Large-scale production of functional and miniaturized spheroids for drug screening

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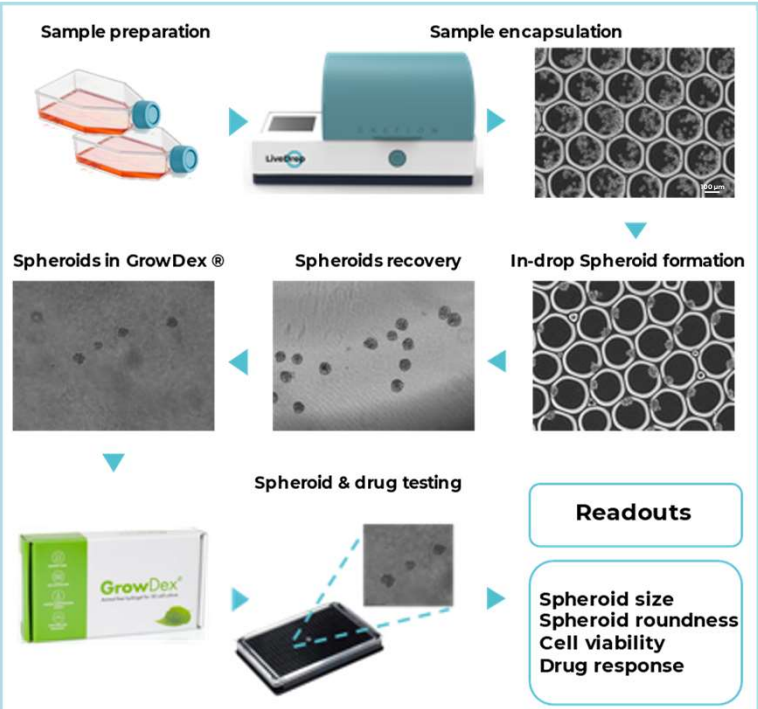
Introduction

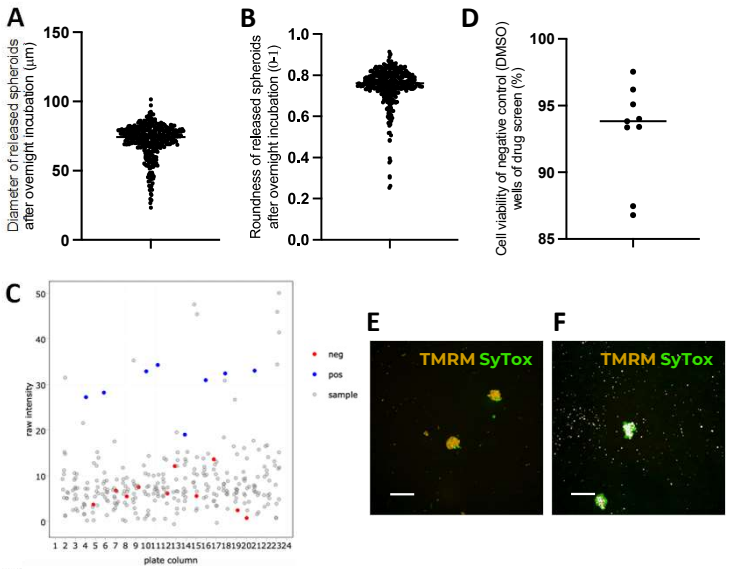
Spheroids have emerged as indispensable tools for both research and industry, offering improved physiological relevance, accelerated drug screening, and enhanced toxicity assessment in comparison to traditional 2D cultures. However, current methods like hanging drop micro-plates suffer from labor-intensive processes, high costs, and poor reproducibility, limiting their scalability for large-scale screening studies. Moreover, traditional large spheroids pose significant challenges for reproducible analysis when using microscopy imaging techniques.
To overcome these challenges, we have developed a streamlined workflow combining the high-throughput miniaturized spheroid production capabilities of the OneFlow™ or ModaFlow™ LiveDrop's microfluidics instruments with the advantageous properties of GrowDex® hydrogel from UPM Biomedicals.

Standardized mini-spheroids as an innovative solution to address HTP toxicity testing



Overview of the drug screening workflow

Results



After 48-hour-incubation in GrowDex hydrogel, the average diameter of spheroids is 70 µm **(A)** and average roundness is 0.72 out of 1 **(B)**. By introducing the drugs to 384-well spheroid culture for 48 hours, we were able to distinguish the negative (DMSO) and positive (Benzoyl chloride) controls **(C)** by measuring the cell viability by TMRM (orange, staining for live cells)-SyTox (green, staining for dead cells) staining. The average cell viability of native control wells is 94% **(D)**, with example TMRM-SyTox staining of negative **(E)** and positive **(F)** control-treated spheroids (scale bars = 200 µm).

Materials and Methods

Using the OneFlow™ instrument, we successfully produced compact and homogeneous spheroids suitable for high-throughput drug screening assays. Approximately 75 HepG2 cells were encapsulated in 3 nL droplets at a rate of 100 µl/min. Following an overnight incubation, we recovered and resuspended the spheroids in 0.2% GrowDex-medium dilution. The overall recovery rate was about 90%, and the spheroids remained compact, and maintained their integrity even when exposed to physical manipulation, such as pipetting during the recovery process.
Subsequently, the collected spheroids in GrowDex were pipetted into 384-well plates. Using Cybio Felix liquid handler technology (Analytik Jena) drugs were introduced to the spheroids. The efficacy of the drugs was evaluated through cell viability measurements like CellTiter-Glo, TMRM and SyTox live cell staining with high throughput imaging.

Conclusions

This collaborative work introduces an innovative approach to efficiently produce miniaturized spheroids, yielding 20,000 spheroids from 0.8 million cells suspended in 100 µL medium in just 3 minutes, with each spheroid measuring approximately 70 µm in diameter. These spheroids exhibit robustness, homogeneity, and functionality.

The incubation of cells in nanoliter-scale droplets promotes cell contact, facilitating cell aggregation and accelerating spheroid formation. Additionally, the use of GrowDex hydrogel provides steric hindrance to spheroids, preventing clustering during drug testing and cell staining.

The workflow is characterized by its robustness and simplicity and holds potential for further development to accommodate various cell types, thereby exploring new therapeutic avenues and personalized medicine applications.

GrowDex® OneFlow™ _ ModaFlow™