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APPLICATION NOTE

C.BIRD | An Innovative Cell Culture Method for Hybridoma in Early Stages

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Abstract

Hybridoma technology is the primitive methodology when it comes to monoclonal antibody (mAb) isolation. As the other methods for developing mAbs suffer from the low growth rate and poor productivity under the low initial density of the cells, hybridoma technology faces the same obstacles. By growing single colonies in microwells, the cells endure an extended time in static culture, in which the concentration of nutrients and oxygen in the medium decreases with time. Low cell density and productivity results in a challenging situation for either clone expansion or proceeding with the early-stage analysis. Here, we introduce the C.BIRD™, which offers continuous mixing in 96-well plates, to improve the culture environment for hybridoma technology. This method provides a higher diffusion rate of oxygen and a more homogeneous culture environment for suspension culture. With the help of the C.BIRD method, the cell growth and mAb productivity were significantly improved, which allowed the possibility of early-stage analytic studies and shorten the timeline of hybridoma development.

Introduction

Being one of the most common treatments used to target diseases, mAbs have played a fundamental role in biomedical industries for the past decades. The high specificity, affinity, and homogeneous nature of mAbs show their strengths in various fields, including flow cytometry, immunoassays, diagnostic, therapeutic, or other applications. Among other methods, hybridoma technology stands out due to the native pairing of constant and variable regions gene recombination, and the robust and efficient production once the clones are established.

Nevertheless, the time-consuming process of selecting and expanding desirable clones has slowed down the development of hybridoma workflow. After fusing the spleen cells and myeloma cells, hybridomas were distributed into microwell plates with low cell density to develop single colonies. Due to the lack of nutrients and oxygen supplements during the long culture period, the poor growth rate of the cells was recognized as one of the major causes for the long-developing process of the technology.

In this study, we proposed a new method to distribute the nutrients and increase the oxygen diffusion in the culture medium by the C.BIRD method. The continuous mixing of the C.BIRD method provides a homogeneous suspension culture in microwell plates. In our result, hybridomas possessed a higher growth rate and mAb productivity by using

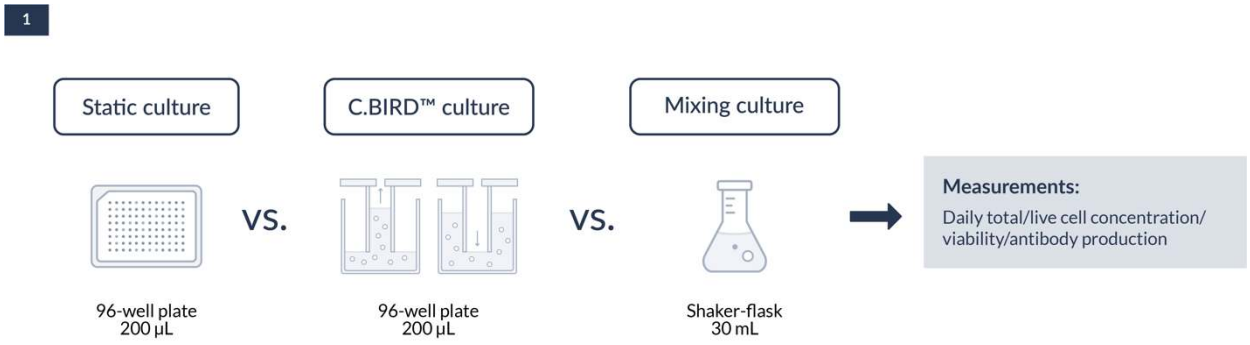

Figure 1. Diagram of experiment design.

the C.BIRD method compared to the conventional culture methods. Undoubtedly, our method shortens the expanding process, which starts from single colonies or low cell density. The C.BIRD method has opened a window for early-stage analytic screening, such as toxicology and First in Human (FIH) studies and also shortens the timeline of mAb production.

Materials and methods

A hybridoma mouse mAb-expressing cell line was used in this study. Cells were cultured in Dulbecco's Modified Eagle Medium, High glucose (#CC103-0500, GenedireX) with 10% of FBS (Gibco) and 1% of penicillin/streptomycin. Standard 96-well plates (#0030730011, Eppendorf) were applied in the study, while shaker-flasks were placed on a shaker (shaking speed: 90 rpm; orbital: 20mm) as a positive control for the C.BIRD and static microwell cultures. All the cells were cultured in 37°C, 5% CO2 water-jacketed incubator.

The initial cell concentration was 2.5 x 10^3 cells/mL in all cell culture methods. Cell number and viability were counted with an automatic cell counter (TC20 from Bio-Rad) from Day 4 to Day 7. To compare antibody production in each cell culture method, we performed IgG (Total) Mouse Uncoated ELISA kit (# 88-50400-88, Invitrogen) to measure the supernatant from the cell cultures collected on Day 7. Data were analyzed by 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 <0.0001(****). Data are shown as mean ± SD.

