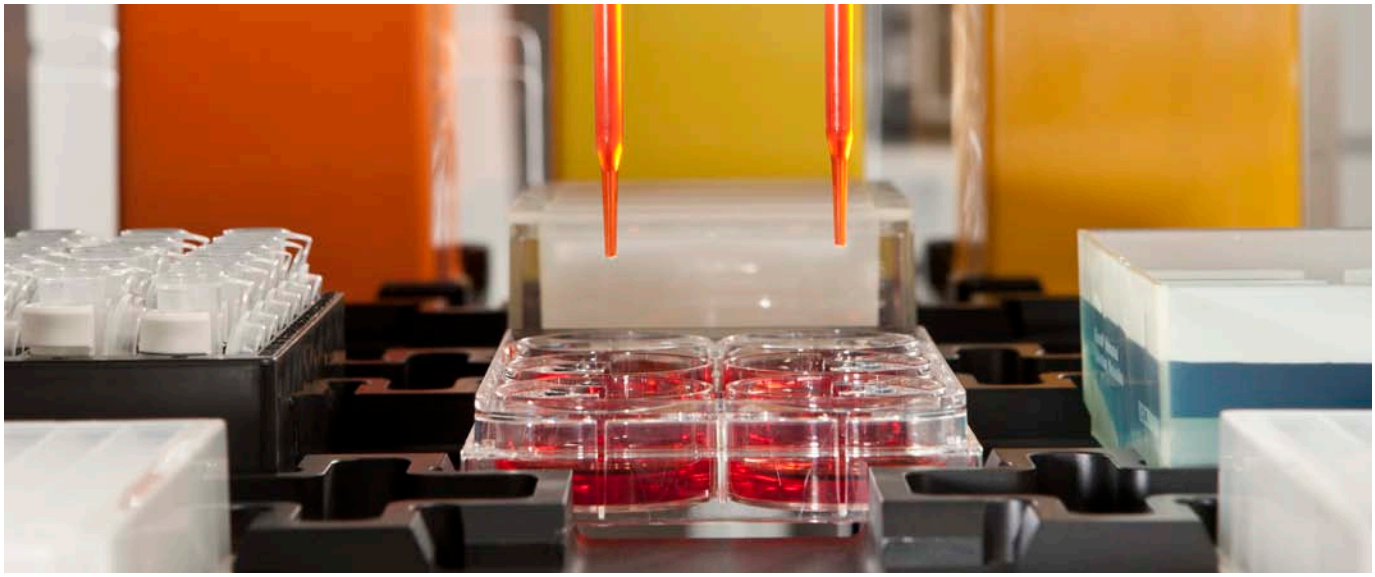
Automated Cell Culture

Improving the consistency of cell maintenance



Abstract

Cell culture has long been an essential aspect of both basic biological research and drug discovery. As the complexity and throughput of both of these tracks have increased, the need for automated solutions has increased correspondingly. In this application note, we illustrate the capabilities for automating a standard workflow for cell line maintenance and demonstrate this process using mouse embryonic stem cells. By automating the feeding and passaging of cells, the active time required for these daily tasks can be significantly reduced and variations in how different users handle cells is eliminated.

Introduction

Cultured cells have played a significant role in numerous scientific discoveries, and their use in drug discovery continues to increase as the cost for cellular assays is much lower than animal testing. The unquestioned value of this technique has justified the significant manual labor that is required to maintain cells for cellular assays. Whether short- or long-term assays, the maintenance of cells is frequently a daily occurrence and the workload increases as the number of cell lines being used by a laboratory increases. A majority of cell culture efforts involves the sterile liquid handling aspects of media exchange, trypsinization of adherent cells, and passaging these cells into a fresh vessel. In addition, cell viability and concentration must be monitored regularly and at the time of passage to ensure the culture is healthy and the desired number is taken forward and/or plated for use. The standard steps involved in cell line maintenance—seeding, feeding, passaging, and expansion of cells—must be optimized before cells can be used for genetic manipulation or endpoint assays.

