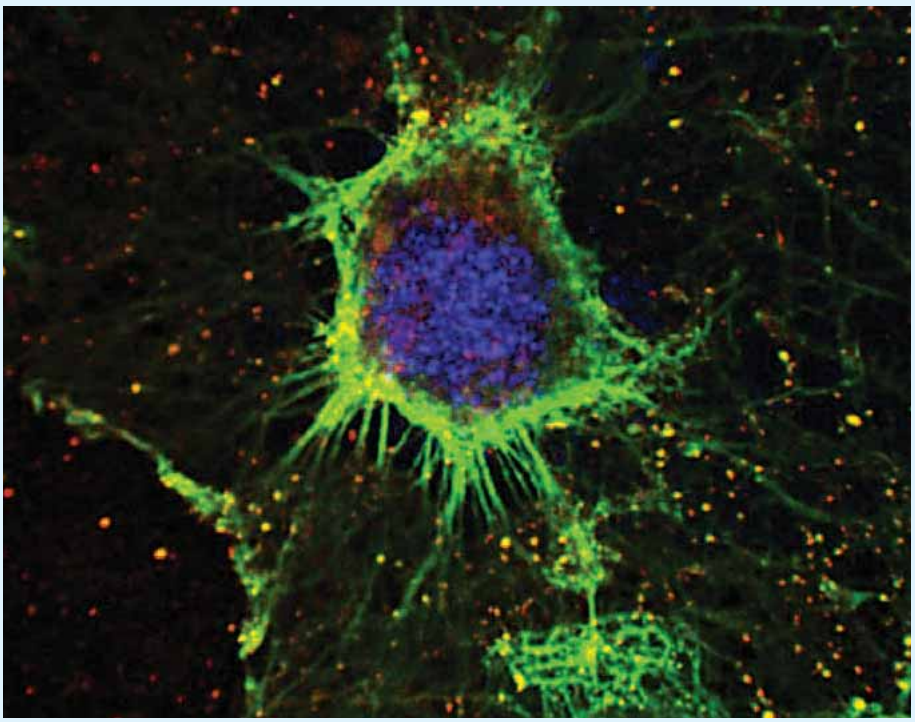
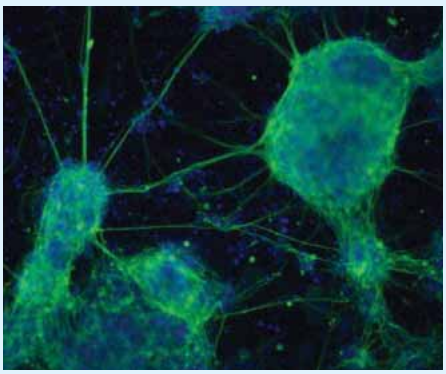
Consistency and reproducibility in stem cell culture

Researchers in the Department of Biochemical Engineering, University College London (UCL), UK, have automated stem cell culture on a custom-built Freedom EVO® 100 platform. Benefits compared to manual methods are higher consistency and reproducibility, better protection of cells from contamination, and improved operator safety.

Research in the Department of Biochemical Engineering, led by Gary Lye and Farlan Veraitch, aims to develop reliable processes for preparation of plates with homogeneous stem cell populations, and to screen them against compound libraries for use in drug discovery applications. One project, funded by the UK's Technology Strategy Board, focuses on the production of pluripotent or differentiated stem cells in microplate formats. This requires stem cell culture which, until very recently, was manually performed with high variability and low consistency, making it difficult to reproduce experimental protocols in stem cell research. Gary explained: "This lack of consistency in culturing stem cells is a real problem in furthering our understanding of the basic science of stem cell biology, and a major obstacle in commercializing applications of stem cells. Most commercial products based on stem cells and cell therapies use manual processes with reproducibility and contamination issues, which are major problems because stem cells are very difficult to grow. Automation is the obvious route for solving these problems and improving consistency of stem cell culturing, because every step is performed in the same way, for the same duration, every time."

