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C.BIRD | Incorporation of Optimized Cell Culture Environment in 24-well Plates for Suspension Cells

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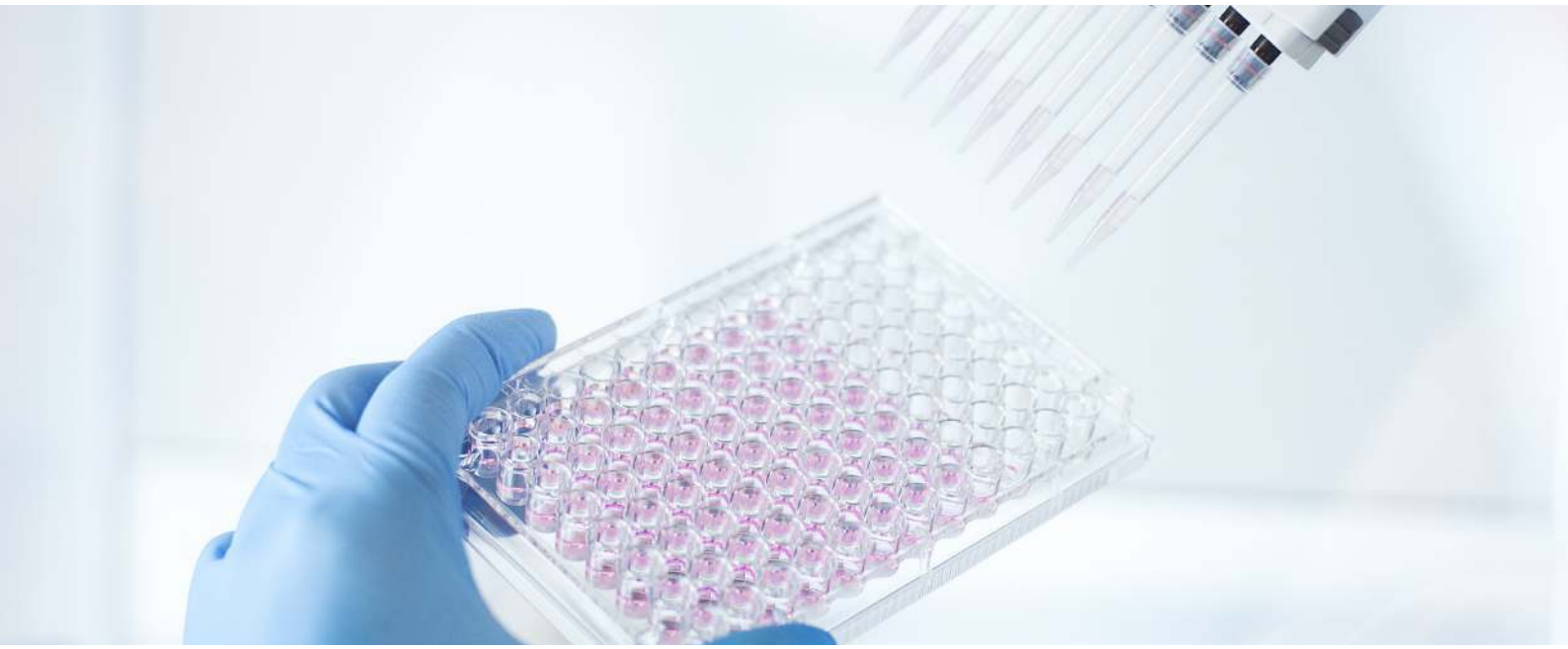

Abstract

Researchers in biopharmaceutical industries have been searching for optimal cell culture methods that provide consistent cell culture conditions from 96-well plates to bioreactors. Having consistent cell profiles of desirable cell lines during this upscaling process provides predictable outcomes and expedites cell line development (CLD). In this study, we aimed to improve the cell proliferation rate of suspension cell lines in 24-well plates by introducing a novel C.BIRD™ cell culture method. Our C.BIRD method provides automatic agitation in each well, creating a suspension culture environment and better circulation of the nutrients in the medium. In this study, Chinese hamster ovary (CHO) cell lines were adopted as models to present the difference in cell profiles under variable cell culture methods, including conventional static culture and shaker flask culture. This study showed that the C.BIRD cell culture method improves cell growth and achieves protein production profiles comparable to traditional cell culture methods.

