Monitoring a Successful Dissociation of Spheroids

Dissociation of mono- and co-culture spheroids into single cells for subsequent flow cytometric analysis

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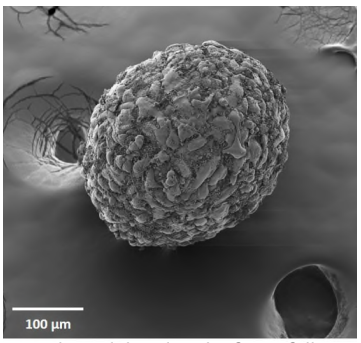
CASY was used in this study to monitor the success of spheroids dissociation quantitatively. CASY enabled the straightforward quantitation of cell aggregates as well as the mean diameter of single cells.

STUDY

Cultivating cells in 3D allows for a more physiological environment and enables intensive direct cell-cell contacts and contact to interstitial ECM (extracellular matrix). For rapid and easy analysis by e.g. flow cytometry, the dissociation of spheroids into single cells is requested. In this work, the authors report optimal dissociation conditions for spheroids.



SEM image of a spheroid consisting of 10,000 fibroblasts (NHDF), similar to the spheroids used in the original paper.

(Image courtesy of Dr. Wolfgang Metzger, Molekularbiologisches Forschungslabor der Abt. für Unfall-, Hand- und Wiederherstellungschirurgie Universitätsklinikum des Saarlandes, Homburg/Saar, Germany)

RESULTS

The authors evaluated several dissociation conditions of spheroids.

The success of dissociation was analyzed by CASY. An only slightly increased aggregation factor of approx. 1.2 was found for spheroids, compared to 2D cultures, also after prolonged incubation.

Regarding cell diameter, the authors observed slight effects from staining the spheroids. Remarkably, after only 1 day spheroid culture, single cell size was significantly reduced compared to 2D culture, resulting in an approx. 20% size decrease on day 6.

Spheroids can be successfully dissociated into single cells for subsequent flow cytometric analysis. CASY analysis was ideally suited to monitor the optimal conditions for dissociation, depending on age, size and cellular composition of the spheroids.