A Novel Approach to Single Cell Identification, Isolation and Characterisation

Prof Leon WMM Terstappen

Scientia

A NOVEL APPROACH TO SINGLE CELL IDENTIFICATION, ISOLATION AND CHARACTERISATION

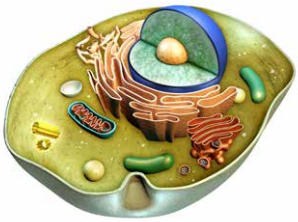
The establishment of cell lines capable of producing high-quality monoclonal antibodies is imperative for the development of therapeutic agents and the advancement of biomedical research. **Prof Leon WMM Terstappen** and his team from the University of Twente in the Netherlands have developed a highly efficient method of identifying and isolating cells that produce high concentrations of specific antibodies. With his team, Prof Terstappen has further honed this method to aid in the refinement of molecular cloning techniques to increase the yield of superior quality monoclonal cell lines.

Optimising Cell Line Production

Monoclonal antibodies that recognise particular antigens are routinely used for therapeutic and research purposes and are predominantly manufactured using mammalian cells. There is an increasing need for rapid, cost-effective and accurate methods to isolate and characterise single cells to create and maintain stable cell lines. Cell populations can be notoriously diverse, and the necessity to ensure that cell lines are derived from a single cellular source is crucial when producing high-quality monoclonal antibodies.

Traditional methods for screening and isolating candidate cells include limited dilution sedimentation, micropipette cell-picking, fluorescence-activated cell sorting and so-called 'lab-on-a-chip' technologies. All have proven to be valuable contributors in the field of single-cell analysis, but are time-consuming, labour intensive, and limited by the requirement for large cell numbers, many of which are lost during processing. Additionally, since these approaches tend to utilise entire cell colonies, the probability of achieving monoclonality is greatly reduced. This discounts their use clinically and hampers the swift detection of rare cells which may ultimately impact patient diagnosis and treatment. Indeed, the understanding of resistance to certain drugs and the identification of alternative therapies is vital to improving disease outcomes.

Prof Leon WMM Terstappen of the University of Twente leads the renowned Medical Cell BioPhysics Group at the Techmed centre. The group strives to overcome the limitations of other cell isolation and characterisation methods by investigating and implementing innovative techniques, with the specific aim of isolating and clonally expanding rare cells. Consequently, the research undertaken by Prof Terstappen and his colleagues has led to the development of an extremely effective procedure for

