International Journal of
Medical Nano Research

Wu and Kisaalita. Int J Med Nano Res 2015, 2:1
ISSN: 2378-3664

Editorial: Open Access

Micro and Nanofabrication: Is a Practical Three-Dimensional Cell Culture Platform for Drug Discovery Achievable?

Ze-Zhi Wu[1]* and William S. Kisaalita[2]

[1]Key Laboratory of Biorheological Science and Technology of the State Ministry of Education, College of Bioengineering, Chongqing University, PR China

[2]Cellular Bioengineering Laboratory, College of Engineering, University of Georgia, USA

***Corresponding author:** *Ze-Zhi Wu, Key Laboratory of Biorheological Science and Technology of the State Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, PR China, Tel & Fax: 0086-23-65102507, E-mail: zzwu@cqu.edu.cn*

Direct and indirect evidence, in support of the notion that biological activity of three-dimensional (3-D) cultures may more closely mirror what happens *in vivo*, has appeared in the literature for the past three decades. This is probably best exemplified in the field of experimental oncology that adopts 3-D multicellular tumor spheroids (MCTS) to mimic the *in vivo* situation. Early seminal work in this section was represented by Sutherland and colleagues who used MCTS as an *in vitro* model for systematic studies on tumor cell responses to therapeutic treatments [1]. Multicellular spheroids have been able to fully recapitulate the multicellular mediated drug resistance of EMT6 tumors, which was inherently induced *in vivo* but completely lost when cancer cells were dissociated and cultured as monolayers. It has been shown that the phenotypic transformation of malignant cells in a 3-D collagen gel configuration is achievable upon treatment with integrin antibodies, while this has never been possible in monolayer cultures. Researchers have also noted that HT-1080 fibrosarcoma and MDA-MB-231 carcinoma cells showed protease-independent amoeboid movement within 3-D collagen matrix while, in 2-D cultures, this movement is totally dependent on proteases like matrix metalloproteinases. This challenges the traditional screening for anti-metastatic agents against proteolytic activity with 2-D monolayer cultures. In fact, Mueller-Klieser [2] has actually proposed that 3-D spheroids should become mandatory test systems in cancer therapeutic screening programs.

Although 3-D cell culture is promising for drug discovery or therapeutics development, establishment of a practical 3-D cell-based platform is actually challenging. Up to now, the few successful examples that demonstrated the advantages and translational potential of 3-D culture in drug discovery were still limited to the fields related to cancer treatment, like those mentioned above. Superiority of the 3-D culture for other cell types, e.g., neural cells, in drug discovery programs has not been experimentally verified. Further advancement of this field is not likely to come from simple scaling-down of the approaches adopted in tissue engineering or regenerative medicine practices, due to the incompatibility of these methodologies with current drug screening modalities. For example, in screening, high throughput needs call for high-density well formats accessible with current readout devices.

The potential of micro- and nanofabrication technologies in engineering 3D and physiologically more relevant cellular microenvironments has been successfully demonstrated in the fields of tissue engineering, regenerative medicine and biopharmaceutical manufacturing. In the past decade, several promising micro- or nano-fabricated culture plates, suggested to be promising for physiologically relevant screening, have appeared on the market. These include Extracel™ hydrogel from Glycosan, AlgiMatrix™ from Invitrogen, Extracel™ sponge from Glycosan and Ultra-Web from Corning. However, it has not been conclusively shown that use of these platforms improves the rate of success of the hits/leads identified.

