Application Note

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Complete and efficient spheroids dissociation using the Dissociation Buffer for Spheroids

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Abstract

Spheroids accurately replicate the natural and complex environment of cell-cell and cell-matrix interaction, making them more effective for studying cellular behaviours and biological processes compared to traditional 2D cell cultures. Over the last decades, numerous models and assays have been developed and used over a wide range of biological processes as tissue development, cancer progression or drug metabolism. All kind of read outs have been developed to analyse those complex structures, looking at the spheroid as a single object, or at each cells composing it. For accurate analyses in this case, the spheroid must be properly and completely dissociated to release all cells, and the viability of the cells must be preserved.

In this study, we demonstrated the superior effectiveness of the Dissociation Buffer for Spheroid, on different spheroids, by comparing it to the current gold standard dissociation method (trypsin - EDTA 0.05%). Those two methods have been compared both on the dissociation efficiency and the post-dissociation cell viability.

Introduction

In the field of biology, cell culture systems are indispensable tools, widely used for *in vitro* studies in basic and clinical research. The development of 3D models, such as spheroids, are increasingly common in translational biology as they have the advantage of more faithfully reproducing certain aspects of human tissues such as cell organisation, tissue architecture, cell-matrix and cell-cell interactions as well as the diffusion characteristics of oxygen, nutrients and cellular metabolism *[1]*. Spheroids are used to study proliferation, cell death, differentiation and metabolism of cells in tumors, or the response of tumors to radiotherapy and chemotherapy *[2]*.

The adoption of these 3D cell models relies on the development of suitable solutions to create, manipulate and analyze them. Many methods have already been developed to create and manipulate spheroids. However, there is still a need to develop methods to enable the detailed analyzes of these models, particularly at the single cell level, to provide valuables biological insights. Indeed, single cell analyses of 3D cell models extend our understanding of these models by assessing the cellular heterogeneity within a single spheroid *[3]*.

Cellenion has developed the **Dissociation Buffer for Spheroids** that allows the rapid, complete and efficient dissociation of spheroids, at low temperature, of different cell types to obtain clean and homogenous cell suspension. Coupled to dedicated solutions, single-cell Omics analyses can be performed to facilitate the transition to tomorrow's medical research.

Materials and methods

Cell culture and Cell Lines

Cell lines were cultivated under standard cell culture conditions (37°C, 95% humidity, 5% CO_2) in the appropriate cell culture medium. HEK 293T, HeLa, CHO, MCF-7 and NIH 3T3 were cultivated with DMEM F-12 GlutaMAX™1(1X) (Gibco) supplemented by 10% of Fetal Bovine Serum (Dutscher) and 1% antibiotic-antimycotic solution (Dutscher). HepaRG™ (Biopredic) were cultivated with basal hepatic cell medium (Biopredic) supplemented by 10% of HepaRG™ Growth Medium Supplement with antibiotics ADD710C (Biopredic).

Spheroid formation

Spheroids were produced using ultra low attachment plate method. For each cell line, about 2000 cells were seeded per well in Nucleon Sphera 96U-well plate (Nunc), and then incubated for 4 days at 37°C with 5% CO_2. HEK 293T, HeLa and CHO spheroids had diameters between 120-150µm whereas NIH 3T3, MCF-7 and HepaRG™ spheroids had diameters between 270-300µm.

Spheroid dissociation

In this study, the Dissociation Buffer for Spheroids was compared with trypsin EDTA 0.05% (w/v) (Gibco), which is commonly used in laboratories for spheroids dissociation. For each cell line, single spheroids grown for 4 days were dissociated directly in Nucleon Sphera 96U-well plate (Nunc) with the two methods described below (Figure 1).

