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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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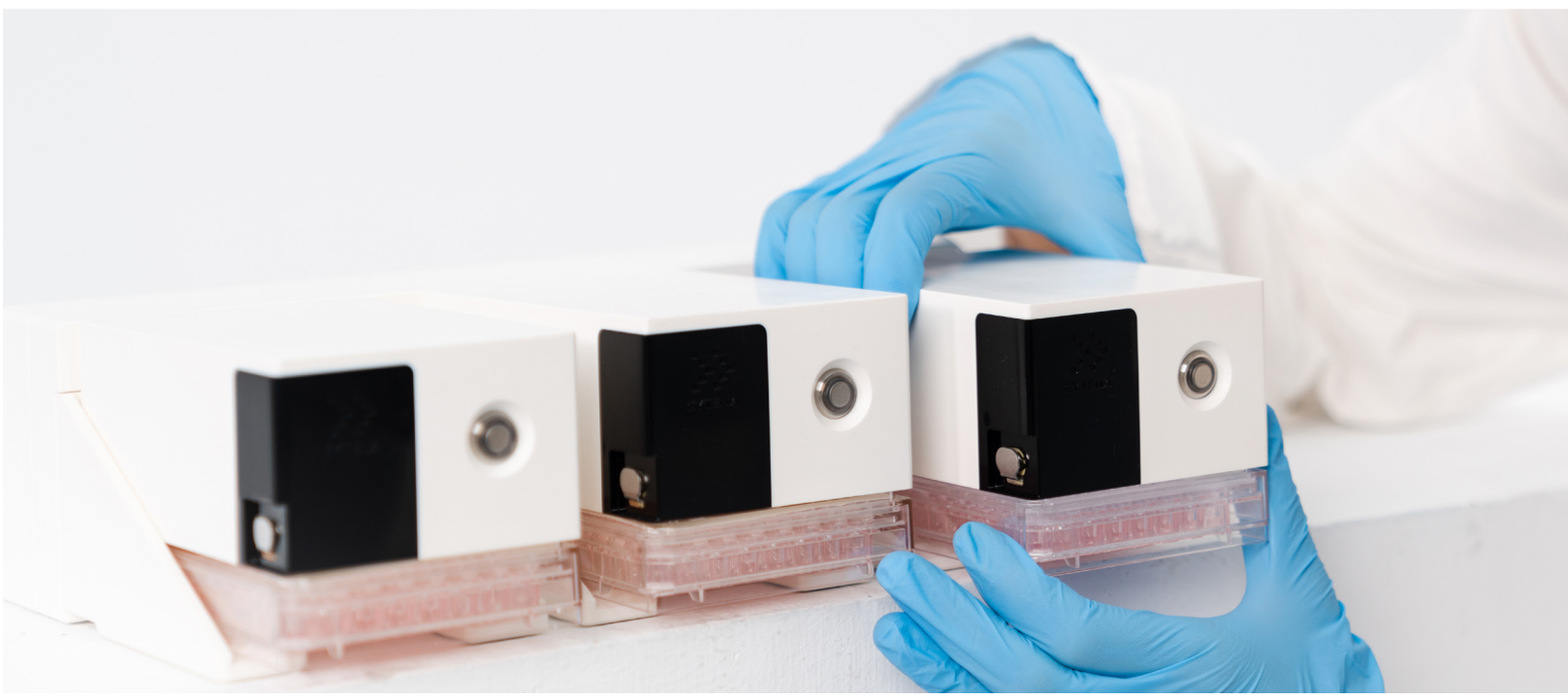

Abstract

Our study demonstrates that the C.BIRD™ microbioreactor for cell line development improves mammalian cell growth in 96-well culture environments and closely imitates the shaker flask culture for cell growth profiles and protein yields. Traditional cell line development (CLD) processes require significant times to find optimal clones due to inconsistent cell line profiles generated between early and late stage development. The C.BIRD system, a new cell culture innovation, provides solutions to this problem by reducing the cell profile differences between the two stages and providing an optimal suspension culture condition earlier in the CLD process. In this study we cultured Chinese hamster ovary (CHO) cell lines that produce monoclonal antibodies with and without the C.BIRD system, and compared cell profiles generated with those of cells cultured in a shaker flask culture environment. The results show that C.BIRD improved live/total cell growth and cell viability, and had superior comparability with those of large-scale shaker flask cultures. In addition, cell doubling time and relative protein yields improved significantly, with no statistical difference when compared to shaker flask cultures.

Introduction

The biopharmaceutical industry is asking for accelerations to cell line development (CLD) workflow so that the new biologics can gain faster regulatory approval. Traditional CLD, however, can take months or up to a year to identify optimal cell clones. One reason for such a long timeline is the difficulties encountered when picking the right clone early in CLD. 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.). That, unfortunately impedes identification of correct 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 process is crucial for enabling early identification of cell clones with correct and sustainable profiles during the CLD process.

