Automating cell culture to optimise cell line generation and selection

Cell culture is and has historically been an essential component of the drug discovery toolbox. Cell culture provides the proteins, membrane preparations and other raw materials required for biological research. In recent years, with the emergence of high-throughput screening technology and binding and live, cell-based functional assays, the demand for cells and new cell lines with varying growth characteristics and expression profiles has grown substantially, both in terms of volume and variety.

By Tim Ward

Cell generation and selection methodologies continue to improve, yet the level of experimental sophistication also continues to advance, and researchers are increasingly demanding better, more robust cell lines with particular characteristics, fine-tuned for specific applications. Not only are researchers, who may be working in many different capacities and departments scattered throughout a drug discovery organisation, often quite specific and uncompromising on the make-up of the cell lines they request, they typically want the cells quickly, virtually on-demand. This scenario, played out daily in large pharma and biopharmaceutical companies around the globe, has precipitated a significant bottleneck across the spectrum of drug discovery research.

An insatiable demand for the rapid production and selection of optimised cell lines, particularly for high-throughput, cell-based screening applications, has out-paced the capacity of traditional cell culture production groups. Growing and maintaining cells in culture in flasks and roller bottles for research purposes has historically relied on the skills of laboratory technicians, who manually feed and expand cells, transfer culture containers back and forth from the lab bench to the incubator and monitor cell growth and culture conditions. These individuals are responsible for cloning, propagating and selecting cell lines, with each step in the process dependent on human skills and subjective decision-making.

To optimise and accelerate the process of cell line generation and selection and to resolve an important and growing bottleneck in drug discovery research, automation of cell handling and computer-based decision-making paradigms is essential. Cells have an increasing relevance in a broad range of applications in discovery research. Automation of cell culture procedures and integration of those automated processes across the spectrum of operations involved in growing, maintaining and expanding cell lines and preparing cell samples for use in assays and other experimental applications will increase the quality and variety of available cell lines and expand the scope of research opportunities.

Automation, and the substantial increase in throughput that it enables, will also allow for the

production of cells on a scale that not only meets the needs of well-established research applications, such as large-scale screening, but is also capable of satisfying the demand presented by emerging technologies and novel research techniques such as interference RNA (RNAi), and *in vitro*, cell-based drug transport, metabolism, and toxicology studies.

Demand for cells and information drives need for change

At present, a number of key factors are contributing to the growing and evolving demand for automated cell culture technology, including the following: an increased reliance on mammalian cells, which typically have more complex growth requirements; the use of a broad spectrum of expression systems and experimental conditions in drug discovery research; and the importance of selecting optimal cell lines for diverse applications. As cell-based experiments become increasingly common beyond the realm of basic drug discovery research and play an increasingly prominent role farther down the development pipeline – in pre-clinical studies and ADME/tox testing, for example – the demands on automated cell processing become even greater, with the need for aseptic handling and the regulatory requirements for data collection and handling and quality control procedures.

Maintaining even a small number of different cell lines – with differing media needs, feeding schedules, growth characteristics and expansion requirements – is a time-consuming, labour-intensive chore. If not done properly, diligently and with appropriate adherence to aseptic technique, a company risks the loss of valuable time and resources. The need for on-demand availability and selection of high quality clones is the driving force behind the development of parallel, automated approaches to cell culture. Historically, such strategies have relied on linking a series of robots, each with a specific function, to perform a series of disjointed processes that must be overseen and monitored by a technician. The availability of a fully integrated, automated cell culture system would help companies avoid costly, organisation-wide bottlenecks in scheduling, resource availability, decision-making and strategic planning.

The numbers of cell lines a company can clone and propagate and provide to its research groups will determine the scope of experimental conditions and expression systems, that may be investi-

gated. Bottlenecks in cell line generation will reduce the size of the pool from which researchers can select the most optimal cell lines for their application. Identifying the best cell line for a particular application and having the means at hand to optimise specific phenotypic characteristics and to select for optimal protein expression levels will not only offer researchers greater flexibility in experimental design, but will also facilitate downstream screening and production operations and have a tremendous impact on the overall productivity of drug discovery and development.

The bottom line in drug discovery is the need to improve productivity across a broad range of technology areas, from target identification and optimisation, to screening of compound libraries, lead discovery and optimisation, and protein production and purification. Across the span of the discovery pipeline, emerging strategies for increasing accuracy, efficiency, reproducibility and overall productivity have largely relied on the introduction of automated processes and protocols, advanced robotic systems and sophisticated algorithms and software solutions.