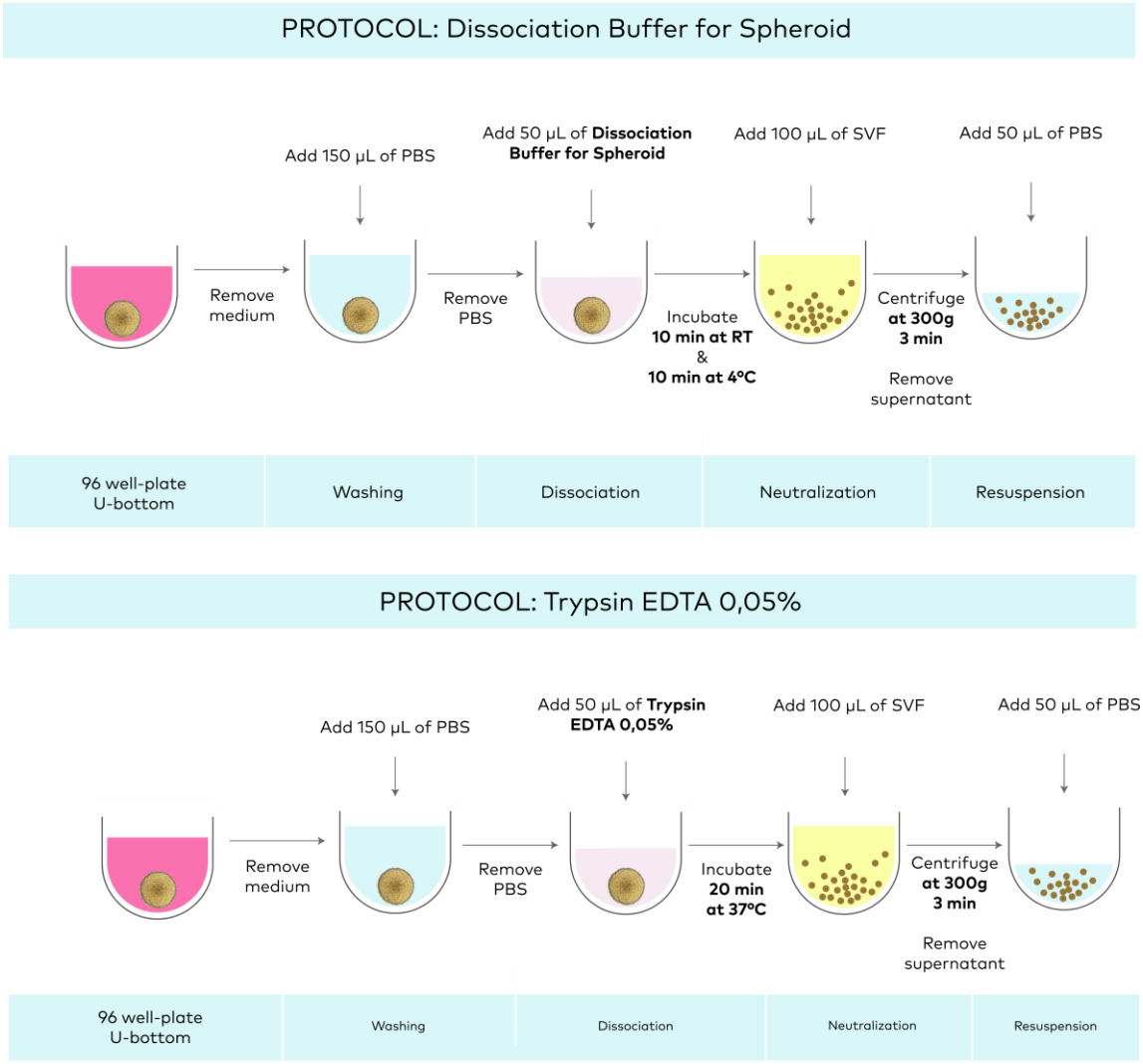

Figure 1: Spheroid dissociation protocol using Dissociation Buffer for Spheroids (top) and Trypsin EDTA 0.05% (bottom).

Spheroid dissociation efficiency

After dissociation, efficiency of spheroid dissociation was assessed by evaluating the percentage of spheroid dissociation by eyes under a microscope (Axio observer Z1, Zeiss). 0% corresponded to a fully intact and undissociated spheroid; 100% corresponded to fully dissociated spheroid; and 50% corresponded to 50% of cells being free of any interaction with other cells, and 50% of cells still being part of a cluster.