Introduction

Scientists face increasing pressure to accelerate CLD as more biologics, like engineered bispecific antibodies and antibody-cytokine fusions, are being introduced. However, CLD is notorious for its tedious and time-consuming steps. A challenge to speed up the laborious upscaling process is the inconsistent cell growth profiles of clones and the low predictability of clones at various stages of CLD. Another challenge includes overcoming a low proliferation rate of cells in early stages because of inferior culture environments for suspension cells. Conventional suspension cell culture methods include static cultures, shaker flask cultures, and bioreactors, which vary in volume, oxygen transfer rate, shear stress, and nutrient perfusion efficiency. The differences in cell growth environments also create unaligned cell profiles in cell growth, post-translational modifications, and titer, which lead to poor predictability and consistency in selected clones during the upscaling process.

The CYTENA BPS C.BIRD microbioreactor for 96-well standard plates has already demonstrated remarkable improvements in cell growth and protein production. Subsequently we optimized the C.BIRD's suspension cell culture method in 24-well standard plates to connect the entire CLD process for enabling suspension cell cultures. In the C.BIRD cell culture method for 24-well standard plates, the same principles used with the C.BIRD for 96-well standard plates were applied and helped establish an optimized suspension culture environment for suspension cells.

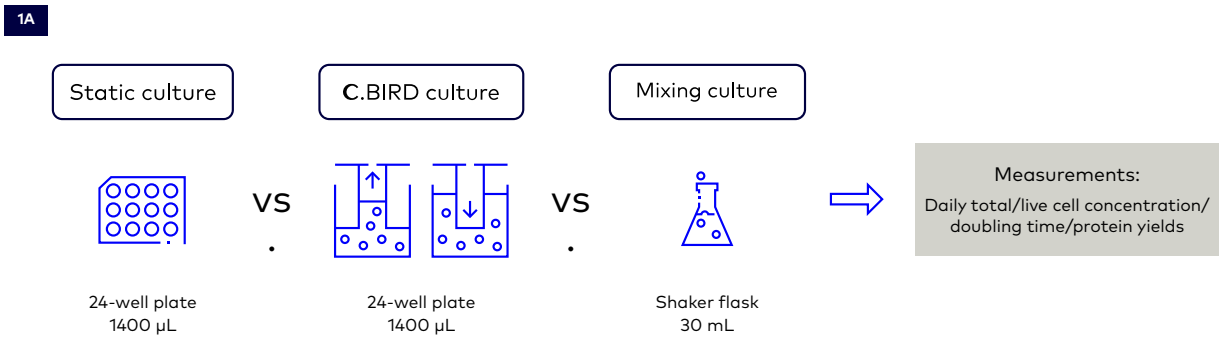

Figure 1. *Diagram of experiment design. Comparing three different types of cell culture methods. The measurements in these groups provided individual cell profiles under different culture environments.*

Materials and methods

Suspension type CHO-K1 and CHO-S mAb expressing cell lines were used in this study. The cells were cultured in chemically defined and animal-component-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 24-well plates (Greiner, #662102) were used as 24-well standard plates for the C.BIRD and static cultures with 1,400 µL of total volume per well. While the shaker flask (Corning, 431143) culture was set on an orbital shaker with a diameter of 19 mm at 130 rpm with 30 mL of total volume. The cells and C.BIRD devices were cultured in 37°C, 5% CO_2 water-jacketed incubator for 3 days of continuous culture. Cell numbers and cell viability were counted with a cell counter (Bio-Rad, TC20). Cell numbers on each day were measured independently in different wells in the same plate to avoid additional perturbations during the cell culture process. Each well, after cell count, was supplemented with the same amount of culture medium as those taken for the cell count and were not measured again during the days that followed. This helped maintain total liquid volume throughout the experiment to lower variations resulting from the evaporation effect.

