Successful isolation and expansion of human primary cells

A network project jointly funded by the Swiss Confederation CTI (Commission for Technology and Innovation) and Tecan, bringing together industrial, academic and clinical partners such as the Zurich University of Applied Sciences (ZHAW) and the University Hospital Balgrist in Zurich, has developed a successful automated system based on the Freedom EVO® platform. The system isolates and cultivates cells from human intervertebral disks, improving reproducibility and encompassing all-important integral quality control.

The Cell Biology and Tissue Engineering Division at ZHAW. Front row left to right: Ursula Graf-Hausner, Epifania Bono, Stephanie Mathes, Marta Kley; back row left to right: Nicola Franscini, Diego Santini

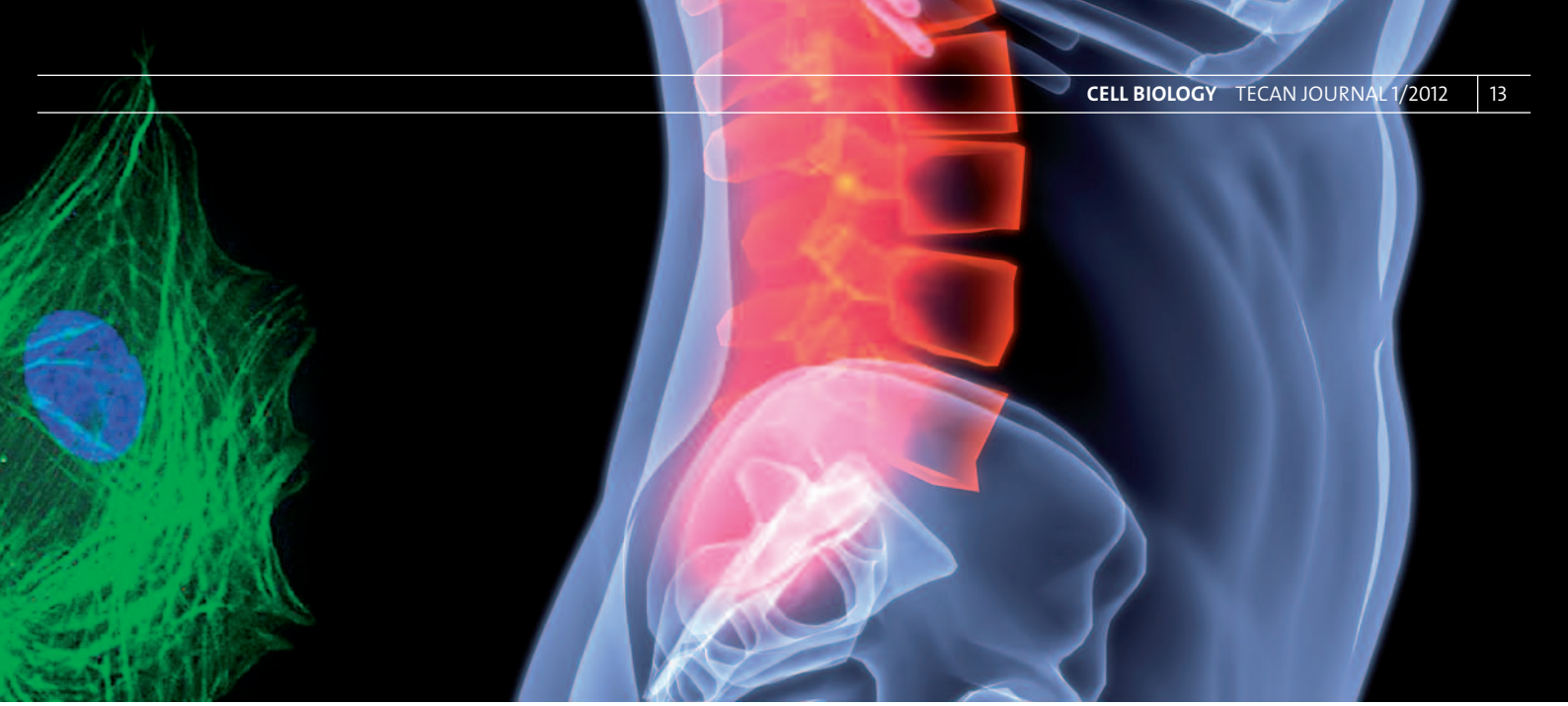
As more laboratories around the world target the growth of human cells for applications in tissue engineering, there is an urgent need for improved consistency in cell culture processes and automation is playing an increasingly important role. Professor Ursula Graf-Hausner, Group Leader of the Cell Biology and Tissue Engineering Division at ZHAW, explained: "Tissue engineering for regenerative medicine is a challenging and promising technology, but it is expensive and many hurdles must be overcome before the technology reaches the clinical arena. In 2007, my clinical colleague Professor Norbert Boos and I first approached Tecan as an industrial partner to look at the advantages automation might bring to cell-based technologies, with the long term aim of making them more standardized and, effectively, more suited to clinical applications."