Cell viability post dissociation

After spheroid dissociation, cells were stained using Acridine Orange/Propidium Iodide (AOPI) following manufacturer recommendations (Logos Biosystems). Then, both cell concentration and cell viability were evaluated using the Luna™ Dual Florescence Cell Counter, (Logos Biosystems).

Results and discussion

Spheroid dissociation efficiency

The dissociation efficiency was compared using 6 different cell lines, 3 proliferative and 3 not proliferative in 3D culture, with either the Dissociation Buffer for Spheroids or the traditional trypsin method.

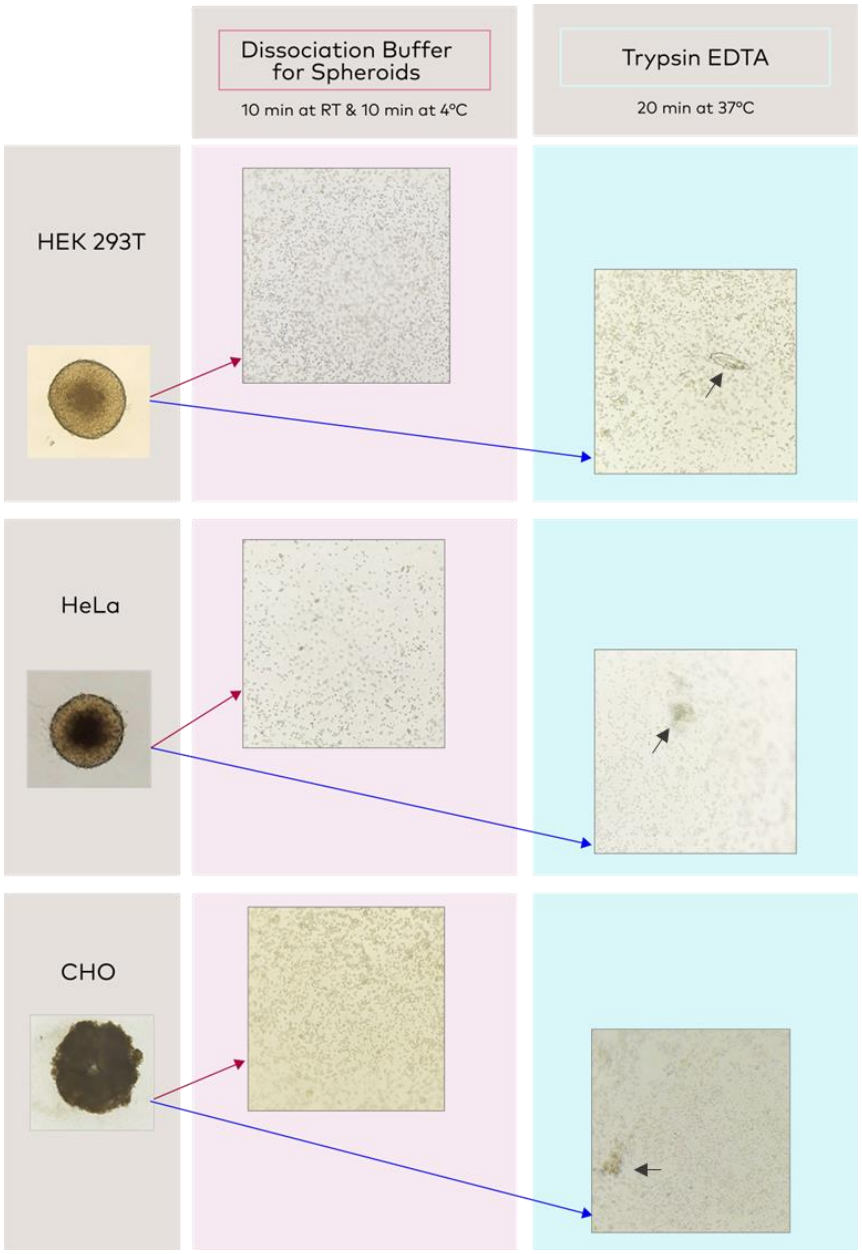

Figure 2: HEK 293T-, HeLa- and CHO-derived proliferative spheroids after dissociation using the Dissociation Buffer for Spheroids or the trypsin-based method.

