A new dimension in plate preparation for 3D cell assays

Scientists at the AstraZeneca Innovation Center China have developed a novel technique using a Freedom EVO® system for temperature-dependent automated preparation of cell culture plates for candidate compound screening. By pre-coating microplate wells with agar prior to pipetting the BD Matrigel™, the team is able to perform 3D cell-based assays in a 96-well microplate format.

AstraZeneca Innovation Center China

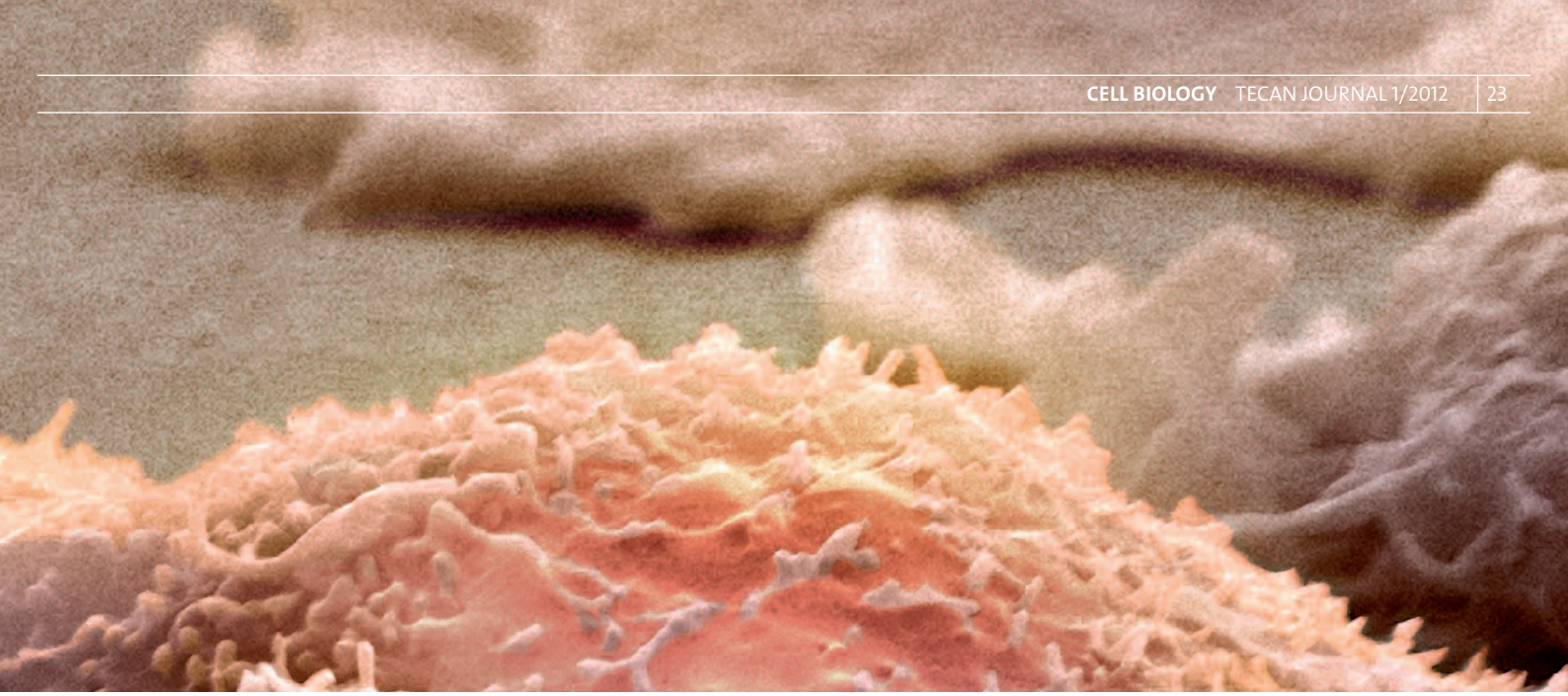
The Innovation Center China (ICC) is an AstraZeneca research and development facility in the Zhangjiang Hi-tech Park, Shanghai. Opened in 2007, the Center specializes in the investigation of diseases affecting the Asian population, including gastric, liver and lung cancers. State-of-the-art technology is used to elucidate the genetic mechanisms of these cancers and identify clinically relevant biomarkers and drug targets.

The Center's Cell Science Discipline, led by Dr Yi Gu, runs an active drug discovery program targeted towards these diseases, using a range of compound screening techniques to identify novel drug candidates. One of the secondary screening techniques used by the group is a 3D cell-based assay where cells are grown on a BD Matrigel substrate (BD Biosciences). Matrigel is a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. Matrigel induces the growth of colonies of cells in a different manner to the standard cell culture surface (Figure 1). Its heterogeneous composition stimulates the formation of colonies, making it ideally suited to the study of tumor cell-cell contact, growth, migration and metastasis. Since the colonies mimic the growth and formation of tissues in the body, they give the researchers the potential to identify the right compound in the very early phase of the drug discovery process.

Manual plate generation for the Matrigel assay is both problematic and time consuming, and so automation of the process with the Freedom EVO workstation was an obvious solution in order to increase throughput and improve consistency. The platform was already being used for compound serial dilutions and a number of other cell-based assays. It could quickly and economically be modified for this protocol without interfering with other applications.