Traditionally, across diverse industries, automation and computerisation have yielded tremendous gains in quality and output as well as substantial reductions in cost. Among the main challenges for introducing automation into the pharmaceutical arena are the need to translate manual techniques into robot-guided functions, and the need for organisation-wide integration of distinct technologies enabling a smooth flow through the pipeline from materials input to 'product' output. Other important issues include the following: security; safety; quality control; tracking of raw materials, cell lines and cell samples; and data collection, archiving, reporting and analysis.

Ultimately, for a process such as drug discovery, automation and computerisation need to do even more than increase productivity and improve product efficacy and safety. They need to make available greater amounts of high quality data that readily translate into knowledge about a system, process or compound. That knowledge must be directly applicable to decision-making paradigms that guide corporate thinking. The industrialisation of key processes such as cell culture,

assay development and screening of compound libraries, combined with optimal utilisation of data mining and analysis tools and data-based decision-making algorithms based on user-defined business rules, will provide the foundation to enable a significant increase in the speed and efficiency of drug discovery.

The evolution of a solution

The automation of cell culture has followed a stepwise, evolutionary path that has largely paralleled the changing demands for cells and cell-based materials in discovery research. In the laboratory, cells have traditionally been grown in T-flasks or roller bottles – derived from conventional labware and modified over the years to support the growth of adherent cells and cells grown in suspension in a sterile environment. The culture vessel itself proved to be one of the first challenges in automating cell processing, as T-flasks and roller bottles are rather large and cumbersome to manipulate. Furthermore, a robotic arm was needed that could remove the screw cap from the vessel in order to gain access to its contents.

The robotic systems originally designed for use in drug discovery (or borrowed from other industries and adapted as needed) were developed primarily for chemistry and screening applications and were designed to handle microtiter plates. Furthermore, these instruments were not designed for aseptic applications and were typically not suitable for cell processing. Preventing cross contamination is a critical issue in cell handling.

The first automated cell culture system was introduced nearly 13 years ago and was designed for scale-up of cell culture in roller bottles under sterile conditions. The system contained a robot arm capable of adding media to cells growing in screw-cap bottles, and it was a self-contained unit that maintained cells in an aseptic environment during incubation and manipulation of the vessels and their contents. This early system overcame many of the basic challenges in automating cell culture. It proved that automated cell processing was

possible, and it provided the pharmaceutical industry with a system that could maintain and expand cells in culture without manual intervention. The system was capable of unattended operation and could be programmed to perform cell maintenance functions overnight. Initially, it was primarily adopted for use in the manufacturing of therapeutic proteins.

As high-throughput screening became a standard tool in drug discovery, and the demand for cellular proteins and membrane preparations increased, researchers in the discovery arena began experimenting with automated cell processing. They found that it enabled them to grow larger quantities of cells more efficiently. As cell-based assays gained in popularity, the demand for whole cells in assay development and large-scale screening grew dramatically. Now, rather than needing larger quantities of an individual cell line, researchers were demanding ready access to many different cell lines, all of which had to be maintained in cell culture simultaneously, available for rapid expansion on demand. In the post-genomic era, the number of drug targets in the discovery pipeline grew substantially, and multiple cell lines, each expressing a different target protein, were needed by target and lead identification and optimisation groups. To find the optimal cell line for any particular assay or application researchers need to sift through hundreds of clones, each with different expression and phenotypic characteristics.

Although the state-of-the-art, fully automated cell culture solutions at that time had the capability to produce a large volume of one cell line in roller bottles or T-flasks, it was not designed to handle multiple cell lines. Modifications were needed which enabled the system to grow multiple cell lines simultaneously in T-flasks and output the cells in microwell plates. The system provided researchers with much smaller volumes of many different assay-ready cell samples in microplate format. This system provided the flexibility to prepare a plate containing samples of a single cell line, or a plate with cell samples representing multiple cell lines. A key achievement of this system was the proven ability to manage multiple cell lines – as many as 30 at one time – aseptically and without cross contamination.

The next evolution in automated cell culture came in response to the continuing increase in the number of drug targets and the resulting demand for more cell lines with various phenotypes, growth characteristics and expression requirements. System requirements included the need to be able to seed, grow and maintain hundreds of cell lines in microwell plates, while minimising the risk of cross-contamination between wells and ensuring sample tracking at the level of an individual well. The switch to microplates as the culture vessel and the logistics involved in processing hundreds of cell lines necessitated changes in both the hardware and the software – the level of intelligence – of existing automated cell culture systems.