HEK 293T, HeLa and CHO cells continue to proliferate when grown in 3D conformation. The resulting spheroids are therefore rather large (600-800µm) and non-compact after 4 days of culture. Our tests showed that both the Dissociation Buffer for Spheroids and the trypsin could both dissociate efficiently this type of spheroids (*Figure 2, Figure 4)*. However, with trypsin (*Figure 4*, black arrow), small cell aggregates were remaining after dissociation whereas the Dissociation Buffer for Spheroids achieved a perfectly complete and uniform dissociation of these spheroids.

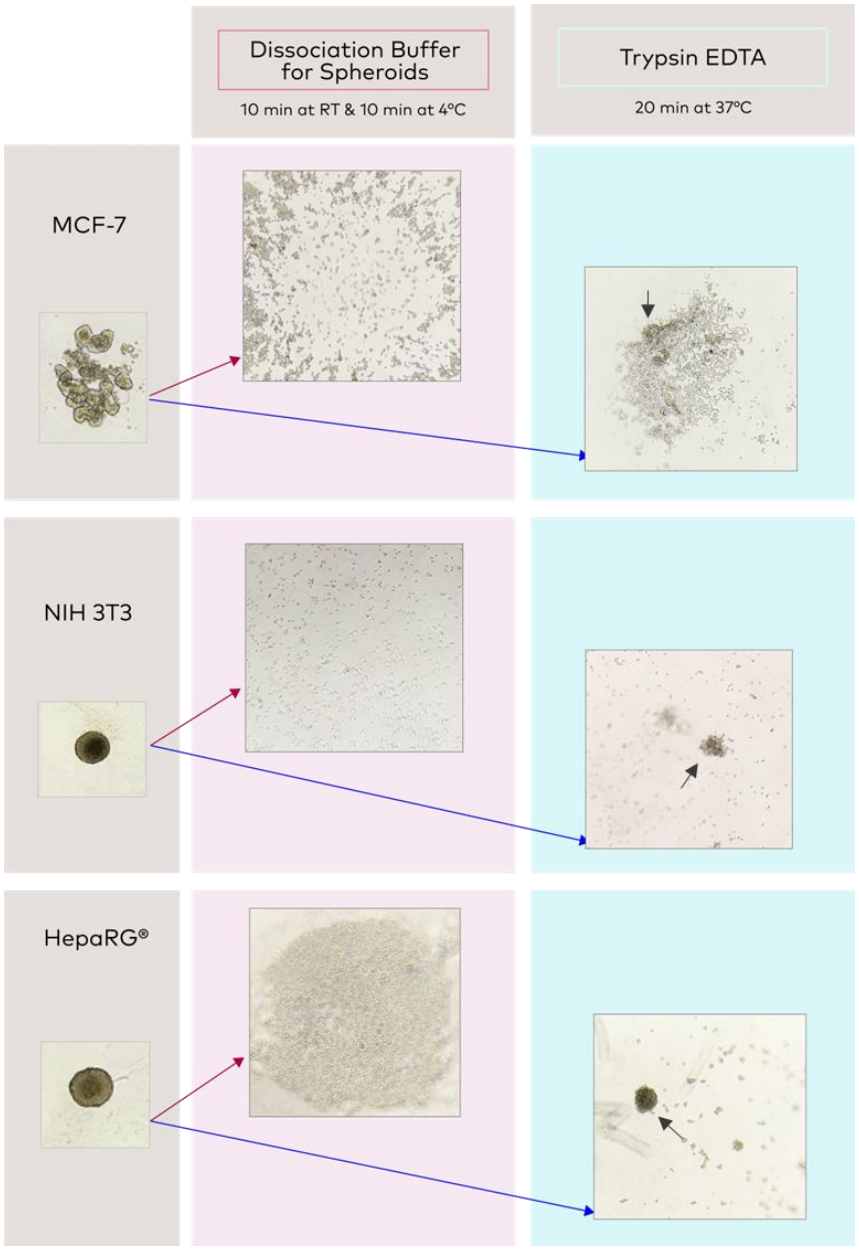

Figure 3: NIH 3T3-, MCF-7- and HepaRG™-derived non-proliferative spheroids after dissociation using the Dissociation Buffer for Spheroids or the trypsin-based method.

