Automated isolation of semi-adherent macrophage-like cells from a fibroblast-contaminated culture using the cell separation robot CellCelector™

Macrophages are basic effector cells of the immune system and involved in pathogenesis of autoimmune diseases. Homogeneity in cell type is desired for almost every application in research or diagnostics. Contaminants can diminish the reliability, reproducibility or validity of obtained results. Since fibroblasts are common contaminants of primary macrophage cell cultures a method for separation of the cells of interest is required. Using the CellCelector™ to automatically detect and harvest semi-adherent cells provides a simple alternative for enrichment of macrophages from a fibroblast-contaminated culture without the use of additional substances such as antibodies or proteolytic enzymes.

Technology

The CellCelector™ (fig. 1a) is a robot for automated cell harvest. The patented system consists of an inverted microscope (1) equipped with a motorized stage (2) and a CCD camera (3), an exchangeable robotic arm (4) as main functional tool and a deck tray for disposable tips (5), capillaries and destination plates (6). The imaging software (fig. 2) enables detection of cells by predefined spectral and morphological parameters. After the culture vessel is scanned, harvest and documentation can be done automatically, based on the generated particle list. The harvest tools provide the collection of adherent or suspension cells as well as colonies in semi-solid media via mechanically detachment and aspiration. The single cell tool (fig. 1b) works with a glass capillary attached to a pipetting system filled with mineral oil. The glass capillary is available in different diameters up to 220 µm. The tool enables the aspiration of small volumes in microliter range. Parameters for the cell harvest can be fine-tuned for the users special application.

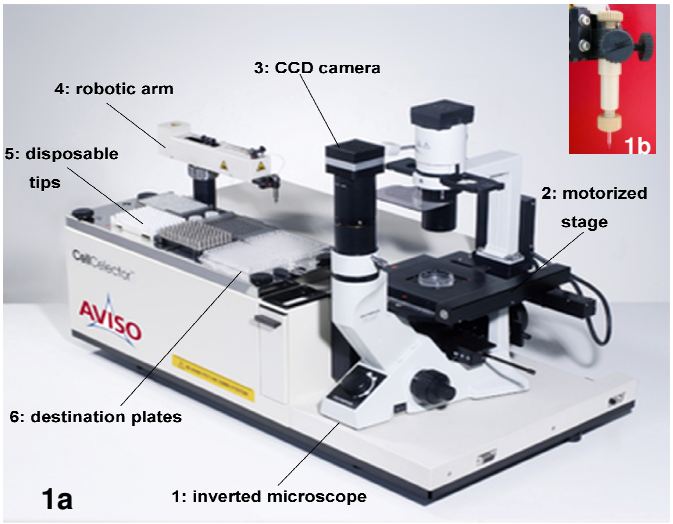

Figure 1: a: Assembly of AVISO's CellCelector™, robot for automated cell harvest **b:** Single cell tool, harvest tool for semi-adherent or suspended single cells.

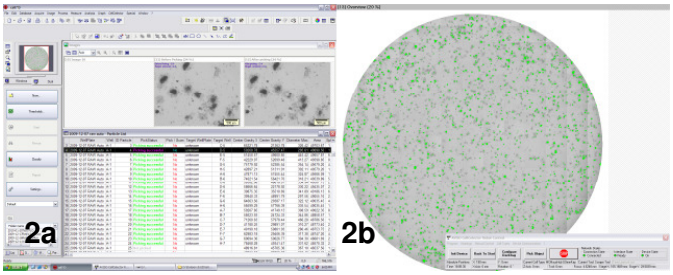

Figure 2: Screenshot of the CellCelector™'s control and imaging software, **a:** Picking list and automatically documented snapshots before and after picking the cells, **b:** Overview image of all detected macrophage colonies in the culture vessel.

Automated detection and harvest of macrophages from a fibroblast-contaminated culture

Macrophages are effector cells of the immune system and contribute to pathogenesis of autoimmune diseases, e.g. rheumatoid arthritis (RA), a chronic inflammatory joint disease. Bone and cartilage destruction are triggered by interactions of synovial fibroblasts and macrophages [1,2,3].

