Standardized Mini-spheroids Manufacturing and Dispensing for HTP Testing

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Introduction

Spheroids offer several advantages over traditional 2D cultures, including better mimicking the *in vivo* environment of tissues. In recent years, it has become evident that spheroids are indispensable tools for both research and industry.
While structurally simple, high-throughput (HTP) production of spheroids for drug screening can be a challenge. Indeed, the commonly used processes can be labor-intensive and often lead to spheroids that are heterogenous in size. This, along with the difficulty of seeding a single sphere per well, hinders robust drug screening.

Thanks to LiveDrop's OneFlow™ or ModaFlow™ microfluidics instruments and the SEED Biosciences Dispen3D dispenser, highly homogenous spheroids of desired size can easily be manufactured at high-speed, then be precisely seeded down to one spheroid per well.

Materials and Methods

Compact and homogeneous spheroids suitable for HTP drug screening were successfully produced with the OneFlow™ instrument. Approximately 150 cells of MCF7 (mCherry, red) or of a mixed culture (1:10 of GFP labelled cells, green with neg cells) were encapsulated in 3 nL droplets of complete media at a rate of > 2,000 spheroids/min. Following an overnight incubation of the droplets, spheroids were recovered and resuspended at 150-300 per mL. Spheroids were < 200 µm in diameter and remained compact and undamaged during the recovery process.

Subsequently, collected spheroids were loaded in the Dispen3D dispenser, an impedance-based pipetting robot that allows gentle and traceable single-sphere dispensing. Spheres were seeded at one (GFP) or two spheroids (GFP/mCherry) per well. Images were taken after 24h with a 4x objective.

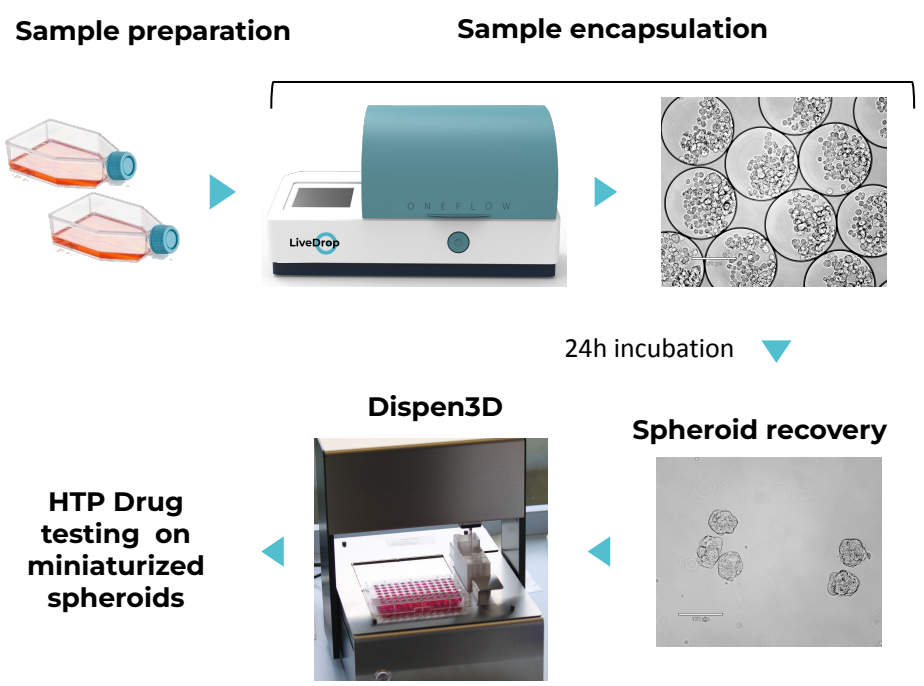


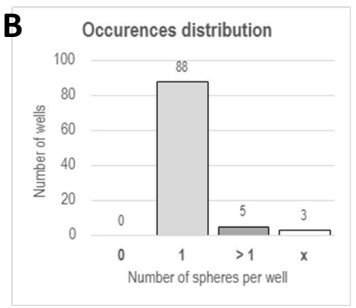
Figure 1: Overview of the workflow for HTP drug screening on spheroids.

Results

A

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	5	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	2	1	1	1	1	1	1	1
F	x	1	2	x	1	1	2	1	1	1	1	x
G	1	1	1	1	1	1	1	1	1	1	1	2
H	1	1	1	1	1	1	1	1	1	1	1	1

B



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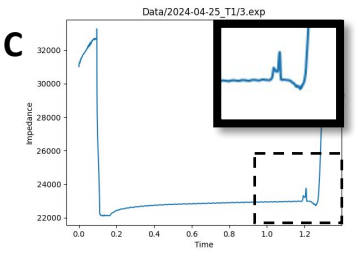
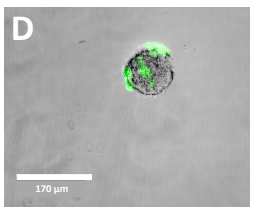


D



E

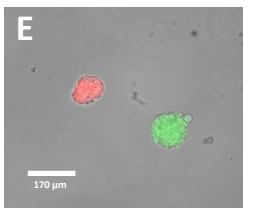


Figure 2: **(A)** Map of a 96 WPshowing the number of spheroids per well as detected by the Dispen3D. "x" indicate wells for which data were not collected. **(B)** Occurrences distribution of the number of spheroids (0, 1 or more than 1) seeded per well. Results show that > 90% of wells were successfully seeded with one spheroid per well. As spheroids were not always located in the center of the wells, image analyses could confirm these results in only > 75% of cases. **(C)** Representative image of the graph obtained for the impedance signal over time for well A4 (red frame in **A**). The solid frame is a zoom of the dash frame and show the impedance signal print of a spheroid. **(D & E)** Representative images of a single seeded mixed spheroid (GFP, green, **D**), and **(E)** spheroids co-seeded in the same well (mCherry, RFP and GFP,). bars = 170 µm.

Discussion and Conclusions

This collaborative work introduces a robust approach to efficiently produce homogeneous miniaturized spheroids, (20,000 spheroids in just 10 minutes) which could then be seeded in less than 7 minutes.

The incubation of cells in nanoliter-scale droplets promotes cell contact, facilitating cell aggregation and accelerating spheroid formation. The automated peak analyser of the Dispen3D ensured a controlled seeding of the spheres with > 90% of reliability demonstrating the robustness of the instrument. The Dispen3D software reports provide the time per plate, the number of elements seeded per plate and the plate-filling rate.

Thanks to its robustness and simplicity, this unique workflow holds potential for reliable exploration of new therapeutics and personalized medicine applications.