ELSEVIER

3D cell culture opens new dimensions in cell-based assays

Bradley A. Justice[1], Nadia A. Badr[1] and Robin A. Felder[2]

[1] Global Cell Solutions, Inc., 770 Harris Street, Suite 104, Charlottesville, VA 22903, USA
[2] The University of Virginia, P.O. Box 801400, Aurbach Building, Room 2325, 450 Ray C. Hunt Drive, Fontaine Research Park, Charlottesville, VA 22908, USA

3D cell culture technologies have revolutionized our understanding of cellular behavior, both in culture and *in vivo*, but adoption by cell-based screening groups has been slow owing to problems of consistency, scale and cost. The evolving field of high content screening technologies will, however, require a rethinking of 3D cell culture adoption to ensure the next generation of cells provide relevant *in vivo*-like data. Three current technologies are presented in this review: membranes, sponges/gels and microcarriers. A short history of these technologies and unique research applications are discussed. Also, the technologies are evaluated for usefulness in modern automated cell-based screening equipment.

Five decades of technology and engineering in traditional cell culture have done little to liberate cells from an unnaturally flat world. As early as 1972, researchers were exploring the differences between cells grown on a flat surface versus three-dimensional formats with novel attachment surfaces, such as extracted extracellular matrix (ECM) [1]. Since then, the striking similarity of *in vivo* morphologies and behaviors of cells grown in 3D culture environments is not only well documented, but also well accepted [2]. Because drug discovery screening continues to transit from high throughput to high content [3], 3D cell culture technologies will become essential efficient methods for increasing assay relevance.

High content screening (HCS) has improved cell-based assays by combining high-resolution digital imaging [4] with powerful software algorithms to increase the amount of data produced per well [5]. Within five years, decreasing capital investment costs and improved software will make HCS the industry standard for drug screening [6]. Several factors will drive the adoption of high content screens, including the ability to perform multidimensional and multiplexed assays generating *in vivo*-like data for all segments of the drug discovery pipeline, such as target validation, screening [7] and toxicology. Also, cost per well savings can be realized by reducing compound use and direct labor. The research community must, however, be equally diligent to adopt and implement 3D cell culture methods globally, along with HCS, truly to realize their return on investment.

3D cell culture will not only empower HCS by supporting *in vivo* morphologies with current cell types, but also enable the use of primary and stem cells in drug discovery [8]. Specifically, primary and stem cells offer this high content biology on the condition they are cultured in an environment that supports *in vivo* 3D-like growth. Primary cells are the dissociated tissue of a human or animal and are often identified by origin like the human umbilical vascular endothelial cell (HUVEC). Stem cells are a subset of primary cells that are more difficult to maintain, but can usually undergo greater expansion in 3D culture. Regardless of the challenges, primary and stem cells will become the focal point of 3D cell culture in the coming years [9].

Research enterprises depend on consistent production of quality cells on a daily basis. Automated cell culture and frozen cells-as-reagents [10,11] are increasing the consistency and availability of research cells. 3D cell culture has failed, however, to be widely adopted because automated methods do not as yet exist. The reality is that 2D culture is entrenched within the drug discovery infrastructure creating a challenge to introducing 3D culture methods. Identifying emerging 3D culture technologies suitable for user-friendly automation is essential to creating a path from 2D to 3D cell culture.

The promise of cell-based therapeutics has stimulated the development of technologies that promote the growth and structure of

Corresponding author: Justice, B.A. (brad.justice@globalcellsolutions.com), (raf7k@virginia.edu)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.11.006

patient-derived primary and stem cell types [12]. Most are scaffold systems using synthetics or animal-derived ECM materials deemed safe for implantation. Unfortunately, these biomaterial technologies are merely adapted to 3D cell culture and, therefore, often fail to meet the demands of scale, cost and format associated with cell-based screening. For the purposes of this discussion, the focus is on currently available 3D cell culture technologies suitable for modern cell-based screening.

