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c.bird™ | Providing Optimal Suspension Culture Conditions in 96-well Plates and Superior Comparability with Large-scale Shaker-flask Culture Environments

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Abstract

Our study demonstrates that the c.bird™ improves mammalian cell growth in 96-well culture environments and closely imitates the shaker-flask culture in terms of cell growth profiles and protein yields. In traditional cell line development processes, it takes a long time to find optimal clones due to inconsistent cell line profiles generated between the early and late stages. The c.bird system, a new cell culture innovation, is created to provide solutions to this problem, reducing the cell profile differences between the two stages and providing an optimal suspension culture condition earlier on. In this study, we cultured CHO cell lines that produce monoclonal antibodies with and without the c.bird system and compared cell profiles generated with that of cells cultured in a shaker-flask culture environment. The results show that the c.bird improved live/total cell growth and cell viability and had superior comparability with those of large-scale shaker-flask cultures. In addition, it significantly improved cell doubling time and relative protein yields with no statistical difference when compared to shaker-flask cultures.

Introduction

There are increasing demands to accelerate the workflow of cell line development (CLD) in the biopharmaceutical industry. Traditional CLD can take months or up to a year to identify optimal cell clones. One reason for such long CLD period is due to difficulties in picking the right clone early in the process. An early cell culture process adopts a static culturing condition because of low cell numbers. Late cell culture processes transition into suspension conditions for the expansion of cell products. The transition from static to suspension cell culture generates different cell line profiles (growth rate, titer, metabolic profile, etc.), which impedes the identification of the right cell clones during early CLD processes. As such, massive numbers of clones are required for testing, increasing the costs and time required for CLD. To speed up the development process, maintaining consistent cell culture conditions throughout the CLD process is crucial for enabling early identification of the right cell clones with the right profiles that are sustainable during the CLD process.

High-throughput technologies are increasingly used in CLD processes to solve this problem, providing suspension culture environment in small-scale wells/flasks with enhanced oxygen supply. Here, we demonstrated a whole-new system with the c.bird microbioreactor that enables early-stage suspension cell culture in standard 96-well plates. The c.bird system is compact and readily fits in a standard incubator. It has a docking station, holding three sets of c.bird systems (Figure 1A). The c.bird system is composed of two parts: an autonomous control box (Figure 1B) on the top and a consumable c.bird lid with 96 cylindrical tubes (Figure

1C). Tubes on the c.bird lid provide 96/24 fluidic channels, allowing air to be pumped into and aspired out of each well in a standard cell culture plate. Pneumatic connection with these channels and actuation by the control system provides continuous reciprocating mixing in each well with working volumes of 150-200 µL in 96-well plates (Figure 1D). With the c.bird, a 3D suspension culture environment can now be achieved for cell culture scales as small as 150-200 µL volume in 96-well plates. As such, the c.bird can imitate a shaker-flask culture environment, providing new possibilities for better cell growth, clone profile predictability and accelerated CLD process from the early stages.

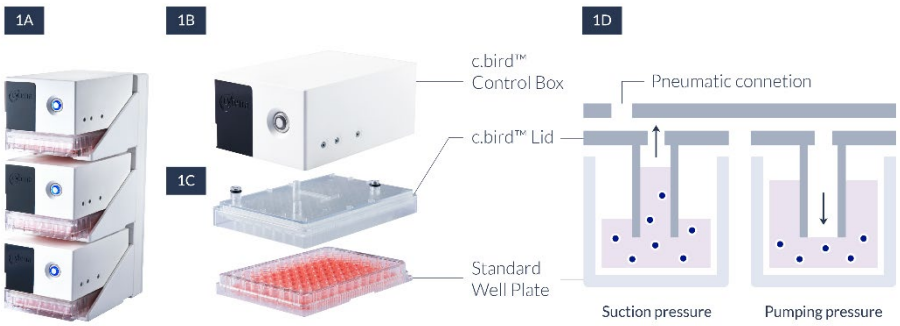


Figure 1. A) The c.bird system includes one docking station and three control units. The control unit is composed of B) a control box and C) a consumable lid and a standard 96-well plate. D) The principle of continuous reciprocating mixing provided by the c.bird system.