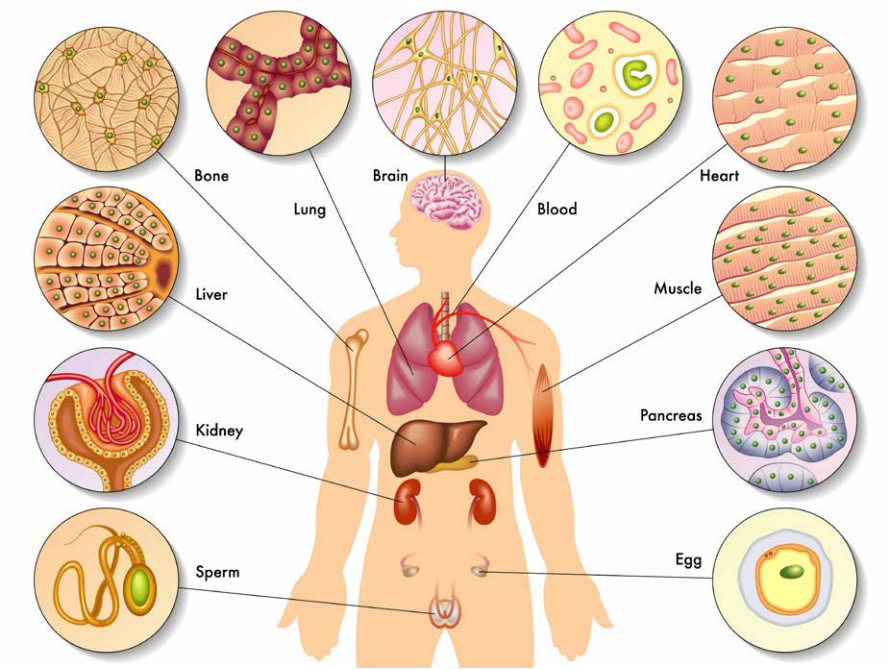


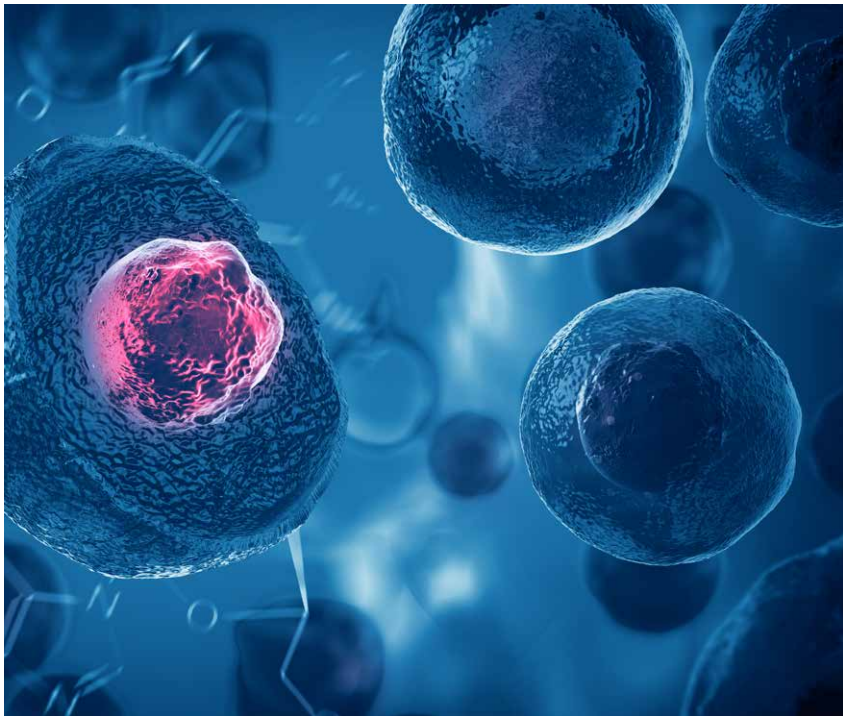
Basic cell structure

the isolation and interrogation of single cells using a self-seeding microwell chip and Fluorescent or Surface Plasmon Resonance imaging (SPRi) readout. Furthermore, Prof Terstappen's group has proposed a novel technology combining several processes which optimises throughput by enabling vast numbers of cells to be precisely screened simultaneously over a significantly reduced time period.

Single Cell Isolation

The preliminary research conducted by Prof Terstappen and his team provides an account of the introduction of a self-





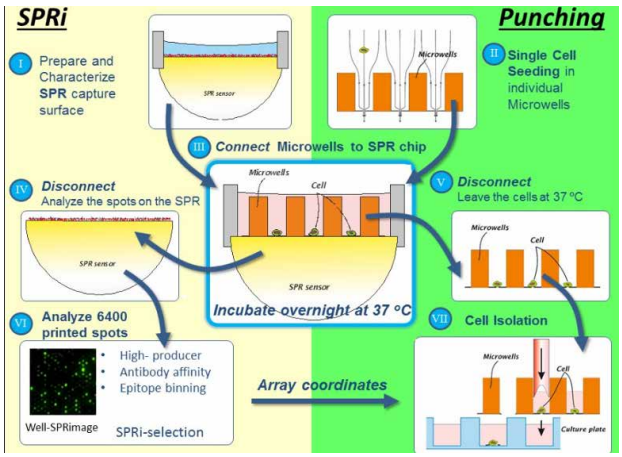
seeding microwell chip to isolate single cells in a semi-automated fashion with minimal manipulation.

Microwell chips comprised 6,400 individual wells, each with a small pore embedded into a thin membrane on the bottom. A solution containing fluorescently labelled target cells was applied to the microwell chip, and a small negative pressure was asserted to gently force the fluid through the well, dragging a single cell to settle on the pore. The fluid then exited via the pore, leaving the cell behind, which effectively sealed the well and prevented further cells from entering. The remaining solution was diverted to the adjoining well, and the next individual cell was deposited. This process was repeated until all microwells contained a single cell, and was completed within approximately 60 seconds. Each cell was then imaged through the membrane on the bottom of the well, using fluorescence microscopy.

The next step of the procedure was to place underneath the microwell chip a thin, high-binding capacity polyvinylidene fluoride membrane which had been pre-treated with a ligand to promote binding with the cell secretions of interest. The microwell chip with the membrane attached had been incubated overnight at 37°C and during the incubation period, the cellular secretions diffused through the pore in the bottom of the well and were captured onto the membrane.

Following incubation, the membrane was detached from the microwell and the membrane stained with fluorescence-conjugated antibodies recognising the secreted products. The cellular secretions could now be seen as tiny spots printed on the surface and quantified by fluorescence microscopy.

