The CELL CULTURE innovations increasing drug discovery success

'Fail early, fail fast' has been a mantra in the pharmaceutical industry for several years and directly impacts the overall strategy of many drug discovery and development programmes. Yet, despite the drive to fail early, many programmes still suffer late-stage attrition, with only 14% of drug candidates at Phase I making it to market[1].

By Helen Stewart-Miller and Abby George

Drugs failing during clinical development results in the loss of significant financial investment, time and resources, which can cause programmes to be abandoned and even force smaller organisations to close. So why are late-stage failures still a problem, and how can innovations in physiologically-relevant cell culture models help increase the chances of success?

The importance of drug target validation

Typically, drugs fail for two reasons: they lack efficacy and/or they have toxic effects. These are often the result of off-target effects, drug-drug interactions and, importantly, poor target validation – a crucial step in the drug discovery process. A good target needs to be efficacious, safe and, of course, druggable. Target validation should therefore elucidate the relationship between the target and the disease and give researchers confidence that target modulation will lead to the desired therapeutic effect.

"Drug target validation is the most crucial initial step in drug discovery. A critical mistake made at the start of this lengthy and complex process starts a sequence of linked events that ends up with a drug that does not work," says Mark Treherne, Non-Executive Director, Cell Guidance Systems and Chairman of Talisman Therapeutics.

To increase the chance of success in the clinic, it is imperative that target validation studies are translational. "Biological data drives decision making, and the more relevant the data generated by those assays are to a given physiological or pathological process, the greater the probability of selecting the optimal compound *in vitro* for subsequent development," Mark adds. As such, there is a drive in the industry to develop more physiologically representative cell-based assays that can give researchers the data they need to make better-informed decisions and increase the chances of drug discovery success.

The limitations of traditional cell culture techniques

2D cell cultures are still the most widely used cultures in drug discovery. During target validation,

they are used to model diseases under carefully-controlled conditions. However, these monolayers of cells lack physiological relevance, as they poorly mimic the complexity of human tissues and the disease they are modelling. As Dr Véronique Schwartz, Venture Team Leader, faCellitate explains: "Although fully automated and scalable, traditional 2D cell culture is restricted to cultivating cells on a flat surface, which does not resemble the way cells grow in the body. It is important to improve the relevance of the chosen model throughout all phases of drug discovery before entering clinical trials."

This limitation could compromise the reliability and significance of the data generated and may be a major contributing factor to the high rate of attrition during clinical development. Instead, it would be beneficial to perform physiologically-relevant studies *in vitro* that better reflect the *in vivo* environment, as Jacob Tesdorpf, Senior Director Life Science Markets, PerkinElmer, explains: "Models that physiologically mimic the human body are more likely to be good predictors of efficacy in humans. This allows scientists not only to measure drug binding to the target of choice but also permits the measurement of multiparametric effects of drug treatment and prediction of both desired and adverse effects."

Tesdorpf continues: "More physiologically-relevant *in vitro* models – primary cells, 3D cell cultures, microtissues and organoids – better represent the cellular/tissue microenvironments, cell-to-cell interactions and biological processes occurring *in vivo*. *In vitro* 3D cell models also exhibit a higher degree of morphological and functional differentiation, representative of *in vivo* environments. Using more physiologically-relevant models could enable drug candidate attrition earlier in the development process."

Greater relevance for greater success

There are two key approaches to make cell cultures more physiologically relevant: using primary cells and 3D models.

"One way of creating a more biologically-relevant model system is to build the cell-based models for assays with human primary cells instead of cell lines," explains Katrin Hoeck, Associate Director, Marketing and Business Development, Discovery Solutions, Lonza. "When establishing an assay with human primary cells, researchers get the opportunity to validate the function of a target and the lead-target interaction in an *in vitro* model closely resembling the *in vivo* situation. The ability to perform these assays in different genetic and epigenetic backgrounds using cells from various donors also helps us to better understand drug-target interactions in early development stages." With the increasing focus on the development of personalised medicine, being able to study the cells of donors with specific characteristics could help to rule out toxic effects early in the discovery process and develop biomarkers to identify specific patient cohorts in which the drug is most likely to show therapeutic effects.

Jan Lichtenberg, CEO and Co-founder, InSphero AG, added:, "The availability of human, primary-cell-derived 3D co-cultures enables target validation with increased predictivity in the laboratory for the first time ever."