What is out there? The ECM and its constituents

The ECM is the frequently used term for the complex mixture of proteins and sugars beyond the membrane of the cell [13]. Compositionally, this variable microenvironment is not simply a scaffold for cells to hold on to, but a communicating structure providing an underpinning to cell behavior, identity and function [14]. The complexity of this environment is difficult to reproduce, but current 3D products are able to reproduce, or mimic, elements of the ECM. A short review of ECM structure and components will add context to this discussion and assist in evaluating 3D cell culture products.

The best known and most widely utilized proteins of the ECM are collagen and laminin. Collagen is the most abundant ECM protein and the term actually refers to a large family of over 25 collagen protein isoforms [15]. Highly purified collagen is available commercially, but many cell culturists use the less expensive gelatin (a mixture of collagens, but primarily Type I). Laminin is available as a purified nondenatured protein and is important for several cellular behaviors [15]. Applied individually or in combination, collagen and laminin represent the most available and best understood protein components of the ECM. Glycosaminoglycans (GAGs), such as chondroitin sulfate and heparan sulfate, are unbranched polysaccharides that usually appear *in vivo* covalently bound to proteins as proteoglycans with the exception of hyaluronic acid. Synthesized at the membrane directly into the extracellular space, hyaluronic acid forms macromolecular complexes with proteins but is not covalently bound to them. The very negative charge of these molecules draws in cations and water to form compressible gels of high excluded volume. GAGs, like the aforementioned proteins, are secreted in a variety of forms by fibroblasts, some of which specialize in certain connective tissue elements like chondrocytes, which produce cartilage. Commercial availability and cost limit the application of GAGs, but new products are making it to market as discussed later.

The basement membrane is a specialization of the ECM required for adhesion of the epithelial cell layer and responsible for a wide range of epithelial cell phenomena including cell identity, wound healing and migration [16]. Fibroblasts synthesize the basement membrane primarily with laminin and type IV collagen to create a sheet of ECM.

ECM is not just a random mix of secreted components, but a specific composition of biochemicals and defined geometrical structure, which stimulates specific cell responses, such as differentiation [17]. For example, epithelial tissue relies on strong cell–cell adhesion to create a polar sheet that is bound to the underlying ECM, which, in the case of epithelial cells, is basement membrane. A dramatic example of the role ECM plays in biology is demonstrated by the connective tissue of the knee that is sparsely populated with cells and mainly composed of secreted fibrous proteins and proteoglycans. Strength in this structure arises from the high fixed-charge density of the proteoglycan gel, which is restrained by a relatively inelastic fibrous network of collagen molecules. The generation of high osmotic pressure and the restriction of fluid flow by this viscoelastic gel enable cartilage, through the arrangement of bundled collagen fibers and compressible gel of proteoglycans, to resist and withstand the daily impact of standing upright [18]. Understanding the ECM that surrounds cells *in vivo* will guide the selection of 3D cell culture products used in a research or screening program.

This review will focus on three commercially available types of 3D cell culture technologies suitable for cell-based assays: filters, gels/sponges and microcarriers. A discussion of each will include a general overview of the technology, examples of representative products and unique advantages or limitations.

Filter well inserts

Filter well inserts are devices that hold a filter membrane in a culture vessel of choice, allowing for an upper compartment and a lower compartment on either side. Microporous filter membranes were adopted by cell culturists in an effort to culture epithelial cells and the filter well insert was the commercialization of this technique. In the 1950s, Grobstein pioneered the use of filters to study the morphogenetic properties of embryonic mouse tissues separated by filters [19]. Filter well inserts provide substrates for cells that allow for their attachment on the basal membrane, at the same time that they support the secretion of various molecules from the basal and apical surfaces. This arrangement allows directional polarized metabolic processes similar to those that occur *in vivo*.