High-throughput technologies are increasingly used in CLD processes to solve this problem. These technologies provide a suspension culture environment in small-scale wells/flasks with enhanced oxygen supply. In this study, we demonstrated a new system with the C.BIRD microbioreactor will enable early stage suspension cell culture in standard 96-well plates. The C.BIRD system is compact and easily fits in a standard incubator. It has a docking station that holds three sets of C.BIRD systems (**Figure 1A**). The 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**).

The 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. The C.BIRD can imitate a shaker flask culture environment and an accelerated CLD process from the early stages, thus providing new possibilities for better cell growth and clone profile predictability.

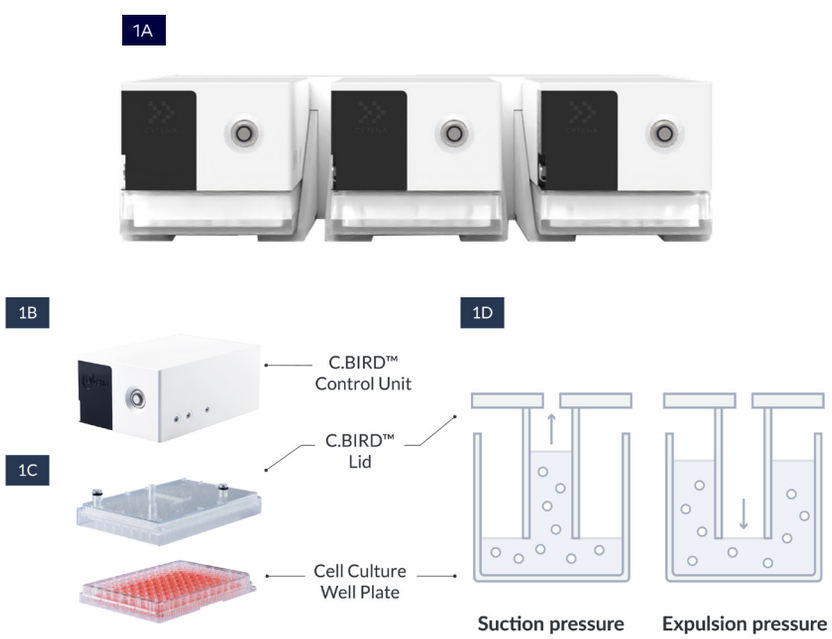

Figure 1. A) *The C.BIRD system includes one docking station and three control units. The control unit is composed of* ***B)*** *a control box and* ***C)*** *a consumable lid and a standard 96-well plate.* ***D)*** *The principle of continuous reciprocating mixing provided by the C.BIRD system.*

Materials and methods

Suspension type CHO-K1 and CHO-S mAb expressing cell lines were used for the study. The cells were cultured in a chemically defined and animalcomponent-free CD Hybridoma medium (Gibco#11279-023) with Glutamine (Gibco #25030-081), Penicillin-Streptomycin (Corning #30-0020-CI), Cholesterol (Gibco #12531-018) and Anti-Clumping Agent (Gibco #01-0057AE). 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 (Corning #431134, 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 (Bio-Rad TC20). Cell counts on different time points for the same conditions were performed on unique wells to ensure no perturbation to cell growth and count accuracy. Cell growth and protein production were compared. Titer measurement was performed with the ELISA kit (Bethyl Lab #E88-104). Data were analyzed by an unpaired t test and one-way analysis of variance (ANOVA). Significance of P value is listed as the following: >0.05 ns, <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****).

Results and discussion

The design of this comparative experiment is shown in **Figure 2A**. We investigated the daily cell growth, doubling time, and protein yields of mAb CHO-K1 and CHO-S cell lines in two different scales, from 200 µL 96-well plates 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 showed 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. Importantly, the C.BIRD suspension culture growth profile closely mimicked a late-stage shaker flask culture profile, an important feature for early cell clone selection and predictability.

CHO-K1

After culturing the cells for five days, 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 showed that the C.BIRD suspension culture provided more optimal growth conditions for maximizing cell growth compared to static culture. Similarly, total cell density per condition showed 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 provided strong evidence that the C.BIRD culture offered the most optimal cell growth conditions at the 96-well scale.

Viability comparison among three conditions showed that the C.BIRD culture outperformed static culture by maintaining high cell viability that is comparable to the 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, 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 was observed between shaker flask culture (35.1 hours) and C.BIRD culture (38.5 hours). The results specified the high performance level of the 96-well C.BIRD and its ability to provide shaker flask growth conditions at an early stage (**Figure 2E**).

Lastly, the protein production of the cell lines in each condition exhibited the same pattern. Fold change of protein yields found with C.BIRD was significantly higher than that of static culture (0.7-fold higher), while fold change between the C.BIRD and shaker flasks had no significant difference (2.86-fold vs. 3.21-fold), as shown in **Figure 2F**.

