AUTOMATED ANALYSIS AND SORTING OF HUMAN IPS CELL CLUSTERS BY LARGE PARTICLE FLOW CYTOMETRY

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Introduction

The generation of induced pluripotent stem cells (iPS cells) from somatic cells is one of the most dynamic fields in biomedicine. Frequently, iPS cell clones are identified by microscopy and iPS cell quality is judged by visual inspection by an experienced operator. Individual iPS cell clones are then isolated by conventional picking. This represents a major bottle neck when it comes to isolation of hundreds or even thousands of iPS cell clones on an industrial scale.

The StemCellFactory project (www.stemcellfactory.de) aims to fully automate by robotics (i) the generation of human iPS cells and (ii) their differentiation into cardiomyocytes and neuronal cells. Here we evaluated large particle flow cytometry technology (BioSorter®, Union Biometrica) for iPS cell isolation, including multiparameter quality assessment of isolated cells.

Methods and Materials

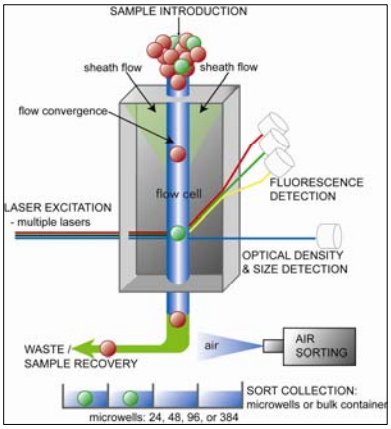


Figure 1: BioSorter analyzes and sorts cell clusters and large cells (1-1500 µm diameter) in a continuous flow stream at high rate (up to 100 ev/sec). The device measures object size (TOF), optical density (EXT) and multiple fluorescent markers.

Sorting is by a pneumatic device located below the flow cell. Fluid pressure (up to 6 psi) is significantly lower than in conventional flow cytometers, thus providing gentle sorting conditions.

The BioSorter has interchangeable fluidics and optics core assemblies (FOCAs), allowing the instrument to analyze and dispense objects across a large size range.

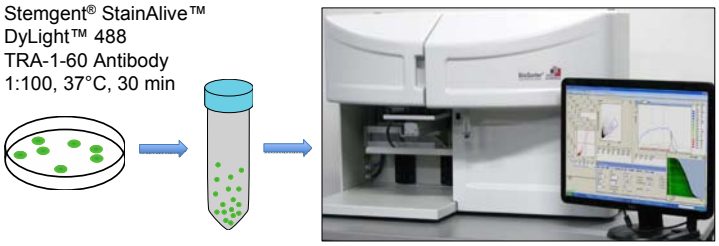


Figure 2: Experimental scheme of the sorting process and the subsequent evalutation of the hiPS cell clones.

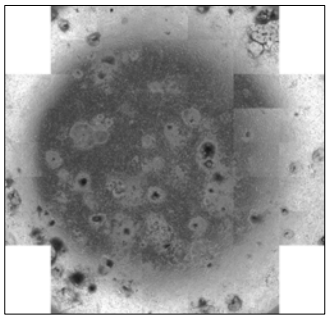


Figure 3: Human iPS cell colonies (21 days post infection) used for sorting.

Human iPS cells were established from fibroblasts with OKSM reprogramming factors and stained with TRA-1-60. iPS cells were collagenase treated and cell clusters subjected to sorting with the BioSorter instrument, by simultaneously assessing size, TRA-1-60 expression and further parameters (optical density etc.). Individual cell clusters were deposited in 96-well format.

Results

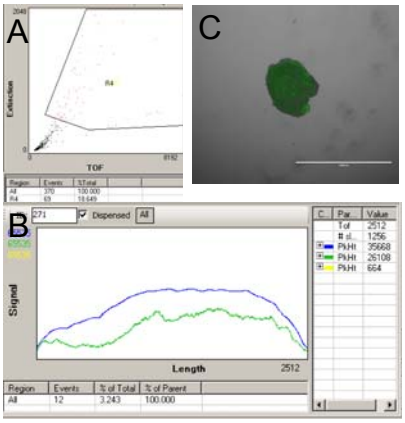


Figure 4: Dot plot analysis of the human iPS cell clusters on BioSorter (A). Profiler graph shows the distribution of TRA-1-60 expression (green) and optical density (blue) along the axis of the iPS cell cluster (B) and the respective iPS cell cluster after sorting in 96-well format (C).

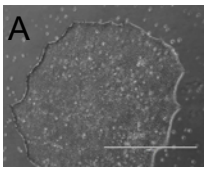
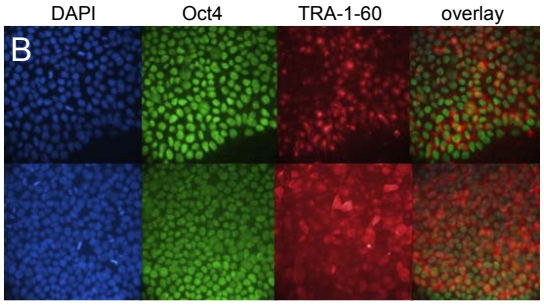


Figure 5: Phase contrast image of a human iPS cell colony (passage 10) after automated sorting with the BioSorter instrument in mTeSR1/Matrigel (A). Expression of pluripotency-associated markers TRA-1-60 and SSEA4 (red) and Oct4 (green) of sorted iPS cell colonies (B).



Conclusions

Large particle flow cytometer BioSorter® represents a powerful tool for measuring, selection and collection of human iPS cell clones in a highly standardized manner and with high throughput.

Automated analysis and sorting is gentle and does not compromise iPS cell viability, morphology and pluripotency. The Profiler II software tool measures fluorescence distribution within cell clusters, which provides valuable information on clonal heterogeneity. Partially reprogrammed iPS cell colonies are readily detected and removed during the sorting process.

This instrument brings the advantages of flow cytometry – multi-parameter and statistical analysis of a large number of events, fast sorting and high throughput – to iPS cell analysis and isolation.

This work was co-funded by the German federal state North Rhine Westphalia (NRW) and the European Union (European Regional Development Fund: Investing In Your Future).

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