Automated neurosphere sorting and plating by the COPAS large particle sorter is a suitable method for high-throughput 3D in vitro applications

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Methods and Materials

Normal human neural progenitor cells used in this study were purchased from Lonza Verviers SPRL (Verviers, Belgium). Human neurospheres were cultured in proliferation medium (DMEM and Hams F12 (3:1) supplemented with B27 (Invitrogen GmBH, Karlsruhe, Germany), 20 ng/mL epidermal growth factor (EGF; Biosource, Karlsruhe, Germany), 100 U/mL penicillin, and 100 μg/mL streptomycin) in a humidified 92.5% air/7.5% CO2 incubator at 37 °C in suspension culture. Differentiation was initiated by growth factor withdrawal in differentiation medium (DMEM and Hams F12 (3:1) supplemented with N2 (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin) and plating onto poly-D-lysine (PDL)/laminin–coated chamber slides.

Flow Cytometry using COPAS PLUS HTS for analysis and dispensing of neurospheres

Neurospheres were sorted in two ways: 1) manually under a binocular and plated with a 100µl pipette or 2) using the COPAS PLUS HTS Flow Cytometer (Union Biometrica, Holliston, MA). The COPAS PLUS HTS with Advanced Acquisition Software and Profiler II was used for the acquisition, analysis and sorting of the samples. The COPAS instrument is equipped with a 1000 micron diameter flow cell. The ProfilerII option creates a digital profile for each object with the 488nm laser. It shows the location and intensity of the optical parameters and allows for extended sorting abilities with user definable sort criteria: peak heights, widths, locations and number for each optical parameter (size, density, 3 channels of fluorescence) in the profile.

Results

Assessment of sorting accuracy. Microscopic images of the human, neurosphere culture before sorting. Scale bar=500 μm (A). 40-50 neurospheres were sorted in a 96-well plate by the COPAS instrument (standard settings) and manually under a microscope . The diameter of the neurospheres was analyzed with the metamorph program (Molecular Devices Corporation). One representative experiment is shown (B).

Shown are the means ± SEM of 4 independent experiments (20 spheres per condition and experiment) in % DCB positive cells (H). Representative histograms of the DCF-fluorescence within the NPCs after the different treatments (I).

Conclusions

We showed that the COPAS large particle sorter instrument is suitable for the fast sorting and dispensing of neurospheres in 96-well plates. None of the parameters tested in COPAS vs. manually sorted and plated neurospheres was affected. The COPAS sorted same sized spheres as the lab worker picked by hand. The COPAS sorted spheres were as viable as the manually selected ones and none of the functional endpoints – NPC proliferation, migration and differentiation - which build 'The neurosphere assay' was affected. We found no additional ROS generation by COPAS high-throughput sorting. Therefore, our work revealed that the COPAS large particle sorter is, amongst others, a suitable method for high-throughput 3D in vitro applications, which contain automated neurosphere sorting and plating.