Importantly, 3D cell cultures allow researchers to make better-informed predictions, as they exhibit many characteristics unseen in the monolayers of traditional 2D model systems. For example, 3D cultures have more architecturally-relevant barriers for compounds to cross, and they better reflect human physiology in terms of morphology and structural complexity. They also simulate the extracellular matrix, maintain cell viability and retain tissue microenvironmental cues – enabling correct cellular differentiation, communication and function.

Over the years there have been various developments resulting in different forms of 3D models, which are broadly categorised as scaffold or scaffold-free systems. This is echoed by Terry Riss, Senior Product Manager, Cell Health, Promega Corporation. "There's a broad spectrum of 3D cell culture models, from scaffold-free spheroids of cancer cell lines to complex microfluidic devices containing several organoids representing a 'human-on-a-chip'."

To date, scaffold-based methods have been widely used. "3D cell culture typically involves co-culture of several cell types, often using a scaffold or matrix in which cells form aggregates or spheroids as they divide," states Hoeck. "Depending on the desired biological model, various technologies can be used to build a 3D model culture. 3D technologies range from simple Matrigel models to spheroids, organ-on-a-chip and bioprinting technologies."

Graeme Macluskie, Head of R&D at Reprocell Europe, further explains that cell culture consumables can take the form of scaffolds, aggregates/spheroids and hydrogels/ECM matrices. He also notes that scaffolds are available in different formats, which can be chosen based on the structure required in culture to mimic the cells' natural physiological environment.

By incorporating hard polymeric structures in labware as scaffolds, cells remain close to nutrient sources, which provides a suitable microenvironment of soluble growth factors, hormones and other molecules that cells interact with in an *in vivo* environment. Despite these benefits, there has been a recent shift toward scaffold-free techniques, as Lichtenberg comments: "Historically, scaffold-based 3D cell culture was dominant. Over the past year, however, scaffold-free 3D models have outperformed their scaffold-based counterparts – in disease modelling especially. Hanging-drop and non-adhesive cell culture plates enable the formation of physiological 3D models, which express their own extracellular matrix and do not require hydrogels to retain their 3D structure."

Non-adherent surfaces can be used to induce cells to self-aggregate into spheroids. Labware with specialised proprietary coatings help decrease surface adherence, and such coatings can be applied to standard microplates as well as specialist labware through photolithography techniques to yield micropatterned surfaces.

Spheroids can also be created in drops of media suspended from the bottom of labware, as cells naturally self-aggregate when there is no surface for them to adhere to. Hoeck notes that spheroids are frequently used to create tumour models, as they mimic the environment of a naturally-occurring tumour, with proliferating cells at the exterior exposed to media, and dormant cells in the interior due to a limited supply of oxygen and nutrients.

There are various benefits and challenges associated with each of the 3D cell culture techniques, and which is most relevant will depend on the drug discovery project in question. Regardless of the technique used, though, 3D models better reflect the *in vivo* environment, offering more relevant disease models that ultimately enrich target validation studies by providing researchers with greater insight. Using the data generated, better-informed decisions can be made in terms of which targets are most efficacious and druggable, helping increase the chances of successful drug discovery and development.

We are having to navigate a new normal at work and at home, while helping to keep our friends, loved ones and wider society as safe as possible.

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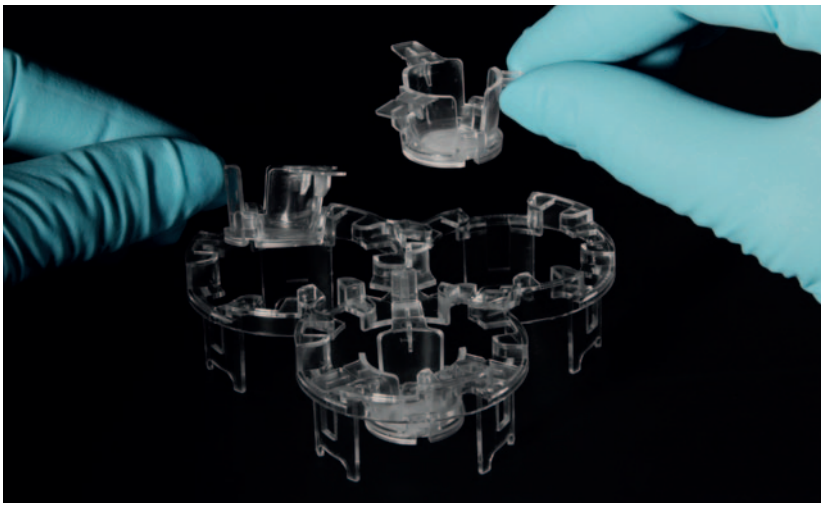

Figure 1: Alvetex 3D cell culture scaffolds allow individual cells to maintain their normal shape and structure with minimal exogenous support and interference

