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Shining a Light on 3D Cell Culture

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The development of techniques to grow cells in culture was a major breakthrough for the field of biology providing an instrumental tool in drug discovery, developmental biology, stem cell studies and cancer research. More recently, cell culture is being used to produce cells within a highly-controlled environment for therapeutic applications, to repair or replace damaged tissues within the body. Traditionally, cell culture techniques have relied on 2D growth models, in which cells are propagated in simple monolayers on the flat surfaces of culture vessels, covered by a few millilitres of growth media. However, this system does not represent the most efficient way of growing cells, as the large internal volume of a culture receptacle is not fully exploited by cellular monolayers. To address such shortcomings, researchers have begun to turn to 3D bioreactor-based methods of cell culture that take advantage of the entire space available in a culture system. Optimisation of these techniques would help maximise the efficiency of cell growth, making bioreactor culture an attractive proposition for producing commercially viable quantities of cells for therapeutic and drug discovery applications.

Optimising cell culture

There are two major factors to consider when growing cells in culture: cell growth rate and cell viability. In order to study cellular processes or create commercial, cell-derived products, it is necessary to grow a large number of cells in a relatively short time. Therefore, maintaining a high cellular growth rate is particularly important. In addition, the usefulness and reliability of these cells is severely impacted by a drop in cellular viability, making it essential that the cells remain as healthy as possible during the culture process.

Researchers at LGC, the UK's designated National Measurement Institute for chemical and bioanalytical measurement, are currently working to optimise offline image-based monitoring techniques for use in bioreactor-based cell culture. The methods being developed can help facilitate process scale-up for use in bioprocessing and high-volume mammalian cell production. The bioreactor cultures use human cells grown attached to the surface of small microcarrier beads, which are 200 µm in size and capable of supporting around 40 cells each. Millions of beads are suspended in hundreds of millilitres of growth media with continual agitation inside a stirred-tank culture flask. These conditions help increase metabolic activity and cellular growth rate by facilitating efficient nutrient and waste exchange between the cells and the growth media. Such an approach also maximises the efficiency of space utilisation. To monitor growth and viability, LGC has developed a novel 3D fluorescent analysis technique, which allows the cells to be labelled with fluorescent dyes, imaged on the surface on the beads and then mapped to show the number of cells growing on each bead (*Figure 1*). This method allows the growth rate of the cells in the culture at a given time point to be measured. These results are combined with data for glucose and glutamine levels within the cell culture media, as these factors are rapidly utilised by growing cells and provide a useful profile of cellular metabolic activity.

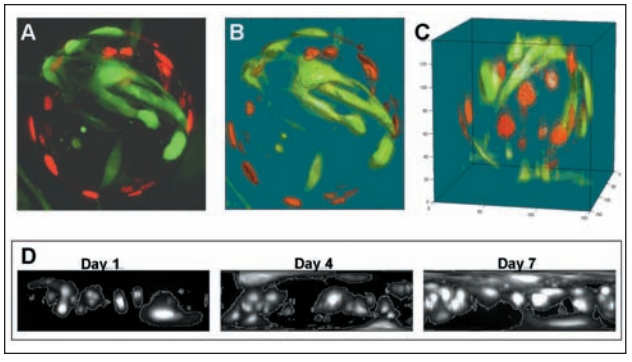

Figure 1. A novel method for measuring cells on the surface of microcarrier beads. Live cells (green) and dead cells (red) are fluorescent labelled and imaged (A), and the images used to create 3D reconstructions (B-C). The 3D images are then used to create cell maps (D) that can be used to track cell growth over several days in bioreactor culture.

Real-time monitoring

Although extremely useful, offline methods of assessing culture status are not without their drawbacks. Sampling only provides a static window into the health and activity of the cells at any given time, and importantly, continual invasive sampling increases the risk of culture contamination and wastes potentially expensive and precious culture material. To investigate ways of minimising the need for unnecessary sampling, researchers at LGC decided to collaborate with Stratophase, a company that designs solutions for monitoring production and biological processes in real-time. Stratophase's technology works by monitoring changes in the refractive index (RI) of a liquid in contact with an optical microchip.

