, for example, testing of compounds for their potential to induce corneal irritation.

For the retina, there are no adequate *in vitro* models mainly due to its complex structure which consists of multiple cells types, including glia, neurons and the retinal pigment epithelium cells (RPE). There are some simple preclinical models available - for example, retinal explant organotypic cultures from early postnatal rodents resemble the retinal morphology to a high degree and contain the relevant cell types including Müller cells and RPE. However, they do not fully mature when cultured *in vitro* and therefore their translation to the mature human retina is limited. Human tissue is not readily available (Caffe A.R. *et al.,* 2000, Pinzón-Duarte G *et al.,* 2002) for these studies. Human-based *in vitro* models using primary or cell lines such as the RPE cell line ARPE19 (Dunn K.C. *et al.,*1996; Seigel G.M. *et al.,*1999) and the Müller cell line (MIO-M1) (Limb G.A. *et al.,* 2002) do provide useful tools but again, their relevance to the mature human retina is limited.

Complex human stem cell-derived models (Gonzalez-Cordero A. *et al.,* 2013) are beginning to emerge which form retinal spheroids *in vitro* but these resemble embryonic retinal development whereas mature phenotypes for example a ganglion cell can take up to 30 days to reach maturity. The spheroids often lack Müller and RPE cells which are key to a fully predictive model. Throughput is another concern as the generation and maintenance of these spheroids is often not amenable to industry use.

In summary, the currently available *in vitro* models have a number of limitations:

- They are typically based on a single cell type in culture and do not reflect the architecture of the mature human retina and the complex interplay between multiple cell types (e.g. photoreceptors, glial cells, endothelial cells, RPE cells).
- Integrated and functional measurements such as electrophysiology and high content imaging other than single cell behavior cannot typically be performed.
- Organotypic cultures of retina explants are typically not stable beyond a maximum of two to three weeks.

As a result of the current limitations, most pharmacological and toxicological studies to assess effects of drug candidates on the retina need to be performed *in vivo*. The degree of visual acuity and structure of the retina differs between animals and humans, reflecting the distinctive role of visual function between species (e.g. rats have more rods for low light scotopic vision, whilst humans have more cones for bright light phototopic vision, Jacobs *et al.,* 2001). For models more representative of human visual function, it would therefore be highly desirable to have access to a human-relevant retinal cell model to support compound testing *in vitro.*

Many other retinal diseases have a genetic background and are lacking suitable animal models. Utilising patient-derived iPS retinal cell models could integrate a disease background and phenotype for compound testing which would address these concerns and provide the opportunity for the development of novel safety testing strategies.

Recent advances in the field of tissue engineering, such as by bioprinting, now provide the capabiity to address the development of a 3D model comprising the three major retinal cell types: Müller and microglia, neurons and RPE. This is the aim of this CRACK IT Challenge.

Such a model would enable the *in vitro* assessment of drug candidate-induced alterations on the cellular phenotypes and functions which have relevance *in vivo*, and which can be used not only for pharmacological and early toxicological assessment but also for disease modelling. This would also provide the potential for a leaner early drug development programme for ophthalmology indications as, for example, optimisation related to mechanistic toxicity could be performed earlier in the cycle, shortening preclinical development and enabling faster time to the clinic.

3Rs benefits

Worldwide, there are currently more than 600 R&D projects in the field of ophthalmology and the market for therapies targeting retinal disorders is expected to grow to $14.8 billion by 2022 (Pharmaventures, 2015). The majority of studies for efficacy and safety testing in opthalmological drug development are performed in animals (mainly rodents and rabbits) that provide both functional and histological readouts. For example, *in vivo* studies investigating ocular safety of new compounds use at least 20 animals per compound.

