AVISO life&brain

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

Recent new technologies enabling the genetic modification of human embryonic stem cells and the derivation of patient-specific induced pluripotent stem 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 collection, thereby bypassing tedious manual selection procedures.

The technology

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

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 dialog can be easily customized for individual applications. The CellCelector provides a quality check of the selected cells at any time via live imaging that is supported by a special motorized stage (manufactured by AVISO). The collection process itself is based on a direct, noninvasive mechanical aspiration process without any need for proteolytic digestion or other pretreatment of cells or colonies.

The CellCelector uses two different kinds of application modules for collection: the scrape module uses specially manufactured autoclavable stainless steel capillaries (available diameters, 300–1,000 µm), and the single-cell module uses borosilicate glass capillaries (available diameters, 50–220 µm).

The entire process from imaging to collection is documented via real-time images and via 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 export of numerical data to standard analysis packages.

Automated collection of hESC colonies

The ability of human embryonic stem cells (hESCs) to differentiate into specialized cells of all three germ layers (pluripotency), their capability for unlimited cell division (self-renewal) and their amenability to genetic modification provide fascinating prospects for the

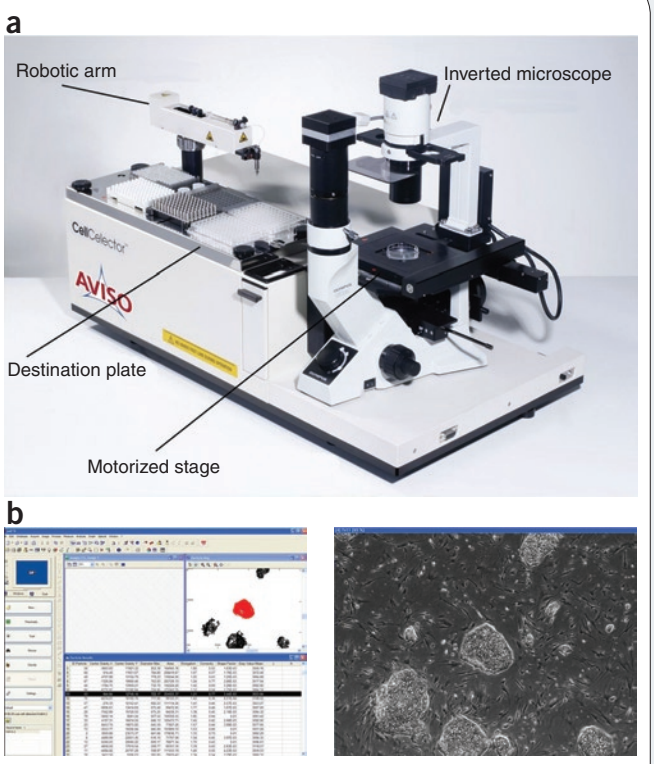

Figure 1 | CellCelector and software screenshots. (**a**) Assembly of the AVISO CellCelector. (**b**) A screen shot of a typical particle result list with corresponding particle result map (left) and phase-contrast image (right) after detection of hESC colonies. Selected colonies are highlighted in red in the particle result map and corresponding detection parameters are marked (black bar) in the particle result list.

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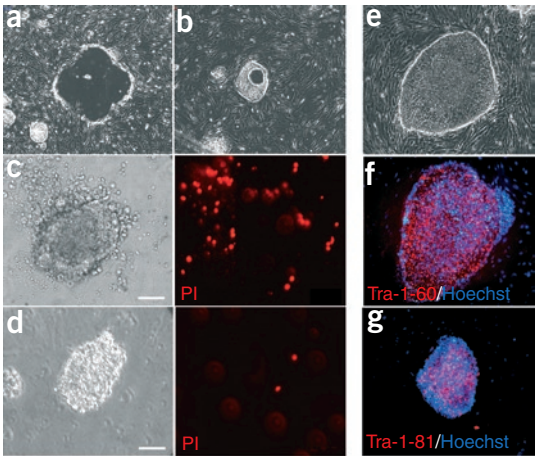

Figure 2 | H9.2 hESCs cultivated on a layer of feeder cells and automatically collected by the CellCelector. (**a**,**b**) hESC cultures after picking the selected colony with the scrape module (**a**) or with a 220-µm glass capillary (**b**). (**c**,**d**) Clusters of hESCs directly after manual picking with a pipette tip (**c**, left) or the CellCelector (**d**, left) with corresponding propidium iodide (PI) staining to visualize dead cells (**c**,**d**, right). (**e**) Phase-contrast micrograph of a replated hESC 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 Hoechst. Scale bars, 50 µm.

generation of genetically modified human cell lines for biomedical and pharmaceutical research.

Recently, induced pluripotent stem cells (iPSCs) have emerged as an additional source of pluripotent cells, which can be derived from adult somatic tissues. Researchers have shown that viral transduction of defined transcription factors (Oct4, Klf4, Sox2 and c-Myc) suffices to reprogram adult fibroblasts and other somatic cell types into pluripotent stem cells with embryonic stem cell properties[1,2].

Both the selection of engineered hESCs and the derivation of iPSCs depend on the collection of individual stem cell colonies, which are then expanded to obtain homogenous cell lines.

Previous studies had shown that mouse embryonic cell colonies grown on feeder cells can be collected and transferred with the AVISO CellCelector[3]. Here we describe the applicability of the CellCelector for automated selection and collection of hESC colonies. Two different modules can be applied to pick hESCs: the scrape module, using autoclavable metal capillaries, and a module working with glass capillaries. For the picking process shown here, we used a 220-µm glass capillary, which enables isolation of small colony fragments and thus a highly selective collection process (**Fig. 2a**,**b**). Transfer rates (81% ± 6%) and replating efficiencies (58% ± 6%) of the automatically picked hESCs were comparable to those of a manual process. To assess potential detrimental effects of the procedure on cell viability, we used propidium iodide staining performed directly after manual and automated colony picking (**Fig. 2c**,**d**). The low extent of propidium iodide incorporation, maintenance of the typical hESC colony morphology, continued proliferation and expression of the pluripotency-associated surface markers Tra-1-60 and Tra-1-81 (**Fig. 2e**–**g**) showed that the cells tolerated the automated transfer comparably to manual picking.

Conclusion

Our data demonstrate that the CellCelector from AVISO enables the automated selection and collection of human pluripotent stem cell colonies. The technology is a powerful tool for the isolation of hESC colonies in a highly selective and standardized manner. Using a 220-µm glass capillary, isolation of specific areas within individual colonies was feasible. We observed no differences from manually picked colonies with respect to vitality, proliferation and pluripotency. 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 for imaging in phase-contrast, brightfield or immunofluorescence modes to identify target cell populations.

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

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

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2. Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872 (2007).
3. Schneider, A. *et al.* "The good into the pot, the bad into the crop!"—a new technology to free stem cells from feeder cells. *PLoS ONE* **3**, e3788 (2008).