Application Note

c.bird™ | A microbioreactor that enables suspension culture in 96-well plates for improved cell growth and recombinant protein yield

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Introduction

In the biopharmaceutical industry, many biological drugs are produced in mammalian cells. Cell line development (CLD) is used to determine which cell lines have the highest recombinant protein production and are also stable during large-scale manufacturing. In the CLD workflow, cell lines undergo incremental scaling of culture from static (in 96- or 384-well plates) to suspension (in 125-mL or 250-mL flasks) prior to banking or further scaling up in bioreactors. Cell culture early in CLD is limited to static as the size of the well plates and number of cells limit the ability to agitate the culture. However, the static culture environments in 96- and 384-well plates are restricted to a 2D surface and oxygen supply can be limited. Additionally, cell line performance in static culture may not correlate with its performance in suspension [1]. Therefore, the ability to introduce suspension culture and mimic the culture environment of later stages (e.g., shaking flask) from the beginning of the CLD workflow can help pharmaceutical companies shorten the CLD process and better predict cell line behavior.

The c.bird™ microbioreactor enables early-stage mammalian cell culture suspension in standard 96-well plates. The c.bird™ device (**Fig. 1a**) consist two parts, 1. an autonomous control system and 2. a consumable functional lid for mixing culture media and cells. The device is compatible with a standard 96-well plate. The novel functional lid with 96 fluidic channels is inserted into a well plate. Pneumatic connection with these channels and actuation by the control system provides continuous reciprocal mixing in each well with working volumes of ~100 µL (**Fig. 1b**). The c.bird™ system is compact, and a docking station for three c.bird™ control systems can readily fit a standard incubator (**Fig. 1c**). With the c.bird™, suspension culture in standard 96-well plates and incubators are now possible. Additionally, the c.bird™ has significantly higher cell concentration and recombinant protein yield compared to static culture.

Materials and Methods

CHO-K1 mAb expressing cell line was used for the study. The cell line was adapted to the culture suspension in a chemically defined and animal-component-free medium (CD Hybridoma medium #11279-023 by GibCo). Standard 96-well plates (Eppendorf, Germany) were used for all experiments. Comparison studies were performed with cells cultivated in standard static culture and c.bird™ suspension culture in a 37°C, 5% CO_2 incubator environment. Resulting cell growth and protein production were compared. Abcam ELISA kit (Ab10047) and PAIA Biotech kit (PA-104) were used for titer measurements. The PA-104 is a fast, high-throughput assay (384 samples) that only requires 5 µl of sample and is performed in 15 minutes. For measurement readout, the SpectraMax iD3 and SYNENTEC NyONE® were used.

Results and Discussion

The c.bird™ suspension culture in 96-well plates can improve cell proliferation, recombinant protein yield and volume-specific productivity (QP) compared to traditional static culture. We investigated two different cases with different initial conditions. However, in both cases, the c.bird™ with suspension culture exhibited better performance than static culture.

Case 1: The initial cell concentration for both plates (static culture and c.bird™ suspension culture) was $1.5x10^6$ cells/mL. Both plates were placed into an incubator and cultured for 3 days. Cell density was measured by Bio-Rad TC20™ Automated Cell Counter. For protein yield determination, supernatant samples were measured using Abcam Ab10047 ELISA kit and SpectraMax iD3.

Case 2: The initial cell concentration for both plates (static culture and c.bird™ suspension culture) was $0.5x10^6$ cells/mL. Both plates were placed in an incubator and cultured for 5

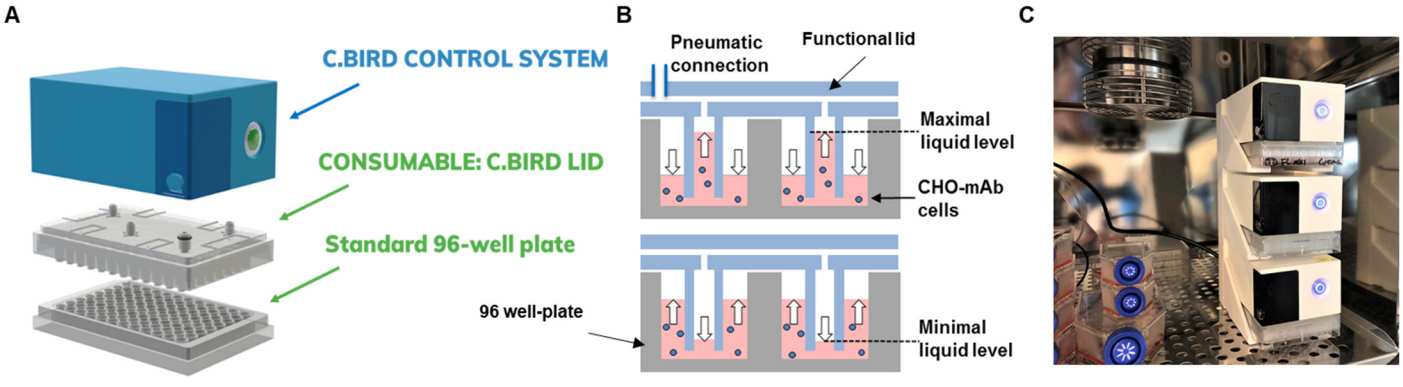

Figure 1. **A.** c.bird™ for cell clone early-stage suspension culture, including c.bird™ control system, c.bird™ lid (consumable), and a standard 96-well plate. **B.** c.bird™ working principle **C.** Picture of three c.bird™ control systems and its docking stations inside a standard incubator.

days. Cell density was measured using a hemocytometer. For protein yield determination, supernatant samples were measured with PAIA Biotech PA-104 kit and SYNENTEC® NyONE.

