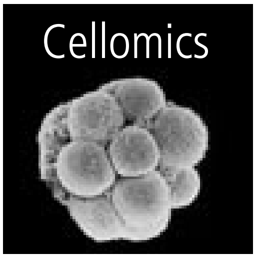

Automation of Adherent Cell Culture Maintenance

The demand for mammalian cells in pharmaceutical screening and cell biology research is constantly increasing. The evaluation of drug candidates is based on their biological effect on cell cultures in high-throughput screening (HTS) while metabolic behaviour and toxicity of new compounds are tested in cell-based ADME-T assays. Basic research relies on numerous cell lines and primary cells, which constitute a tool for the elucidation of basic mechanisms of cell proliferation, differentiation and function. In this domain, embryonic stem cells are gaining importance as a model system. In addition to their enormous differentiation potential, these cells possess the capacity for unlimited self-renewal, which facilitates their efficient genetic modification. Limitations of the currently used cell culture procedures include the lack of standardization associated with poor reproducibility and insufficient throughput. Thus, the availability of cells can become a bottleneck in the logistic chain of experiments. HAMILTON and Life & Brain have joined their expertise in the development of a system for the automated culture of primary cells, cell lines and embryonic stem cells which provides high-quality cells in large numbers.

Figure 1: *Cell^host system. The core of the system is a MICROLAB® STAR pipetting robot. Cell cultures and nutrient solutions as well as growth factors are kept in Kendro incubators at different temperatures (37°C incubator for cell cultures; 4°C incubator for media). The equipment is contained in a sterile housing with laminar air flow.*

Equipment and Materials

Hardware

The core of the Cell^host system is the MICROLAB® STAR pipetting workstation equipped with monitored air displacement pipetting technology. This eliminates use of tubing, pumps or system liquids, thus significantly reducing the risk of contamination by bacterial growth. An internal robotic hand – the iSWAP – handles SBS standard cell culture plates on the deck. Two Kendro incubators are fully integrated into the system (Figure 1). Typical manual processes like plate agitation are perfectly mimicked by analogous robotic movements. The system is controlled by a standard PC and HAMILTON's open and flexible MICROLAB Vector Software for process control and 3rd party component integration.

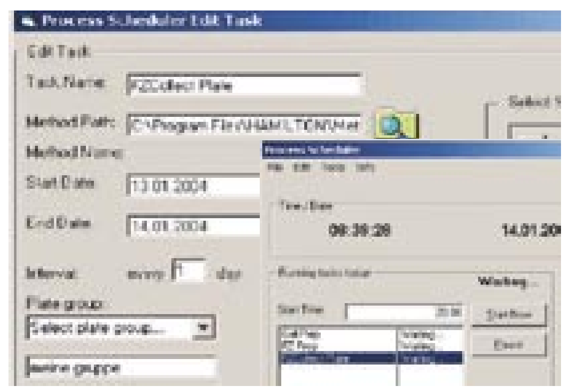

Figure 2: *HAMILTON Cell^task software enables repetitive task management, e.g. changes of media every n days.*

Software

Any automated system devised for handling large numbers of plates, with each plate undergoing complex operations, requires sophisticated operating and data tracking capability. The HAMILTON Cell^task software allows easy scheduling of repetitive processes, enabling customer-friendly cell culture automation (Figure 2).

The Cell^track software monitors and tracks every operation on every single plate and enables data documentation in conformity with CFR 21 Part 11 requirements.

Results

The Cell^host system was validated using embryonic stem cells, one of the most sensitive cell culture paradigms.

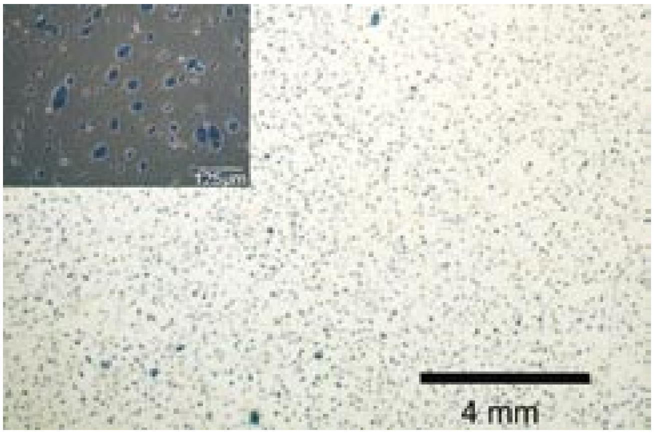

Figure 3: *Microscopic views of murine embryonic stem cells plated and incubated in the cell host system. The inset shows a detail enlargement. The cells were stained for alkaline phosphatase activity, a marker of pluripotency.*

Plating

Plating with Cell^host leads to homogenous distribution of murine embryonic stem cells on a feeder cell layer (Figure 3). An even distribution of cells is particularly important in the case of embryonic stem cells, since formation of cell clumps leads to uncontrolled differentiation. To achieve this result, the typical manual movements for distributing the cells across the plate are simulated by the robotic hand.

Media Change

One of the most frequently occurring processes in cell culture is media change. In order to minimize the residual volume, cell culture plates are raised at one side (Figure 4), and the medium is carefully aspirated at the lowest point of the well. The new medium is slowly dispensed at the edge of the well, thus avoiding disturbance of the cell layer. Microscopic investigation shows the integrity of the cell layer (Figure 5a) - in marked contrast to a less cautious manual experiment (Figure 5b).

Figure 4: *HAMILTON plate lifter mimics human manipulation of cell culture plates thus facilitating the complete removal of old medium.*

Cell Growth

Embryonic stem cells propagated with cell host retain their typical morphology after plating and media change (Figure 3, inset). The degree of spontaneous differentiation does not exceed the extent observed in manually treated cultures. Maintenance of the pluripotent status is verified by histochemical detection of alkaline phosphatase activity. Cell viability after a culturing time of 48 hours is comparable to that determined in the manual control experiment. No contamination is detectable.

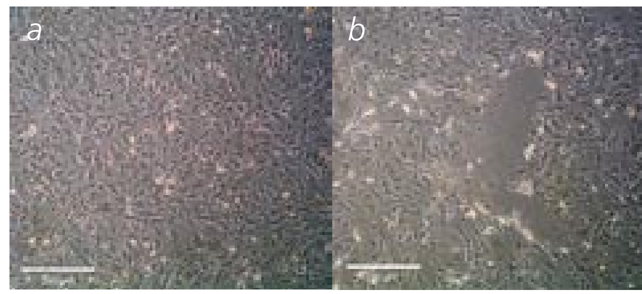

Figure 5: *Microscopic picture of a fibroblast layer immediately after a media change with the MICROLAB® STAR in the Cell^host system (a) and after a less careful manual media change (b). The robotic media change leaves the cell layer undamaged.*

Discussion

The results of this study show the feasibility of automating embryonic stem cell culture. The system used here has the advantage of being based on manual methods and on being free from liquid-filled components. Cells easily survive various pipetting procedures such as mechanical plating and repeated changes of media. Microscopic observation shows that the typical morphology of embryonic stem cells is retained. Furthermore, no increase in spontaneous differentiation compared to the manual control can be observed – a definite indication for the gentle handling of the cells by cell host. The system operates in a contamination-free way. It is to be expected that the processes developed for the delicate culture of embryonic stem cells can be translated to less sensible cell types commonly used in toxicological tests and pharmaceutical screening. The way is now open to further automation of cell lines like CaCo2 and downstream applications such as reporter gene assays.

With the automation of cell cultures, a significant reduction of manual workload for pharmaceutical and biotech companies comes into reach.

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