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Closer-to-Real 3D Cell Culture Advances

New in vitro technologies have a real chance of replacing animal models for drug testing, yet some challenges remain.

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As the 360 government-owned chimpanzees officially retired on Wednesday, June 26, the hope is that in vitro technologies will replace animals in some drug testing and other biochemical and physiological studies.

The animals' release comes as more sophisticated platform-based in vitro technologies, supported by private organizations as well as investors, are being developed and applied to study human drug metabolism. And animal rights organizations and investors are backing cell and tissue culture methodologies that could fill the long-needed in vitro void in validated drug testing with cash. Companies have introduced innovative products ranging from unique scaffolds to support cell growth to platform systems.

Priming the introduction of these platforms, according to Stefan Przyborski, founder and CSO of Reinnervate, may be the accumulation of research showing that conventional cell culture models involving growth of cells on two-dimensional (2D) substrates don't provide an accurate in vitro picture of the real 3D world.

Demand has increased, he says, for new 3D cell culture models that allow cells to acquire a natural 3D phenotype and permit increased cell proliferation, differentiation, and function.

Reinnervate currently markets Altavex™, a polystyrene scaffold designed as a platform technology to enable routine 3D cell culture. The company is developing an artificial 3D skin construct for cosmetic and topical drug testing, a liver toxicity assay using 3D cultured hepatocytes, a 3D platform for enhanced cell differentiation by various stem cell types, and a cell invasion model to evaluate cell migration in 3D to assess various anticancer compounds. The company says it is now focused heavily on preparing its commercial facilities to support quality controlled procedures for the scaled manufacture and production of Alvetex, due for formal product launch in various formats later this year.

Obstacles Remain

But John Comley, Ph.D., managing director of HTStec, an independent market research consultancy focused on assisting clients delivering novel enabling platform technologies, wrote in a 2010 report that based on vendor descriptions, "One might conclude that 3D cell culture was a done deal and tissue generation is readily achievable. However, HTStec's survey uncovered many problems and unmet needs."

Among these were poor reproducibility between batches of biomimetic scaffolds, 3D matrices with too many components, and limited ability to scale up or down a single 3D format. Further, he noted, users reported that post-culturing processing/cell extraction proved difficult to handle and that proven automated solutions with a higher throughput were required. In particular, he noted, there was room for improvement with more physiological substrates, and greater stability in long-term experiments was needed.

He added that in 2010 at least, the state-of-the-art "seems some way off from providing fully validated or robust 3D culture solutions, and the field is clearly open to major improvements at this point in time." 3D remains challenged as comparable results from different culture systems continue to plague investigators.

Investigators at the department of engineering science, National Cheng Kung University, Taiwan, reported that they had developed a high-throughput perfusion, 3D microfluidic cell and described the development of an integrated system aimed at providing a "user-friendly cell culture tool" for biologists to perform assays, "but also to enable them to obtain precise data."

Noting that while microfluidic cell culture systems are versatile tools for cellular assays, their use has yet to set in motion an evolutionary shift away from conventional cell culture methods, SB Huang and colleagues commented that the "situation is mainly due to technical hurdles." The operational barriers to the end-users, the lack of compatible detection schemes capable of reading out the results of a microfluidic-based cellular assay, and the lack of fundamental data to bridge the gap between microfluidic and conventional cell culture models all pose issues to the use of such systems, they said.

Technical features of their culture system included integration of a heater chip based on transparent indium tin oxide glass that provides stable thermal conditions for cell culturing. The platform also features a microscale 3D culture sample loading scheme that is both efficient and precise, a nonmechanical pneumatically driven multiplex medium perfusion mechanism, and a microplate reader-compatible waste medium collector array for the subsequent high-throughput bioassays.

To determine the effects of cell culture models on cellular proliferation, and the results of chemosensitivity assays, the investigators compared their data with that obtained using three conventional cell culture models. They found that the nature of the cell culture format could lead to different evaluation outcomes, cautioning that when establishing a cell culture model for in vitro cell-based assays, it might be necessary to investigate the fundamental physiological variations of the cultured cells in different culture systems to avoid any misinterpretation of data.

But overall, they say, their integrated microfluidic cell culture system overcame several technical hurdles associated with the practical application of microfluidic cell culture systems, and obtained fundamental information to reconcile differences found with data acquired using conventional methods.

Organ on a Chip

Hurel has focused on producing “organ on a chip” in vitro 3D tissue cultures that simulate and predict in vivo function of the liver and other organs. Last month, the company announced that it is receiving an investment from the Humane Society of the United States (HSUS) to support original scientific research into new applications of Hurel’s cell culture technologies, as well as the commercial launch of Hurel’s first series of products.

On April 3 Spring Mountain Capital organized and closed a $9.2 million Series A private equity financing facility for the company.

H*μ*REL® perfusion-based biochips and instrumentation, the company says, enable parent compound clearance and metabolite generation significantly greater than that afforded by hepatocytes cultured under static conditions, the company says.

Hurel’s microfluidic “biochip” comprises an arrangement of separate but fluidically interconnected “organ” or “tissue” compartments, each containing a culture of living cells drawn from, or engineered to mimic primary function(s) of, the respective organ or tissue of a living animal. Microfluidic channels permit a culture medium to recirculate as in a living system, driven by a microfluidic pump. The geometry and fluidics of the device simulate the values of certain related physiological parameters found in vivo, according to the company. Test compounds are added to the culture medium and allowed to recirculate through the device.

A Hurel official says the firm’s technology can be adapted to numerous experimental applications and is compatible with virtually any type of in vitro assay modality, including mass spectrometry and immunohistochemical, immunofluorescent, and gene expression assay formats, among others.

Last April, the results of toxicological evaluations performed on H*u*relStaticDog™, Hurel's new, patent-pending method of culturing canine liver cells, were highlighted as a part of UCB Pharma’s keynote presentation at the ADME and Predictive Toxicology 2013 meeting in Barcelona. The presentation focused on the current state of the art of in vitro models for early toxicology assessment to detect human hepatotoxic drugs.

The study results derived from H*u*relStaticDog represent a culmination of a multi-year collaboration between UCB and Hurel, under which this new pre-clinical analytic tool was developed. UCB funded the development of H*u*relStaticDog and has collaborated with Hurel in its characterization and validation testing.

Franck Atienzar, Ph.D., associate director and head, in vitro toxicology unit, at UCB Pharma, and his colleagues compared hepatotoxicity prediction among different models including the HepG2 (glu/gal) cultured hepatocyte model, primary hepatocytes (rat/Human) HepaRG (tumor derived human hepatocyte) model, and the Hurel dog co-culture model. The hepatocytes in the Hurel model maintained Phase I and II gene expression and metabolic activities. They were also maintained after two weeks of culture and allowed identification of drug metabolites comparable to that seen in vivo.

Wayne Pacelle, president of HSUS, said in announcing his organization’s commitment to Hurel that the funding “reflects our confidence that technologies like Hurel’s have the potential to greatly reduce the use of animals in drug development and chemical safety testing.”

This 3D in vitro platform and others in development may advance that goal.

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