Progress made in applying 3D cell culture technologies

Three dimensional (3D) cell culture has attracted considerable attention and headlines in recent years owing to its potential to deliver higher quality culture information that is more representative of tissue morphology and predictive of drug responses in vivo. Three years ago we concluded in DDW that 3D cell culture was still open to major improvements, so what has changed? By far the biggest difference has been in the number of technologies that now support or drive the formation of spheroids. Spheroids are now the number one 3D structure users want to generate in culture. As with any maturing assay technology, we are beginning to see the emergence of 3D optimised assays, protocols and kits, which should hasten the wider adoption of 3D technologies. Compatibility with automated lab equipment and suitability for HTS feature highly in current 3D offerings although to date very little HTS/primary screening has been done using 3D cell culture. Another trend is the increasing number of convincing examples of biomimetic tissue constructs that reproduce and model organotypic 3D structure. These are derived using a variety of hydrogel and structural scaffolds and from different types of culture/fabrication techniques. The majority of respondents surveyed thought that 3D cell culture had still not reached its full market potential. In conclusion, 3D technology offerings have certainly multiplied, diversified and are probably more enabling than in previous years. However, there still appears to be some latent hesitancy in market such that it does not appear to be developing at quite the same pace as one might have predicted given the level of interest there seems to be in 3D at the bench.

T

Three dimensional (3D) cell culture has attracted considerable attention and headlines in recent years owing to its potential to deliver higher quality culture information that is more representative of tissue morphology and predictive of drug responses in vivo. However, three years ago we concluded in DDW that 3D cell culture was easier said than done and the state-of-the-art at that time was still some way from providing fully validated or robust 3D culture solutions and tools and the field was open to major improvements1. So has anything changed in the intervening period? With this mind HTStec carried out an update of its market report on this 3D cell...
The main objectives of this year’s benchmarking study were to comprehensively document continuing interest in, experience gained and progress made in applying 3D cell culture technologies in academic research, drug discovery and tissue engineering/regenerative medicine settings, and to understand what 3D scaffolds/approaches respondents were most interested to utilise in the future.

Current opinion of 3D cell culture
Survey respondents’ current opinions on various statements about 3D cell culture were as follows: most in agreement was with the statement ‘In certain areas or assays 3D cell culture has major benefits (eg stem cells, oncology etc)’ and moderate agreement with the statement ‘3D cell culture is significantly changing how I plan my research’. Survey respondents were only very marginally in agreement with the statements ‘3D cell culture lacks a generic technology suited to most applications’ and ‘There are too many alternatives and most lack validation/reproducibility’. Survey respondents were least in agreement with the statement ‘I would not adopt a new 3D scaffold without independent validation in peer-reviewed publications’ (Figure 1).

Advantages of 3D cell culture
Survey respondents ranked better mirrors the environment experienced by normal cells in the body as the most important advantage of 3D cell culture. This was closely followed by mimics the typical organ microarchitecture and in vivo-like morphology, more predictive of disease states and drug responses, and then more relevant phenotypic response. Rated the least important advantage was shorter production times relative to current 2D monolayer cultures (Figure 2).

Main applications of 3D cell culture
The main application of 3D cell culture reported by survey respondents was cancer therapy (45% using). This was closely followed by cell-to-cell interactions (43% using), cell-to-matrix interactions (41% using), high throughput screening (40% using) and then model development/tissue modelling (39% using) (Figure 3).

3D structures most interested in generating culture
Spheroids/3D microtissues were the 3D structure most (45%) survey respondents were interested in generating in culture. This was followed by organotypic co-cultures (25% interested), biomimetic
tissue constructs (18% interested), and then directional cultures and other (5% interested in each) (Figure 4).

3D approaches that have demonstrated most promise
All respondents ranked hydrogel scaffolds as the approaches that had demonstrated most promise to date in facilitating 3D cell culture. Ranked next was biomimetic tissue constructs followed by scaffold-free. Ranked showing least promise was magnetic nanoparticles (Figure 5).

3D scaffold requirements
Survey respondents had the following mean requirements for 3D scaffolds with different types of properties: 37% scaffolds through which the cells can be fluorescently imaged; 36% scaffolds that are representative of the human extra-cellular matrix; 35% scaffolds from which the cells grown in 3D can be harvested for downstream analysis; 33% scaffolds that must be amenable to high throughput and automation; 20% scaffolds that must be biologically inert and/or transplantable; 20% scaffolds that are mounted into a removable permeable microplate well insert/Transwell; and 17% scaffolds that must be fully biodegradable (Figure 6).

