Sorting and dispensing of single spheroids for drug evaluation

Introduction

It is widely accepted that the two-dimensional monolayer cell assays have little resemblance with the complex three-dimensional multicellular organization found in living organisms. Instead, multicellular spheroids are more appropriate cellular models to better reconstitute the microenvironment that cells would find in the body or in a tumor[1]. Therefore, it is expected that spheroids would respond to drugs in a more representative manner. In order to implement spheroids in drug discovery processes, it is necessary to isolate them into uniformly sized population. This application note describes how the combined use of our microfluidic chip-based cell sorter, On-chip Sort, and our single cell dispenser, On-chip SPiS, facilitated the investigation on the effect of using protease inhibitor MG132 on spheroids formed by HT-29 human colon adenocarcinoma cells.

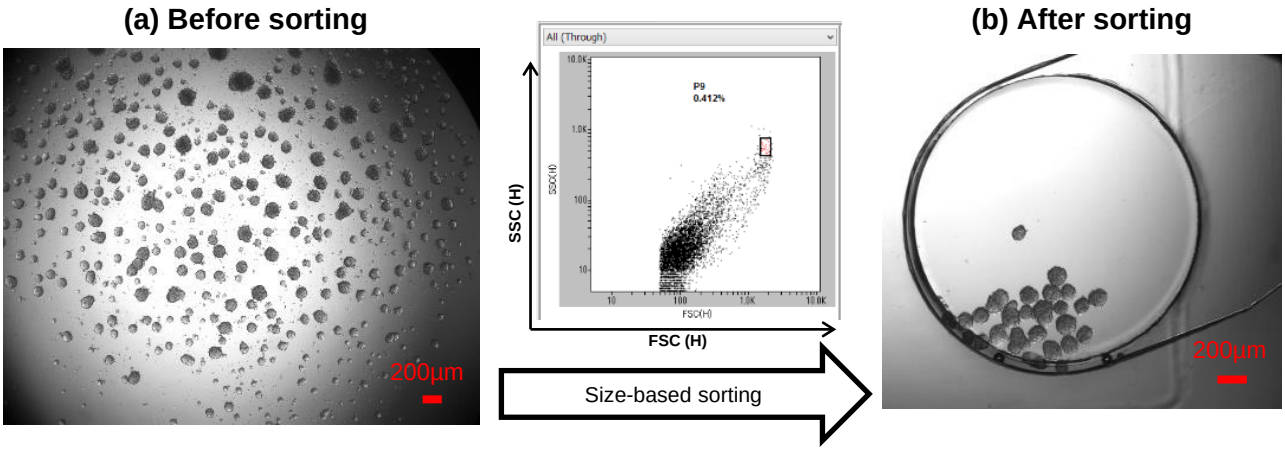

Fig. 1. Images of cultured HT-29 human colon adenocarcinoma cells before (a) and after (b) sorting based on size on On-chip Sort.

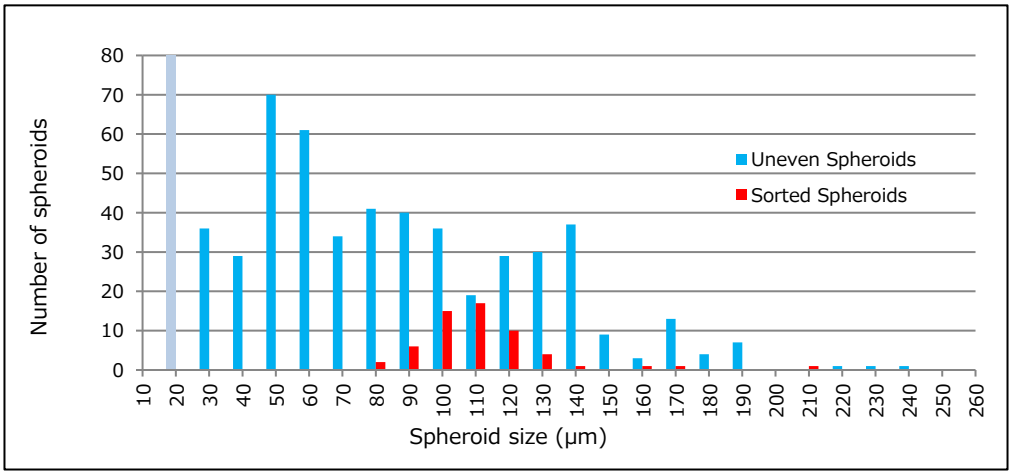

Fig. 2. Size distribution of spheroids detected on On-chip Sort. Red bars represent spheroids that were sorted.

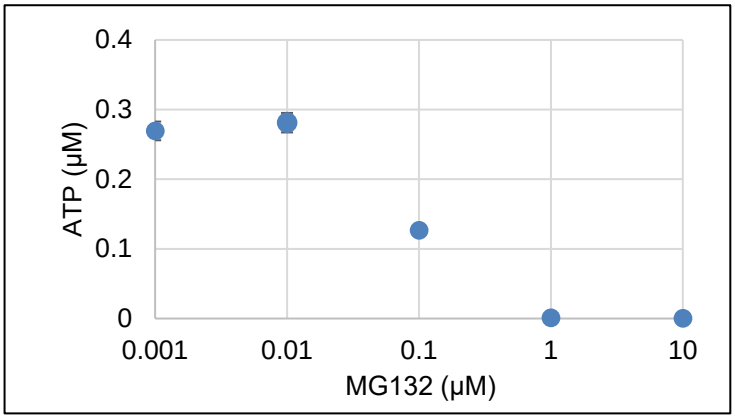

Fig. 3. ATP assay to assess the effect of MG-132 on isolated spheroids.

Methods

HT-29 human colon adenocarcinoma cells were cultured into spheroids over three to four days on the NanoCulture Plate (NCP-LH96) following the spheroid formation protocol provided by SCIVAX Life Sciences, Inc. (currently sold by Medical & Biological Laboratories Co., Ltd.). About 1800 spheroids sized around 100 μm were isolated using On-chip Sort with 150 μm channel. Culture medium was used as sheath fluid for sorting. Isolated spheroids were suspended in 500 μL culture medium and dispensed into wells of a 96-well plate using On-chip SPiS (one spheroid per well). Protease inhibitor, MG132, at 0, 0.01, 0.1, 1, or 10 μM concentration were directly added to the spheroids and cultured for three days. Spheroid viability was analysed by measuring ATP present using CellTiter-Glo® luminescent cell viability assay（Promega Co., Ltd., Wisconsin, USA) and Infinite 200 PRO microplate reader (Tecan Co., Ltd., Kanagawa, Japan).

Results

Spheroids cultured for three to four days (Fig. 1a) were sorted by size using On-chip Sort. The sizes of spheroids present ranged from 30 to 190 μm, and only those within 80 and 120 μm were isolated (Fig. 2). Isolated spheroids had approximate size of 100 μm (Fig. 1b). The effect of MG132 concentration on the viability of spheroids was confirmed using ATP assay (Fig. 3).

Conclusion

In summary, On-chip Sort and On-chip SPiS were capable of assisting in drug evaluation.

References

Ivascu, A. and M. Kubbies. (2006). Rapid generation of single-tumor spheroids for high-throughput cell function and toxicity analysis. *Journal of Biomolecular Screening*, 11(8): 922-932.

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