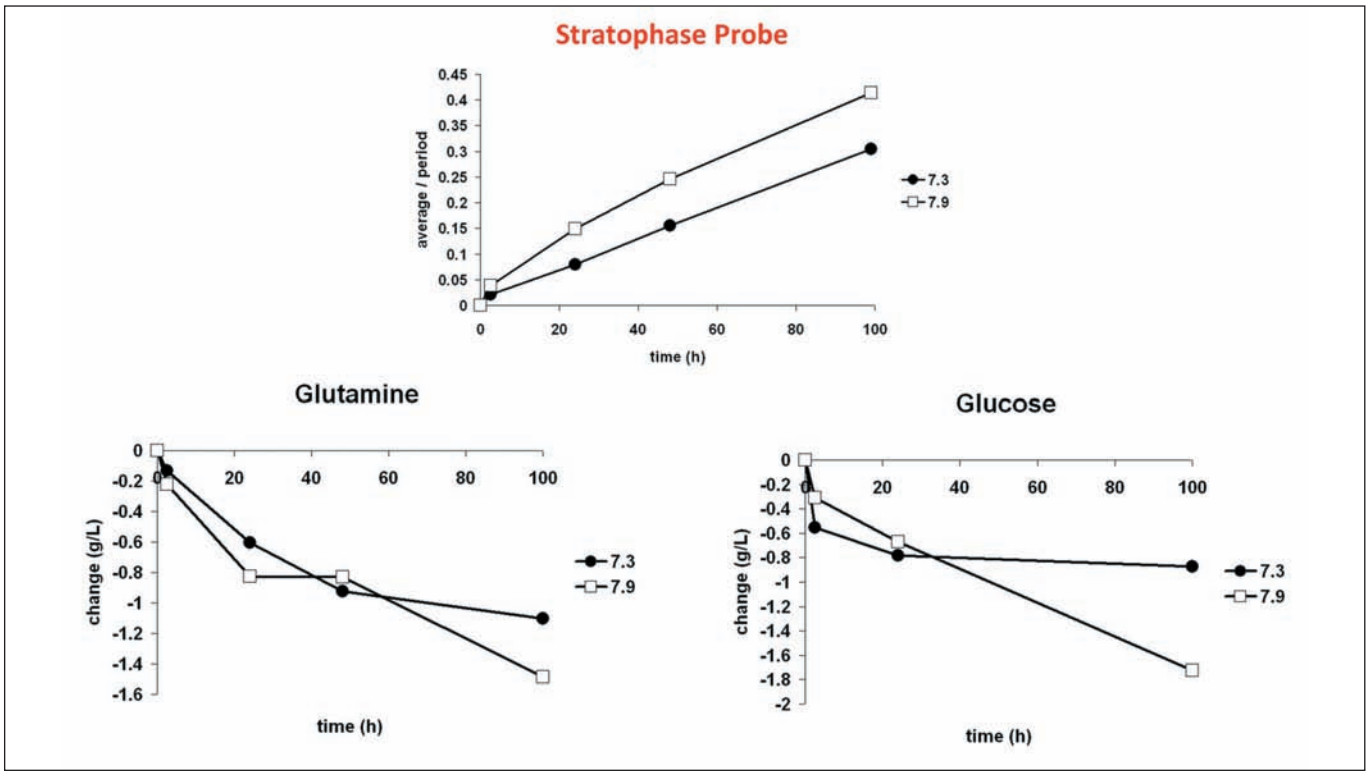

Figure 2. The 3D cell culture grown at optimal growth conditions (pH 7.9) rapidly utilised the glucose and glutamine substrates, implying faster cell growth in this culture. This data was reflected in the RI changes measured by the Stratophase probe, with a greater RI change indicating higher metabolic activity.

RI is influenced by modulations in liquid composition, such as variations in glutamine and glucose concentration. By introducing these sensors into the cell culture receptacle, it should be possible to monitor the status of the culture in real-time by recording alterations in the RI of the media.

In order to test this idea, Stratophase's optical sensor probes were placed directly into growth media to provide real-time monitoring of RI, with simultaneous offline analyses of glucose and glutamate levels carried out at specific time points for direct comparison. Two separate cultures were established, each growing for 4 days under different conditions, one at pH 7.9 and one at pH 7.3. A pH of 7.9 provides the optimal environment for cell growth while pH 7.3 restricts cell growth. This enables the activity of fast and slow growing cells to be compared. As expected, the cells grown at pH 7.9 consumed more glucose and glutamine than the cells grown at pH 7.3 (*Figure 2*). These findings were mirrored by the data generated using the optical sensor, with RI changing at a greater rate in the faster growing culture (*Figure 2*). These results indicated that the optical sensor provided real-time information that could be used to differentiate between a fast growing and slow growing culture. Although changes in RI do not provide direct insights into the levels of particular metabolites, pilot studies such as this can be used to create an idealised RI profile that reflects optimal culture conditions. This can then be used as a benchmark with which to monitor future cultures. Therefore, continuous RI monitoring is a viable alternative to invasive sampling for following cell culture performance, and could be used to optimise growth conditions. Utilising this approach, the system can be calibrated to monitor any liquid-based research or production process.

Conclusions and future perspectives

The work described here is a proof-of-principle study, illustrating that real-time monitoring using an optical sensor can provide useful insights into the status of a cell culture. Real-time monitoring has several potential advantages over offline sampling methods. By providing a continuous stream of data, the optical sensor instantly alerts the user to any unexpected deviation in culture conditions. This facilitates informed decision-making, while saving time and raw materials, as the process can be stopped or the culture conditions changed immediately as required. In addition, a reduction in offline sampling reduces culture wastage and helps minimise potential contamination. Such an approach is necessary to optimise 3D cell culture for use in research and to facilitate efficient scale-up, making the method an attractive option for adoption by pharmaceutical and bioprocessing manufacturers.

The ability to make instant decisions during the production process could save companies vast sums of money when undertaking batch bioproduction and may drive the uptake of real-time monitoring solutions over the next decade. The efficient monitoring of cell culture conditions is also an important step in developing methods for the mass production of therapeutic stem cells. Such a treatment strategy will be reliant on a source of readily available, high quality cells for use in the clinic, something that will only be possible by adopting the processes pioneered by researchers such as those at LGC. The Stratophase real-time optical sensor is one such solution, designed to make the real-time assessment of cell culture performance simple, rapid and informative, both in research and in bioproduction.