The proposed new model set by the Retinal 3D Challenge has the potential to replace the use of animals in the discovery of new opthalmologic drugs as well as screen out a significant number of compounds that would fail later in the pipeline, thus avoiding unnecessary animal use. The *in vivo* studies that are still required will be designed with improved mechanistic toxicological and pharmacological knowledge to enable more relevant dosing and group numbers and potentially deliver more refined humane endpoints.

Need for collaboration

The structure of the retina is complex, comprising many different cell types including neurons, glia and epithelial cells. To establish a retinal 3D model, a sound background in ophthalmology and retinal physiology will be key. Expertise in tissue engineering and/or bioprinting to build in the 3D element to the model is also important. Depending on the model proposed, knowledge in stem cell research would be beneficial, as well as comprehensive expertise in the application of state-of-the-art assays for functional characterisation of a retinal model, (e.g. imaging and electrophysiology).

Overall aim

The overall aim is to establish a 3D retinal cell model which is physiologically-competent and predictive of human physiology. The model should consist of all the major cell types of the retina and enable their interplay: Müller and microglia, RPE and neurons (including photoreceptors). The model needs to resemble key morphological and functional features which can easily be addressed with a panel of functional readouts.

Key deliverables

The retinal 3D model should replicate:

- The morphology of the mature human retina (e.g. resemble the ‘barrier properties’ of an inner limiting membrane, proper layering of cell types).

- The human physiology of the retina to the extent required for the specific assay endpoints, including readouts (e.g. electrophysiological, immunohistochemical) of the cell types used.

Phase 1 deliverables

- Establish an *in vitro* culture system which enables stable co-culture of the required cell types in 3D as evidenced by:
    - Basic morphological (and functional) characterisation of cell types using biomarker expression and indicators of maturation stage by e.g. immunohistochemistry and gene expression.
    - Reproducibility between different experiments.
- Demonstration of cell phenotype stability and viability for at least (72 hours) as indicated by relevant biomarkers/readouts.
- Robust plans to deliver Phase 2 of the Challenge including commercialisation and dissemination.

Phase 2 deliverables

Essential:

- Extensive functional and morphological characterisation of the retinal model.
- Development of additional methods to address function and/or phenotypic changes of the different cell types in the culture system as a function of both maturity and drug treatment.
- Recapitulation of retinal toxicities of known drugs in the system based on morphological and functional readouts, down to mechanistic phenotypes and the cellular level.
- Assessment of inter/intra-laboratory reproducibility.
- Provision of accessible morphological and functional readouts and be compatible with standard microscopes.
- Amenity to the testing of compounds in parallel (multi-well-plate setting), easy to implement in an industry laboratory setting, reproducible and easily transferrable between laboratories.
- Evidence of cost efficiency and amenity to individual and longer term experiments.
- Adaptability to other species relevant for safety assessment (rodent and/or non-rodent).

Desirable:

- Demonstration of the barrier function of outer and/or inner-limiting membrane.
- A functional blood-retina barrier.
- Vasculature (endothelial cells) to mimick neo-vascularization and leakage of blood vessels to model disease phenotypes such as Diabetic Macular Edema.

The model should not:

- Require complex and time consuming set-up, impeding parallel testing of multiple (10-20) conditions.
- Incorporate only cytotoxicity as a readout.

- Incorporate material which is know to show strong compound adsorption (e.g. PDMS).
- Require specific legal work for acquisition of source cells and materials (i.e. individual informed consent for each study/experiment).

Proposals that utilise primary cell, cell lines or stem cells are welcome.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Sponsors will provide:

- Expertise in ophthalmology and *in vitro* models including specifications for an *in vitro* model which is fit for purpose for drug testing in an industry setting.
- Compounds and knowledge of compounds where available for evaluation of both the pharmacological and toxicological performance of the *in vitro* retinal test system.
- Potential for in-house testing using the system to test transferability and reproducibility of the retinal *in vitro* model.

Duration

Phase 1: six months Phase 2: Up to three years

Budget

Phase 1: £100k, Phase 2: £1million

Sponsors

Roche, Merck and Novartis

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