NIH 3T3, MCF-7 and HepaRG™ cells do not proliferate once they are in a spheroidal conformation. The resulting spheroids were therefore rather small (150-200µm) and compact. For those three cell lines, a clear difference of dissociation efficiency was observed between both conditions (*Figure 3, Figure 5)*. The Dissociation Buffer for Spheroids successfully achieved complete dissociation and provided a very homogenous single cell suspension for each cell types, whereas with the trypsin-based approach numerous cell clusters were remaining (*Figure 3*, black arrow).

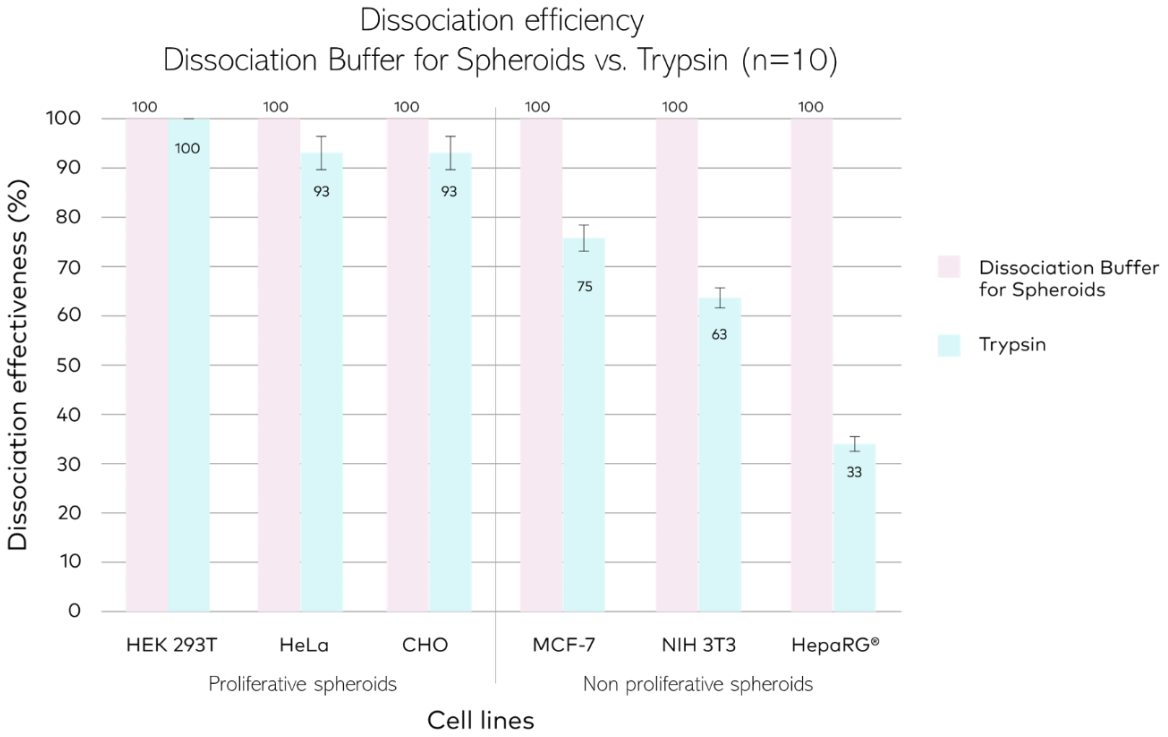

Figure 4: Average spheroids dissociation efficiency, Dissociation Buffer for Spheroids (pink), and trypsin (blue). Mean +/- SEM error bar, n=10 per condition.

These experiments demonstrate that the Dissociation Buffer for Spheroids outperformed the widely used Trypsin protocols by providing more complete spheroids dissociations with an obvious superiority for non-proliferative and compact spheroids.

Cell viability post dissociation

Cell viability was measured using AOPI staining after spheroid dissociation to evaluate the stress induced on cells by the different dissociation protocols.