Assay types most demonstrated with 3D cell culture
The assay types most used or successfully demonstrated in a 3D cell culture matrix by survey respondents was cell proliferation and cell viability. This was followed by cell differentiation and cell migration and then cell signalling assay and high content screening assay. The assay types least used or successfully demonstrated in a 3D cell culture matrix were patch clamping and transport assays (Figure 7).

Analytical technologies most applied to 3D cell culture
The analytical technologies most used or successfully applied to 3D cell culture by survey respondents today were fluorescence microscopy, brightfield/phase contrast microscopy and plate readers. This was followed by PCR/qPCR, histology and flow cytometry. The analytical technologies least used or successfully applied in 3D culture were label-free readouts and next-gen sequencing (Figure 8).

3D approaches respondents are most interested in purchasing
Survey respondents rated hydrogel 3D scaffolds
and microplates designed to encourage/support micro-tissue/spheroid generation as the 3D consumables they are most interested in purchasing. This was closely followed by microwell inserts/Transwells incorporating 3D scaffolds and structural 3D scaffolds (purchased separate of culture vessel). Least interested was for purchasing magnetic nanoparticles for levitation of cell and microcarriers/magnetic beads with 3D scaffold core/coating (Figure 9).

Main barriers to the adoption of a new 3D matrix
Survey respondents rated budget constraints – cannot afford to change formats as their main barrier to adoption of a new 3D matrix. This was closely followed by desired analytical technology will not work with 3D cell cultures; and then lack of consistency between wells, lots, batches or results generated; and difficult to dispense/insert scaffold/matrix into microplate wells. Rated least limiting was no perceived need – what I have is working okay (Figure 10).

Some recent developments in vendor 3D cell culture offerings
The following vendor snapshots provide an overview of the very latest 3D cell culture technologies for use in biomedical research and drug discovery and the key application areas for these technologies.

The Perfecta3D® Hanging Drop Plates from 3D Biomatrix (www.3DBiomatrix.com) are 96- and 384-well plates that facilitate the formation, culture and testing of 3D spheroids and embryoid bodies without cellular contact with artificial surfaces or matrices. With only one spheroid forming in each well, 3D spheroids formed in Perfecta3D Hanging Drop Plates are consistent and diameter can be controlled by the number of cells seeded in each well. Media and compounds can be added or removed from the top of the plate, and cells can be added for patterned co-cultures. The well plate format means that the plates are compatible with manual or automated liquid handling equipment. Products complementary to the Perfecta3D Hanging Drop Plates are available specifically for conducting assays and HTS with 3D spheroids. The Perfecta3D Spheroid Transfer Tool facilitates transfer of spheroids from the Hanging Drop Plates to a receiving plate. This is especially useful for HTS as the tool allows for the transfer of all drops from the Hanging Drop Plates at once. 3D Biomatrix has also partnered with Cayman
Chemical to commercialise the Perfecta3D Cell Viability Assay Kit, an assay for studying cell proliferation in 3D spheroids generated in the Perfecta3D Hanging Drop Plates. The assay is one of the first commercialised assay kits designed for 3D cell culture (Figure 11).

3D Biotek (www.3dbiotek.com) has been developing its patented 3D micro-fabrication technology to fabricate 3D scaffolds of biodegradable and non-biodegradable materials with well-defined fibre and pore size for 3D cell culture applications including stem cell research, in vitro 3D tumour model and tissue engineering. 3D scaffolds have been frequently used not only in creating normal healthy tissue for tissue repair but also in creating in vitro disease models for drug screening. Recently, the importance of doing cell culture in 3D has been recognised. It has been indicated that cells cultured in 3D scaffolds show different growth profile and protein expression characteristics than those in 2D. When cells grow in 3D, compared to 2D monolayers, the extracellular matrix (ECM) produced by cells is high. The increased ECM environment will change the cell-cell interactions and cellular behaviour of the 3D cultured cells. Hence the cellular response of the cells to drugs and other stimuli are significantly changed. Abundant literatures have shown that cells cultured in 3D are more similar to cells in vivo. Therefore, 3D cell culture systems are especially useful for conducting screening assays for new drug candidates. Polystyrene (PS) and Polycaprolactone (PCL)
scaffolds from 3D Biotek showed excellent properties in 3D cell culture. 3D Biotek’s PCL scaffold is the reference scaffold selected by US National Institute of Standards and Technology. Cells cultured in these scaffolds showed increased level of ECM synthesis (Figure 12).