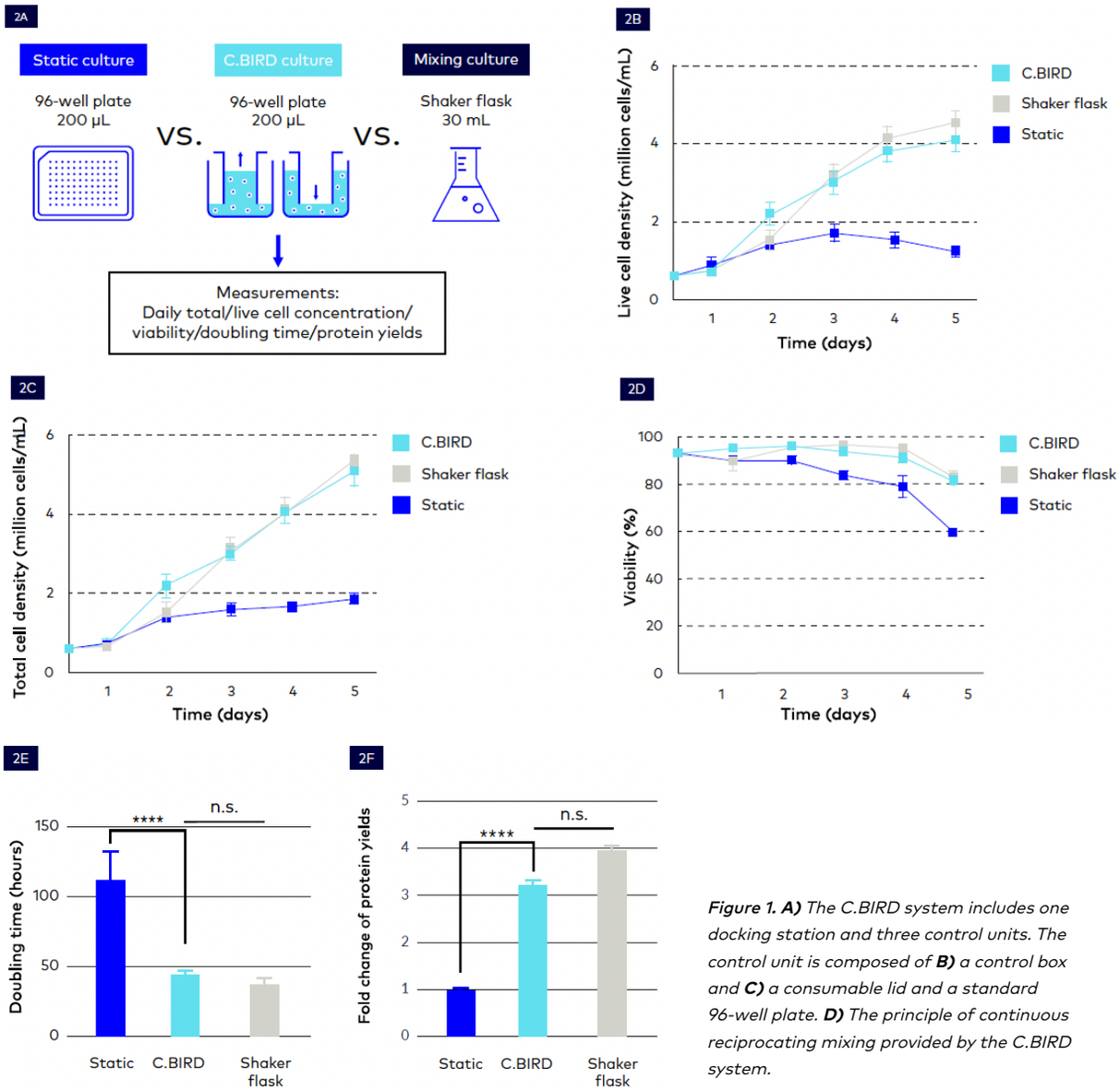

Figure 1. A) *The C.BIRD system includes one docking station and three control units. The control unit is composed of* ***B)*** *a control box and* ***C)*** *a consumable lid and a standard 96-well plate.* ***D)*** *The principle of continuous reciprocating mixing provided by the C.BIRD system.*

CHO-S

On day 5, the C.BIRD suspension culture achieved live-cell densities of 5.54 x 106 cells/mL, shaker flask reached 7.07x106 cells/mL, while the static culture only reached 1.68 x 106 cells/mL. (Figure 3A). In terms of total cell density, the 96-well C.BIRD culture achieved 6.48 x 106 cells/mL, shaker flask reached 7.34 x 106, while the static culture only reached 2.33 x 106 cells/mL on day 5 (Figure 3B). The approximately 3-fold difference in cell density between C.BIRD suspension culture and static culture further confirmed that the C.BIRD suspension culture provided more optimal growth conditions than static culture.

