Capturing the 'dynamics' of automated cell culturing

Researchers at the innovative stem cell company Cellular Dynamics International (CDI) have developed a novel, serum-free directed differentiation protocol, based on Tecan's Cellerity™ automated cell culturing system. The new method enables the maintenance of a uniform starting population of human induced pluripotent stem cells (hiPSCs), leading to the generation of hematopoietic precursor cells (HPCs).

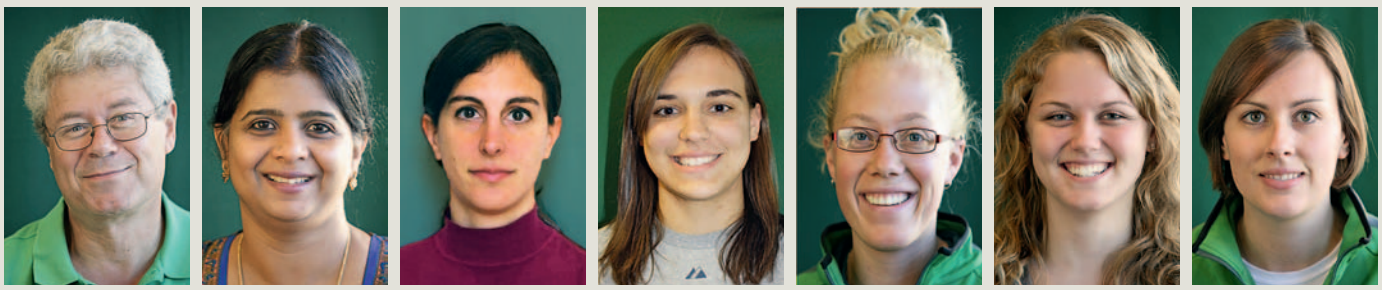
CDI, based in Wisconsin, USA, is currently the world's most innovative stem cell company, as voted by The Wall Street Journal in its 2011 Technology Innovation Awards. Founded to commercialize ground-breaking technology developed by stem cell pioneer James A. Thomson, CDI produces industrial quantities of differentiated human cells for basic research, drug discovery and development, and potential therapies to advance personalized medicine. To date, CDI has had great success launching iCell® Cardiomyocytes, iCell Endothelial Cells and iCell Neurons, with hepatocytes and many other cell prototypes already in development. The Company will also soon be launching its MyCell™ Custom Services for custom reprogramming.

Scaling up the production of pluripotent stem cells and directing differentiation to different cell types is technically challenging, labor-intensive and has inherent process variability. From an economic, regulatory and process control perspective, manual processing of stem cells is not a viable long-term solution. With these challenges in mind, researchers at CDI began to work with automated systems in order to standardize the maintenance and expansion of hiPSCs, and the subsequent differentiation into HPCs. To improve technical limitations, mainly the lack of throughput, a more sophisticated liquid handling system, Tecan's Cellerity, was purchased for the task.

The system, a Cellerity500, is based on a Freedom EVO® 200 liquid handling platform, and includes a LiCONiC™ STX 500 automated incubator with a capacity for 500 plates, a media storage fridge, an AutoLoader™ for loading flasks, a Cedex™ cell counter (Innovatis, Roche), spinner flasks for expansion and seeding of suspension cells, a Robotic Manipulator Arm to handle plates and an eight-channel fixed tip Liquid Handling Arm. The Cellerity also includes a positively pressured HEPA filtered environment in a BSL2-compliant class 100 sterile room for operations under GMP guidelines. Cells are maintained under sterile conditions in automation-friendly Corning® RoboFlasks®, which have an area of slightly less than 100 cm^2 and have a pierced septum for automated access. The system is disinfected using a sporicidal solution at the end of each run to prevent cross-contamination between cell lines.

Nick Seay, Chief Technology Officer at CDI, explained: "We are using the unique capabilities of the Cellerity robotic cell culture system to successfully generate blood precursor cells from hiPSCs that are often found only in sparse numbers in post-natal tissues. This robust and efficient automation system delivers consistency and other systematic improvements to our processes, allowing us to optimize differentiation to produce multipotent (CD43+/CD34+) HPCs and other defined cell types from blood."

