Automation of Multi-Day Cellular Applications

Abstract

To study complex phenomena such as cancer invasion and metastasis, or embryonic development and stem cell differentiation, long-term cellular applications are required. Automation of these cellular experiments can greatly reduce the active time required for their execution and minimize the likelihood of errors that will occur in these complex workflows. Here we demonstrate the use of SAMI Process Management software to schedule and execute the automated liquid handling steps on a Biomek SAMI EX Workstation, for overlapping 8-day embryonic stem cell differentiation experiments.

Introduction

Cultured cells are used as model systems to investigate numerous biological processes. While many cellular experiments are interested in the short-term response to stimuli (i.e., phosphorylation kinetics), many studies require long-term experiments to observe more complex phenomena of interest. This is particularly true as the field of cell culture moves away from simple monolayer cultures into three-dimensional cultures, in an attempt to better mimic actual cellular organization and interactions. One common example of such long-term cellular experiments is the differentiation of stem cells.

Cellular differentiation is a complex process that frequently requires numerous interventions over multiple weeks. These interventions include changing the cell media to alter the growth factors and other signaling molecules that are present, as well as changing the format in which the cells are cultured—from suspension to adherent cultures. Many stem cells are cultured in a "hanging drop" format to induce the formation of 3-dimensional spheroids known as embryoid bodies (EBs). Once established, EBs may be cultured on adherent tissue culture plates to induce further cellular differentiation.

The complexity of these frequent interventions increases with the throughput of a given experiment or when multiple experiments are occurring simultaneously—a near certainty with long-term assays. Keeping track of what actions are required for which experiments or cell lines on any given day is a significant challenge—one that is often met with suboptimal solutions such as jotted notes and reminders. In contrast, automating the scheduling and execution of these interventions can reduce the

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likelihood of forgetting steps and the burden on scientists' time, while improving the continuity of data across the steps of an experiment.

Automation Solutions

Biomek SAMI EX Workstations provide an open platform that can be used to automate a wide variety of applications and throughputs. For long-term cellular assays, it is essential that sterility of the cultures be maintained. In addition to using sterile pipette tips, custom enclosures can be utilized to maintain a sterile work surface for liquid handling steps. While automating the liquid handling steps alone provides significant utility for these long-term assays, the integration of additional instruments, such as incubators and analyzers, allows for the automation of a complete cellular workflow. This reduces the opportunity for error, as the plates do not need to leave the integrated system during the multi-day or -week process.

While integrating instruments provides the opportunity to automate complete long-term experiments, the organization of multiple interventions that are separated by long incubations is still essential. SAMI Process Management software is a scheduling program that facilitates this organization. This software links the required interventions of a long-term process and reserves the Biomek Workstation for usage during the required times. This allows the interleaving of multiple experiments with a visual schedule of instrument usage and availability—along with indications of storage capacity limits and weekend activities that can either be rescheduled or set up in advance. The result is a calendar of scheduled activities that replaces the jotted lists for organizing manual experiments.

Demonstration

Here we illustrate the use of an integrated cellular system with SAMI Process Management software to automate the differentiation of mouse embryonic stem cells (mESCs). We utilized a modified hanging drop method[1] that requires 4 cellular manipulations over an 8-day time course to direct differentiation of mESCs into cardiomyocytes. The workflow is shown in Figure 1. Briefly, cells were trypsinized and counted and the cell suspension was added to 384-well polypropylene plates to induce EB formation. Additional medium with growth factors was added on day 2; on day 5, the EBs were transferred to 96-well tissue culture plates with additional medium to allow for further differentiation over an additional 3 days.