The viability comparison on day 5 showed that the C.BIRD culture outperformed static culture by maintaining a higher cell viability. The C.BIRD culture maintained high viability at 86% and was relatively similar to the shaker flask culture at 99%. However, static culture dropped to a low viability at 72% (Figure 3C).

The C.BIRD significantly shortened cell doubling time on day 4 from 48.6 hours to 28.1 hours compared to static cultures, while the doubling time of shaker flask culture was 26.2 hours. These results confirmed the high performance of the 96-well C.BIRD and its ability to provide shaker flask growth conditions at an early stage (Figure 3D).

Lastly, protein production of the cell lines in each condition exhibited the same pattern that validated our statement. On day 5, the CHO-S cells in C.BIRD suspension culture produced 23.52 µg/mL of mAb, while the cells in shaker flask culture produced 24.68 µg/mL of mAb. The protein yield of C.BIRD was also significantly higher than static culture (15.08 µg/mL) by 1.5-fold (Figure 3E).

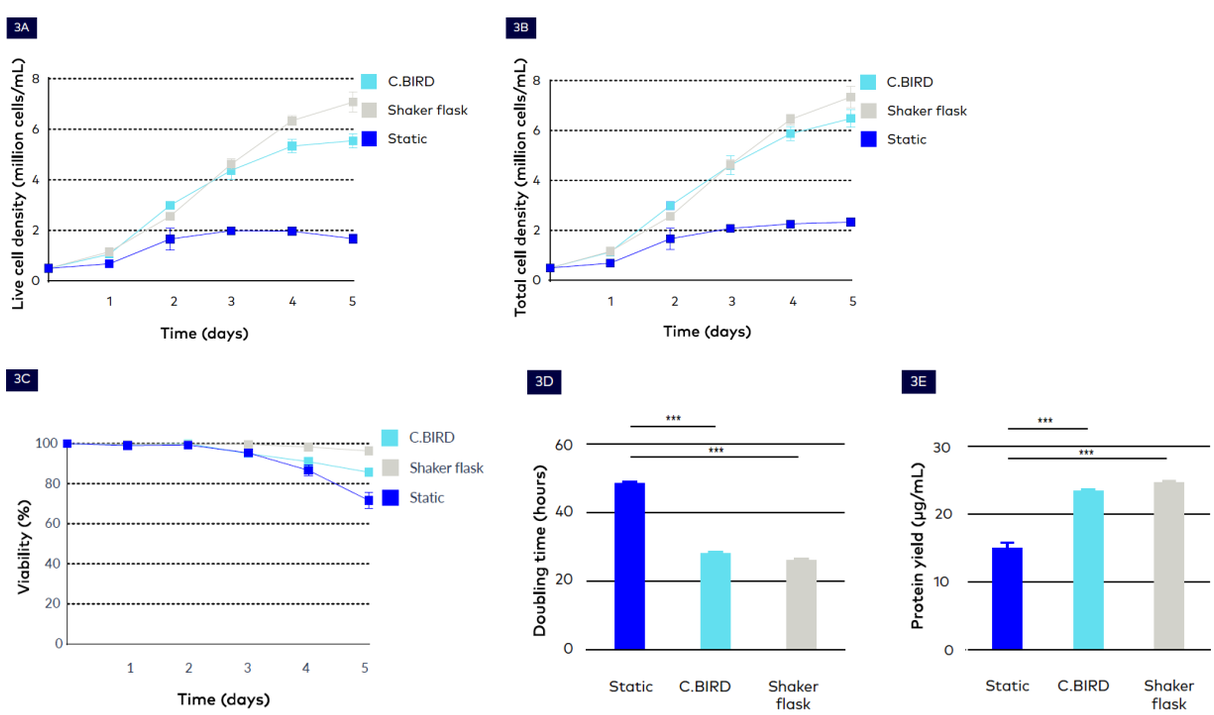

Figure 3. A), B) *and* ***C)*** *daily live cell/total cell density and cell viability of three groups: 96-well static culture, 96-well C.BIRD culture and shaker flask culture.* ***D)*** *and* ***E)*** *doubling time and protein yields comparisons within three culture conditions.*

Conclusion and future direction

This study showed that the C.BIRD microbioreactor for cell line development improves mammalian cell growth in a 96-well culture environment in three important ways: 1) cell growth, 2) doubling time and 3) protein yields.

We also demonstrated that the C.BIRD system closely mimicked the shaker flask culture in cell growth profiles and protein yield. In summary, the C.BIRD enables earlier transitions to suspension cell culture with a higher cell growth rate in standard 96-well plates, potentially providing better translation to large-scale shaker flask/ bioreactor conditions for later CLD processes.

Reference

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