Filter well inserts were one of the first technologies that began to approach a 3D-like exposure of cells to a substrate by allowing all membrane sides to interact with the environment. This design also allows for the study of both surfaces of a cell monolayer, which has shown to be invaluable in the study of epithelial cell line migration, development and tissue modeling. Handler *et al.* devised some of the original filter-containing dishes [20], and demonstrated greater epithelial differentiation on their filter substrates than standard tissue cultures dishes [21]. Polarity has been established using filter substrates in MDCK cells, when Cereijido *et al.* demonstrated an electric potential across basal and apical membranes [22]. Transepithelial transport has also been examined, and its polarity established, with the aid of filter substrates for cells [23], and the field of tumorigenic invasion has been greatly advanced with the use of this technology [24].

The type of filter well insert selected is the most crucial step in the success of this technique. Filter well inserts come in a vast array of formats, sizes, coatings and pore sizes, just to name a few of the characteristics that play a large role in the choice of filter insert. All of these choices depend on the cell type used and the assay performed. The different manufacturers of filter well inserts include selection guides that help determine the filter insert best suited for the nature of research. Two major commercially produced filter well inserts and the options they provide are discussed here: (1) Millicell® by Millipore and (2) Transwell® by Corning.

Millicell® filter inserts come with an option of four different membrane types each particularly suited for specific functional

assays or observable phenomenon: (1) biopore membrane, consisting of hydrophilic poly(tetrafluoroethylene) (PTFE), particularly suited for live cell viewing or immunofluorescent applications because of its transparency. (2) MF-Millipore™ membrane made of mixed cellulose esters, optimal for studies requiring exceptional polarization. Studies using cellulose acetate and cellulose nitrate millipore filters have helped establish the toad kidney epithelial cell line A6 as a representative model system for studying apical entry pathway for sodium in tight junction epithelia [25]. (3) Isopore™ membrane is a polycarbonate membrane used for the growth of attachment dependent cells without the use of a matrix. This membrane type is best suited for transport and permeability applications. Finally, (4) polyethylene teraphthalate (PET) is a thin, microscopically transparent membrane, allowing for better visualization of cells and can be used in a vast array of applications.

Transwell® provides a similar selection with the addition of the Transwell-COL inserts that are collagen-treated PTFE with a proprietary collagen coating to enhance cell adhesion.

Both sources of filter well inserts provide the option of a variety of coatings, such as collagen, fibronectin, laminin and Matrigel™, as well as detailed guides to the selection, use and preparation of filter well inserts culture. The inserts come in a variety of formats ranging from 6-well to 96-well formats. Millicell® products are available as single-well inserts and multi-well inserts. Single-well inserts run less risk of contamination but are not well suited for high-throughput screening (HTS). The Transwell® line of products include HTS Transwell-24 and HTS Transwell-96 insert systems amendable to automation.

Sponges and gels

Gels and sponges use purified ECM molecules and biopolymers to recreate *in vivo* cues for cells. Gels are poured by the user or purchased precast on the culture flask or assay plate and the most common are gelatin, collagen and laminin. Sponges, such as AlgiMatrix™, are generally lyopholized gels with large pores for cellular microenvironments. Cell substrate interactions range from complex cell survival signaling to practically nonexistent depending on the product chosen. We will consider three commercial offerings: (1) Matrigel™ from BD Biosciences, (2) Extracel™ from Glycosan Biosciences and (3) AlgiMatrix™ from Invitrogen.

Matrigel™ is a reconstituted basement membrane collected from the Engelbreth–Holm–Swarm (EHS) tumor grown in mice and is uniquely suited for the culture of epithelial cells. The isolation of the basement membrane components [26] and their characterization [27] identified the major components as type IV collagen, laminin and heparin sulfate. Matrigel™ was and continues to be an essential and well-cited element of cell culture. Dr. Mina Bissell's work with breast cancer using Matrigel™, or an equivalent, demonstrated the enabling power of 3D culture for creating *in vivo* model systems [28] and the importance of integrin signaling in cancer [29] (for a review see [30]).

