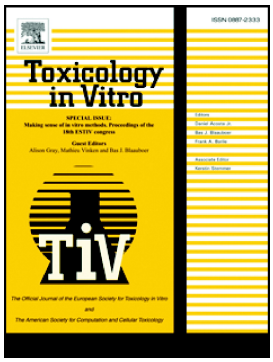

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High-Content Imaging Assays on a Miniaturized 3D Cell Culture Platform

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Abstract

The majority of high-content imaging (HCI) assays have been performed on two-dimensional (2D) cell monolayers for its convenience and throughput. However, 2D-cultured cell models often do not represent the *in vivo* characteristics accurately and therefore reduce the predictability of drug toxicity/efficacy *in vivo*. Recently, three-dimensional (3D) cell-based HCI assays have been demonstrated to improve predictability, but its use is limited due to difficulty in maneuverability and low throughput in cell imaging. To alleviate these issues, we have developed miniaturized 3D cell culture on a micropillar/microwell chip and demonstrated high-throughput HCI assays for mechanistic toxicity. Briefly, Hep3B human hepatoma cell line was encapsulated in a mixture of alginate and fibrin gel on the micropillar chip, cultured in 3D, and exposed to six model compounds in the microwell chip for rapidly assessing mechanistic hepatotoxicity. Several toxicity parameters, including DNA damage, mitochondrial impairment, intracellular glutathione level, and cell membrane integrity were measured on the chip, and the IC_{50} values of the compounds at different readouts were determined to investigate the mechanism of toxicity. Overall, the Z' factors were between 0.6 – 0.8 for the HCI assays, and the coefficient of variation (CV) were below 20%. These results indicate high robustness and reproducibility of the HCI assays established on the miniaturized 3D cell culture chip. In addition, it was possible to determine the predominant mechanism of toxicity using the 3D HCI assays. Therefore, our miniaturized 3D cell culture coupled with HCI assays has great potential for high-throughput screening (HTS) of compounds and mechanistic toxicity profiling.

Keywords: High-content imaging, high-throughput screening, mechanistic toxicity, 3D cell culture, miniaturization, microarray chip platform

1. Introduction

Current drug development has become an expensive business with costs around $2.6 billion per FDA-approved drug and development time nearly 10-12 years. The cost of drug development has increased tremendously over the past 20 years with the approval rate of new drugs rapidly declining (Paul et al., 2010). With the current attrition rate standing at 90% for new molecular entities, decreasing the high attrition rate is a major challenge for pharmaceutical industries. Toxicity is one of the leading causes for attrition of lead candidates in drug discovery processes. Lack of highly predictive *in vitro* toxicity assay platforms in an early preclinical drug discovery stage has been attributed to the high failure of lead compounds in animals and humans (Astashkina et al., 2012). To overcome this issue, multi-parametric mechanistic toxicity assays, also known as high-content imaging (HCI) assays, have been implemented *in vitro* to weed out potentially toxic compounds *in vivo*. Its capability to analyze multiple endpoints such as target specific signals (e.g., mitochondrial impairment, DNA damage, glutathione level, oxidative stress, calcium homeostasis, apoptosis/necrosis, *etc.*) as well as morphological changes in nuclear structure and organelle structure along with various reporter signal has enhanced our understanding of the mechanism of action of drug candidates. Thus, HCI assays have become an important tool in drug discovery process in pharmaceutical industry (Vliet et al., 2014). Nonetheless, the majority of HCI assays have been still carried out on two-dimensional (2D) cell monolayer culture for their low cost, high throughput, and convenience, despite of the enormous reports on the lack of morphological, physiological, protein/gene expression, and metabolic properties along with limited cell-cell and