

Automated selection and harvesting of pluripotent stem cell colonies using the CellCelector™

Recent new technologies enabling the genetic modification of human embryonic stem cells (hESC) and the derivation of patient-specific induced Pluripotent Stem cells (iPS cells) provide attractive opportunities for the development of new cell-based bioassays. Both technologies critically depend on the selection of pluripotent cell colonies. AVISO's CellCelector™ enables automated colony detection and harvesting, thereby bypassing tedious manual selection procedures.

The ability of hESC to differentiate into specialised cells of all three germ layers (pluripotency), their capability for unlimited cell division (self-renewal) and their amenability to genetic modifications provide fascinating prospects for the generation of genetically modified human cell lines for biomedical and pharmaceutical research.

Recently, induced pluripotent stem (iPS) cells have emerged as an additional source of pluripotent cells, which can be derived from adult somatic tissues. Shinya Yamanaka and co-workers have shown that viral transduction of defined transcription factors (Oct4, Klf4, Sox2, c-myc) suffices to reprogram adult fibroblasts and other somatic cell types into pluripotent stem cells with ESC properties^{1,2}.

Both, the selection of successfully engineered hESC and the derivation of iPS cells depend on the harvesting of individual stem cell colonies, which are subsequently further expanded to obtain homogenous cell lines.

The Technology

The patented CellCelector™ is a multifunctional automated robotic system combining sophisticated imaging software with a high-precision but gentle harvesting process of single cells and cell colonies (Fig.1). The harvesting process is supported by an inverted microscope that offers phase contrast and bright field observation as well as fluorescence illumination. The CellCelector's working process can be divided in three independent steps: imaging, harvesting and documentation.

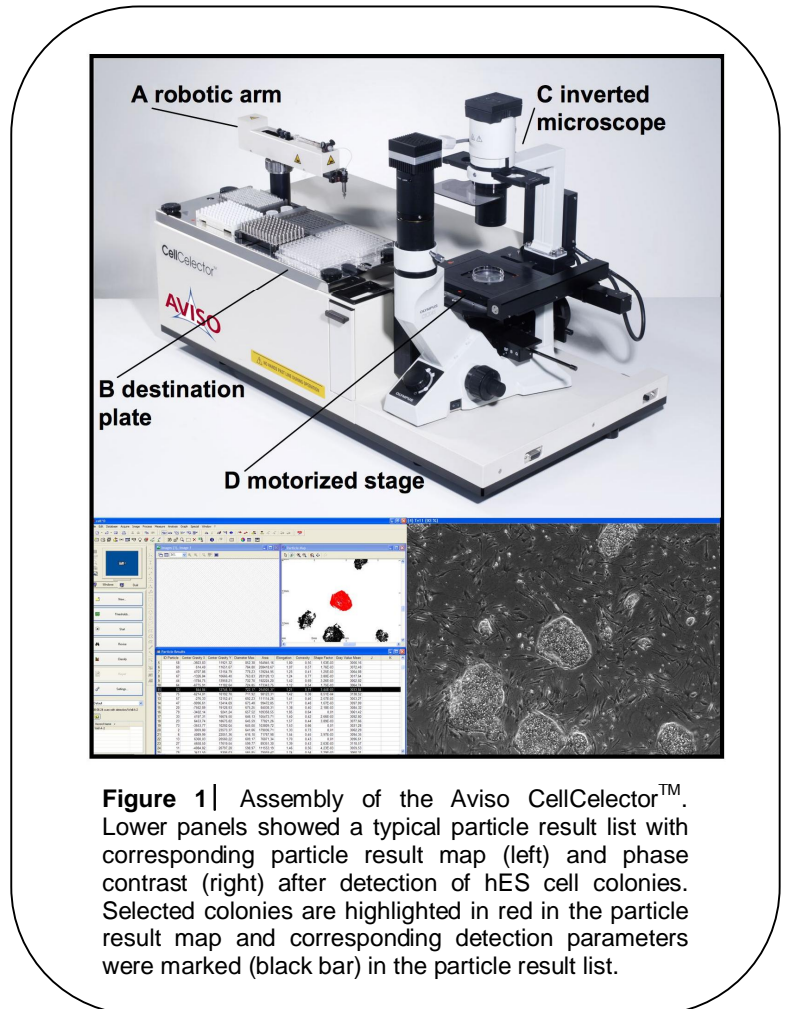


Figure 1 | Assembly of the Aviso CellCelector™. Lower panels showed a typical particle result list with corresponding particle result map (left) and phase contrast (right) after detection of hES cell colonies. Selected colonies are highlighted in red in the particle result map and corresponding detection parameters were marked (black bar) in the particle result list.