Basement membranes are continuous sheets of specialised extracellular matrix that form an interface between endothelial, epithelial, muscle or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound healing. They not only support cells and cell layers, but they also play an essential role in tissue organisation that affects cell adhesion, migration, proliferation and differentiation. Basement membranes provide major barriers to invasion by metastatic tumour cells. Cultrex® Basement Membrane Extract (BME) from Amsbio (www.amsbio.com) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumour. The extract gels to form a reconstituted basement membrane. The major components of BME include laminin, collagen IV, entactin and heparin sulfate proteoglycan. BME can be used in multiple applications, under a variety of cell culture conditions, for maintaining growth or promoting differentiation of primary endothelial, epithelial and stem cells. BME can also be utilised in cell attachment, angiogenesis, in vitro cell invasion and in vivo tumourigenicity assays. Recently Amsbio have developed an additional formulation of Cultrex® BME known as Cultrex® BME 2. Cultrex® BME 2 provides a proprietary formulation that is higher in tensile strength when compared to its original BME. This new technology is already used to support the growth of primary organoids from healthy and cancerous tissues. BME 2 is particularly advantageous in generating complex 3D cultures. This new technology brings BME to the forefront of personalised medicine by enabling the study of individual primary materials in both basic biology and drug development applications (Figure 13).

Corning (www.corning.com) continuously strives to improve efficiencies and develop new products and technologies for life science researchers. Its 160-year legacy of innovation and manufacturing excellence puts it in the forefront as a leading supplier of laboratory consumables. Combining its expertise in plastic consumables and surface chemistries, Corning has developed and launched multiple new products for 3D cell culture applications. Corning® Transwell® permeable supports have become a standard method for culturing cells by permitting cells to uptake and secrete molecules on both its basal and apical surfaces, and thereby carrying out metabolic activities in a more natural fashion. Recent technology advances have resulted in an increased use of spheroid-based functional assays in target validation and drug evaluation. Spheroids, which are 3D cultures of cells that bind to each other instead of attaching to a substrate, can be grown in microwells with surfaces that inhibit attachment. New 96-well and 384-well spheroid microplates from Corning feature the
The company’s Ultra-Low Attachment surface combined with a clear round bottom to enable the formation and growth of a single spheroid per well with reproducible size. The black sidewalls of the microplate reduce cross talk and background noise in fluorescence and luminescence assays without the need to transfer spheroids to a different assay plate. Additional Corning technologies for 3D cell culture include Matrigel® Matrix, a natural hydrogel; PuraMatrix™, a synthetic hydrogel and Transwell® Permeable Supports (Figure 14).

The Electrospinning Company (www.electrospinning.co.uk) has launched a 96-well plate incorporating the Mimetix® scaffold for 3D cell culture. Mimetix is highly porous and mimics the extracellular matrix. Breast cancer primary cells have been shown to proliferate more slowly and to be more resistant to drug-induced apoptosis in Mimetix than in 2D, demonstrating a true 3D phenotype in this environment. Mimetix is created by electrospinning the medical grade polymer poly-L-lactide (PLLA), a material which does not degrade or alter over the course of an experiment, into nanofibres. The Mimetix scaffold is incorporated into a standard 96-well plate frame with a base having superior optical clarity, by proprietary laser-welding technology which provides minimal base distortion.

3D Cell Culture Solutions from Corning

ECMs | Surfaces | Permeable supports | Screening plates

If your research would benefit from 3D cell cultures, Corning offers a wide range of 3D cell culture tools, including our new Spheroid Microplates that feature optically clear, round bottom wells for uniform, single spheroid formation across all wells. Call us today at 800.492.1110 or email CLSCustServ@corning.com to request a free sample. For additional information, visit www.corning.com/lifesciences
Our highly-consistent and easy-to-use 3D platform holds great promise to reduce the number of costly drug failures in clinical trials, enabling more realistic tumour and toxicology models.

The Mimetix scaffold is highly porous and mimics the extracellular matrix, providing an ideal environment to support the 3D growth of cells. The Mimetix multi-well plate is designed to be easy to use and compatible with industry-standard automated handling and imaging equipment. The synthetic scaffold, which is 50 microns thick and available in a range of pore sizes, is laser-welded into the plate. Mimetix has been validated with a number of primary cells and cell lines, including breast cancer cells and hepatocytes, and supports highly consistent cell performance in 3D.