The complexity of scheduling processing steps for hundreds of cultures, ensuring the availability of adequate amounts of a variety of different media and reagents, expanding and outputting individual cell lines on demand, and tracking the location of each cell line in the system and keeping a historical record of the functions performed on each cell line could no longer be managed by a laboratory technician. For optimal efficiency, the system software needed to take over scheduling functions.

In addition, decision-making algorithms were developed that offered significant benefits for clone selection. When empowered with a set of user-defined business rules, the software could evaluate input data derived from assay results and combine that with information about the growth characteristics and robustness of a cell line to make an objective determination.

Optimising cell line selection

Breaking down the process of stable cell line generation and propagation into its component parts reveals a high degree of compatibility with robotics-driven, computer-controlled functions. The most obvious advantages of replacing manual operations with automated protocols are increased precision and high reproducibility – not to mention that robots do not get sick or arrive to work late, and they do not become bored performing tedious, repetitive tasks. A cell culture system capable of unattended operation frees highly qualified personnel previously needed to perform clone generation and selection to apply their training and skills to more challenging and stimulating work.

Beyond these direct benefits of automation lies a perhaps even more important advantage – particularly as it affects productivity on an organisation-wide scale. An automated cell culture system capable of parallel, high throughput growth, selection and expansion of cell lines can meet the needs of multiple research and development groups spread throughout the sprawling infrastructure of a large pharmaceutical company, avoiding the all-too-common problems associated with unanticipated scheduling conflicts. While one group may want to investigate a particular receptor and will need to transfect the receptor into various cell lines, grow

up clones and determine which yields the best expression level, another group may be optimising a new assay, while yet another research team may be working on the production of a monoclonal antibody, performing RNAi experiments, doing pharmacokinetic or drug toxicity studies, or developing knock-out cell lines.

An automated cell culture system can meet all of these diverse needs by offering a high degree of flexibility in batch size, in the numbers of plates processed at each stage and in the variety of functions performed. It would be capable of seeding transfected cells from prepared cell suspensions into the system's handling reservoirs and performing the necessary dilutions to transfer a single cell into each well of a microplate. The plated cells would be transferred to an incubator and undergo periodic media changes according to pre-programmed, user-defined operating rules. The system would periodically measure colony size as a determination of confluence and decide when the culture needs to be divided. This process of seeding, growth and expansion is repeated until the cells are ready for offline assaying to test for protein expression. The system would then prepare replica plates or select specific cell lines to transfer to the assay group. Assay data are imported into the system's database, and based on the data, the system would selectively expand the appropriate cell lines, which may in turn undergo one or more round of subcloning. Finally cells are output in plates ready for scale-up and for cell banking.

Throughout this process, the system records a detailed transaction history at the level of an individual well, allowing users to mine the historical database to track the activity and results of a particular cell line. It also provides the user with updated information on available system capacity, the contents of the incubator, cell suspensions available for seeding operations, and sample plates ready for outputting. Additionally, the system alerts the user of consumables requirements, and any resource conflicts or scheduling issues that might arise.

Eliminating bottlenecks, improving productivity

The goal of automating cell culture is not necessarily to mimic the steps previously performed manually; rather, the aim is to introduce robotic systems and computer controls that will improve the efficiency, accuracy and reproducibility of the customer's process. Of critical importance for any large pharmaceutical company pursuing drug discovery and development across multiple therapeutic areas, with disparate research and technology groups working on diverse projects that have variable resource needs and timetables, is the ability of centralised scheduling software to manage and organise a host of individual demands.

With far greater speed and accuracy than would be possible manually, the software is able to plan and optimise the complex logistics of culturing multiple cell lines in parallel and managing multiple projects simultaneously. An automated system can organise the workflow to adapt to divergent growth rates and schedule processing steps in a way that avoids overlap and conflicts while optimising throughput and resource utilisation.

Mammalian cells have become a vital tool in drug discovery and development, especially in the areas of cell-based assays for target identification and optimisation, high throughput screening of chemical libraries to assay for biological activity, ADME/tox studies and the generation of large quantities of therapeutic proteins. Optimising protein expression is critical to enhancing research and discovery efforts and to maximising the productivity of drug development. The flexibility to optimise culture conditions for individual cell lines significantly impacts protein expression. The ability to use data-based algorithms to select optimal cell lines for specific applications contributes to improved overall efficiency and productivity. Automated cell culture systems provide the flexibility, standardisation and robustness needed to eliminate what has become a key bottleneck in the discovery pipeline. DDW

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