Materials and methods

CHO-K1 mAb expressing cell line was used for the study. The cell line was adapted to suspension culture in a chemically defined and animal-component-free medium (CD Hybridoma medium #11279-023 by GibCo). Standard 96-well plates (Eppendorf #0030730011, Germany) were used for all experiments. Comparison studies were performed with cells cultivated in standard static culture, 30 mL shaker-flask culture (shaking speed: 130 rpm; orbit:19 mm) and c.bird suspension culture in a 37°C, 5% CO_2 incubator environment.

Cell numbers and cell viability were counted with an automated cell counter TC20 from Bio-Rad. Cell counts on different time points for the same conditions were performed on unique wells to ensure no perturbations to cell growth and count accuracy. Cell growth and protein production were compared. Titer measurement was performed with the ELISA kit (E88-104) from Bethyl Lab. Data were analyzed by an unpaired t-test, one-way analysis of variance (ANOVA.) Significance of p value is listed as the following: 0.12(ns), 0.033(*), 0.002(**), 0.0002(***) and <0.001(****). Data are shown as mean ± SD.

Results and discussion

This experiment is designed as shown in Figure 2A. We investigated the daily cell growth/doubling time/protein yields of mAb CHO-K1 cell line in two different scales from 96-well plates (200 µL) to 30 mL shaker flasks and compared two different culture conditions in 96-well plates: standard static culture and c.bird suspension culture. Our results show that the c.bird system with suspension culture exhibited superior performance compared to static culture and exhibited comparable cell line profiles to shaker-flask culture.

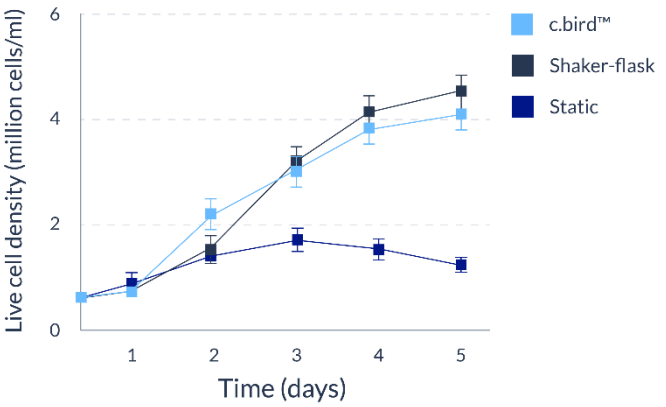
After culturing the cells for 5 days, both the c.bird suspension culture and the shaker-flask culture achieved live cell densities of 4.4 x 106 cells/mL and 4.9 x 106 cells/mL, respectively, while the static culture only reached 1.2 x 106 cells/mL on Day 5 (Figure 2B). The 3.5-fold difference in cell density shows that the c.bird suspension culture provided more optimal growth conditions for maximizing cell growth compared to static

culture. Importantly, the c.bird suspension culture growth profile closely mimics late stage shaker-flask culture profile, a feature that is important for early cell clone selection and predictability. Similarly, total cell density per condition shows the same tendencies. The 96-well c.bird culture and shaker-flask culture achieved total cell densities of 5.2 x 106 cells/mL and 5.7 x 106 cells/mL, respectively, while the static culture only reached 2.0 x 106 cells/mL on Day 5 (Figure 2C). The data provides strong evidence that the c.bird culture offered the most optimal cell growth conditions at 96-well stage.

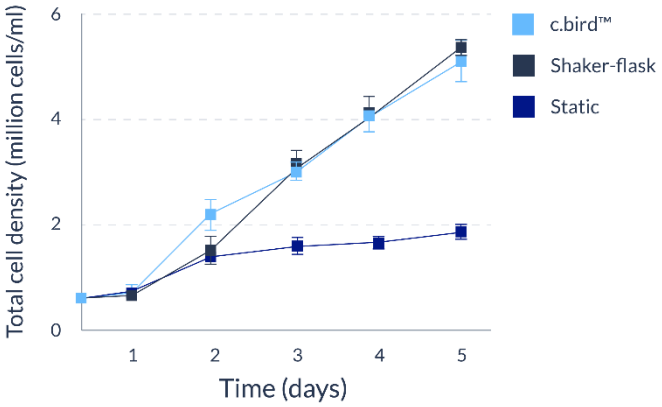
Viability comparison among three conditions shows that the c.bird culture outperforms static culture by maintaining high cell viability that is comparable to shaker-flask condition. The c.bird culture and shaker-flask culture maintained high viability until Day 5, at 84.3% and 86.3%, respectively. However, static culture dropped as low as 60.3% on Day 5 (Figure 2D). Unsurprisingly, we can show that the c.bird significantly shortened cell doubling time from 114 hours to 38.5 hours compared to static cultures. No significant difference in doubling time of cells is observed between shaker-flask culture (35.1 hours) and c.bird culture (38.5 hours). The results indicate the high performance of the 96-well c.bird and its ability to provide shaker-flask growth conditions at an early stage (Figure 2E).