In case 1, the c.bird™ suspension culture achieved a cell density of $4.2x10^6$ cells/mL, but the static culture only reached $1.8x10^6$ cells/mL after 3 days of culture. And in case 2, c.bird™ suspension culture achieved a cell density of $3.1x10^6$ cells/mL, but the static culture only reached $1.9x10^6$ cells/mL after 5 days of culture (**Fig. 2**).

The c.bird™ also demonstrated ~2.5-3.8 times higher recombinant protein yield than static culture. (**Fig. 3**). For the relative volume-specific productivity (QP), the c.bird™ suspension culture generated ~1.8-2.6 times higher QP than static culture (**Fig. 4**).

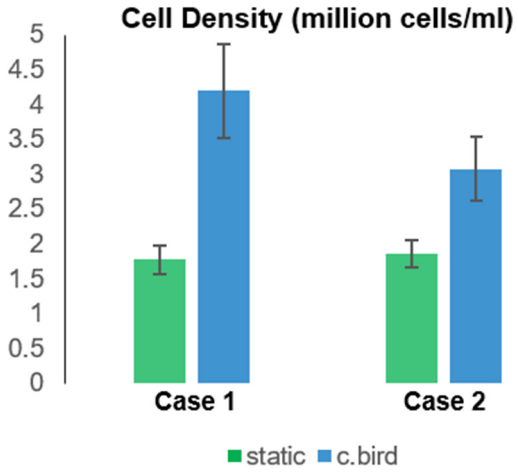

Figure 2. Experimental result of CHO mAb cell density of static culture and c.bird™ suspension culture.

Conclusion

This study demonstrates that the c.bird™ improves mammalian cell line culture conditions over static culture in the early stages of CLD. The c.bird™ achieves early transition to cell growth in suspension and increased oxygen transfer rate in standard 96-well plates with only 100 µL cell suspension. Compared to traditional static culture, the results show that c.bird™ suspension culture enhanced cell growth and significantly increased recombinant protein yield and volume-specific productivity (QP) per cell. Overall, the system is compact and can easily integrate into established CLD workflows for improved cell line performance.

Reference

1. A. Porter, Selection Strategies for Isolating Desirable GS-CHO Cell Lines, CLD & Eng. 2008.

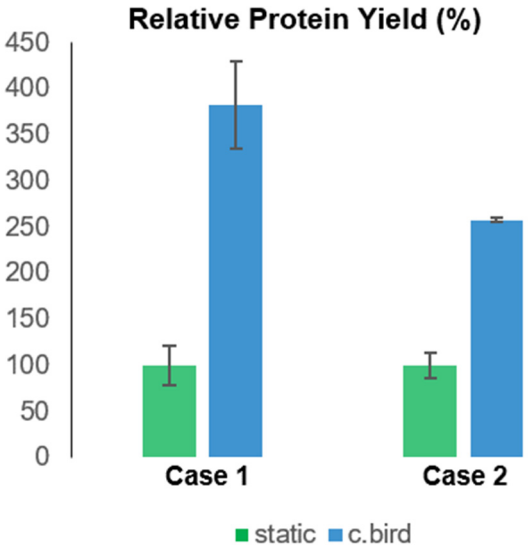

Figure 3. The comparison of protein yield between static culture and c.bird™ suspension culture.

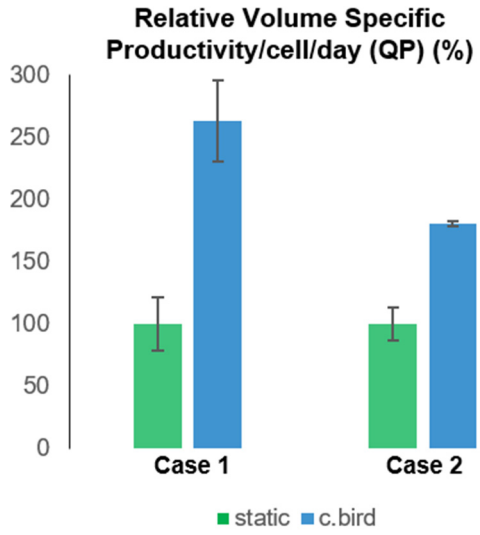

Figure 4. The comparison of relative volume-specific productivity between static culture and c.bird™ suspension culture.

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