The amount of antibody secreted per cell per day was determined and based on this analysis, the cells could be categorised as high-, medium-, low- or non-producing cells. The data from these experiments confirmed that the majority of cells produced no or only a small number of antibodies, and only a few cells produced high quantities. This further emphasises the need for accurate cell isolation techniques with minimal cell destruction. Since the coordinates of the spots matched those of the microwells containing each cell, secretions could be accurately tracked to a specific cell, the wells containing high antibody producing cells could be



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identified, and a thin needle was used to punch the bottom of the microwell containing the desired cell into a small cup positioned below the microwell. Each individual cell could then be expanded to develop monoclonal cell lines with increased antibody production.

Introducing a Novel Technology

Having refined this pioneering technique, the team turned their attention to SPRi. Conventionally, SPRi is used to measure label-free real-time biomolecular interactions to provide insights into the affinities of interactions between antibodies and their targets (ligands). The SPRi process occurs in real time and uses a primed sensor to capture secretions of interest for analysis. However, only recently has SPRi been utilised for cell analysis and protein secretions from intact single cells quantified.

Prof Terstappen and his collaborators were able to combine the self-seeding microwell chip with SPRi technology to precisely isolate single cells and not only accurately characterise the quantity of antibodies produced by the individual cells, but also their affinity – a previously unreported feat. More specifically, following self-seeding of the wells with individual cells, the microwell chip was attached to the SPRi sensor and incubated overnight. The cellular secretions diffused out through the pore and were captured by the ligands on the sensor, creating a series of small spots printed on the surface directly below each well. Using imaging software, the kinetics of antibody production including the pg antibody produced per cell per day and their affinity to the ligand was determined and revealed those cells that likely could be expanded into monoclonal cell lines with high quantity and quality of antibodies.

Characterising Cell Secretions and Monitoring Dynamics

A further extension of Prof Terstappen's work includes the development of a microwell printing method that circumvents production issues encountered with other cell line manufacturing methods. Having tirelessly researched effective single-cell selection and isolation methods, and the successful capture and analysis of specific cellular secretions, the group proceeded to categorise cells into high-, medium-, and low-producing cell populations. They also monitored cell cultures over a period of 48 hours to evaluate whether their secretion status remained constant over time.

Following the determination of the cell secretion concentrations, Prof Terstappen and the team then investigated the secretion dynamics of the same cells at different time periods. Although the group has previously shown that cells from different cancer cell lines can be successfully isolated and expanded, further research is needed to determine whether those cells deemed high producers maintain their secretion levels over an extended time period. However, the success of these experiments is largely dependent on the ability to maintain optimal sterile culture conditions.

For the secretion dynamics experiments detailed here, the antibody production of each cell was measured at 8-hourly time intervals, using a fresh membrane for each time point. The data demonstrated that the amount of antibody secreted by the cells remained relatively steady, and that cells initially characterised as high producers remained so throughout the culture period, even after cell division. Prof Terstappen and his colleagues are actively conducting experiments to establish whether cells that produce high levels continue to do so after isolation and expansion. Importantly, this proposed new technology could reduce the cell selection process from 21 days to a matter of hours, with the possibility of screening >30,000 cells per day.

The Microwell Cell Selection Printer

The pinnacle of the group's research thus far is the creation and ongoing advancement of the Microwell cell Selection PRinter (McSPRinter, NWO grant #15327), a ground-breaking technology combining the described techniques which will no doubt have a huge impactf on the discovery of targeted therapeutic agents.

The concept of the McSPRinter has been met with enthusiasm and has received support and funding from several high-profile collaborators.

Predicted uses of this technology, and the focus of future work for Prof Terstappen and his team, include the screening of rare cells for the development of therapeutic antibodies, and the discovery of new treatment targets. The experiments summarised in this article have the potential to be adapted for the screening of many different cell types, including circulating tumour cells, B-cells encoded to produce antibodies against desired targets and T-cells for the detection of various molecules of interest, including cytokines, which may prove invaluable in the development of highly effective targeted drug regimes.

Meet the researcher

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Prof Leon WMM Terstappen obtained his PhD in Biophysics from the University of Twente in the Netherlands, and in 2007 he founded the Department of Medical Biophysics at the same institution. Having held various research positions around the globe, his research is now primarily focused on circulating tumour cells (CTC), and he remains intrigued by solving medical problems using ground-breaking technologies. Indeed, Prof Terstappen was instrumental in developing the FDA-cleared CellSearch system for the quantification of CTC in blood. His more recent work has resulted in the invention of the McSPRinter, a tool designed to determine the secretions produced by individual cells, with the ultimate aim of extending bespoke medical care to all cancer patients. The recipient of several prestigious awards, Prof Terstappen is an internationally lauded expert in the field of cytometry and the detection of rare cells.

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FURTHER READING

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