The invasive potential of cancer cells can be measured by using migration and chemotaxis assays. Such phenotypic, functional cell-based assays are designed to observe the physiological behaviour of single cells or multicellular units. The measurement of the phenotypic functionality has many advantages. For example, compensation effects caused by redundant mechanisms are already included in the cellular response, due to the nature of the experiment. However, the outcome of phenotypic assays depends strongly on the environment in which the cellular behaviour is imaged. Therefore, the in vitro conditions need to mimic the in vivo conditions as close as possible, in order to improve and optimise the comparability and predictability of such assays. Based on this knowledge, ibidi (www.ibidi.de) developed a chemotaxis assay that enables the quantitative measurement of the directed migration of cancer cells within 3D gel matrices. Following the standard protocol, cells are seeded out in a small observation field with an area of 2mm² and a resulting volume of 140 nanolitres. The chemical gradient is set up by diffusion and then maintained over days, giving sufficient time to measure the migration parameters using video microscopy with optimal accuracy. The present version of the µ-Slide Chemotaxis 3D is optimised to observe single cell migration. Currently, ibidi is working on a new version of the slide that will allow for the investigation of the invasive potential of cancer cells. To achieve this, a cancer cell spheroid is placed in a chemical gradient. 3D-invasion is then measured by imaging the cells that migrate into the surrounding collagen I gel (Figure 16).

Hanging drops form a nearly perfect vessel for culturing cells as 3D spheroids, as no unwanted surface interactions occur. However, conventional hanging-drop culture was limited by manual turning of the culture plate, low drop volumes and the missing possibility of sampling from the drop.
InSphero’s (www.insphero.com) patented GravityPLUS™ platform was introduced in 2009 as the first standard SBS hanging-drop multiwell plate to allow top loading using pipettes or robotic liquid handlers. A microchannel connecting the top filling port and the culture volume below the plate allows dispensing into and aspiration from the drop to refresh medium, to add compounds or to run a biochemical assay. In addition the channel’s capillary force holds the drop tightly, effectively preventing spillage or contamination of neighbouring drops. Spherical 3D microtissues form within two to four days for the majority of cell types. To facilitate microtissue maintenance and long-term culture, InSphero added a second plate to the GravityPLUS™ kit, the GravityTRAP™ plate. The microtissue is kept inside a pocket at the bottom of the well, which is coated with a special, 3D compatible ULA film to prevent adhesion. In the GravityTRAP™ plate, medium exchange and assays are easy and robust without the risk of aspirating the microtissue; a flat, optically clear 1mm window allows for high-quality microscopy and size analysis. A growing number of instrument manufacturers such as Perkin Elmer and Tecan support this assay platform, so that in combination with 3D optimised assays that InSphero works on with Promega, 3D cell-based assays can be performed as easily as a 2D experiment. In addition to its patented culture platform in 96-well and soon also in 384-well format, InSphero offers a broad portfolio of assay-ready 3D microtissues for toxicology and oncology applications. The 384-well system will be presented at SLAS 2014 (Figure 17).

To define new paradigm in detecting drug-induced cardiotoxicity before entering clinical trials, in collaboration with pharmaceutical companies, US Food and Drug Administration (FDA), Cardiac Safety Research Consortium, US Environmental Protection Agencies (EPA), National Institute of Health (NIH) and non-profit Cardiovascular Research Institute (CVRISI), InvivoSciences (IVS) (www.invivosciences.com) is establishing a highly predictive preclinical cardiac safety-testing platform using 3D engineered heart tissues (EHTs) fabricated with cardiac cells derived from human-induced pluripotent stem cells (iPSCs). Human EHTs represent diverse populations with different susceptibilities to external stimuli, including drugs and toxicants (ie personalised treatment). Excitation-contraction (E-C) coupling describes a fundamental process that regulates physiological function in cardiac muscle.
Periodical inductions of action potentials at plasma membrane trigger subsequent molecular events leading to contraction and relaxation of cardiomyocytes. In addition, to the hERG channel, there are numerous cellular proteins including those expressed in sarcoplasmic reticulum, mitochondria and cytoskeleton that are indispensable in E-C coupling. Therefore, it is critical to analyse effects of drug candidates on E-C coupling comprehensively using a system in which physiological and pathological properties of tissues that drug’s target are much higher than with isolated cells (Figure 18).

Using human EHTs, action potential, Ca²⁺ transient, and cardiac contractility are measured concurrently to analyse compound-induced changes in E-C coupling. Based on these technologies, IVS offers contract research and development services for analysing on- and off-target effects of pharmaceuticals, cosmetic products, food and household chemicals. The predictive capacity of assays using 3D tissues that mimic physiological and pathological properties of tissues that drug’s target are much higher than with isolated cells (Figure 18).