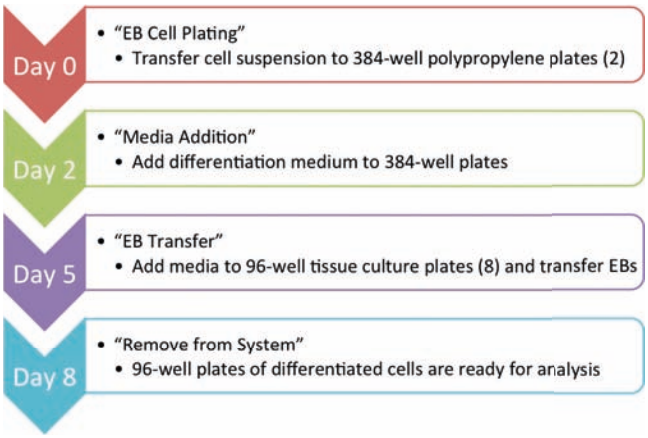
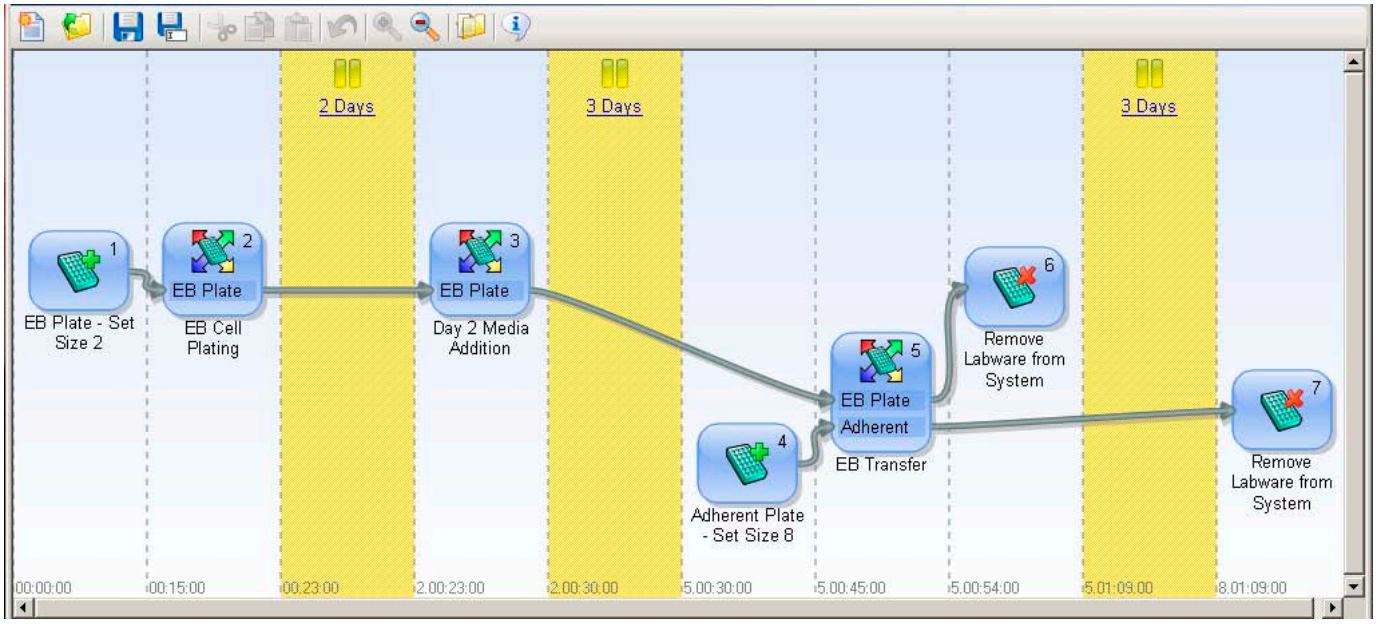


Fig. 1. Workflow for automated murine embryonic stem cells (mESCs) differentiation.

To automate this workflow, a Biomek FX^P liquid handler was integrated with a 37°C incubator and an ambient plate hotel, along with a Vi-CELL XR Cell Viability Analyzer, as shown in Figure 2. The liquid handling steps were created in SAMI EX Workstation software, and SAMI Process Management software was utilized to link these steps across multi-day incubations (Figure 3a). SAMI Process Management was then used to schedule 2 differentiation experiments of 2 plates each, staggered by 1 day in their start time. Figure 3b shows the calendar indicating which steps are required for which plates on the system for each day, thereby reducing the chance of forgotten steps. The automated steps can be initiated directly from the calendar so the user can be walked through their setup and execution.