A fully automated procedure was established on a Freedom EVO 150 workstation, configured using typical modules for automated cell culture. Freedom EVOware® was used for script generation and device control. All steps from enzymatic isolation of cells to proliferation of the isolated cells – including harvesting and seeding – and on-system quality control were automated. Parameters such as yield of isolated cells, viability, aggregation and growth rates, as well as phenotype, were analyzed to compare the automated and manual procedures.

The procedure used biopsy samples taken from patients undergoing spinal surgery (disk herniation). In the manual procedure, tissue was cut into small fragments whereas in the automated procedure, the tissue was homogenized using the Dispomix® system (Medic Tool, Switzerland). Incorporating this early stage of sample preparation into the automated workflow was a main goal and aimed to avoid inconsistencies in tissue handling by different laboratory personnel. The homogenized tissue was then further digested and washed to obtain purified single disk cells. Finally, cells were seeded into Corning RoboFlasks® using proliferation medium. To ensure the optimal conditions for cell proliferation, the medium was changed twice a week and, when about 70 % confluence was reached (typically after 5-6 days), cells were harvested and re-seeded.

In the manual procedure, regular determination of cell number was carried out after trypsination using an image-based Cedex™ Analyzer (Roche) for cell counting. Confluency measurement was implemented in the automated method using an integrated Roche Cellavista™ System. The Cellavista System is non-invasive and can be used to accommodate different growth kinetics for different donors. This is a huge advantage, since cells from one patient may take longer to grow than those from another patient. Another real benefit of the system was the ability to predict the number of cells expected at a certain time; by calibrating the system with specific cell growth parameters, the team was able to non-invasively correlate confluence with an expected cell number after harvest. This online monitoring also served as a warning option if predicted growth curves did not correspond to the measured values.

Establishing intensive quality control at each step and comparing the manual and



automated processes were absolutely essential for the project to succeed. Phenotype comparison was achieved by immunostaining (collagen I, collagen II, versican and total proteoglycan) of cells grown on glass slides for two days, either manually or on the Freedom EVO using the same staining protocol. Immunofluorescent analysis was performed using the Cellavista System (automated procedure) or an Axioskop® 2 Plus microscope (Zeiss) manually.

The analysis of isolated primary cells and of cellular characteristics demonstrated that the automated process resulted in the same number, viability, phenotype and even growth rate of cells as the manual method. Overall, the procedure allowed successful isolation of human intervertebral disk cells from biopsy and their expansion over several passages. Professor Graf-Hausner concluded: "The automated system achieved all our aims; we achieved excellent levels of reproducibility and standardization, which is essential when you're working with human material that could potentially be used therapeutically. From the technical and scientific point of view, the challenges of cell-based therapy have effectively been solved; the next step will be to look at these techniques in animals and then pre-clinical studies. We continue to have an excellent collaborative working relationship with Tecan, which is essential for success because we are biologists and not equipment experts. We have several small projects underway and the Freedom EVO is so flexible that we can adapt it depending on the questions we want to answer."

Full details of this study can be found in: Franscini, N. *et al*. J Lab Autom, 2011, 16(3), 204-13.

To find out more on Tecan's Freedom EVO liquid handling workstations, visit **www.tecan.com/freedomevo**

For more information on the Zurich University of Applied Sciences (ZHAW), visit **www.zhaw.ch/en.html**

1. LiHa Arm with four standard tips and four wide bore tips for cell pipetting **2.** Robotic Manipulator (RoMa) Arm equipped with eccentric gripper fingers **3.** Flipper module with four positions for RoboFlasks **4.** Centrifuge Rotanta 46 RSC Robotic (Hettich, Germany), mounted under the worktable **5.** Clean air hood **6.** Cellavista System **7.** Orbital shakers **Not shown:** Optional incubator for fully automated system (37 °C, 5 % CO_2, humidified atmosphere)