The Elplasia plate (www.elplasia.com), also called the Micro-Space Cell Culture plate, has been developed by Kuraray with its micro-fabrication technology. The plate has a number of micro-spaces regularly arrayed on the surface which allow cells to self-assemble into spheroids. It is suitable for high-throughput screening due to simple handling and its standard SBS 96-well and 384-well plate formats. Some cancer cell lines form hundreds of spheroids in one well, each with uniform size. For drug screening, cell culture and viability assays can be done in the same plate and additionally the plate can be read by a general plate reader. The bottom of the plate is a thin film made by a transparent material, polystyrene, so that it is suitable for microscopic observation and imaging study. The difference of drug sensitivity between 2D and Elplasia culture has been observed which indicates its possibility for oncology drug screening. The cell culture procedure involves applying a surface coat of poly-HEMA immediately prior to use to make the surface low adhesion. Depending on coating condition, these spheroids generated in each micro-space can be easily harvested so that RNA or protein analysis can also be performed. A 24-well format is also available and a six-well plate is nearing commercialisation for those confirmative analyses (Figure 19).

Life Technologies (www.lifetech.com) is a leader in providing high quality cell culture reagents, including an extensive line of extracellular matrices and scaffold materials enabling researchers to create culture environments that better model in vivo biology. More recently, it has facilitated a ‘do it yourself’ approach enabling researchers to use its standard human primary keratinocytes, specialty media and collagen matrix to create more physiologically relevant epidermal skin models. This simple yet elegant approach to 3D cell culture applications does not require any specialised products or instrumentation to create useful models for skin biology including applications in basic research, wound healing, drug delivery and absorption as well as for assessing potential corrosive and irritant chemicals. The 3D Epidermal Skin Model Generation protocol simply requires the treatment

**Figure 18:** Predictive preclinical cardiac safety-testing platform using 3D human engineered heart tissues from InvivoSciences

**Figure 19:** Multicellular tumour spheroids growing on the Kuraray Elplasia plate
of trans-well inserts with Gibco® Coating Matrix followed by the addition of Human Epidermal Keratinocytes (HEK) prepared in supplemented EpiLife® Medium plus three additional components. Cells are incubated, submerged for two days and then raised to air liquid interface for an additional 8-10 days, with media changes every 48 hours, upon which model morphology and stratification are suitable for testing. While ready to use commercial skin model products are available from other companies, Life Technologies offers researchers greater flexibility and control in building 3D primary cell models at a significantly lower price (Figure 20).

MatTek (www.mattek.com) specialises in development and commercial production of in vitro human tissue models. MatTek’s models are produced from normal (non-transformed) human cells that are cultured at the air-liquid interface (ALI), leading to development of 3D organotypic structure and function that reproduces the structure and function of in vivo tissues. Currently available model systems include skin (EpiDerm™, EpiDerm FT™), ocular (EpiOcular™), bronchial airway (EpiAirway™) and vaginal (EpiVaginal™) epithelium. Compared to traditional submerged culture systems, the ALI culture format allows for more realistic treatment conditions and application of water insoluble materials, finished formulations of creams and lotions, as well as aerosol sprays and gaseous and particulate components of environmental agents or smoke. MatTek’s organotypic models have demonstrated outstanding intra-lot and lot-to-lot long term reproducibility, and have been accepted as animal replacements by the OECD for regulatory use in skin and ocular safety testing. Advanced model systems employ co-culture of epithelial cells with melanocytes, dendritic cells and/or stromal cells. Tissue models of diseases such as melanoma and psoriasis are also available. Newer products being developed at MatTek include co-culture models of the human alveolar epithelium (EpiAlveolar™) and the human small intestine (EpiIntestinal™). The EpiIntestinal™ model (Figure 21) currently at the beta testing stage, is expected to find utility in pharmaceutical applications for screening intestinal adsorption and metabolism of orally administered drugs.

Since 2009, a few pharmaceutical companies have successfully integrated QGel’s (www.qgelbio.com) MT 3D Matrix, a synthetic extracellular matrix for 3D cell culture, facilitating attachment, growth, differentiation and migration of any cell type. Based on the solid science obtained by our partners and internal developments in the past years, QGel® Matrix can now be used on a routine basis for regular cell-based screening campaigns. Since QGel products are designed for miniaturisation in plates as small as 1536-wells, customers can bring 3D matrix-based models into an industrial setting for screening campaigns of several 10,000s compounds per day, with no need for any manual steps. QGel Matrix has enabled the development of disease models that are defined by concrete, physiologically relevant parameters. Such parameters include biomarkers, target receptors and other biological characteristics that mimic in vivo physiology and other key disease features. Experimental readouts can be acquired using conventional detection devices and include, for example: 1) analysis of biomarkers to measure RNA expression and protein activities; 2) image-based analysis to capture cellular and tissue organisation and morphology.
immunofluorescence, etc; and 3) cell proliferation quantified by metabolic activity, DNA-based assays and cell count. Thanks to QGel Matrix properties, standard screening equipment can be used throughout the whole process from liquid-handling to read-out acquisition, while at the same time guaranteeing data reproducibility. On a case-by-case basis QGel also advises its customers on how to upscale tissue- and disease-specific cell-based assays with QGel Matrix on an executional and analytical level for large-scale compound screening campaigns (Figure 22).