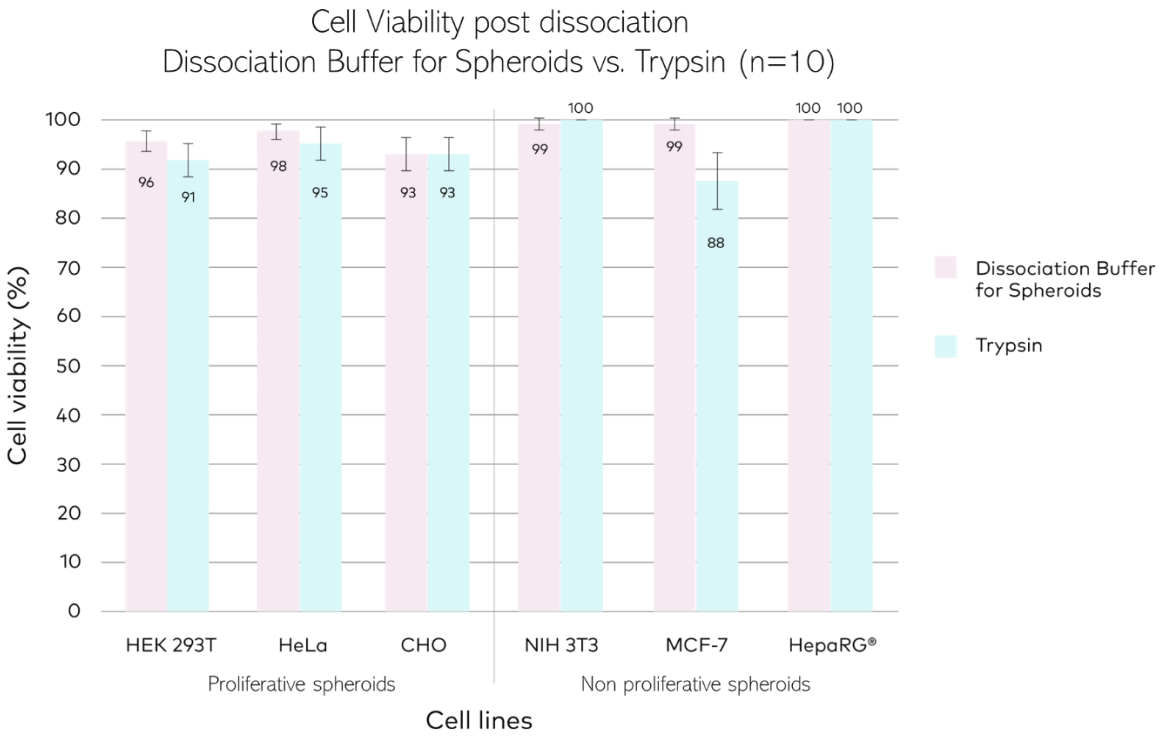

Figure 5: Average cell viability after spheroid dissociation, Dissociation Buffer for Spheroid (pink), and trypsin (blue). Mean +/- SEM error bar; n=10 per condition.

For proliferative cell lines which gave larger spheroids (270-300µm diameter), cell viability after dissociation was between 93% and 98% and was between 91% to 95% for the Dissociation Buffer for Spheroids and trypsin, respectively.
For non-proliferative cell lines which gave small spheroids (120-150µm diameter), cell viability after dissociation was between 99% and 100%, and between 88% and 100% for the Dissociation Buffer for Spheroid and trypsin, respectively.

Importantly, both the Dissociation Buffer for Spheroids and trypsin minimally affected cell viability post dissociation. However, the Dissociation Buffer for Spheroids demonstrated slightly better results for HEK and MCF-7 cell lines.

Conclusions

In this study, we have demonstrated, using a range of different spheroids, that the Dissociation Buffer for Spheroids outperformed the more traditional Trypsin-based method both in terms of efficiency of dissociation and cell viability post dissociation.

Importantly, it was shown that this new solution achieved complete spheroids dissociation to obtain uniform single cell suspensions, in a short time and at low temperature (10 minutes at RT plus 10 minutes at 4°C).

Taken together, these properties make this new buffer an ideal candidate for preparation of single cell suspensions from spheroids suitable for single cell Omics analysis.

Acknowledgement

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References

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