NOVEL PROCESS CONTROL IN A CLOSED SYSTEM BIOREACTOR FOR CULTURE OF ADHERENT CELLS

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INTRODUCTION

As cell therapies move to clinical practice, there is a need to simplify and optimize production protocols and move away from traditional culture techniques in flasks. Production costs and risk of contamination can be lowered by reducing handling, while process optimization using multi-parameter control increases quality, reproducibility and traceability.

A new perfusion bioreactor system is introduced that employs rigorous process control for the cultivation of adherent cells. Novel monitoring (of biomass) and control (of DO and pH) technology is integrated for optimal environmental parameters.

MATERIALS AND METHODS

The Scinus Cell Expansion system (figure 1, right) is a perfusion bioreactor for GMP-grade culture of adherent cells. Cells are cultured from biopsy to harvest on microcarriers in a single use bioreactor bag (figure 1, left). The volume is minimized to support growth of minimal cell concentrations and can be expanded as more culture surface is required. The cabinet and bag are fitted with sensors, allowing control of critical parameters at the site of culture.

Figure 1 The Scinus Cell Expansion perfusion bioreactor (right) and the single use bioreactor bag fitted with sensors for process monitoring and control (left).

A unique oxygenator was designed for the tight control of DO and pH (figure 2A). Homogeneity of the culture environment is maintained by means of a rocker that keeps microcarriers in suspension and prevents aggregation (figure 2B). Accuracy was determined vs traditional control (combined oxy- carboxygenator) over a period of 96 hours.

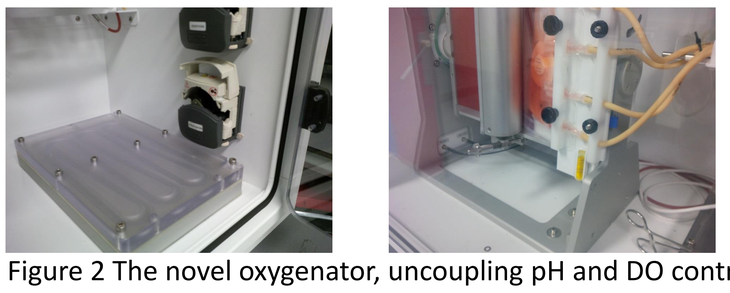
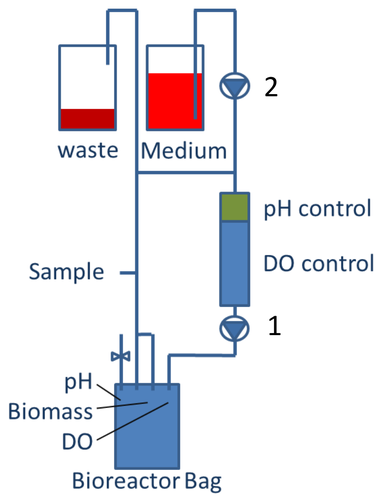


Figure 2 The novel oxygenator, uncoupling pH and DO control (A) and the single use bioreactor bag in the rocker system (B).

Human bone marrow-derived MSCs were cultured to confluency (see poster #360). The ability of the biomass sensor to measure viable biomass in the Scinus and in a stirred vessel was assessed. Radio frequency impedance (RFI) measurements were compared to offline cell counts and tested for correlation.

RESULTS



Process control was achieved by integrating the novel oxygenator with separate pH and DO control into a perfusion (figure 3). A pump head (1) controls the perfusion to the bioreactor bag. Fresh medium is added by a second pump (2). Excess medium is led to a waste container. Environmental parameters are monitored at the site of culture (inside the bioreactor bag), and controlled using controller software (Applikon BV).

Figure 3 Schematic overview of the perfusion process inside the Scinus Cell Expansion system

Traditional control of DO and pH using pressurized air, N_2 and CO_2 resulted in large fluctuations around setpoint (figure 4, left). Uncoupling of the pH and DO control led to highly improved stability of both parameters (figure 4, right). The accuracy of pH control improved from 0.1 to <0.05 around setpoint (figure 4, top right). DO was maintained within 3% around setpoint, compared to approximately 15% using traditional control.