Extracel™ is a 3D cell culture product combining hyaluronan, gelatin and the crosslinker polyethylene glycol diacrylate (PEGDA) [31]. Considering the range of ECM components, the inclusion of hyaluronan creates a compressible hydrogel similar to the structure of a joint, as opposed to Matrigel™ that mimics the basement membrane under epithelial cells. This hyaluronan-based gel may be modified by the addition of other ECM components, such as laminin, and the gel stiffness adjusted by the fixation crosslinking procedure. Extracel™ provides some ECM components for cell attachment with the advantage of a defined composition that can be modified by the researcher.

Unlike the former products, AlgiMatrix™ is an animal-free product as a ready-to-use sponge made from lyophilized alginate gel [32]. Alginate is a polymeric sugar from brown seaweed that gels in the presence of divalent cations to form a negatively charged hydrogel like the GAGs. The intent of AlgiMatrix™ is to allow cells to invade the pores and secrete endogenous ECM components that support *in vivo*-like morphologies, structures and behaviors. These small microenvironments are well suited for the growing popularity of primary and stem cell spheroid culture (see Fig. 1) [9,33].

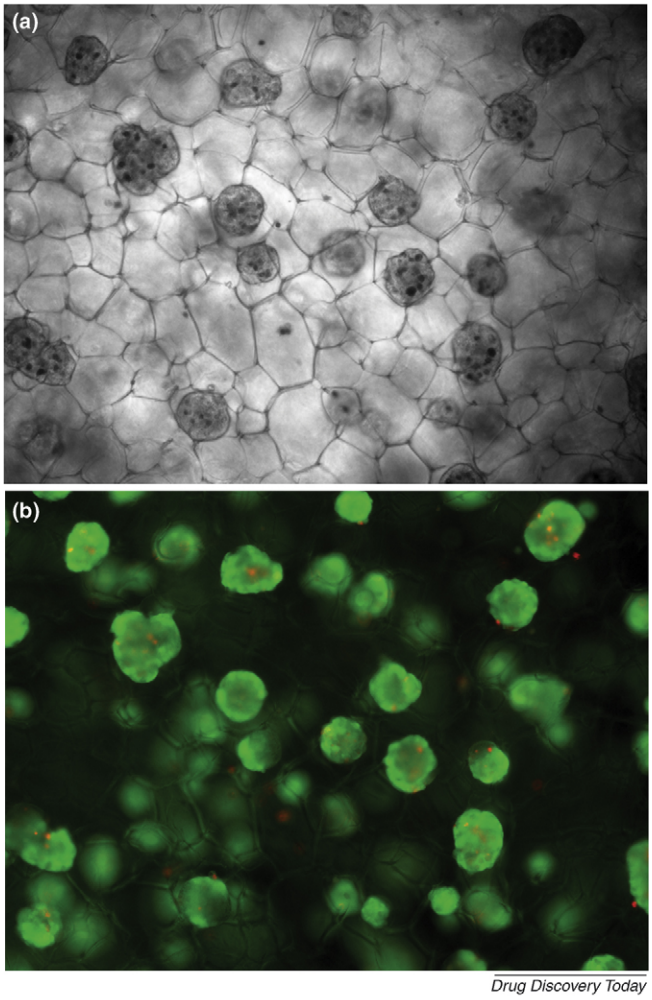

FIGURE 1

C3A human hepatocytes form spheroids cultured in AlgiMatrix after four weeks. **(a)** Phase contrast of the spheroids within the matrix. **(b)** Spheroids collected after dissolving the alginate matrix. Live cells in green and dead cells in red.

Gels and sponges offer the largest and richest range of 3D environments, but come with inherent limitations for use in drug discovery. First, the animal-origin of Matrigel™, laminin and collagen leads to inherent variability and makes them impractical for therapeutics. Second, pouring the gels in-house is impractical for screening, but the assay plates are relatively expensive at over $100 each. Third, assaying cells growing in 96-well microplates in 3D presents the challenge of maintaining well-to-well consistency. Finally, cells in gels and sponges may be difficult to observe without a confocal microscope. This challenge is being surmounted though with new HCS technologies and advances in microscopy such as light-sheet-based fluorescent microscopy [34].