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Each scanning process runs according to predefined parameters such as cell size, morphology, spectral features, intensities or shape to achieve an optimized detection of the cells of interest. This setting dialogue can be easily customized for the individual application. The CellCelector™ provides a quality check of the selected cells anytime via live imaging that is supported by a special motorized stage (manufactured from AVISO). The harvesting process itself is based on a direct, non-invasive mechanical aspiration process without any needs of pre-treatment of cells or colonies,

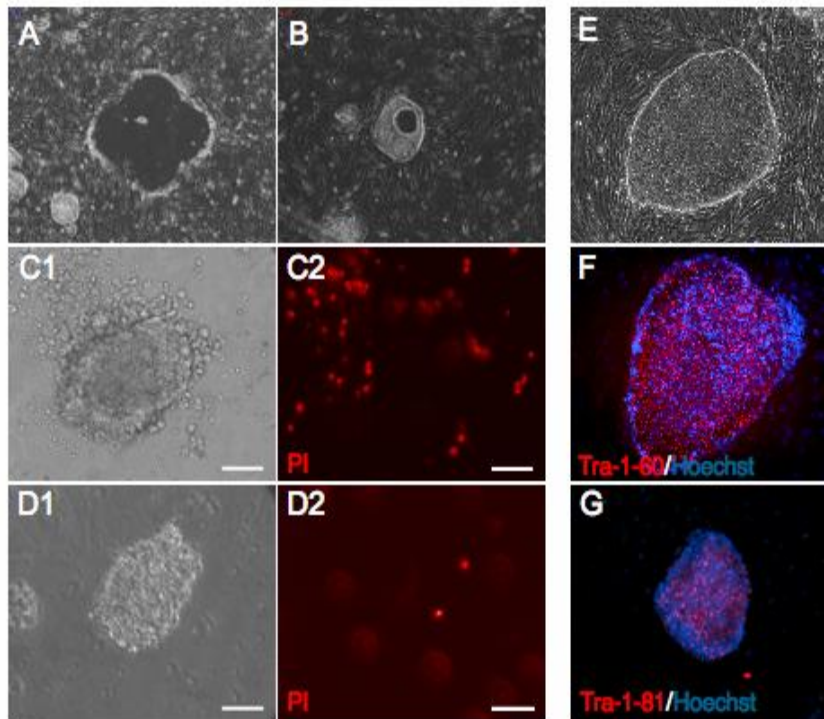


Figure 2 | H9.2 human embryonic stem cells (hESC) were cultivated on a layer of feeder cells and automatically harvested by the CellCelector. **(A-B)** hES cell cultures after picking the selected colony with the scrape module **(A)** or with a 220 µm glass capillary **(B)**. **(C1-D2)** Clusters of hES cells directly after manual picking with a pipette tip **(C1)** or the CellCelector **(D1)** with corresponding propidium iodide (PI) stainings to visualize dead cells **(C2, D2)**. **(E)** Phase contrast micrograph of a replated hES cell colony 5 days after picking with the CellCelector. **(F-G)** Immunocytochemical stainings for the pluripotency-associated surface markers Tra-1-60 **(F)** and Tra-1-81 **(G)** 5 days after automated selection and recultivation, visualized with Alexa-555-coupled secondary antibodies. Cell nuclei were counterstained with DAPI. (Scale bar 100 µm)

even without any proteolytic digestion. The CellCelector™ employs two different kinds of application-modules for harvesting: The scrape-module utilizes special manufactured autoclavable stainless capillaries (available diameter from 300 µm to 1000 µm) and the single cell-module utilizes borosilicate glass capillaries (available diameter from 50 µm to 220 µm). The entire process from imaging to harvesting is documented via real-time images and a corresponding particle result list, which contains all predefined settings and filing positions. The CellCelector™ software translates result lists into user-defined graphics and allows exporting numeric data in standard analysis packages.

Automated harvesting of hES cell colonies using the CellCelector™

Previous studies had shown that mouse embryonic ES cell colonies cultivated on feeder cells could be successfully harvested and transferred with the Aviso CellCelector™³. Here we studied the applicability of the CellCelector™ for selection and harvesting of human ES cell colonies. Two different modules can be applied to pick hES cells, the scrape module utilizing autoclavable metal capillaries and a module working with glass capillaries. For the picking process a 220 µm glass capillary was utilized, which enables isolation of small colony fragments and thus a highly selective harvesting process (**Fig.2 A-B**). Transfer rates ($81\% \pm 6\%$) and replating efficiencies ($58\% \pm 6\%$) of the automatically picked hES cells were comparable to those of the manual process. Propidium iodide (PI) staining performed directly after manual (**Fig.2 C2**) and automated colony picking was used to assess potential detrimental effects of the procedure on cell viability. The lower extend of PI incorporation after automated transfer clarified maintenance of the typical hES cell morphology (**Fig.2 G**), continued proliferation and expression of the pluripotency-

associated surface markers Tra-1-60 and Tra-1-81 (**Fig.2 F-G**) showed that the cells tolerate the automated transfer at a level comparable to manually picked hES cells.

Conclusion

Our data demonstrate that the CellCelector™ from Aviso enables the automated selection and harvesting of human pluripotent stem cell colonies. The technology represents a powerful tool for the isolation of hES cell colonies in a highly selective and standardized manner. By utilizing a 220 µm glass capillary isolation of specific areas within individual colonies is feasible. No differences to manually picked colonies with respect to vitality, proliferation and pluripotency were observed. As a major advantage the technology provides standardization that can be combined with image recognition and documentation for identification and filing of the selected cells. Furthermore the CellCelector™ provides different modules operating at the phase contrast, bright field or immunofluorescent level to identify target cell populations.

This technology should also facilitate the isolation of genetically modified pluripotent stem cell clones as well as the harvesting of newly generated iPS cell colonies for biomedical applications.

Additional information is available online at the company website (<http://www.aviso-gmbh.de>)

References

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