Results and discussion

The C.BIRD method provides a perpendicular mixing in the multi-well plates, which introduced a better circulation of the nutrients and oxygen by the constant flows. To compare, if the mixing of the C.BIRD method could give advantages to the cell culture of hybridoma, we designed the experiment as shown in **Figure 1**. We investigated daily total cell density, viable cell density, and viability of the three cell culture methods. The volume of both static

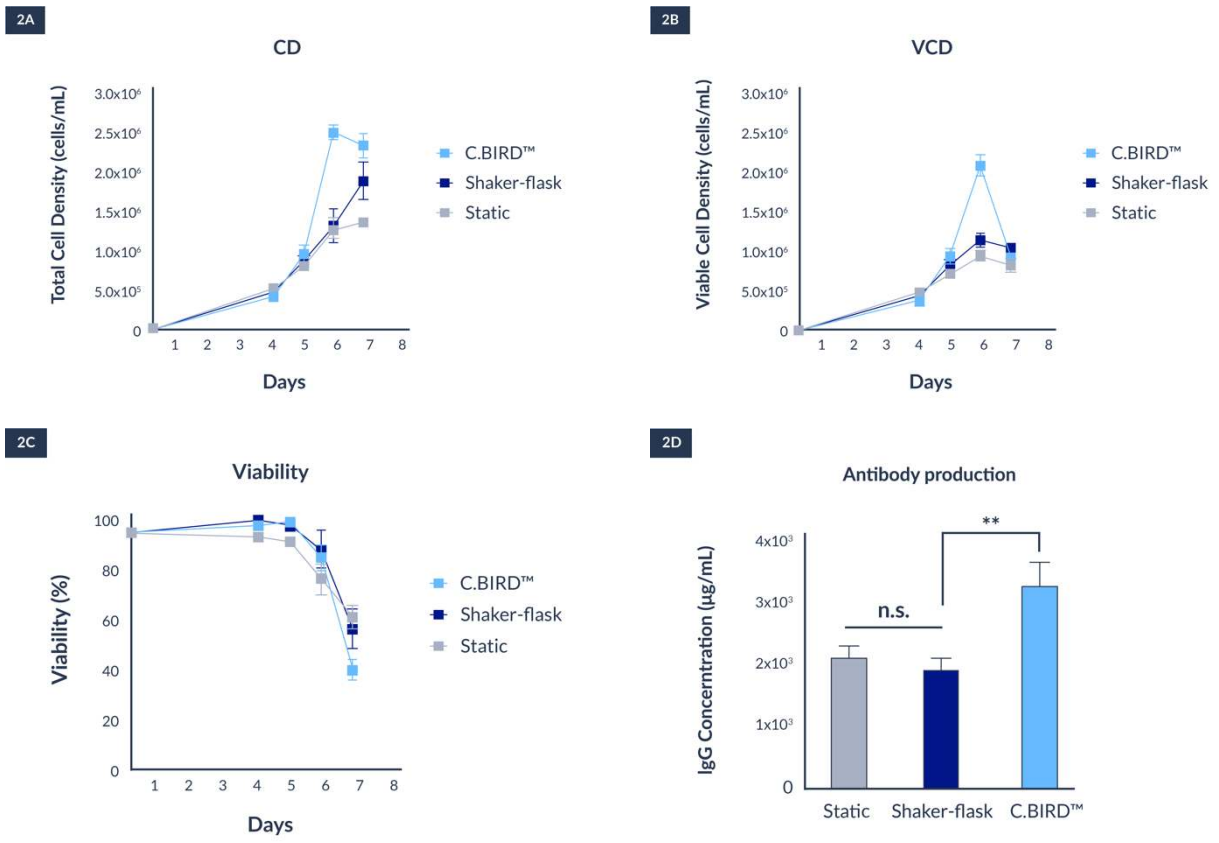

*Figure 2. The cell growth and antibody production of three culture methods in 7 days of continuous culture. The C.BIRD method facilitated the cell growth both in **A)** total cell density and **B)** viable cell density while maintaining the same **C)** viability as the other two groups. In addition, the C.BIRD group showed a significantly higher **D)** antibody production on Day 7, comparing to both static and shaker-flask groups.*

and the C.BIRD groups was 200 µL/well medium in standard 96-w plates, while shaker-flask culture was 30 mL in total.

Under continuous mixing (25s/cycle) in the C.BIRD culture in the standard 96-w plate, the total cell density peaked at 2.44 x 10^6 cells/mL on average on Day 6, which were 1.99-fold and 1.89-fold higher compared to the static and shaker-flask groups, respectively (**Figure 2A**). Additionally, viable cell density of the C.BIRD group reached an average of 2.04 x 10^6 cells/mL on Day 6, and the other two groups had only an average of 9.22 x 10^5 cells/mL and 1.11 x 10^6 cells/mL in static and shaker-flask groups, respectively. **Figure 2B**). The results showed a significant improvement in cell growth under the C.BIRD method. The method provided a more efficient way to expand the hybridoma cells from a relatively low cell density, thereby dramatically speeding up the research process after transfection.

On the other hand, the viability in the C.BIRD group was not compromised, regardless of the high cell density (**Figure 2C**). On Day 6, while sustaining the highest viable cell density within three groups, the C.BIRD group had 83% of viability on average, close to that of the shaker-flask group (87%) and was higher than the static group (75%). The result demonstrated that the C.BIRD method could increase environmental carrying capability and helped to expand the population. While the viability hit around 50% in either group, mouse mAb was collected from the supernatant on Day 7. We observed a high concentration of mAb in the C.BIRD group, which reached an average of 3309.2 µg/mL, and outcompeted that in both static and shaker-flask groups (**Figure 2D**).

Conclusion

While many researchers are suffering from low productivity and cell density, the C.BIRD method provided an innovative way to boost cell growth in an extremely low seeding cell density. Our study showed a magnificent improvement in 1) total cell density, 2) viable cell density, 3) maintenance of viability during long-term culture, and 4) high productivity of mAb, comparing to static culture in 200 µL/well conditions. More excitingly, the C.BIRD group outperformed the shaker-flask group in cell growth and antibody production. By using the C.BIRD method, researchers can expedite antibody selection and shorten the expansion timeline for hybridoma development.

Reference

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