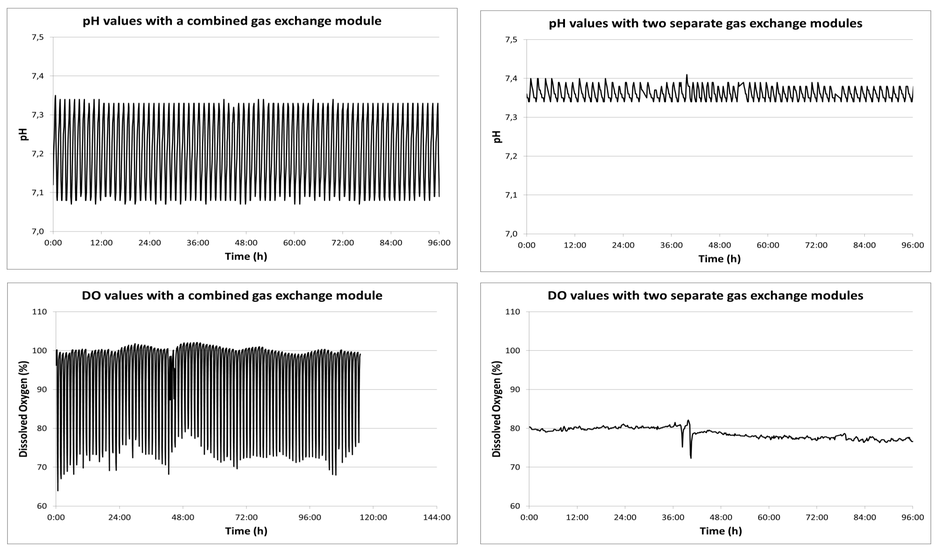


Figure 4 Enhanced environmental control using the novel oxygenator design. The control of pH was dramatically improved (compare top left, traditional control, with top right). Similarly, DO control saw a major improvement with the new oxygenator (bottom left vs bottom right). Fluctuation around setpoint were minimized, creating a highly stable culture environment.

Correlation of measured RFI and viable biomass was performed in a stirred vessel and on Scinus-grown MSCs. For stirred vessel culture, measured impedance was determined during eight days of MSCs culture and compared to offline cell counts (figure 5, left). For biomass measurement inside the Scinus's single-use bioreactor bag, confluent microcarriers inside a bioreactor bag were serially diluted with empty carriers. The impedance measured by the integrated biomass sensor correlated highly (linear correlation, $R^2 = 0.998$) with offline cell counts (figure 5, right) performed using a standard particle counter (Coulter Counter Z1).

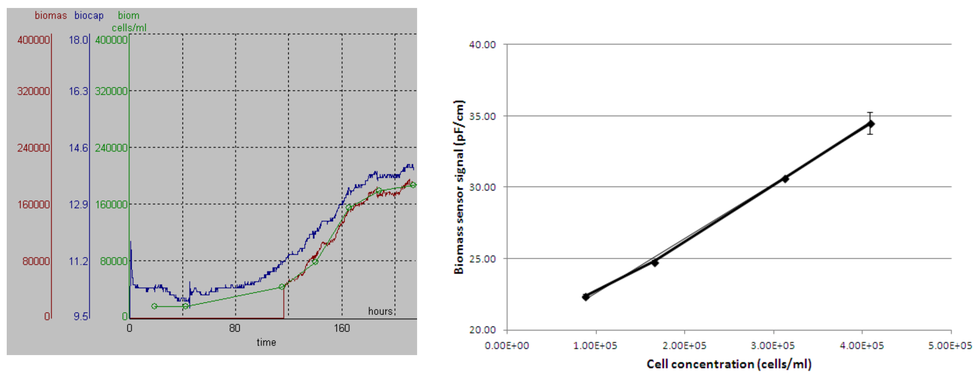


Figure 5 Correlation between online and offline measurement. Left, measured impedance and counted MSC concentration in a stirred vessel. Right, biomass measurement inside a single-use bioreactor bag. The high correlation (r^2=0,998) indicates a linear correlation between the measured impedance (pF/cm^2) and the viable cell concentration.

CONCLUSION AND DISCUSSION

Production of cell therapy can benefit from culture procedures that minimize operator involvement and reduce cost. The presented bioreactor enables one operator to handle one entire culture with minimal labor. Precise monitoring and control increases reproducibility and quality of the final cell product. The newly developed oxygenator resulted in a precise regulation of the critical parameters, DO and pH, without introducing unwanted factors. Moreover, biomass can be monitored without the need for destructive sampling.

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