

A framework for success

Freiburg-based Oncotest GmbH specializes in preclinical pharmacological contract research, and has successfully automated 3D cell culture on a Freedom EVO® workstation using polystyrene-based Alvetex® Scaffold 96-well plates from Reinnervate.



Sumeer Dhar

Conventional 2D cell culture formats are used extensively for high throughput *in vitro* assessment of the efficacy of anti-cancer agents, but the limitations of this approach have now been widely recognized. As more targeted drugs are being developed, the need to use well-characterized and validated, physiologically relevant *ex vivo* systems has become the hallmark for drug response studies using the tumor micro-environment.

German company Oncotest, founded in 1993 by Professor Heiner Fiebig, has successfully incorporated a novel 3D cell culture technology, Alvetex Scaffold 96-well plates from Reinnervate, into its service offering, enabling automation of 3D cell culture assays. Dr Sumeer Dhar, head of the Assay Development, Robotics and Screening Division at Oncotest, explained: “When Oncotest was founded, drug development scientists were mostly using cell lines as

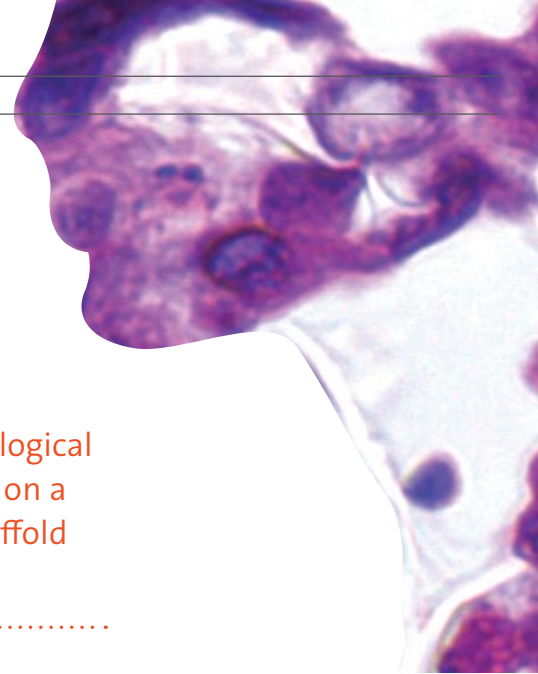
models for screening, developing and characterizing anti-cancer therapeutics. Professor Fiebig had the idea of using primary cells more extensively and in a more efficient manner than was the case at that time.”

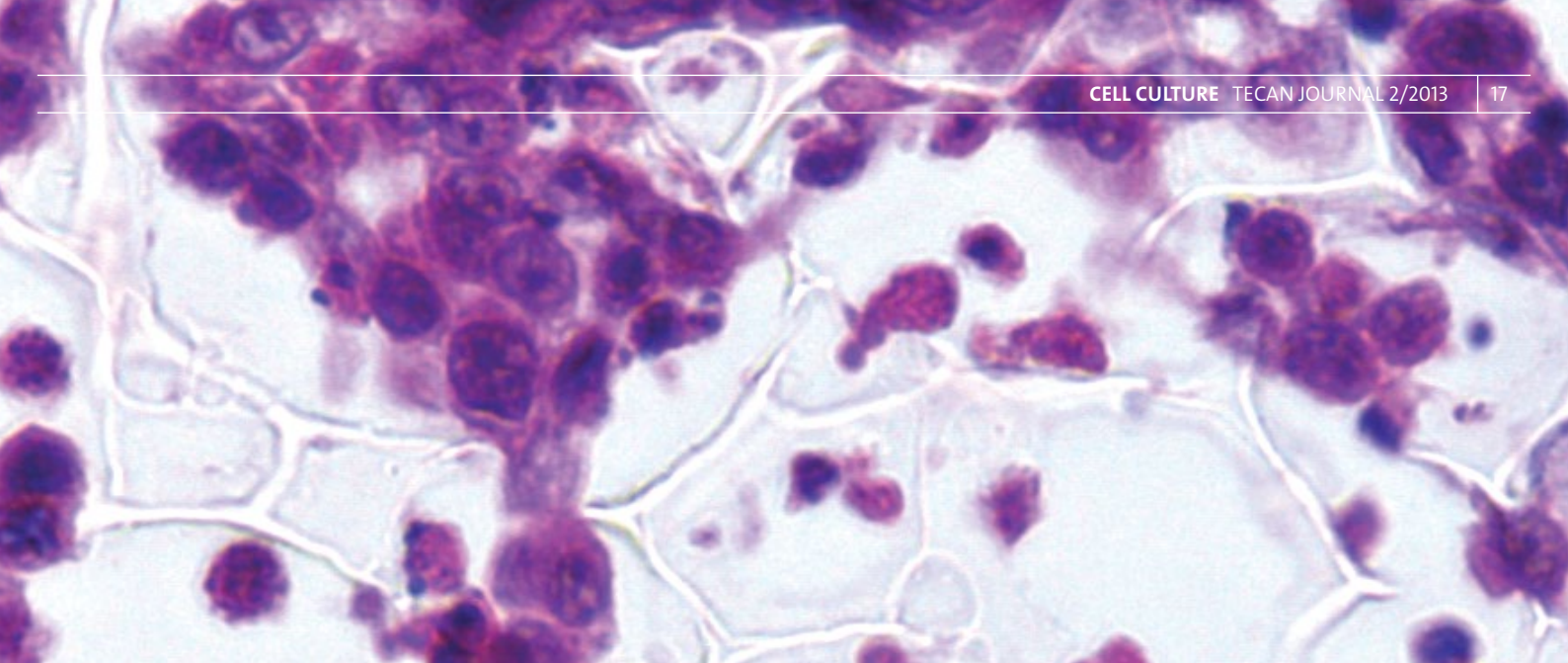
Today, Oncotest uses xenograft-derived primary cells for drug discovery and development, and has established a large repository of patient-derived xenograft tumors containing at least 80 cell lines and 300 well-characterized tumor models, including pancreatic, colon, breast, lung and skin cancers. The Company obtains tissue samples/biopsies from patients’ tumors, which are fragmented and transplanted into mice. The resulting tumors are then retrieved for future use in candidate screening and drug development. This means that Oncotest is not dependent on the clinical availability of tumor tissue, and can immediately perform screening studies or assays to meet its customers’ needs.