Reinnervate (www.reinnervate.com) produces Alvetex®, a highly porous polystyrene-based scaffold, developed exclusively for in vitro applications in 3D cell culture. Alvetex® materials are composed of voids and inter-connecting pores, engineered into 200 micron thick membranes. Void dimensions are controlled during manufacture and tailored toward specific applications in cell culture. Voids in Alvetex®Scaffold allow cells to freely enter the interior of the material. Unlike cells grown on conventional flat polystyrene substrates, cells in Alvetex®Scaffold do not flatten and retain their native 3D structure and form 3D interactions with neighbouring cells. This is a fundamental issue in cell biology since cells that change and adopt flattened morphologies also alter their function – Alvetex® is designed to address this issue creating cell-based assays that more closely mimic the structure and function of cells in real tissues. Alvetex®Strata has smaller void dimensions so entry of cells into the material is impeded such that the majority of cells are retained on the surface of the membrane. Cells readily form compact 3D structures on the surface and are readily accessible. Alvetex® is available in multi-welled culture plates and well inserts. The technology is very flexible and versatile. Applications have been developed for its use in many areas of cell biology, including cancer cytotoxicity, stem cell biology, neuroscience, bone, liver toxicity, skin, 3D cell migration assays, co-culture and 3D organotypic cultures. Alvetex®Strata is also suitable for the long-term maintenance of intact tissue slices, retaining the nature 3D architecture of tissue in vitro (Figure 23).

Scivax (www.scivax.com/usa) NanoCulture® Plate (NCP) is a micro-patterned surface type 3D cell culture plate. Of the scaffold-based 3D cell culture systems available today it is one which is highly suited for HTS. Unlike the suspension or hanging drop culture, which force cells to form aggregates by gravity, cells actively form multi-cellular spheroids on NCP, just like gel-based 3D cell culture. The advantage of NCP is not only its high throughput, but its capability for live imaging of such cell behaviour, under both bright-field and fluorescence imaging. Due to this character, NCP is capable to develop various assays, which were difficult with monolayer or other 3D cell culture systems. The latest application with NCP is an epithelial-mesenchymal transition (EMT) inhibitor screening system. A549 cell line form spheroids on NCP, while EMT is induced by the stimulation with TGF-β, which causes the spheroids to disintegrate into single cells. This cell behaviour, morphological change can be observed by a microscope under bright field, and can be quantitated by fluorescence measurement using HCS (high-content screening) equipment, after staining with Hypoxia Probe LOX-1. Scivax verified 1,330 compounds (Sigma LOPAC®) with this EMT screen assay using NCP, and found EMT inhibitory effect with several
compounds. It was able to discover several compounds with novel mechanism of action, other than the inhibitor of TGF-β. NCP is also suitable for co-culture, which enables anti-cancer drug screening under the condition mimicking tumour microenvironment (Figure 24).

A variety of 3D cell culture technologies have been developed since the recent recognition that 3D culture has many advantages over conventional 2D culture. Among the most widely used are suspension of anchorage-dependent cells in non-adherent culture ware, encapsulation in hydrogels, pellet culture and hanging drop culture. A common feature of all of these methods is the static nature of the culture environment. In the early 1990s a new type of cell culture technology was developed at NASA’s Johnson Space Center for the purpose of studying the effects of microgravity on cultured cells, but it was soon recognised that the system readily facilitated 3D culture. The technology, which has been commercialised by Synthecon (www.synthecon.com) as the Rotary Cell Culture.
System™ (RCCS), consists of a cylindrical culture vessel completely filled with media and rotating about the horizontal axis. The rotation of the vessel suspends the cells with very low shear stress allowing individual cells to aggregate into 3D tissue-like constructs. Unlike static 3D culture technologies, where mass transport of nutrients, oxygen and wastes are limited by diffusion, the RCCS employs a dynamic culture environment in which the cell aggregates are constantly moving in the culture medium. These conditions promote improved mass transport and reduce the incidence of necrotic cells inside the aggregate. The RCCS was originally conceived as a batch culture system, but more recently, externally perfused versions have been developed allowing long term cultures to be maintained without disturbing the contents of the culture vessel (Figure 25).