For the purpose of cell-based drug discovery, the ultimate goal that the functionality of cultured cells reflects those under the *in vivo* conditions has been termed complex physiological relevance (CPR) [3]. Realization of CPR relies on the micro- and nanoengineering of the microenvironment factors (MEFs) that regulate cellular phenotype outcomes. These MEFs include [4]: (1) chemical or biochemical composition, (2) spatial (geometric 3D) and temporal dimensions, and (3) force and substrate physical properties. For the chemical and biochemical composition, the current patterning techniques, such as contact printing, and microfluidic technologies offer accurate control. However, the challenge raised with these technologies is that they entail specific instrumentation and additional cost to the screening programs that conventional screening laboratories can not afford, especially for high throughput applications. For spatial and temporal dimensions, an immediate challenge for adoption of cellular aggregates in the screening activities is the weak adhesion of those aggregates or spheroids for withstanding physical handling like medium changes. Gel embedded aggregates do indeed provide a solution in this regard. However, the mass transportation limitations need to be carefully evaluated, especially in case of long culture duration, even if the resultant hypoxia may sometimes be considered (patho) physiologically relevant. Micro- and nanofabrication does indeed provide huge potential for tailoring substrate physical properties such as topography and mechanical stiffness for use in cell-based assays. In this regard, researchers have fabricated a number of substrates, in

ClinMed International Library

Citation: Wu ZZ, Kisaalita WS (2015) Micro and Nanofabrication: Is a Practical Three-Dimensional Cell Culture Platform for Drug Discovery Achievable?. Int J Med Nano Res 2:008e

Received: March 30, 2015: **Accepted:** April 03, 2015: **Published:** April 06, 2015

the form of either porous polystyrene scaffolds, microwell patterned poly-l-lactic acid and SU-8 substrates, or Cytodex and polystyrene microbead arrays. Responsiveness of voltage-gated calcium channels (VGCCs) of SH-SY5Y human neuroblastoma cells, ENStem-ATM neural progenitor cells (Millipore, Billerica, MA) and primary mice superior cervical ganglion (SCG) cells on these substrates have thus been evaluated. Although it was found that physiologically relevant differences existed between VGCC responsiveness/expression for cells on these fabricated substrates and those from 2D counterparts, the verification of the superiority of 3D over 2D culture of neuronal cells in terms of drug discovery against VGCCs still need further verification.

It is a great challenge to simultaneously engineer the three MEFs in a single effort to create physiologically relevant cell culture microenvironments. A survey of the literature has shown that in such a scenario some MEFs may not be as important as others. For example, *in vitro* cultured HepG2 cells showed similar phenotypic outcomes if cell-cell interaction or the formation of cellular aggregates predominates, even if the substrate or scaffold mechanical stiffness varies within a range of orders of magnitudes. It is therefore helpful to evaluate the relative importance of those MEFs and understand the minimum and necessary MEFs for cultured cells to initiate self organization of prototype tissues and to establish their own physiologically relevant 3D microenvironments. The evaluation of physiologically relevance of the target responses can then be carried out. To serve as an example, we have recently adopted a microwell pattern to induce the peeling of C17.2 cells or cellular strips for the self assembly of multicellular aggregates [5]. Such aggregates were tethered to the pattern for withstanding medium handling while continuing to grow in suspension as cell aggregates.

References

1. Sutherland RM, Inch WR, McCredie JA, Kruuv J (1970) A multi-component radiation survival curve using an in vitro tumour model. Int J Radiat Biol Relat Stud Phys Chem Med 18: 491-495.
2. Mueller-Klieser W (2000) Tumor biology and experimental therapeutics. Crit Rev Oncol Hematol 36: 123-139.
3. Kisaalita WS (2010) 3D Cell-Based Biosensors in Drug Discovery Programs: Microtissue Engineering for High Throughput Screening. CRC Press, Taylor and Francis Group, Boca Raton, London, New York.
4. Yamada KM, Pankov R, Cukierman E (2003) Dimensions and dynamics in integrin function. Braz J Med Biol Res 36: 959-966.
5. Zhang LG, Zhong DH, Zhang Y, Li CZ, Kisaalita WS, et al. (2014) A microwell pattern for C17.2 cell aggregate formation with concave cylindrical surface induced cell peeling. Biomaterials 35: 9423-9437.