Protein yields were measured by ELISA Kit (Bethyl Lab, E88-104). Statistics were performed by one-way ANOVA. Significance of *P* value is listed as follows: >0.05 ns, <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****). Data are shown as mean ± SD.

Results and discussion

CHO-K1

The experiment was designed as shown in **Figure 1**. Total cell density, live cell density, doubling time, and protein yields of monoclonal antibody (mAb) CHO-K1 cell lines were measured from static culture, C.BIRD culture, and shaker flask culture. Starting from 0.33 x 10^6 cells/mL, total cell density and live cell density of each group were measured daily in triplicate during a 3-day experiment.

On day 3 of the experiment, live cell density and total cell density of the C.BIRD group reached an average of 2.87 x 10^6 cells/mL and 3.06 x 10^6 cells/mL, respectively, which were both significantly higher than measurements in the static culture group (**Figure 2A** and **2B**). This indicated that the C.BIRD group improved cell culture environment in a 24-well plate, hence providing a higher carrying capacity compared to the static culture.

Additionally, the C.BIRD group significantly shortened the average doubling time of CHO-K1 cells from 35 hours to 24 hours compared to the static group. However, there was no significant difference when compared to the shaker flask group at 23 hours (**Figure 2C**). This suggests that the C.BIRD method enhances the cell proliferation rate in CHO-K1 cells and provides cell proliferation profiles similar to shaker flask culture in the experiment's early stages.

The C.BIRD group showed a protein yield comparable to the shaker flask group and was nearly 4-fold higher than that of the static group (**Figure 2D**). Rapid growers, like those in the C.BIRD group, achieved higher C.BIRD/shaker flask ratio with an average of 0.91 for titer and 1.10 for VCD on day 3. Slow growers, like those in the static group, reached only 0.26 for titer and 0.53 for VCD at the same time point. This showed the C.BIRD method on 24-well standard plates could enable screening of desirable clones with cell profiles that retain high consistency in late stages of the CLD process. The C.BIRD method also improved predictability of clones at the 24-well stage by mimicking cell culture environment as in shaker flask.