RAFT (Real Architecture For 3D Tissues) technology from TAP Biosystems (www.tapbiosystems.com) enables 3D cell cultures to be made from collagen – at tissue-concentration – in standard microwell plates simply, consistently and reproducibly. Innovative solid hydrophilic absorbers gently concentrate cell-seeded hydrogels without cell viability loss. The process takes less than one hour and has been automated on a Tecan liquid handling robot. RAFT cultures remain attached to the well during extended culture periods and for assay. Many common assay kits and analytical methods, including cell proliferation assays, immunostaining, imaging and ‘omics techniques, have been used successfully. Cells can be recovered using collagenase to dissociate the collagen matrix, or whole cultures removed for staining and sectioning. RAFT kits (24-well, 96-well and cell culture inserts) allow scientists to explore the influence of extracellular matrix composition, and even topography, on cell behaviour and cell signalling in 3D. The extracellular matrix can be modified by adding laminin, fibronectin or even Matrigel to the physiological-strength collagen, so providing cell signalling molecules important in oncology and angiogenesis. More complex, but well defined, organotypic models and co-cultures can be made easily and maintained for days or weeks. These are useful for studying stem cell behaviour and differentiation, cell signalling, cell-cell and cell-matrix interactions, and creating epithelia/endothelia and barrier models. Recent published examples include creation of a brain endothelial barrier to study nanoparticle transport, enhancing iPS hepatocyte maturity while maintaining CYP expression over several weeks in culture, and cell signalling analysis in oncology (Figure 26).
The study of cell biology \textit{in vitro} via monolayer cell culture systems is not always an accurate representation of the complex environment \textit{in vivo}. Significant interaction that occurs between cells and with the extracellular matrix is often not reflected in these simplified culture systems. 3D cell culture systems better mimic complex interactions and are extremely useful in broad applications of cell biology. For example, in human cancer biology, 3D cancer spheroid culture systems can simulate the structure of tumour growth for the purpose of studying tumour cell progression and sensitivity to anticancer agents. Another area where 3D culture plays an irreplaceable role is the formation of an embryoid body as an intermediate step in pluripotent stem cells differentiation. However, variability in 3D spheroid-forming cultures has been linked to changes in medium composition, volume, cell density, duration of culture and, most importantly, the cellular interactions with the culture dish itself. The cellular attachment to the culture surface must be prevented to promote the cell-cell aggregation in spheroid culture. Although several low cell adhesion surfaces are

\textbf{RAFT™}

\textbf{Only 3 steps to create complex 3D cell cultures}

\textbf{Step 1. Mix}

- Cells & neutralized collagen are mixed, dispensed & incubated

\textbf{Step 2. Make}

- The RAFT process creates cell cultures with strong tissue-like properties

\textbf{Step 3. Measure}

- Cell cultures can be analyzed using a range of techniques

For more information about RAFT visit www.raft3dcellculture.com or email info@tapbiosystems.com
available, spontaneous cell attachment is still a challenge to many researchers. Furthermore, in order to fully preserve their low adhesive property, these surfaces are usually aseptic, a step down from being sterile. The novel Thermo Scientific™ Nunclon™ Sphera™ surface by Thermo Fisher Scientific (www.thermoscientific.com) with thoroughly validated sterility demonstrates superior performance consistency over other low adhesion surfaces by preventing protein adsorption and allowing cells to grow in suspension with virtually no cell attachment (Figure 27).

Xanofi (www.xanofi.com) will soon release its first entry product into the 3D cell scaffold market — XanoMATRIX™. XanoMATRIX™ is the closest biomimic to human ECM, using an advanced combination of staple nanofibres to create a chaotic architecture substrate. A key feature of its short fibre structure is that it allows cells to reshape the scaffold to establish more robust, true 360-degree nutrient gradients, and provides more native ECM building blocks. It can be very easily shaped into almost any design and can even be sprayed through 3D printers. With XanoMATRIX™, the pore size of the scaffold can be controlled while achieving overall surface areas that are an order of magnitude higher than other substrates. Our material can be made from a wide array of polymers and can be implantable for in vivo applications. XanoMATRIX™ is an extremely versatile platform for tissue culture support and an ideal candidate for cancer and regenerative medicine (Figure 28).

Discussion

Table 1 lists the 3D cell culture technologies discussed in the article, and attempts to summarise them with respect to the main basis of the 3D technology described and the attributes individual vendors have assigned to their technology. This list by no means covers all vendors working in the 3D space, but is reasonably representative of the current market offerings.