Fig. 2. Representation of the integrated cell culture system used to automate the differentiation of mESCs.

a.



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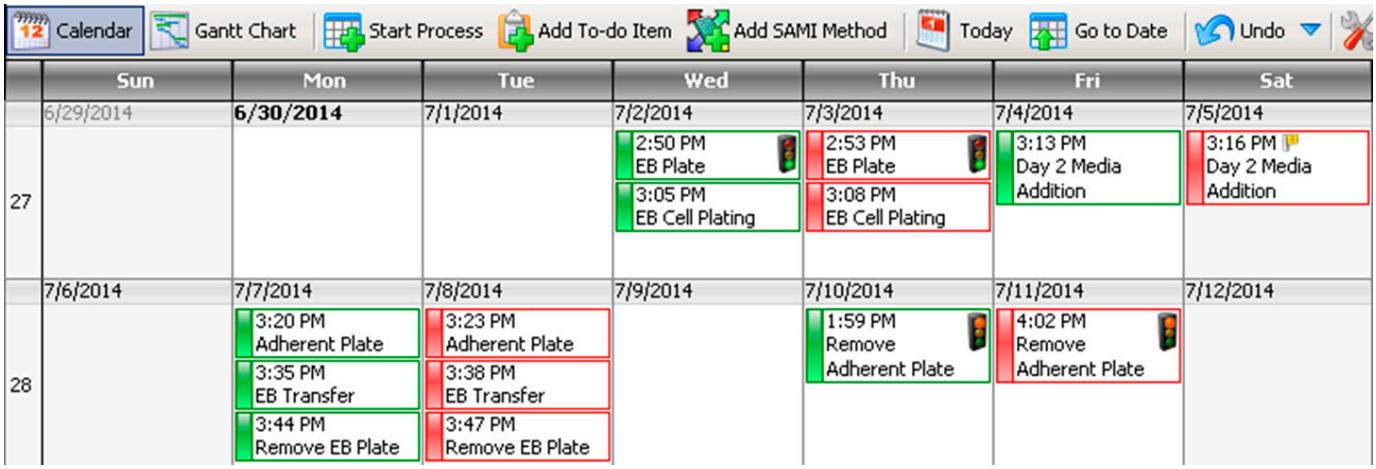

Fig. 3. a. Screen capture of SAMI Process Management software illustrating the linked liquid handling steps separated by multiple multi-day incubations. **b.** Screen capture of the calendar function of SAMI Process Management software that indicates the usage and availability of the Biomek SAMI EX Workstation during interleaved experiments. An intervention on a Saturday has been flagged.

The 768 wells per batch were visually inspected for the presence of an EB following the transfer at day 5; and the presence of cardiomyocytes was confirmed visually on day 8 by detection of spontaneously contracting cell clusters. Table 1 shows the results of the 2 batches, with averages and CVs calculated across the 8 96-well plates for each batch. The automated system achieved excellent reproducibility in EB growth and transfer, and beating cardiomyocytes were seen in the majority of the wells. In addition, there was no contamination detected in any of the plates—despite the lack of antibiotics in the media—indicating the enclosure and sterile tips were sufficient to maintain sterility of the cultures over long-term experiments.

Table 1. Results of Automated Embryoid Body and Cardiomyocyte Formation.

		Average	CV
Batch 1	EB Present	99.3%	0.5%
	Beating Cells	75.5%	9.5%
Batch 2	EB Present	99.5%	1.1%
	Beating Cells	66.1%	9.5%

Conclusion

The results shown here illustrate the ability of an integrated Biomek SAMI EX Workstation to automate a complex and long-term workflow through the use of SAMI Process Management software. The automation of the liquid handling steps can reduce the time scientists spend on these labor-intensive tasks while also eliminating user-to-user variability. However, the true value for long-term experiments is the ability to accurately schedule workflow steps for overlapping experiments in advance, thereby reducing the likelihood of forgetting a step. In addition, the continuity that comes from maintaining the plates on the system helps to eliminate plate mix-ups that stem from user intervention.

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References

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