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Editorial

Miniature Bioreactors for Rapid Bioprocess Development of Mammalian Cell Culture

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There is a growing need to accelerate the bioprocess development for mammalian cell culture. Major pharmaceutical and biotech firms are facing challenges to reduce the process development costs and cultivation times. The conventional method for mammalian cell lines development usually involves a series of shake flasks for screening the cell lines prior to large scale cultivation (Doig *et al*., 2006). The shortcomings of this method are the long development times; laborious operation and limited experimental throughput which resulted in slow bioprocess development of mammalian cell culture.

For improvement, various scale-down miniature bioreactors had been designed to speed up the bioprocess development of mammalian cell culture. Generally, state of the art is to perform the small scale experiment in a high throughput and highly parallel manner (Hanson and Rao, 2010). Current technology endeavours to enable high throughput process development include the use of microtitre plates (MTPs), miniature stirred tank bioreactors and microbioreactor (Bareither and Pollard, 2011).

There have been an increasing number of studies focussing on microtitre plates (MTPs) as screening tools alternative to shake flasks (Micheletti and Lye, 2006). Microtitre plates have traditionally been applied for medical diagnostic tests such as enzyme linked immunosorbent assays, chemistry and biotechnology applications (Lye *et al*, 2003; Betts and Baganz, 2006). Typical formats of MTPs in mammalian cell process development are 24, 48 or 96 well plates made from various plastics and polymers. The advantages of microtitre plates are that they provide a miniaturised system and high throughput (HT) solution that is amenable to automation (Micheletti *et al*., 2006; Barrett *et al*., 2010). Automation of the MTP technology has been achieved by integration with robotic equipment for liquid handling to allow sampling and feeding, and monitoring of pH and dissolved oxygen (DO). The liquid mixing in MTPs is usually achieved by shaking the entire plates housed in a temperature-controlled incubator (Micheletti and Lye, 2006). The agitationapplied to the plates provides the centrifugal rotational movement that mixes the liquid in the wells (Bareither and Pollard, 2011).

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At present, there are a number of commercial small scale shaken systems available on the market with instrumented controllable microbioreactors such as Micro-24 Microreactor System (Pall Corporation, Port Washington, NY) and M2P Biolector, (M2P Labs GmbH, Aachen, Germany). The Micro-24 system is basically an orbital shaken 24-well plate that operates at working volume 3 – 7 mL with 24 independent reactors (deep wells, shaken and sparged) running simultaneously. Each reactor is designed as single use reactor that has the ability to continuously monitor and control the pH, DO and temperature. The reactor aeration is supplied by sparging air from gas feeds that can be controlled individually. Furthermore, pH can be controlled by gas sparging using either dilute ammonia or carbon dioxide directly into the culture medium through a membrane at the bottom of each reactor. Chen *et al*., (2009) evaluated the Micro-24 system for the mammalian cell culture process development and found the Micro-24 system is suitable as scale-down tool for cell culture application. The result showed that intra-well reproducibility, cell growth, metabolites profiles and protein titres were scalable with 2 L bioreactors.

By contrast, the Biolector system is based on a specially design shaken flower-shaped MTP, that has an automated liquid handling system integrated with the 48 or 96 reactors (scales of 100 – 2000 µL). The Biolector system runs in parallel mode with real time monitoring of pH, dissolved oxygen (DO) and temperature. One of the applications is illustrated in fermentation of *Escherichia coli* under pH control by Büchs group (Funke *et al*., 2010). In this work a combination of Biolector technology with a fibre optic online monitoring system for MTPs that have a

microfluidic control for feeding was used. It suggests that the microfluidic Biolector could work successfully for scale-down applications which allows for parallel and high-throughput operation and could also be investigated for bioprocess development of mammalian cell culture.

Another contemporary design using slightly larger volumes is the miniature stirred tank bioreactor. It is based on the conventional stirred tank reactor to enable a rapid and scalable experimental process development. Experiments are usually carried out in 4 - 16 parallel reactors running simultaneously at scales of 10 mL to 500 mL. The main advantages of these reactors are reduced cultivation times and costs, and its ability for continuous monitoring and real time visualisation of key process parameters in each single bioreactor (Gill *et al*., 2008a). Moreover, the capacity of miniature stirred bioreactor to monitor on line and control the pH, DO and temperature could make these reactors an excellent alternative to shaken systems for early stage mammalian cell culture bioprocess development (Ge *et al*., 2006).

The Automation Partnership (TAP) recently developed an advanced automated microscale bioreactor for cell culture, the ambr^{TM} system. The ambr^{TM} system operates with 24 to 48 disposable cell culture reactors of 10 -15 mL working volume that have similar features to conventional bioreactors in terms of mixing and aeration. Moreover, the ambr^{TM} culture vessels are integrated with a liquid handling system operated in a sterile workstation enabling automation for sampling and feeding with little risk of contamination (Bareither and Pollard, 2011).

Another alternative scale-down model for mammalian cell culture is the microbioreactor. An example is the commercially available SimCell system that has been developed by Seahorse Bioscience Inc. The SimCell system is based on a cassette containing 6 reactor chambers with 300 to 700 µL working volume. Each cassette is arranged on a rotating wheel that is placed in an incubator. The mixing in microbioreactors is achieved through the continuously rotated chambers in the innovative “rotissierre” arrays design. The impact from rotation within the cassettes creates a hydrodynamic environment that simulates mixing similar to larger stirred tank reactors. Furthermore, these microbioreactors are complemented with an automated robotic system for measurement and control of e.g. pH and DO in hundreds of microreactors for parallel experimentation (Legmann *et al*., 2009). One major disadvantage of the system is high cost which means that only few large companies have bought this device.

Even though each miniature bioreactor system is designed to fulfil the requirements for bioprocess development, there usually is a trade off in terms of information content process performance and throughput (Doig et.al, 2006). For example, there are a number of challenges using microwell-based mini/micro reactors systems such as high evaporation rate, limited sample volume, different hydrodynamic conditions, and cell reproducibility that need further investigation. In addition, another key challenge is the scale translation of these systems to mimic large scale process condition and maintain the full functionality of conventional bioreactors. Overall, further development of miniature bioreactors is required to enable automated, highly parallel, high-throughput operation and direct translation to large scale and thus offer major benefits for the rapid bioprocess development of mammalian cell culture.

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