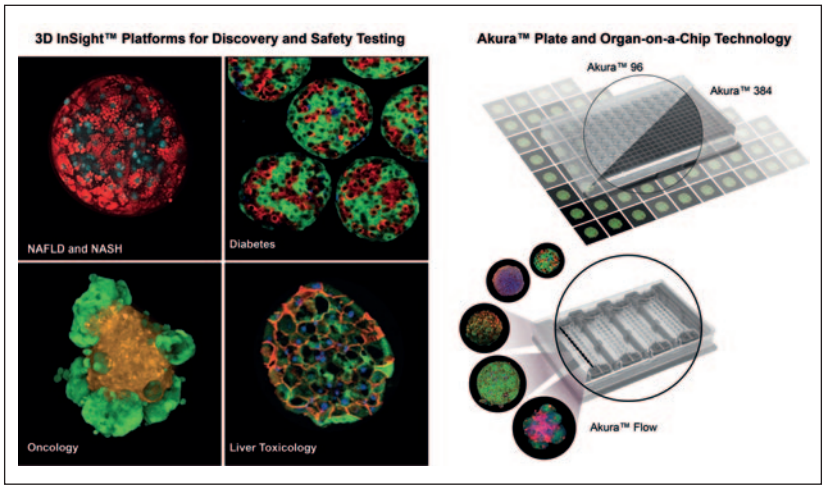

Figure 2: InSphero scalable 3D cell technology for modelling complex diseases underlies the company's 3D InSight™ platforms for modelling human diseases, such as NAFLD, NASH, diabetes and cancers

Figure 3: faCellitate's BIOFLOAT™ FLEX coating solution and 96-well plates support rapid and reproducible spheroid and organoid culture

The future of 3D cell cultures for enhanced target validation

The use of 3D cell cultures offers many advantages in the drug discovery process, but there is still room for improvement, and future models could be even better at mimicking human and disease physiology.

"Now that everybody realises that there is the world to win in terms of increasing the physiological relevance of cell culture, a large number of academic groups and companies are developing advanced tissue culture platforms," states Paul Vulto, Chief Executive Officer, Mimetas.

However, more complex models can prove challenging to routinely generate in the laboratory, taking time and requiring much skill. Indeed, whether 3D cell cultures replace 2D cultures altogether will depend on how reproducible these models are. As Schwartz explains: "The progress within the next five years in terms of standardisation and reproducibility of protocols will determine if 3D cell culture methods will be able to completely replace traditional 2D methods."

According to Vulto, it will be important for researchers to identify the complexity of the 3D model required according to the stage of the drug discovery programme. "Particularly in the early stages of drug discovery, including target discovery and validation, highly-relevant systems are needed. Once the biology is understood and one or more targets identified, one can reduce the complexity of the model, while still ensuring that we capture the essence of the disease under investigation."

"Indeed, what we have seen is a tremendous progress in stem cell and organoid techniques. If you consider intestinal organoids, mini brains or kidney organoids, you realise we are in a revolutionary decade where tissue culture is concerned," Vulto concludes.

Commercially-available solutions

There has been an explosion in new tools that allow assays to be conducted using aggregated cells that more adequately resemble whole tissues. Some of these solutions are discussed below.

REPROCELL's Alvetex polystyrene 3D cell culture scaffolds enable 3D cell growth and are available in different formats based on the structure required in culture to mimic the cells' natural physiological environment. A wide range of next generation technologies are available to create induced pluripotent stem (iPS) cells from somatic cells such as REPROCELL's 3rd-Generation RNA technology, which has increased the efficiency and

decreased the timescale involved in obtaining excellent quality iPS cells. These cells can be differentiated into any cell lineage or type with the appropriate differentiation protocols (**Figure 1**).

InSphero 3D InSight™ Platforms for Disease Discovery and Safety Testing include multicellular 3D models that enable researchers to rigorously test compounds early in the preclinical stage. These versatile platforms are based on scaffold-free, 3D microtissue disease models, which can be used in the company's scalable Akura™ 96, 384 and organ-on-a-chip formats. Each microtissue model is engineered using primary human cells and mimics human tissue composition, architecture and functionality. Hallmarks of diseases, such as those seen in non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), diabetes (TD1 and TD2) and many types of cancers, can be recreated *in vitro* in a scalable, automation-compatible format. The scalable Akura™ plate and organ-on-a-chip technology underlying InSphero platforms ensure continuity across experimental programs, from simple screening assays to complex multi-organ networks (**Figure 2**).

faCellitate's BIOFLOAT™ FLEX coating solution provides a homogeneous surface coating which is truly inert, guaranteeing cells are cultivated without being sequestered by any remaining interaction with a plastic surface/compound. This solution is applicable to all relevant laboratory consumables and can be used to tune any equipment to improve the physiological relevance of the assay (**Figure 3**).

