Biomaterials 33 (2012) 9087–9096

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Influence of a three-dimensional, microarray environment on human Cell culture in drug screening systems

Luciana Meli [a], Eric T. Jordan [b], Douglas S. Clark [f], Robert J. Linhardt [a,b,c,d], Jonathan S. Dordick [a,b,d,e,*]

[a] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA
[b] Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA
[c] Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA
[d] Department of Biomedical Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA
[e] Department of Material Sciences, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA
[f] Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:
Received 17 August 2012
Accepted 29 August 2012
Available online 19 September 2012

Keywords:
3D cell culture
Microarray
High-throughput
Drug resistance

ABSTRACT

We have used a modified 3D cellular microarray platform for the high-throughput analysis of growth, cytotoxicity, and protein expression profile of a human hepatocellular carcinoma cell line, HepG2, in alginate. The results obtained were compared to analogous studies in 2D and 3D environments at the microtiter scale. The antiproliferative effects of four drugs, tamoxifen, 5-fluorouracil, doxorubicin, and amitriptyline, were studied as a function of seeding density in the three different culture platforms. The chemosensitivity of HepG2 cells to all four compounds decreased substantially with increasing cell number in the 2D and 3D microtiter-based cultures, while no seeding density dependence was observed in the IC_{50} values obtained in the 3D microarray culture platform. These results can be rationalized based on the development of confluence-dependent resistance in cultures where proliferation is restricted by cell–cell contacts and nutrient availability, as is the case for both of the microtiter-based cultures. Additionally, further development of an on-chip, in-cell immunofluorescence assay provided quantitative data on the levels of specific target proteins involved in proliferation, adhesion, angiogenesis and drug metabolism, and was used to compare expression profiles between 2D and 3D environments. The up-regulation of several CYP450 enzymes, β1-integrin and vascular endothelial growth factor (VEGF) in the 3D microarray cultures suggests that this platform provides a more *in vivo*-like environment allowing cells to approach their natural phenotype.

1. Introduction

A drug candidate in Phase I clinical testing often requires a decade of discovery followed by preclinical evaluation, yet still has only an 8% chance of reaching the bedside [1]. A leading cause for the failure of drug candidates during clinical trials, and even after a drug has been introduced into the market, is adverse toxicity that was not predicted by animal models [2]. Moreover, the ethical issues and financial constraints surrounding the use of animal-based models in drug screening and toxicity testing have placed increasing pressure to transition testing to *in vitro*, human cell-based assays that are inexpensive, faster, and potentially more predictive than the current animal testing paradigm [2–4].

Cell-based assays can facilitate evaluation of a target molecule in a cellular context at an early stage in drug discovery by simultaneously providing information on multiple biochemical and biological end-points, such as proliferation, chemoresistance, motility, differentiation, cell shape, drug absorption, metabolism, and protein expression and localization [5]. These assays are not only rich in information, but are often amenable to automated, high-throughput (HT) screening, reducing screening cost and time, and improving accuracy. However, there remain significant challenges in developing *in vitro* cell-based models that can recapitulate the *in vivo* tissue environment to evaluate biologically complex processes. One step toward developing more realistic culturing models is to constrain cells to a more *in vivo*-like, three-dimensional (3D) environment. This should facilitate cell–cell communication, and can be useful in determining how the cells perceive, interpret, and respond to cues from their microscale environment.

* Corresponding author. Department of Material Sciences, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA. Tel.: +1 518 276 2899; fax: +1 518 276 2207.
E-mail address: dordick@rpi.edu (J.S. Dordick).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.08.065