Author's Accepted Manuscript

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PII: S0039-9140(17)30966-9
DOI: http://dx.doi.org/10.1016/j.talanta.2017.09.022
Reference: TAL17925

To appear in: *Talanta*

Received date: 1 August 2017
Revised date: 7 September 2017
Accepted date: 8 September 2017

Cite this article as: Fakhar Singhera, Emily Cooper, Louis Scampavia and Timothy Spicer, Using Bead Injection to Model Dispensing of 3-D Multicellular Spheroids into Microtiter Plates, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2017.09.022

Using Bead Injection to Model Dispensing of 3-D Multicellular Spheroids into Microtiter Plates

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Abstract
Biomedical translational research has relied on two dimensional (2D) cell cultures for drug discovery over the decades, requiring cells to grow on a flat surface which does not always accurately model *in vivo* biological states. Three dimensional (3D) cell cultures, also known as 3D spheroids or organoids, grow as cellular tissues that are more physiologically relevant especially with respect to emulating cancer tumor-like structutes[1]. While attractive, current methods for generating 3D spheroids has yet to replace 2D culturing methods used for drug discovery efforts that employ high-throughput screening (HTS), having limitations with scalability, reproducibility, and compatibility predominantly associated with conventional microtiter plate usage. Presented is a novel use of bead injection for the reproducible placement of spheroids and beads into high density microtiter plates of a 384- and 1536- well format.
Keywords: Bead Injection; microfluidics, 3D cell culture, spheroid; organoid, cancer; microtiter plate

Introduction

Initial leads for new cancer drugs are often identified by high throughput screening (HTS) campaigns against large chemical libraries as tested against cancer relevant cell lines that are grown as two dimensional cultures within a microtiter plate. Unfortunately, many leads discovered have a poor correlation when advanced to *in vivo* studies [1, 2]; postulating that 2D cultures fail to accurately recapitulate solid tumor architecture and biology. Real cancerous tumors are much more complex with "cell to cell" and "tissue to extracellular milieu" interactions that present a vastly different microenvironment not emulated by 2D cellular cultures. Due to the limitations of the 2D approach, 3D cellular spheroids are now being advanced as a more accurate pre-clinical model for drug discovery and drug testing[3]. A number of methods for generating 3D spheroids are currently employed[4], but most of these methods are limited to bench-top research and not amenable to HTS automation which requires a cost-effective solution to the scalability of spheroid production and reproducibility of analysis within microtiter plates; the standard tool used in translational research and biomedical testing. Microtiter plates used in large scale HTS are typically a 384-well or a 1536-well plate format for large chemical library screenings; ideally requiring the placement of the same quantity of cells in each well to accurately determine a compound's potency as compared to controlled drugs. Large scale production of spheroids, in mass, has been reported using bioreactors[1] and micro-cavity tissue flasks[5] preliminarily answering a need for scalability of production; however the redistribution of these large collection of spheroids into microtiter wells as discrete and equal aliquots remains an unmet challenge. Bead Injection[6] (BI) methodology provides a novel means of automating fluidic handling of micro-beads and presents a technology that can be leveraged for the automated fluidic management of 3D spheroids. Presented is a "proof of concept" using fluorescently labeled Sephadex® beads as a surrogate for 3D spheroid dispensing in 1536-well microtiter plates.

Determining an Analogue to Multicellular Spheroids

The decision to use chromatography gel filtration beads as a surrogate for multicellular spheroids was motivated by cost and testing expediency by negating the need to culture spheroids or organoids (~4 day process). The desired 3D spheroids are derived from the HT-29 cell line (human colon cancer cells); which have a uniform shape and diameter of approximately 210 ± 11um after 96 hours of incubation. Sephadex® G25 (Sigma-Aldrich: G25300) are cross-linked dextran gel filtration beads and are well suited for this emulation task having a particle size distribution between 100 – 300um. The beads were also filtered through a 249um and 297um filter before the dispense step to ensure they were ranging in the same diameter as spheroids.[7] To ensure that the density of the Sephadex® G25 coarse beads matched the density of the HT-29 spheroids, approximately 10ul of