To follow, we describe ways in which cell maintenance can be automated, thereby saving technician time at the hood. However, these potential time savings have no value if automation cannot meet or surpass the quality of manual cell maintenance. This quality can be achieved through fine control of the liquid handling steps while also improving consistency of cell maintenance and cellular assays by removing user-to-user variability. The feasibility of automating cell maintenance was demonstrated by culturing mouse embryonic stem cells (mESCs) as a proof of principle. The stem cell cultures remained sterile in the absence of antibiotics and cell viability and pluripotency of >90% was maintained for 10 consecutive passages.[1]

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Automation Solutions

The liquid handling steps for cell maintenance can all be automated through the use of Biomek Workstations, and the entire workflow can be performed through the use of integrated instrumentation. Beckman Coulter offers a number of liquid handlers that can automate the steps of cell culture. The Biomek 4000 Workstation can fit in a standard laminar flow hood, providing an ideal solution for stand-alone cellular solutions. The Biomek NX^{P} and FX^{P} can be used with HEPA-filtered enclosures to ensure a sterile work surface for cell manipulation. Culture sterility is also maintained through the use of sterile or sterile barrier pipette tips. Automated liquid handling steps can also be designed to mimic manual handling, such as tilting plates to remove media through the use of a specialized active labware positioner (ALP).

To further enhance the capabilities of the Biomek Workstation as a cell culture system, additional instruments involved in the cell culture process can be integrated. Integrated storage devices such as incubators and ambient microplate hotels facilitate higher throughput and longer-term applications, such as a cell culture process that requires repeated accessing of cells (i.e., media exchange or cell passaging). Cell count and viability can be determined with an integrated Vi-CELL XR Cell Viability Analyzer. Other analyzers such as plate readers and imagers can also be integrated to monitor cell cultures or acquire data in endpoint assays. Figure 1 illustrates this type of integrated system, which was used for the stem cell culture. The movement of multiple plates through the integrated system and the data-driven liquid handling steps can be controlled through the SAMI EX Workstation software while multi-day/week applications can be scheduled using SAMI Process Management Software.

Automating cell maintenance and cell-based assays, either with an independent liquid handler or an integrated system, can greatly reduce the time spent in these activities while also eliminating variability that is inherent in the different ways that multiple users perform these tasks.