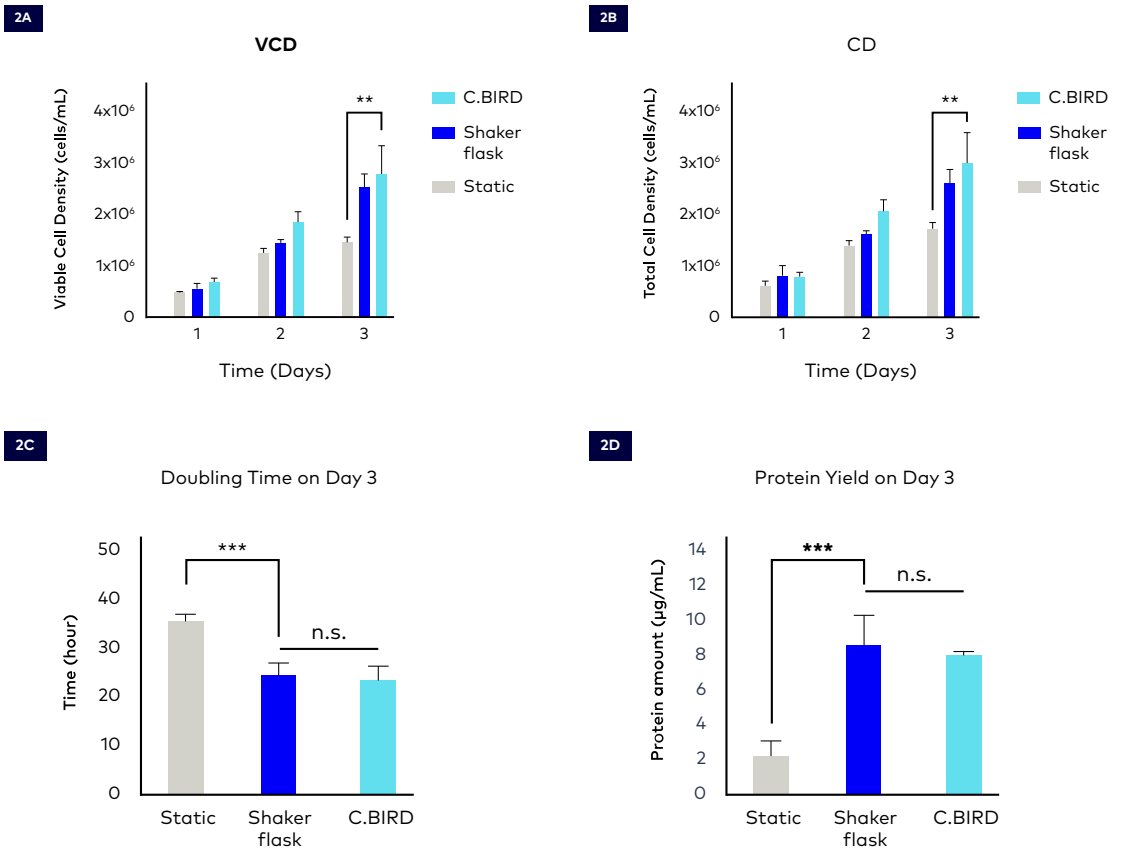

***Figure 2. A** and **B)** Daily live/total cell density of the 3 groups: 24-well static culture, shaker flask culture and 24-well C.BIRD culture. **C)** Doubling time of the three groups on day 3. **D)** Protein production of the three groups on day 3. Statistics were performed by one-way ANOVA. Significance of P value is listed as follows: > 0.05 (ns), ≤0.05 (*), ≤0.01 (**), ≤0.001 (***), and ≤0.0001 (****). Data are shown as mean ± SD.*

CHO-S

As with the CHO-K1 cell line, the total cell density, live cell density, doubling time, and protein yields of the mAb CHO-S cell line were measured from static culture, C.BIRD culture, and shaker flask culture. The initial seeding density of the CHO-S cell line was from 0.5 x 10^6 cells/mL. Total cell density and live cell density of each group were measured daily in triplicate in a 3-day experiment.

On day 3 of the experiment, live cell density and total cell density of the C.BIRD group reached an average of 2.67 x 10^6 cells/mL and 3.03 x 10^6 cells/mL respectively, which were both numerically higher than the live cell density and total cell density of the static culture group, 1.76 x 10^6 and 1.63 x 10^6 respectively (**Figure 3A** and **3B**). Additionally, the C.BIRD group significantly shortened the average doubling time of CHO-S cells on day 3 from 42.43 hours to 29.77 hours compared to the static group (**Figure 3C**). These results suggest that the C.BIRD method is able to enhance the cell proliferation rate in CHO-S cells at the early stages of the experiment more efficiently than the static method.

When protein (mAb) yield was examined, cells in the C.BIRD suspension culture produced 17.01 µg/mL of mAb, while cells in static culture and shaker flask culture produced 10.22 and 21.11 µg/mL of mAb. This result showed that the C.BIRD method on 24-well standard plates can enable efficient protein production compared to the static culture method. The higher consistency of protein yield between the C.BIRD and shaker flask culture methods efficiently supported the screening of desirable clones whose cell profiles possess high consistency in the late stages of the CLD process.