The automated splitting of hiPSCs was made possible by the use of ROCK inhibitors and its effect on improving the cell viability of individualized cells. Cells can be lifted and individualized with trypsin, and plated



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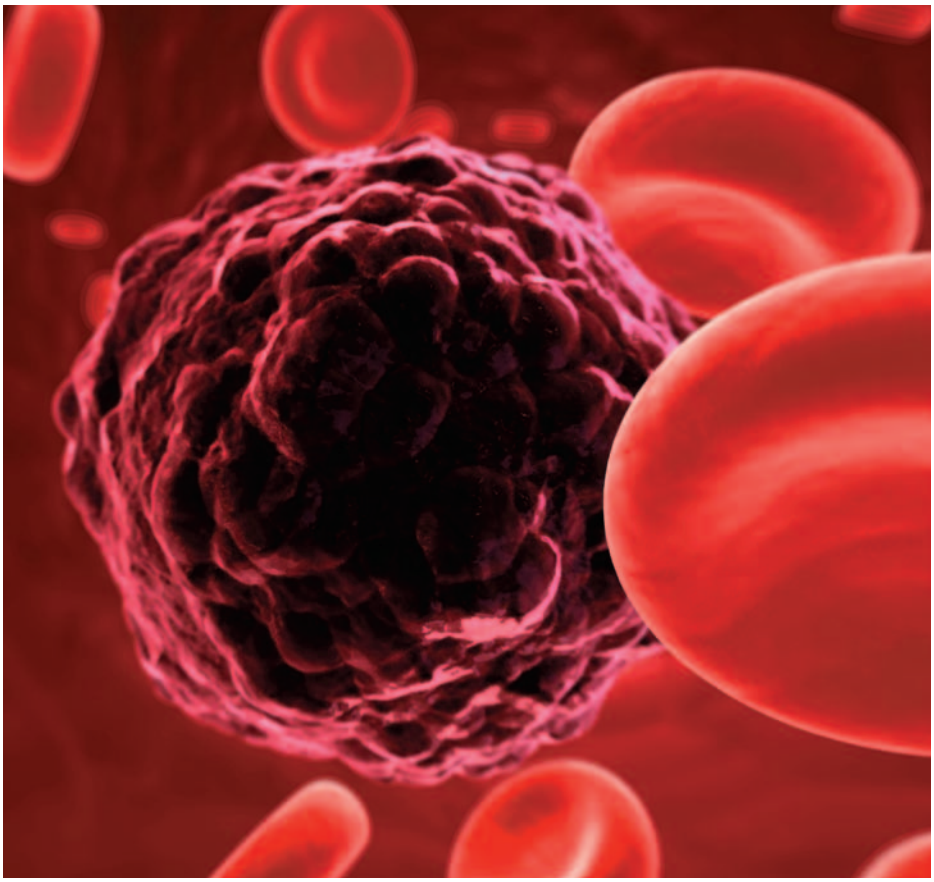

Hematopoietic precursor cell

without centrifugation as single cell cultures in the presence of trypsin inhibitor in mTeSR media. The cells are then expanded under feeder-free conditions on matrigel-coated RoboFlasks. This technique, developed at CDI (patent pending), eliminates the need for mechanically detaching the cells from the surface of the plates, and the system is capable of generating 5-6 billion hiPSCs in a weekly process.

Critically for the process, automation does not alter the genetic stability of these cells, nor their potential to form blood precursor cells. The serum-free differentiation is achieved on matrix proteins in the presence of cytokines. Aliquots of a heterogeneous HPC population are placed in various defined cytokine cocktails for further selective differentiation to a myeloid (generating macrophages and dendritic cells), megakaryocyte (platelets), or erythroid (red blood cells) lineage following an additional two to three weeks in culture.

Nick added: "We have also used the Cellerity to screen over 2,000 small molecules, identifying those that boost the generation of blood-forming precursors from stem cells and improving the efficiency and quality of the blood precursor stem cells even further. Overall, the project has significantly enhanced our scientific understanding, enabling us to control the growth, maintenance and differentiation of stem cells into blood precursor cells, as well as other specialized cell types, with increased yield and precision. The ability to expand stem cells and produce large numbers of differentiated cell types by automation is a real hallmark of our success in this developing field."

To find out more on Tecan's cell biology solutions, visit **www.tecan.com/cellbiology**

To learn more about CDI, go to **www.cellulardynamics.com**

High throughput system overview

Cells maintained in a RoboFlask

Aggregates generated in a 96-well plate

Differentiation for 12 days

Addition of small molecules
(All steps performed on the Cellerity)