MIMETAS' proprietary Phaseguide™ technology allows layering of extracellular matrix, negating the use of artificial membranes, such as those used in transwells. The platform is based on a microtiter plate footprint and is compatible with all microscopes and robots. The OrganoPlate® Graft platform also allows grafting of external tissues on a perfused microfluidic-grown vascular bed and offers improvement in tissue homeostasis, maturation and metabolic capacity (**Figure 4**).

Cell Guidance Systems' PODS® technology aims to solve the issue of degrading soluble growth factors, which are essential for complex cultures to grow effectively. PODS® place proteins in a protective protein crystal lattice, which stabilises cargo proteins even at high temperatures and continuously replenishes active proteins from a local,

Figure 4: MIMETAS' OrganoPlate® Graft is the first *in vitro* cell culture platform that allows vascularisation of 3D tissues like spheroids, organoids, and tumours *in vitro*

Figure 5
PODS® nanocrystals from Cell Guidance Systems maintain steady-state bioavailability of cargo proteins over extended periods and have a range of applications in 2D and 3D cell culture

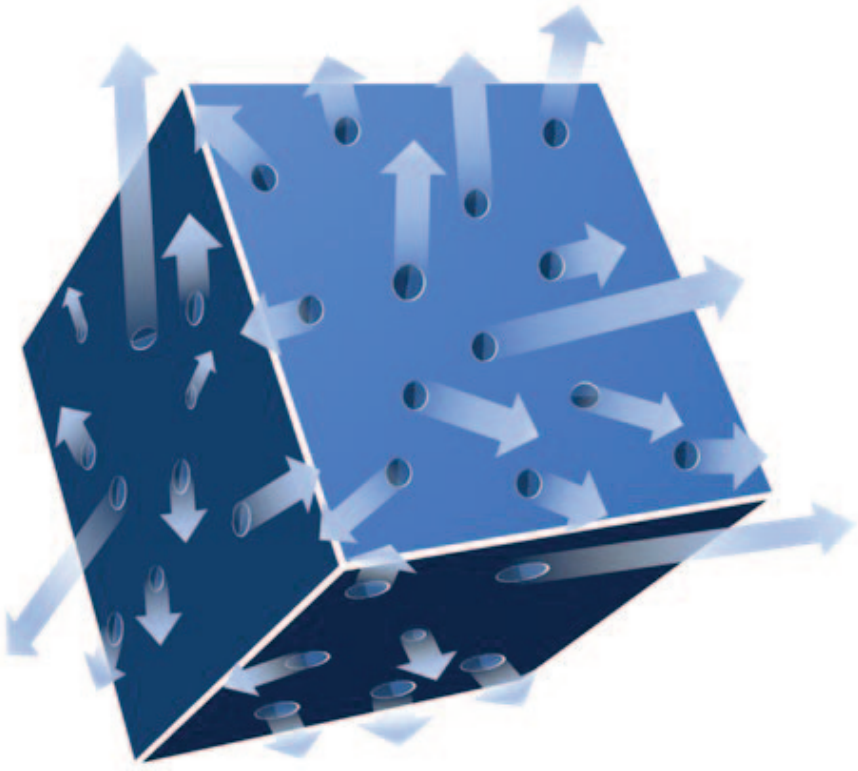

Figure 6
Promega's RealTime-Glo™ Cell Viability Assay allows cell viability monitoring in real-time, saving time, cell culture and reagent costs

microscopic store to maintain bioavailability at steady state levels. The crystals can be readily used for patterning by attachment to surfaces or suspension at specific positions in hydrogels (**Figure 5**).

Promega has several assays specifically designed to meet the challenges that 3D cultures present, such as the CellTiter-Glo 3D Cell Viability Assay. The RealTime-Glo™ MT Cell Viability Assay to measure live cells and CellTox™ Green Cytotoxicity Assay to measure dead cells represent a useful multiplex approach to validate assay results; reagents are added at the start of the experiment and data recorded in real-time over several days. The LDH-Glo™ Cytotoxicity Assay is a bioluminescent plate-based assay for quantifying lactate dehydrogenase release into the culture medium following plasma membrane damage. Being more sensitive than colorimetric or fluorescent methods, it allows accurate detection of LDH from a small number of cells, including primary cells and 3D cell cultures (**Figure 6**).