Microcarriers

The curved surface of the microcarrier may be the simplest, yet most overlooked, 3D substrate for cell culture. Microcarriers are small spheres, typically less than 500 μm in diameter, whose enormous surface area of up to 500 cm^2/g, can culture large numbers of cells in small volumes. The view that microcarriers are designed exclusively for bioproduction is being challenged by those finding new cell culture utility in this technology.

Microcarriers are attracting new users from the field of tissue engineering and biomaterials. A fundamental challenge is expanding the primary or stem cells collected from a patient for reimplantation. Primary chondrocytes grown on microcarriers have been shown to expand efficiently and retain characteristics essential for implantation [35]. The carrier is also an implant scaffold and has been shown to be a useful device for trypsin-free culture and implant of mesenchymal stem cells [36]. Lastly, microcarriers are being used successfully to expand mouse embryonic stem cells 50-fold without inducing differentiation [37,38].

Microcarrier coatings, like 2D tissue culture flasks and plates, can include any number of proteins such as collagen or laminin. Commercially available are gelatin-coated carriers such as GE Healthcare's Cytodex 3 and animal-free coated carriers like the Synthetic Peptide II or ProNectin®F from Solohill Engineering, Inc. In either case, the coating functions, similar to gels, to promote the adhesion of cells to the substrate. The Cytoline microcarriers from GE Healthcare offer a porous substrate for cell growth similar to a sponge. Both types of carrier can be employed in a typical bioreactor, packed bed device or cultured in rocked dish to achieve varying yields and 3D phenotypes.

Global Cell Solutions has developed a novel magnetic microcarrier with a gelatin-coated alginate core (GEM™). The GEM™ takes advantage of the highly negative alginate core to simulate the GAGs but possesses a collagen-derived coating for cell adhesion. The growth of primary human proximal tubule cells demonstrates that these qualities create a desirable *in vivo* phenotype with a large increase in microvilli and the associated protein villin (Fig. 2). Furthermore, the GEM is well suited to adhesion-dependent cells, acting as an inert carrier during sensitive procedures including cell-based assays/HCS, cryopreservation and electroporation. In addition, the BioLevitator™ (Fig. 3) was developed to offer an easy-to-use, compact bench-top device for simple, disposable microcarrier cell culture.