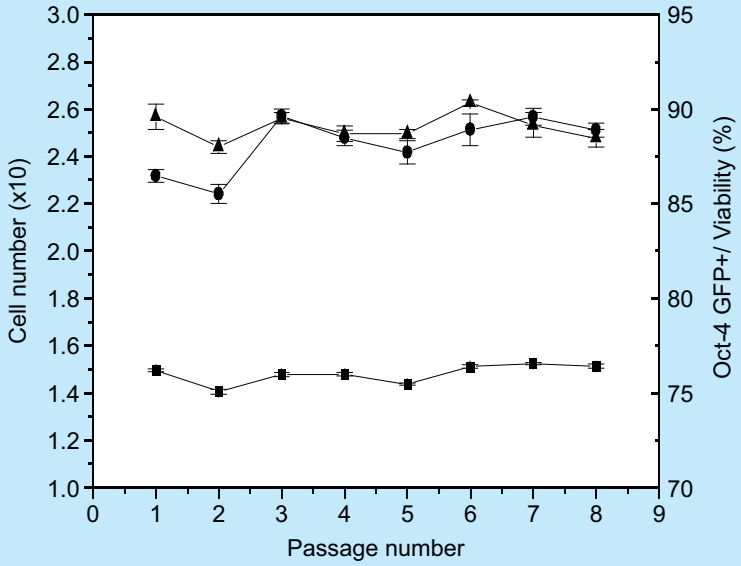
"Our Freedom EVO 100 platform was custom-built for us by Tecan with environmental control, and is set up in a self-contained biological safety cabinet for automated stem cell culture and manipulation. This guarantees both operator safety and non-contamination of the stem cells. Control of temperature, levels of carbon dioxide and oxygen is automatic, so the cells experience no variation in CO_2 levels, and consequently pH, during transport of plates between the automated CO_2 incubator and the Freedom EVO deck, which houses various modules including a microplate centrifuge. Controlling and keeping the environment around the cells constant minimizes fluctuations in the growth and differentiation of these cells, ensuring consistent results. Use of disposable tips on the 4-channel pipetting arm of the Freedom EVO platform enhances consistency, by minimizing crossover and contamination. We can then systematically vary the conditions to improve growth and control differentiation of stem cells from a variety of sources."





Immunocytochemistry of embryonic stem cells after 192 hours of neural differentiation. (above) single staining for bIII tubulin-FITC (green) and (right) double staining for bIII tubulin-FITC (green) and MAP2-PE (red). Neuronal cells were identified in clusters showing typical neural rosette morphology. DAPI (blue) shows cell nuclei.

Farlan Veraitch with the Freedom EVO platform in the Department of Biochemical Engineering at UCL.



Reproducible automated passaging of embryonic stem cells over eight consecutive passages: (■) cell number, (●) Oct-4-GFP+ expression indicating maintenance of pluripotency, (▲) cell viability. Error bars represent 1 standard deviation about the mean (n=4).

Gary continued: "We have to address two questions when automating stem cell culture and differentiation; the ability of these cells to thrive, and outcomes compared to manually performed processes by experienced operators. Manual and automated methods of culturing pluripotent stem cell populations, through several passages, were compared. Each successive passage involves multiple operations, including media exchanges and re-plating of the cells, each with the potential for propagation of errors. Both manual and automated procedures gave similar cell numbers, but automation significantly reduced variability in pluripotency-specific gene expression profiles, producing a more uniform final stem cell population. In addition, after differentiation into neuronal cells, the cells cultured using the automated system gave higher and more consistent levels of neuronal surface markers and neuron-specific gene expression."

"Our role as biochemical engineers is to make the production of pluripotent stem cells or differentiated cell types as reproducible as possible, and at a scale sufficient for commercial applications in industry. Stem cells from a variety of sources can be grown in 24-well plates, and typically run over a series of passages to get either pluripotent stem cells in all the wells, or undergo differentiation into specific cell types, such as neuronal cells. Following expansion, stem cells retain the ability to differentiate into all three germ cell types – neuroectoderm, mesoderm and endoderm – so in principle we could access any cell type. We have automated the process successfully for differentiation of neuronal cells, and it potentially applies to any screening process requiring consistent cell numbers and cell quality." Gary concluded: "Successfully automating stem cell culture on the Freedom EVO platform has reinforced our collaborations in two ways; with academic researchers studying different stem cell types and applications, and with industrial partners developing applications of stem cells for drug discovery or use in cell therapies."

Acknowledgements

Professor Lye and Dr Veraitch would like to acknowledge Dr Waqar Hussain and Mr Paul Mondragon-Teran who performed the work described here, and also the contributions of Professor Peter Dunnill, Professor Chris Mason and Dr Ivan Wall.

For further information on Tecan's stem cell solutions, visit **www.tecan.com/stemcell**

For further information on UCL, visit **www.ucl.ac.uk/biochemeng/industry/regenmed**