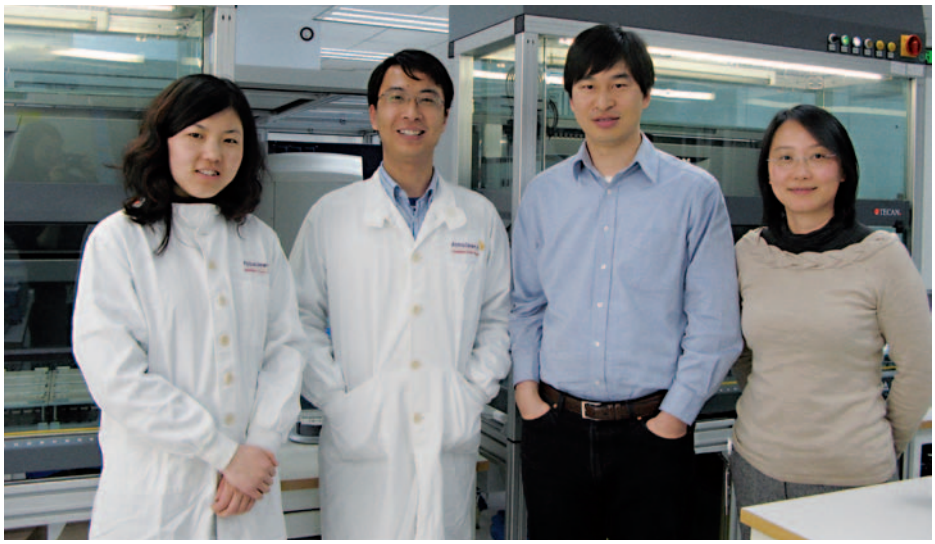
William Shi, Senior Associate Automation Scientist at the ICC, explained: "Historically, these assays have been performed in 6-well plates. Because of the large volumes needed and the complex handling requirements of the Matrigel, the manual production of the plates was rather difficult. Additionally, this format is poorly suited to drug screening due to the large volumes of reagents and candidate compounds required, and so we looked at ways to optimize this assay for cost effectiveness. More importantly, Matrigel is extremely difficult to pipette in low volumes, as its heterogeneous composition leads to high surface tensions and uneven distribution across a well, making analysis of results extremely difficult, and impossible for 96-well plate formats. To overcome this, we developed a protocol to precoat the microplate wells with a thin layer of soft agar prior to adding the Matrigel. This solved the surface tension issues associated with the Matrigel, but further complicated pipetting logistics as agar solidifies below 50 °C and Matrigel becomes too viscous to pipette above 4 °C."

The Freedom EVO platform was then adapted for the assay plate preparation by addition of several temperature-controlled carriers, allowing both the agar and Matrigel reservoirs to be kept at their appropriate temperatures to allow pipetting. All pipetting is carried out using a single channel on the system's Liquid Handling (LiHa) Arm, which is pre-heated or pre-cooled by multiple



aspiration and dispense cycles to avoid the risk of tip blockages. Initially, hot agar (at around 55 °C) is pipetted into pre-warmed 96-well plates using the LiHa Arm's multi-dispense function. This minimizes the risk of premature cooling of the media and allows small media reservoirs to be used, helping to further reduce costs. The plate then cools at room temperature on the deck of the instrument for 15 to 20 minutes, allowing the agar to solidify prior to addition of the Matrigel.

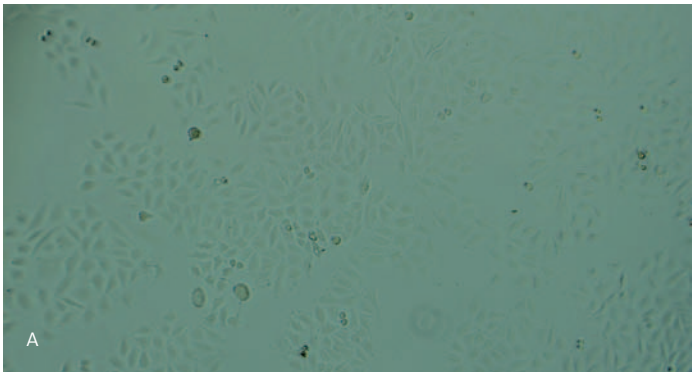
Using this new protocol, six complete plates could be prepared in one and a half hours, compared with only three or four plates in an entire working day when performed manually. The quality of the plates has also improved tremendously using the automated technique, avoiding the batch-to-batch, plate-to-plate and even well-to-well variation that often occurs with manual plate preparation. William concluded: "This consistency is vital for drug screening applications, providing high quality results to help us accelerate the drug discovery process, and the flexibility of the Freedom EVO platform made this easy to achieve."



The Cell Science Discipline team. From left to right: Jenny Xia, William Shi, Bin Xiang, Yi Gu

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

To find out more about the AstraZeneca Innovation Center China, visit **en.astrazeneca.com.cn**



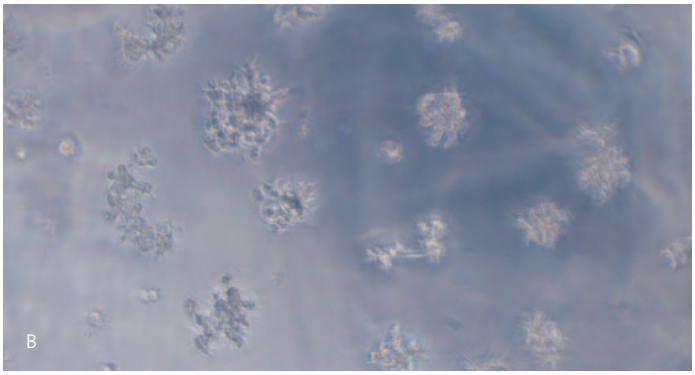


Figure 1: Gastric cancer cell line SNU-668 colonies growing in Corning® 96-well clear flat bottom TC-treated microplate, A) without Matrigel B) on Matrigel