By far the biggest difference and trend in the past three years has been in the number of technologies that now support or drive the formation of spheroids. These are spherical aggregates of proliferating, quiescent and necrotic cells that in culture retain 3D architecture and some tissue-specific functions. The term spheroid typically encompasses what some are calling microtissues, while others, mainly in the cancer area, refer to as organoids or those working with stem cells call embryoid bodies. The real value in spheroids comes when they can be reproducibly produced (ie with zero well-to-well or lot-to-lot variability) and can be easily removed/harvested for downstream studies. The ability to form spheroids of two different cell types, to make hundreds of spheroids of uniform size in a single pipetting step and ultimately to have only one spheroid per microplate well, are all critical to exploiting spheroids, particularly in drug discovery applications. Several of the technologies described (particularly the hanging drop cultures and the low attachment microplates) have made big advances in this respect. Assay-ready spheroid constructs are now increasingly available, and outsourced testing services utilising spheroids offered.

Another trend that is evident from the vendor snapshots is the increasing number of convincing examples of biomimetic tissue constructs that reproduce the organotypic 3D structure and in some cases model its function as well. These are derived using a variety of scaffolds (both structural and hydrogel) and from different types of culture/fabrication techniques. Several new structural scaffolds are now available for 3D applications and it will be interesting to see which emerge as the most enabling and for what applications over the coming years.

As with any maturing assay technology, we are
beginning to see the emergence of 3D optimised assays; of ‘do it yourself’ protocols that allow the user to combine cells, media and matrix to reliably create tissue models; and the availability of the first commercialised assay kits designed for 3D cell culture. All should hasten the wider adoption of 3D technologies.

Several 3D technologies describe their compatibility with automated lab equipment and talk about their suitability for HTS. However, only 36% of survey respondents have so far fully adopted 3D cell culture technologies in their routine work and of those survey respondents actually involved in screening, only 23% have so far run any HTS/primary screens using 3D cell culture. All of which points to a rather limited impact of 3D cell culture technology to date.

The rather upbeat vendor snapshots about 3D potential need therefore to be counterbalanced with the views of end-users. The majority (73%) of survey respondents have concluded that 3D cell culture had not yet reached its full market potential (ie there were still unmet market needs) and gave feedback on gaps/limitations of current 3D technologies. The following is an example of the comments received:

“3D is really still a very immature product and what is offered right now is a very limited panel of choices. The basic models are identical, each with its own faults, limitations and very few advantages. There is no realistic approach to a tissue reconstruction, other than giving a scaffold or constraining the growth to create spheroids. Is a sphere better than a monolayer, not always but probably for some things? Is a cell obliged to grow on collagen feeling better than one grown in suspension? Sometimes yes, but for sure its gene expression levels are completely different from what has been studied until now. Does it make any difference to grow a stabilised cell line in 3D or not? Is it more realistically informative or just blatantly a marketing issue? Nobody has really shown rigorous scientific data that growing cells in 3D is more biologically sound, at least nobody knows if it is always true. 3D is interesting but has still a long way to go to be mature!”

Even if these views were extreme they still point to a vendor and literature disconnect as to the validity of 3D approaches.

When survey respondents were asked to judge the success they had achieved with 3D cell culture to date, only a minority (39%) reported they had achieved major success (ie significant improvement) (Figure 29). All others claimed their success was moderate, minimal or none, and this is from a survey sample that was almost certainly biased towards those labs most interested in 3D cell culture.

In conclusion, 3D technology offerings have certainly multiplied, diversified and are probably...
more enabling than in previous years. However, there still appears to be some latent hesitancy in market such that it does not appear to be developing at quite the same pace as one might have predicted given the level of interest there seems to be in 3D at the bench.

**Figure 29: Current level of success achieved with 3D cell culture**

<table>
<thead>
<tr>
<th>Level of Success</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No success (no improvement)</td>
<td>7%</td>
</tr>
<tr>
<td>Minimal success (limited improvement)</td>
<td>26%</td>
</tr>
<tr>
<td>Moderate success (some improvement)</td>
<td>28%</td>
</tr>
<tr>
<td>Major success (significant improvement)</td>
<td>39%</td>
</tr>
</tbody>
</table>

© HTStec 2013

---

**References**


---

**Dr John Comley** is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation; assay methodologies and reagent offerings) to drug discovery and the life sciences. Since its formation 10 years ago, HTStec has published nearly 100 market reports on enabling technologies and Dr Comley has authored more than 45 review articles in Drug Discovery World. Please contact info@htstec.com for more information about HTStec reports.