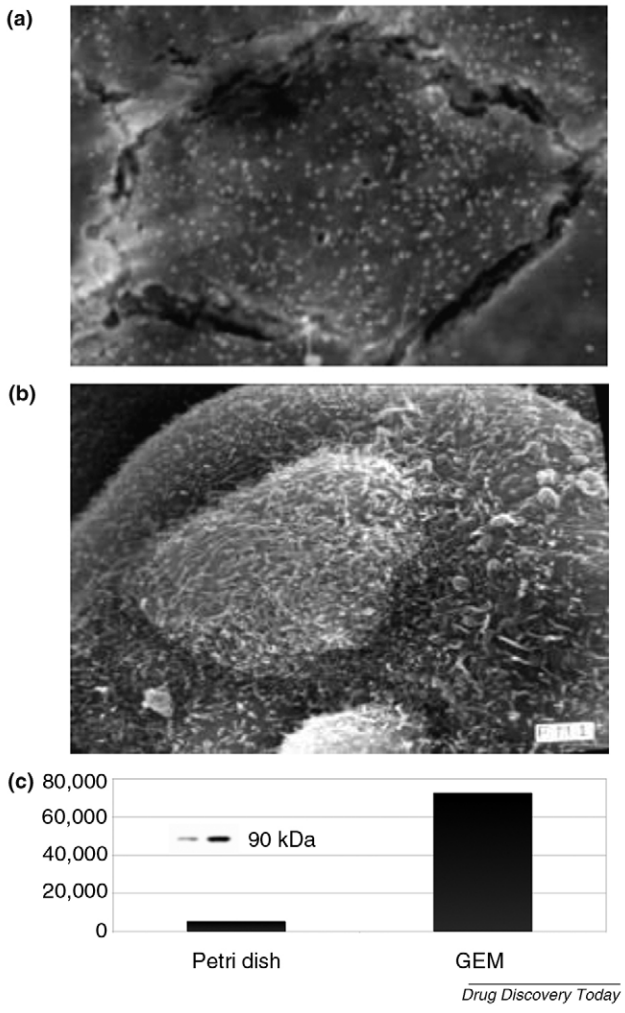

FIGURE 2

Increased microvilli expression in human proximal tubule cells (hPTC). **(a)** hPTCs grown on 2D flasks. **(b)** hPTCs grown on the GEM™. **(c)** Villin expression is greater when grown on the GEM.

3D in practice

When evaluating 3D cell culture technologies one must determine how to select the best technology for optimal biological results combined with the efficiency and convenience of automation (Table 1). The previous sections discussed the fundamentals of 3D biology and cell culture, but the following discussion considers the impact of price, scalability and process integration on technology decisions.

The requirement for consistency across the assay is hindered by animal-derived components and format complications. A current trend in cell culture is to limit or reduce the animal-derived components of cell culture such as serum. Unfortunately, laminin, collagen and reconstituted basement membrane are animal-derived and subject to the inherent production variability. Filters, animal-free products, such as alginate, foam and most microcarriers may prove appropriate for animal-free applications. Format can also complicate consistency. Growing 96 individual cultures on a filter or in gel can

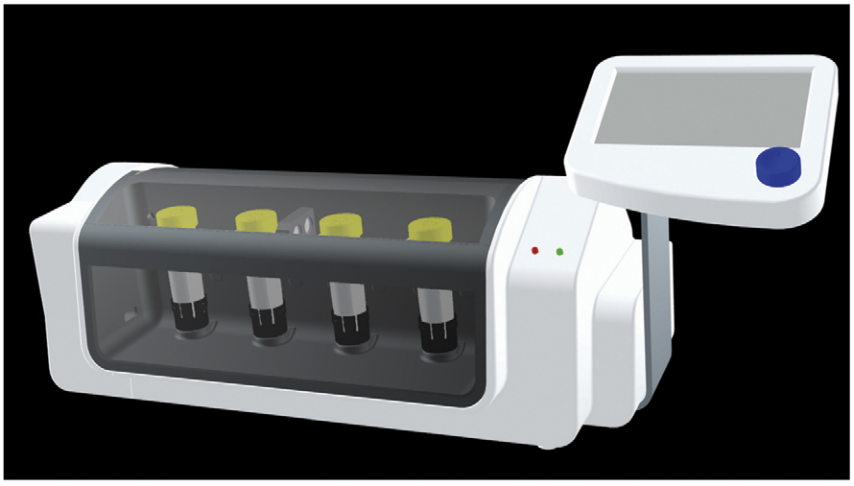

FIGURE 3

The BioLevitator™, a compact bench-top device for simple, disposable microcarrier cell culture.

TABLE 1

A comparison of 3D cell culture formats illustrates the suitability of various 3D cell culture technologies for automated cell-based assays.

	Cost	Plate density	Vendors
Filter wells	+++	96-well	Corning Life Sciences Millipore
Gelatin gel	++	384-well	BD Biosciences Sigma-Aldrich
Purified gel	+++	96-well	BD Biosciences Glycosan BioSystems
Sponges	+++	96-well	Invitrogen
Microcarrier	+	1536-well	GE Healthcare Global Cell Solutions

Cost per well ranging from typically less than US$0.05 (+), less than US$0.50 (++) and greater than US$0.50 (+++). Commonly available plate densities not requiring custom coating and their vendors.

lead to well-to-well variability. One advantage of microcarriers is that cell culture remains in a homogenous liquid state until they are used in your assay.

Most 3D culture technologies to date have catered to research applications and therefore do not scale well for screening applications that require significant 3D culture expansion and consistent cellular response. The filter and gel technologies have been adapted to the 96-well format, but require culture in the specialized filter vessel. Consistency aside, there is no efficient method to maintain such cultures for large-scale screening. The 96-well format is also bested by 384- and 1536-formats that increase screening density dramatically. Microcarriers, though, may prove to be ideal for scaling up 3D culture. Not only can they culture large numbers of cells, but also microcarriers can be dispensed in 384- and 1536-formats where they effectively increase the number of cells per well.