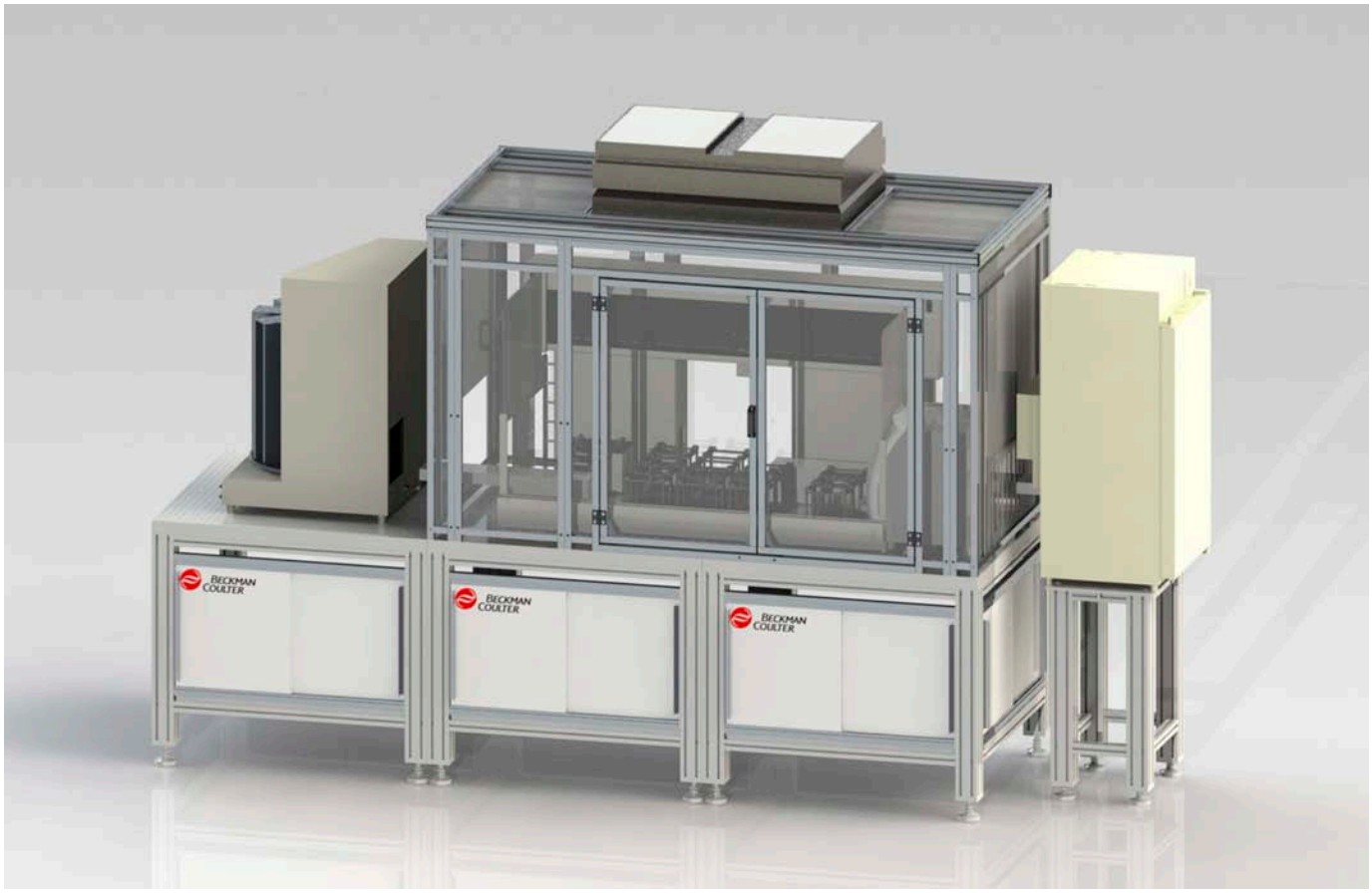

Fig. 1. Rendering of the integrated system used to culture murine embryonic stem cells.

Demonstration

As a proof of principle, we cultured murine embryonic stem cells (129) in 6-well plates on an integrated Biomek FX^{P} Workstation. Cells were passed on the Biomek Workstation every 2–3 days, with automated media exchanges on non-passage days. Trypsinized cells were counted with an integrated Vi-CELL XR and these results drove the volume transfers to achieve passage of 800,000 viable cells. Additional cells were manually stained after each passage for SSEA-1 as a marker of pluripotency.[2] Figure 2 shows the SAMI EX method used for passaging these cells.

Cell viability and pluripotency were tracked over 10 consecutive automated and manual passages. The automated cell cultures maintained >90% viability (Figure 3a) and >90% pluripotency (Figure 3b), showing equivalency with cells cultured manually.[1] In addition, culture sterility was maintained across all 10 passages in the absence of antibiotics in the media indicating the enclosure and sterile tips were sufficient to prevent contamination. These results indicate that even a sensitive embryonic stem cell line can be maintained on an automated system without any detectable loss in fidelity.

