SMALL Editorial

Title: **Advanced *In Vitro* Models for Replacement of Animal Experiments**

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In the last decade there has been an exponential increase in the scientific focus given towards enhancing the physiological relevance of *in vitro* systems and expanding their applications to support the movement towards replacing, reducing and refining (3Rs) the use of *in vivo* experimentation. Beyond socio-economic considerations towards public stakeholders, this has also had a substantial impact upon ongoing changes in regulation surrounding the application of animal testing strategies. Despite the continuing advancements within the field there remains a constant need to develop, enhance and implement *in vitro* model systems in order to; (i) deduce their potential to reduce and potentially replace animal experimentation, as well as (ii) progress their implementation and acceptance by regulatory and legislative stakeholders.

The intention of this Special Issue (SI) was to bring together a wealth of expertise and knowledge regarding the plethora of ongoing research activities regarding *in vitro* model systems, specifically providing current insight into the advances made and to discuss the issues being encountered.

Introducing the topic area there are two essays that discuss the problems and challenges of animal experiments (*Fontana paper*), and then how these problems can be counteracted by new *in vitro* methodologies (*Burden paper*). The paper of Burden and colleagues is further supported and expanded upon by reviews authored by both Bas *et al* (*Bas paper*) that highlights the specific requirements for the development of standardised *in vitro* approaches for validation purposes; as well as Halappanavar and colleagues (*Halappanavar paper*) that highlights non-animal strategies for toxicology testing, particularly engineered nanomaterials, focussing upon adverse outcome pathways. The specific use of *in vitro* models of the liver for nanomaterial genotoxicology testing (*Llewellyn paper*), and the cardiovascular system to assess myocardial infarction (*Sharma* & *Gentile papers*) are then introduced. While Fritsche and colleagues highlight the potential application of models based on embryonic stem cells and induced pluripotent stem cells of human origin within next level *in vitro* toxicology assessments (*Fritsche paper*).

Building upon these expansive reviews of different *in vitro* tools available and how they can be used to support efficacy and safety testing, 11 original articles then highlight the multitude of ongoing activities with *in vitro* systems. With the comparisons continually made towards *in vivo* data sets, and its relevance towards regulatory acceptance of *in vitro* approaches, Ma-Hock and colleagues (*Ma-Hock paper*) focus upon the necessary dose setting for *in vitro* studies based upon previous *in vivo* studies relevant to engineered nanomaterial lung burden. Further to this, studies demonstrating the advantages offered by advanced *in vitro* and *in*

silico models for nanosafety assessment are highlighted by Kampfer and colleagues (*Kampfer paper*), Burgum *et al.* (*Burgum paper*) Chiou and colleagues (*Chiou paper*), as well as Jagiello *et al*. (*Jagiello paper*), utilising a range of approaches from (geno)toxicity testing to microscopic evaluation and predictive modelling based on quantitative structure-activity relationship models for nanomaterials (Nano-QSAR).

In terms of advancing systems towards re-creating the physiological and anatomical human structures *in vitro*, the work of Poventud-Fuentes and colleagues (*Poventud-Fuentes paper*), Kohl and colleagues (*Kohl paper*), as well as Lee and colleagues (*Lee paper*) expand upon organ-on-a-chip technologies and microfluidic devices. Tang *et al.* (*Tang paper*) discusses the possibility of 3D bioprinting *in vitro* glioblastoma models, and Suhito *et al.* (*Suhito paper*) apply the use of spheroid cultures to create a nanostructured platform that facilitates the electrochemical detection of cellular changes in cancer cells. These papers further highlight the expansive possibilities associated with *in vitro* technologies. The final original article published in this SI takes another direction with Hsu and colleagues (*Hsu paper*) reporting the effects of the oxygen microenvironment and matrix composition on the 3D endothelial cell network formation, providing a further example of how to alter variables within *in vitro* systems to more closely mimic human physiology. Recreating the physiological nature of the human body is further discussed in the concept article by Llewellyn and colleagues (*Llewellyn paper*) whereby they re-create acellular lung and gut micro-environments to assess the impact of nanomaterial transformation in such biological compartments, upon toxicological outcomes in advanced *in vitro* models of the gastro-intestinal tract and liver.

This SI therefore highlights the qualities that *in vitro* systems can provide in terms of understanding mechanisms of toxicology, or interactions of xenobiotics with specific cellular structures of a tissue/organ. It further progresses towards introducing the current state of the art in terms of advanced *in vitro* technologies (*e.g.* microfluidics, organ-on-a-chip, bioprinting and re-creation of physiological components). With the expansive introductions at the beginning of the SI, setting the scene of 'alternative models' and advanced *in vitro* systems, this collection of papers provides a fantastic basis for both people being introduced to the field, but also the experienced *in vitro* scientist.