Cost can deflate even the best laid 3D cell culture plans. Filter well plates can range in cost per well from 60 cents to over $11 for specialty-coated membranes. Plates prepared with gels are commonly available, some even in a 384-well format and can range in cost per well from 10 cents for a gelatin coating to about $1.50 per well for a thick coat of reconstituted basement membrane. Finally, microcarriers are generally economical with some being as inexpensive as US$0.17 per T75 flask-equivalent, but may require additional handling and culture equipment.

Finally, consideration must be given to your current automation infrastructure. Although filter well, gels and sponges are automatable within modern liquid handling platforms, their culture still remains a manual process. Most microcarriers possess the same limitations. The GEM™ is designed for culture in the BioLevitator™, a bench-top incubator and bioreactor hybrid capable of four independent high-density 3D cultures co-developed by Global Cell Solutions and Hamilton Company. The GEM™ and BioLevitator™ technologies may be integrated on a modern liquid handling platform, to create the 3D CellHOST™. The 3D CellHOST™ is a next generation automated 3D cell culture system utilizing the GEM™ as a pipetteable culture substrate thereby enabling current liquid handling/robotic systems to easily maintain and dispense 3D cell cultures.

Conclusion

The modern understanding of the ECM and its role in a multitude of cell functions and behaviors has given researchers a growing interest in 3D cell culture [39]. Importantly, for cell-based assays, the evolution of HCS is yielding more insight into the inner workings of the cell. The rising demand for primary and stem

cells points to the future of HCS. Advances in detection technologies are, however, outstripping the advancements in current cell culture technologies. In light of the technologies presented, the modern microcarrier represents a fresh approach to 3D cell culture and an excellent candidate for next generation drug discovery, particularly when fully automated.