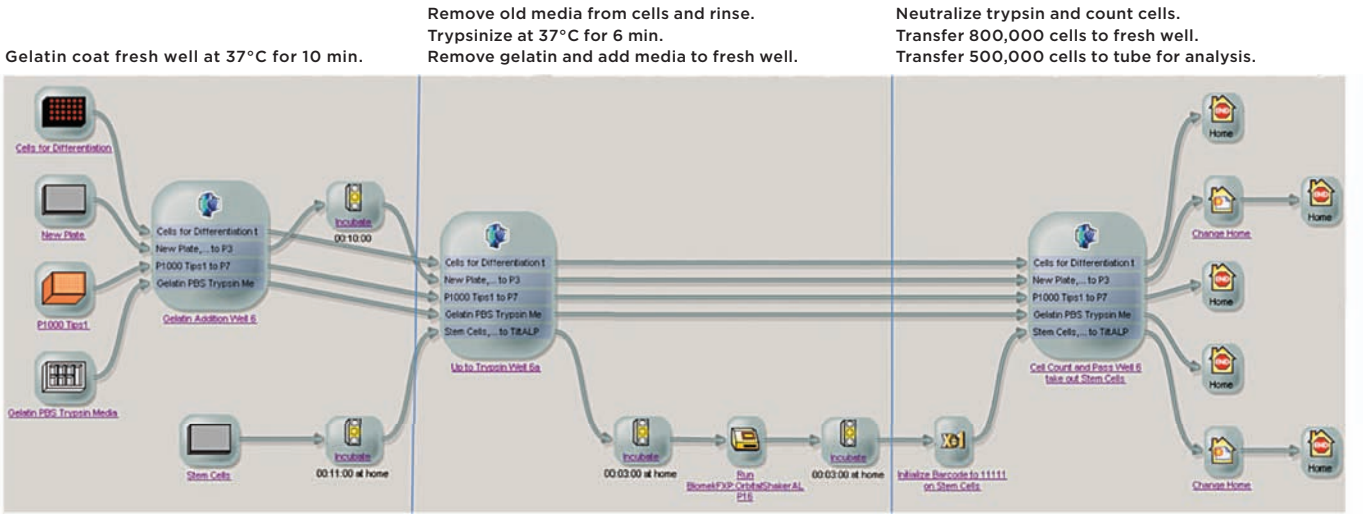

Fig. 2. Screen capture of the automated cell passage method in SAMI EX Workstation software. The software controls the movement of plates between the Biomek Workstation and integrated devices (Cytomat™ 2C incubator, Cytomat ambient storage) and cell counting on the Vi-CELL XR, all while tracking well data. The cell counts were then used to drive distribution of cells to fresh wells and a tube for pluripotency analysis by flow cytometry.

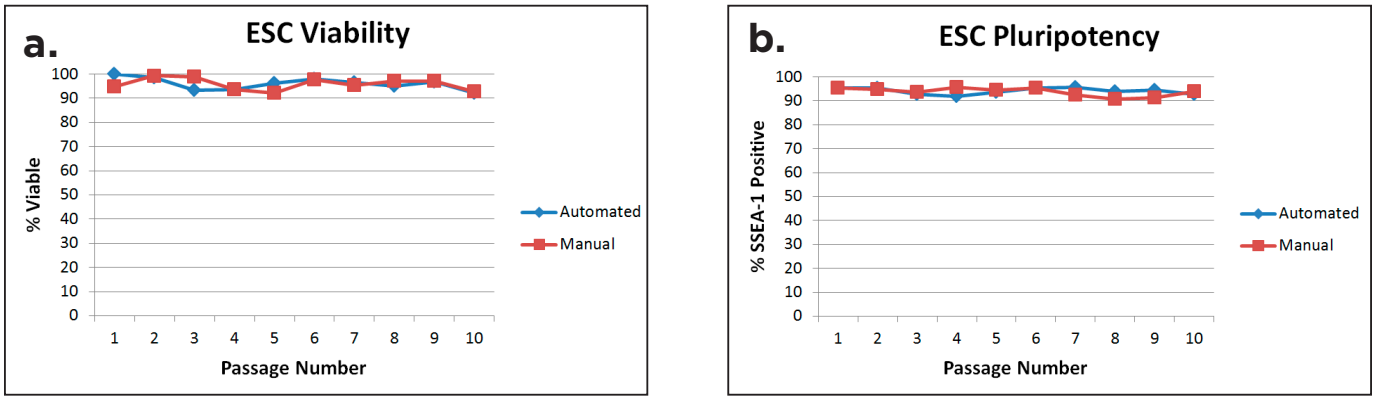

Fig. 3. Comparison of automated and manual stem cell cultures. **a.** Percent viability of stem cell cultures as determined by a Vi-CELL XR Cell Viability Analyzer. Cells were maintained above 90% viability and the average viability between the two methods were not significantly different (Automated Mean = 96.0±2.5%, Manual Mean = 95.9±2.5%, p=0.88). **b.** Percent pluripotency of stem cell cultures, as determined by flow cytometry (SSEA-1^{+}). Cultures maintained a pluripotency rate >90% and the average pluripotency between the 2 methods was not significantly different (Automated Mean = 94.2±1.4%, Manual Mean = 93.8±1.7%, p=0.56).

Conclusion

Here we have described a number of ways of automating the basic steps of cell culture and demonstrated the feasibility of automating this process by successfully maintaining mESCs for 10 passages. In addition, integrating devices such as incubators, centrifuges, or analyzers can facilitate the automation of entire cellular workflows. By automating these processes, one can achieve robust results and greatly reduce the active time that scientists must dedicate to cell line maintenance and assay preparation and allow them to focus on experimental design and analysis of results.

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References

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2. Solter, D, Knowles B B. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci USA*. 75(11); 5565-9: (1978).

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