Sumeer continued: “3D cell culture techniques with soft agar, such as the Tumor Clonogenic Assay (TCA), have been used in drug development for more than 20 years and are seen as the gold standard. However, this method is primarily suitable for cell viability assays, and we needed a robust 3D assay platform that would enable multiparametric analysis. We looked at several different technologies, and found the Alvetex Scaffold platform to be the simplest and most reliable solution. It is ideal for studying downstream targets following drug treatment (assessing changes at post-translational and transcriptional levels), and can be easily adapted to automated liquid handling procedures.”



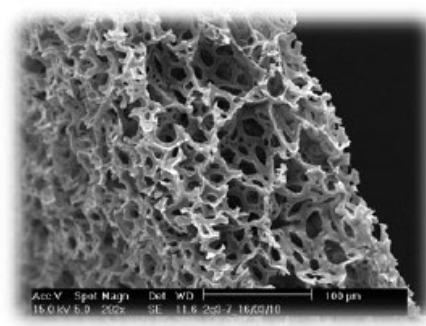
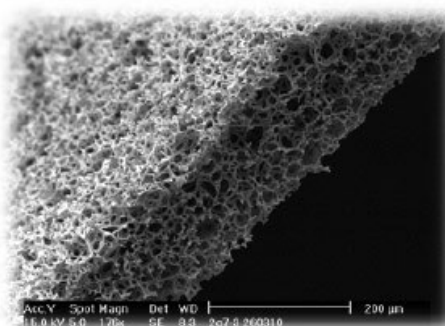
Members of the Oncotest team with the Freedom EVO 3D cell culture workstation (left to right): Eva-Maria Egenter, Fabiola Weggenmann, Hendrikje Weidmann (sitting) and Iris Seybold





Oncotest investigated the use of Alvetex Scaffold 96-well plates as a bridging platform between conventional 3D formats and preclinical *in vivo* models to aid candidate selection and reduce possible false positives. The Alvetex Scaffold system is well suited to studying drug response in an *in vivo*-like tumor micro-environment, and the Company believes this could be an important step forward in reducing the costs and increasing the predictive value of the assay platform. To increase throughput, procedures were automated on a Freedom EVO 200 platform equipped with a Liquid Handling Arm configured for disposable tips, a MultiChannel Arm™ 96 (MCA 96) with an integrated gripper, a Te-Shake™ orbital mixer, a cooling carrier, a LiCONic StoreX 110 incubator and a PerkinElmer EnVision® multimode reader. The Alvetex Scaffold plates are prepared and hydrated according to the instructions provided by Reinnervate. Growth medium is then dispensed into each well, before plating the cell suspensions and incubating overnight at 7.5 % CO₂ and 37 °C. Compound plate preparation is also performed using the Freedom EVO workstation, as well as subsequent addition of compounds to each assay plate using the MCA 96. Plates are incubated for 8 or 13 days, depending on the colony formation status of the tumor cells, and the CellTiter-Glo® luminescent viability assay (Promega) is used to determine candidate drug efficacy.

“The system is in use every day, and provides everything we need for our protocols. Tecan’s application specialists helped us to write the initial Freedom EVOware® scripts, and we quickly gained confidence once we started running the system on a regular basis.



Electron microscope images of the scaffold at different magnifications

We can now easily make any modifications ourselves, giving us a lot of flexibility. One of the biggest advantages of automation is for therapeutic combination studies. The conventional one combination per plate approach requires a lot of material and is a great deal of work when performed manually, but the Freedom EVO enables drug combination studies to be performed in a much more precise way. This saves both time and cost, as far less candidate compound is required, as well as giving us complete walkaway automation,” concluded Sumeer.

To find out more on Tecan’s 3D cell culture applications, visit www.tecan.com/3dcellculture

To learn more about Oncotest, go to www.oncotest.de

To learn more about the use of Alvetex Scaffold technology for cancer research, go to www.reinnervate.com/topic/index/cancer



Freedom EVO 200 with eight-channel Liquid Handling Arm, MCA 96 and integrated gripper, carousel and incubator (StoreX 110, LiCONic)