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These cell types are objectives of various studies. Cells isolated from primary tissue material are often contaminated with undesired cell types. The study of specific interactions of the cells or analysis with molecular biological or biochemical methods necessitates the separation to get a cell population consisting of a single cell type. Especially purification of macrophages can be a complicated procedure. Well-established separation methods, such as magnetic or fluorescence-activated cell sorting (MACS™, FACS™) depend on antibody binding to ligands on the cell surface. This could involve a biochemical influence onto the cell type of interest, which can adversely affect further analysis. Using the CellCelector™ macrophages can be collected via mechanical detachment and aspiration. Macrophage-like RAW264.7 cells were isolated from a co-culture with human synovial RA fibroblasts. RAW264.7 cells were seeded in low density and incubated 3 days to obtain small colonies. After adding the fibroblasts the culture was incubated for additional 4 days. Then, the Petri dish was transferred to the CellCelector™. RAW264.7 cells had formed colonies up to 500 µm in diameter, showed signs of activation, and tended to a semi-adherent growth behavior. The transparent appearance of the fibroblasts in bright-field was advantageous for the selective detection of the RAW264.7 cells (fig. 3). Similar results could be obtained with confluent cultures. Applying an 80 µm glass capillary aspiration of RAW264.7 cells was possible without influencing the stronger adherent fibroblasts (fig. 4). Aspirated cells were transferred into a 96-well-destination plate were they were pooled or distributed to separate wells. Picking and documentation were proceeded automatically. A transfer of the cells was assured with a probability of about 95 %. After Isolation the RAW264.7 cells showed high vitality (fig. 5). Visual controls could not detect any fibroblast contamination of the separated cells after 24 h incubation of the destination plates.

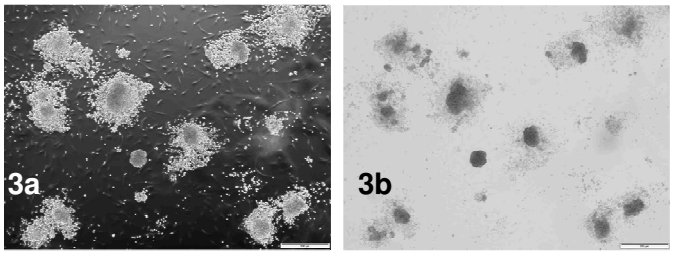

Figure 3: Fibroblast-contaminated RAW264.7 culture **a** under phase contrast and **b** under bright-field microscopic observation. In bright-field microscopy, fibroblasts appear transparent. Macrophage colonies could be detected selectively. Scale bars = 500 µm

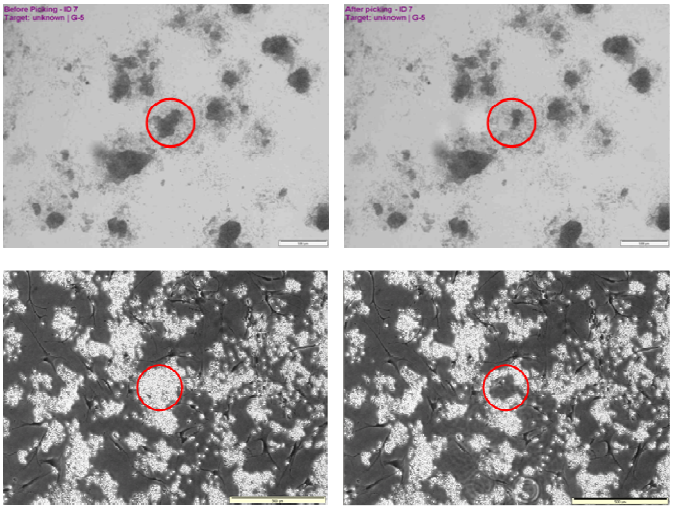

Figure 4: Automatically documented images during the harvest process. The upper bright-field images show the isolation of a colony of RAW264.7 cells. Below the RAW264.7 cells are randomly distributed in the culture. Observation of picking process in phase contrast shows that only the macrophages are aspirated. Scale bars = 500 µm

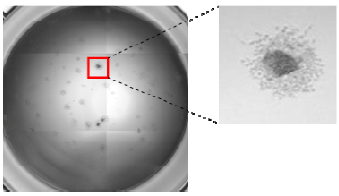

Figure 5: Isolated RAW264.7 cells in a well of a 96-well-destination plate after 5 days of incubation. Cells are vital and free of contaminants.

Conclusion

The application of the CellCelector™ for automated detection and harvest of macrophages from a fibroblast-contaminated culture is a simple and reliable alternative to well-established separation procedures and provides several advantages:

✓Cells are harvested under culture conditions (37 °C, 5 % CO_2).

✓Sterility during separation process is maintained.

✓Harvest and documentation can be done automatically.

✓Cells are mechanically separated. Additional substances (enzymes, antibodies, chemicals) are not required and may thus not interfere with functional integrity of harvested macrophages.

References

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