Lonza's RAFT™ 3D Cell Culture System offers a new way to develop microtissues and organoids. The RAFT™ 3D Culture System is designed to enable self-assembly of cells into a dense natural protein scaffold. The technology can be used to develop microtissues and organoids from cells and cell lines in less than one hour. Drug activity can thus be assessed in collagen-based 3D using imaging, biochemical and histological assays. Lonza's RAFT™ System can be used to incorporate multiple cell types to assess specificity of on- and off-target effects *in vitro* or to set-up *in vitro* barrier models for permeability and toxicity assays (**Figure 7**).

Figure 7: Lonza's RAFT™ 3D Cell Culture System provides a unique solution for scalable organoid culture

PerkinElmer's CellCarrier® ULA microplates ensure minimal cell-plate adhesion, promoting uniform and single-spheroid formation. Resulting cultures can be monitored using the EnSight® multimode plate reader while cell viability can be measured with ATPlite® 3D reagents and target engagement measured with Alpha CETSA® kits. Rich multiparametric data from 3D grown cells can be obtained using the Opera Phenix® Plus high content screening system, which employs a proprietary Synchrony Optics® microlens to simultaneously acquire multicolour confocal images with speed and sensitivity. The microlens-enhanced dual-disk design features a pinhole distance optimised for thick samples such as spheroids and microtissues. The new liquid handling option and fast kinetics mode enable live cell assays in real time (**Figure 8**).

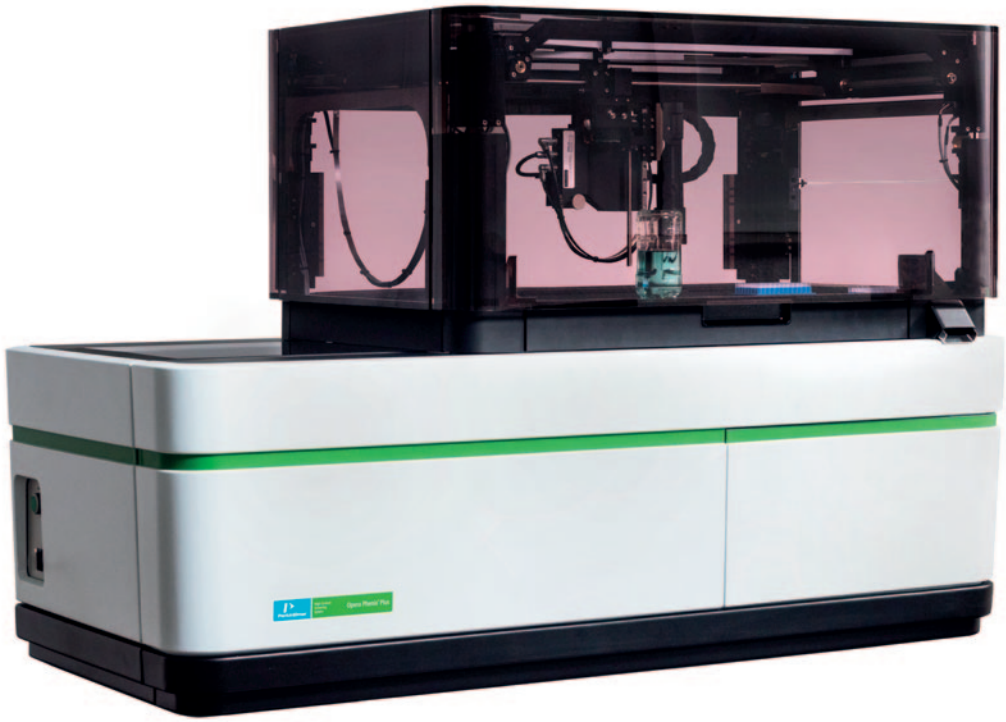

Figure 8: The Opera Phenix® Plus high content screening system from PerkinElmer is particularly well suited for applications using physiologically relevant models

Conclusion

Over the past decade, model systems have become more representative of humans – and therefore more complex. Previously, cell lines or animal lines used in 2D cultures were the dominant tool. Today, however, more complex cultures that use primary cells and multiple cell types in three dimensions provide more physiologically-relevant data for use in target validation. In the future, we are likely to see increasing numbers of ever-more sophisticated cell culture systems, with the possibility that testing using such models becomes almost as relevant as testing in humans. DDW

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Reference

1 Dale, Alex. The success rate of clinical trials is higher than we thought. Labiotech.eu. https://www.labiotech.eu/medical/clinical-trials-success-rate/ (Accessed 13 May 2020).