References

1 Elsdale, E. and Bard, J. (1972) Collagen substrata for studies on cell behavior. *J. Cell Biol.* 54, 627–637
2 Pampaloni, F. *et al.* (2007) The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* 8, 839–845
3 Mayr, M.M. and Fuerst, P. (2008) The future of high-throughput screening. *J. Biomol. Screen.* 13, 443–448
4 Bullen, A. (2008) Microscopic imaging techniques for drug discovery. *Nat. Rev. Drug Discov.* 7, 54–67
5 Starkuviene, V. and Pepperkok, R. (2007) The potential of high-content high-throughput microscopy in drug discovery. *Br. J. Pharmacol.* 152, 62–71
6 Frost & Sullivan. (2007) U.S. High content screening markets, N11C-55
7 Korn, K. and Krausz, E. (2007) Cell-based high-content screening of small molecule libraries. *Curr. Opin. Chem. Biol.* 11, 503–510
8 Bhadriraju, K. and Chen, C.S. (2002) Engineering cellular microenvironments to improve cell-based drug testing. *Drug Discov. Today* 7, 612–620
9 Kim, J.B. (2005) Three-dimensional tissue culture models in cancer biology. *Semin. Cancer Biol.* 15, 365–377
10 Zaman, G.J.R. (2007) Cryopreserved cells facilitate cell-based drug discovery. *Drug Discov. Today* 12, 521–526
11 Cawkill, D. and Eaglestone, S.S. (2007) Evolution of cell-based reagent provision. *Drug Discov. Today* 12, 820–825
12 Lee, J. *et al.* (2008) Three-dimensional cell culture matrices: state of the art. *Tissue Eng. Part B Rev.* 14, 61–86
13 Griffith, L.G. and Swartz, M.A. (2006) Capturing complex 3D tissue physiology in vitro. *Nat. Rev. Mol. Cell Biol.* 7, 211–224
14 Cukierman, E. *et al.* (2002) Cell interactions with three-dimensional matrices. *Curr. Opin. Cell. Biol.* 14, 633–639
15 Miner, J.H. (2008) Laminins and their roles in mammals. *Microsc. Res. Tech.* 71, 349–356
16 Simons, M. and Mlodzik, M. (2008) Planar cell polarity signaling: From fly development to human disease. *Annu. Rev. Genet.* [ePub]
17 Adams, C.A. and Watt, F.M. (1993) Regulation and development of differentiation by the extracellular matrix. *Development* 117, 1183–1198
18 Carney, S.L. and Muir, H. (1988) Structure and function of cartilage proteoglycans. *Physiol. Rev.* 3, 858–910
19 Grobstein, C. (1953) Morphogenetic interaction between embryonic mouse tissues separated by a membrane filter. *Nature* 172, 869–870
20 Steele, R.E. *et al.* (1986) Porous-bottom dishes for culture of polarized cells. *Am. J. Physiol.* 251, 136–139
21 Handler, J.S. *et al.* (1984) Factors affecting the differentiation of epithelial transport and responsiveness to hormones. *Fed. Proc.* 15, 2221–2224
22 Cereijido, M. *et al.* (1978) Polarized monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* 77, 853–880
23 Matlin, K.S. and Simons, K. (1984) Sorting of an apical plasma membrane glycoprotein occurs before it reaches the cell surface in cultured epithelial cells. *J. Cell Biol.* 99, 2131–2139
24 Lu, Z. *et al.* (2001) Epidermal growth factor-induced tumor cell invasion and metastasis initiated by dephosphorylation and downregulation of focal adhesion kinase. *Mol. Cell. Biol.* 21, 4016–4031
25 Sariban-Sohraby, S. *et al.* (1983) Sodium uptake in toad epithelial line A6. *Am. J. Physiol.* 245, 167–171
26 Kleinman, H.K. *et al.* (1982) Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochemistry* 21, 6188–6193
27 Kleinman, H.K. *et al.* (1986) Basement membrane complexes with biological activity. *Biochemistry* 28, 312–318
28 Barcellos-Hoff, M.H. (1989) Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development* 105, 223–235
29 Weaver, V.M. (1997) Reversion of the malignant phenotype of human breast cells in three-dimensional culture an in vivo by integrin blocking antibodies. *J. Cell Biol.* 137, 231–245
30 Bissell, M.J. *et al.* (2003) Tissue architecture: the ultimate regulator of breast epithelial function. *Curr. Opin. Cell Biol.* 15, 753–762
31 Shu, X.Z. *et al.* (2006) Synthesis and evaluation of injectable, in situ crosslinkable synthetic extracellular matrices for tissue engineering. *J. Biomed. Mater. Res. A* 15, 902–912
32 Shapiro, L. and Cohen, S. (1997) Novel alginate sponges for cell culture and transplantation. *Biomaterials* 18, 583–590
33 Kunz-Schughart, L.A. *et al.* (2004) The use of 3-D cultures for hight-throughput screening: the multicellular spheroid model. *J. Biomol. Screen.* 9, 273–285
34 Pampaloni, F. (2007) The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* 8, 839–845
35 Malda, J. *et al.* (2003) Expansion of bovine chondrocytes on microcarriers enhances redifferentiation. *Tiss. Eng.* 9, 939–948
36 Yang, Y. *et al.* (2007) Ex vivo expansion of rat bone marrow mesenchymal stromal cells on microcarrier beads in spin culture. *Biomaterials* 28, 3110–3120
37 Abranches, E. *et al.* (2006) Expansion of mouse embryonic stem cells on microcarriers. *Biotechnol. Bioeng.* 95, 1211–1221
38 Fernandes, A.M. *et al.* (2007) Mouse embryonic stem cell expansion in a microcarrier-based stirred culture system. *J. Biotechnol.* 132, 227–236
39 Prestwich, G.D. (2007) Simplifying the extracellular matrix for 3-D cell culture and tissue engineering: a pragmatic approach. *J. Cell. Biochem.* 101, 1370–1383