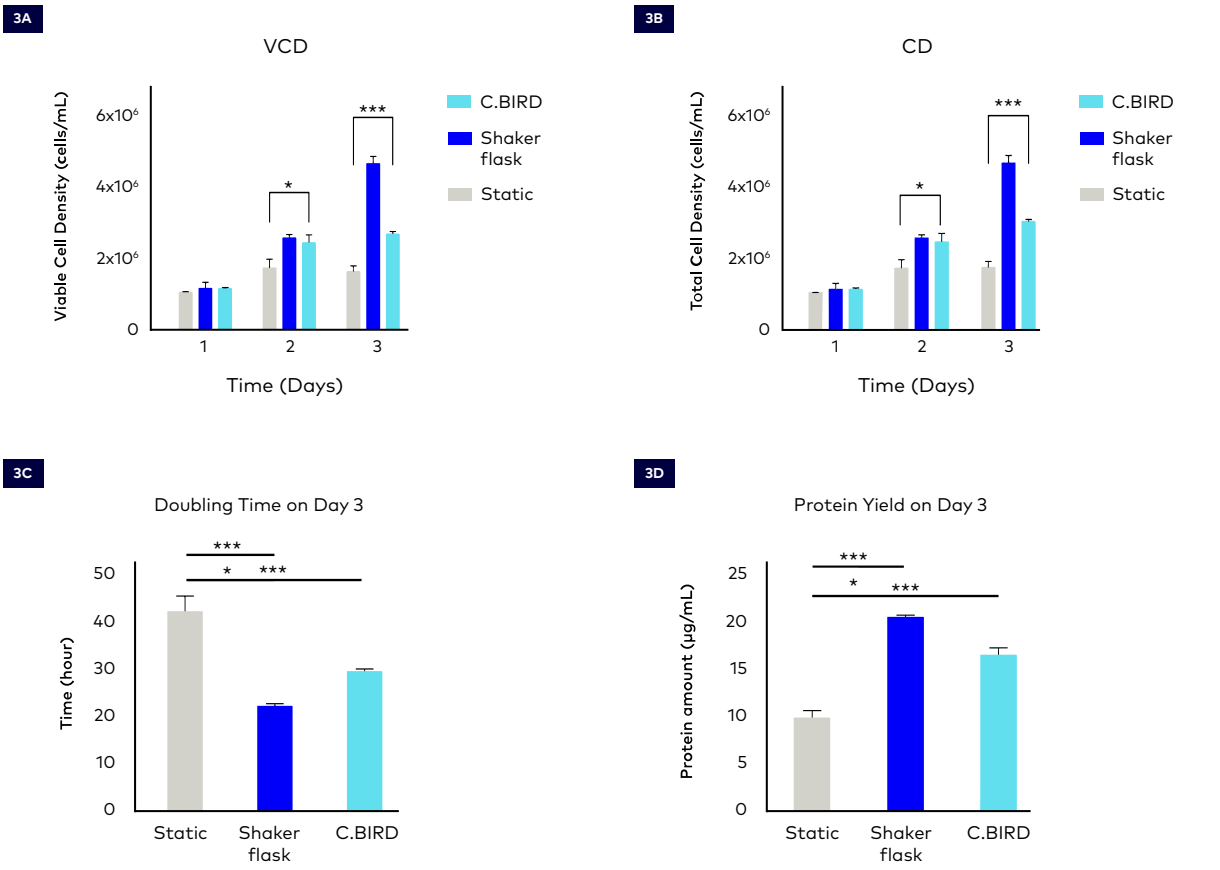

***Figure 3. A** and **B)** Daily live/total cell density of the 3 groups: 24-well static culture, shaker flask culture, and 24-well C.BIRD culture. **C)** Doubling time of the 3 groups on day 3. **D)** Protein production of the 3 groups on day 3. Statistics were performed by one-way ANOVA. Significance of P value is listed as follows: > 0.05 (ns), ≤0.05 (*), ≤0.01 (**), ≤0.001 (***), and ≤0.0001 (****). Data are shown as mean ± SD.*

Conclusion and future direction

Results show that the C.BIRD cell culture method used on 24-well standard plates improves suspension cell culture environment by (1) providing higher environment carrying capability, (2) increasing cell proliferation rate and, (3) sorting out rapid growers with comparable protein production profiles in the late stages. The high consistency of cell growth and protein yield profiles enables better predictability of biological profiles of the clones at early CLD stages. It also avoids additional time and costs spent on picking undesirable clones. In summation, the C.BIRD cell culture method on 24-well plates optimizes suspension cell culture with higher cell proliferation rate and protein production, and selecting rapid growers in the population. This C.BIRD method on 24-well plates finalizes the workflow of CLD and offers a more amenable culture environment for suspension cell lines.

References

1. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proceedings of the National Academy of Science of the United States of America*. 1979; 76(3): 1,274-1,278. DOI:10.1073/pnas.76.3.1274.
2. Lai T, Yang Y, Ng SK. Advances in Mammalian cell line development technologies for recombinant protein production. *Pharmaceuticals*. 2013; 6(5): 579-603. DOI:10.3390/ph6050579.

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