Lastly, we can show that protein production of the cell lines in each condition also exhibits the same pattern. Fold change of protein yields of c.bird is significantly higher than that of static culture (0.7-fold higher), while fold change between the c.bird and shaker flasks have no significant difference (2.86-fold vs. 3.21-fold) (Figure 2F).

2A



2B



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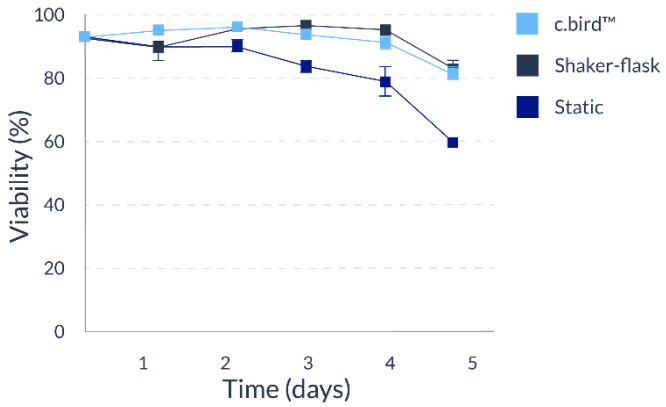
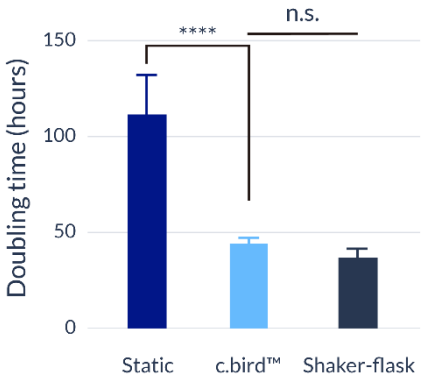
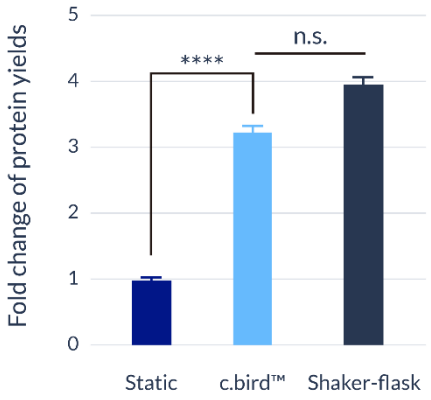


2D



2E



2F

Figure 2. **A)** Diagram of our experimental design. **B)**, **C)** and **D)** Daily total/live cell concentrations and cell viability of three groups: 96-well static culture, 96-well c.bird culture and shaker-flask culture. **E)** and **F)** Doubling time and fold change of protein yields comparisons within three culture conditions.

Conclusion

The study shows that the c.bird improves mammalian cell growth in 96-well culture environment in three important aspects: 1) cell growth, 2) doubling time and 3) protein yields. We also demonstrate that the c.bird system closely mimics the shaker-flask culture in terms of cell growth profiles and protein yield.

In summary, the c.bird enables early transition to suspension cell culture with higher cell growth rate in standard 96-well plates, potentially providing better translation to large-scale shaker-flask/bioreactor conditions for later CLD processes.

References

1. Sandner V, Pybus LP, McCreath G, Glassey J. Scale-down model development in ambr systems: An industrial perspective. *Biotechnology Journal*. 2019; 14(4): e1700766. DOI:10.1002/biot.201700766.

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