APPLICATION NOTE

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C.BIRD- Augment the Human T Cell Activation in Standard 96-well Plates Using the C.BIRD Microbioreactor

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Abstract

T cell-based immunotherapies are popular cancer treatments, and require procuring a large number of T cells. T cells that are isolated from the patient need to be optimally activated and expanded in vitro, then re-infused back to the patient for fighting cancer cells. This study sought to improve the activation level of human T cells in 96-well plates by introducing a novel C.BIRD™ cell culture method. Study results showed that the C.BIRD cell culture method enhanced the activation level of human T cells, but did not cause unwanted activation. This study demonstrates a great potential to improve T cell-based immunotherapies studies by accelerating the process development workflow.

Introduction

Adoptive cell therapy (also known as cellular immunotherapy) is a cancer treatment that uses a patient's immune cells to eliminate cancer. T cell-based therapy is the mainstream immunotherapy today. There are different types of T cell therapies, such as tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, and chimeric antigen receptor (CAR) T cell therapy. All of these T cell therapies require a high number of T cells to be re-infused into a patient. Therefore, expanding the number of isolated T cells, with or without genetic engineering, is the cornerstone of T cell therapies. Accordingly, T cell activation is the critical step in researching or manufacturing T cell-based therapies, which involves gene editing and non-clonal expansion. Efficient activation of T cells may accelerate the workflow of T cell-based cell therapies and reduce development time.

Previous data have shown that the C.BIRD microbioreactor can improve the growth of Jurkat cells, a human T cell line, through continuous mixing mode. To reinforce the T cell expansion workflow, the T cell activation level in the C.BIRD culture system was tested. We activated the Jurkat cell line in a C.BIRD 96-well culture system and found that the gentle mixing mode can improve T cell activation level compared to standard static culture. Results suggest an ideal activation mode or human T cells and a more effective way of process development for T cell-based cell therapy.

Materials and methods

The Jurkat cell line, an immortalized human T lymphocyte, was the model used in this study. Jurkat cells were cultured in RPMI 1640 (Corning #10-040-CV) supplied with 10% FBS (Gibco #10437028), 2 mM L-glutamine (Corning #25-005-CI), 10 mM HEPES (Gibco #15630080), 1 mM sodium pyruvate (Gibco #11360070), 2,500 mg/L D-(+)-glucose (Merck #G7021) and 1x Penicillin-Streptomycin solution (Corning #30-002-CI). Standard 96-well cell culture plates (Eppendorf #0030 730.011) were used for this experiment. Comparison studies were performed with cells cultivated in standard static culture and C.BIRD suspension culture in a 5% CO_2 humidified atmosphere at 37°C.

The cell density of both the static and C.BIRD groups was 5 x 10^4 cells per well in 150 µL medium. The Jurkat cells were activated with DynabeadsTM Human T-Activator CD3/CD28 (ThermoFisher scientific #111.61D). The tested ratio of Dynabeads to Jurkat cells were 0-to-1, 1-to-1, 3- to-1, and 5-to1. The C.BIRD mixing rate was 40 s/cycle continuous mixing mode. The IL-2 expression levels were determined using ELISA MAX™ Deluxe Set Human IL-2 ELISA kit (#431804, Biolegend). Data were analyzed by an unpaired t test. Significance of *P* value is listed as the following: 0.05 (ns), <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****).

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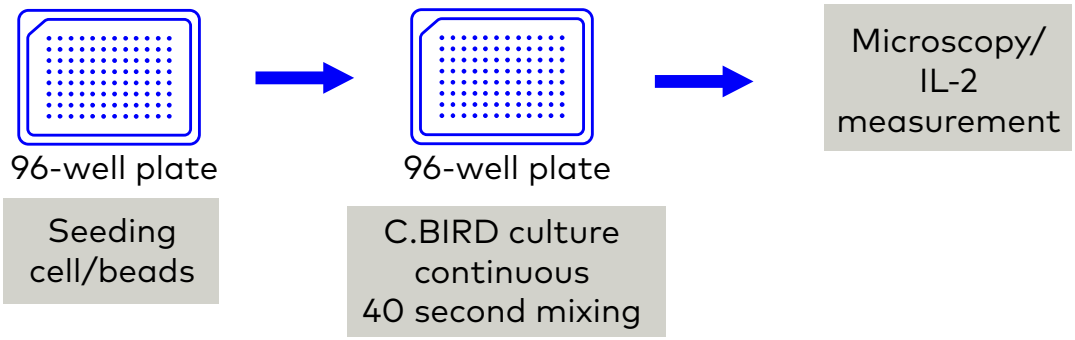

Figure 1. *Diagram of experiment design*

Results and discussion

The human T cell activation experiment was designed as shown in **Figure 1**. The human T cells formed clusters and started to proliferate during activation. We observed the morphology of Jurkat cells after Dynabeads Human T-Activator CD3/CD28 treatment for 24 hours (**Figure 2A**). Results show the Jurkat cells formed larger cell clusters as the ratio of CD3/CD28 Dynabeads to Jurkat cells increased. And cell clusters in the C.BIRD culture were larger than in static culture.

The other indicator of T-cell activation is the specific cytokines production, such as interleukin 2 (IL-2). The expression of secreted IL-2 from CD3/CD28 Dynabeads treated Jurkat cells (**Figure 2B**) was measured. No significant difference was observed in IL-2 expression level between static culture and C.BIRD culture without Dynabeads stimulation (P = 0.2943), suggesting that C.BIRD mixing culture does not affect T-cell activation without stimulation. The expression of IL-2 of C.BIRDTM cultures was significantly higher than in static cultures in all groups of CD3/CD28 Dynabeads-treated Jurkat cells. The P value of IL-2 expressions in CD3/CD28 Dynabeads-to-Jurkat cells co-culture ratio of 1-to-1, 3-to-1, and 5-to-1 were 0.0078, 0.0033, and 0.0002, respectively. These results demonstrated that the C.BIRD culture method could improve the T cells activation.

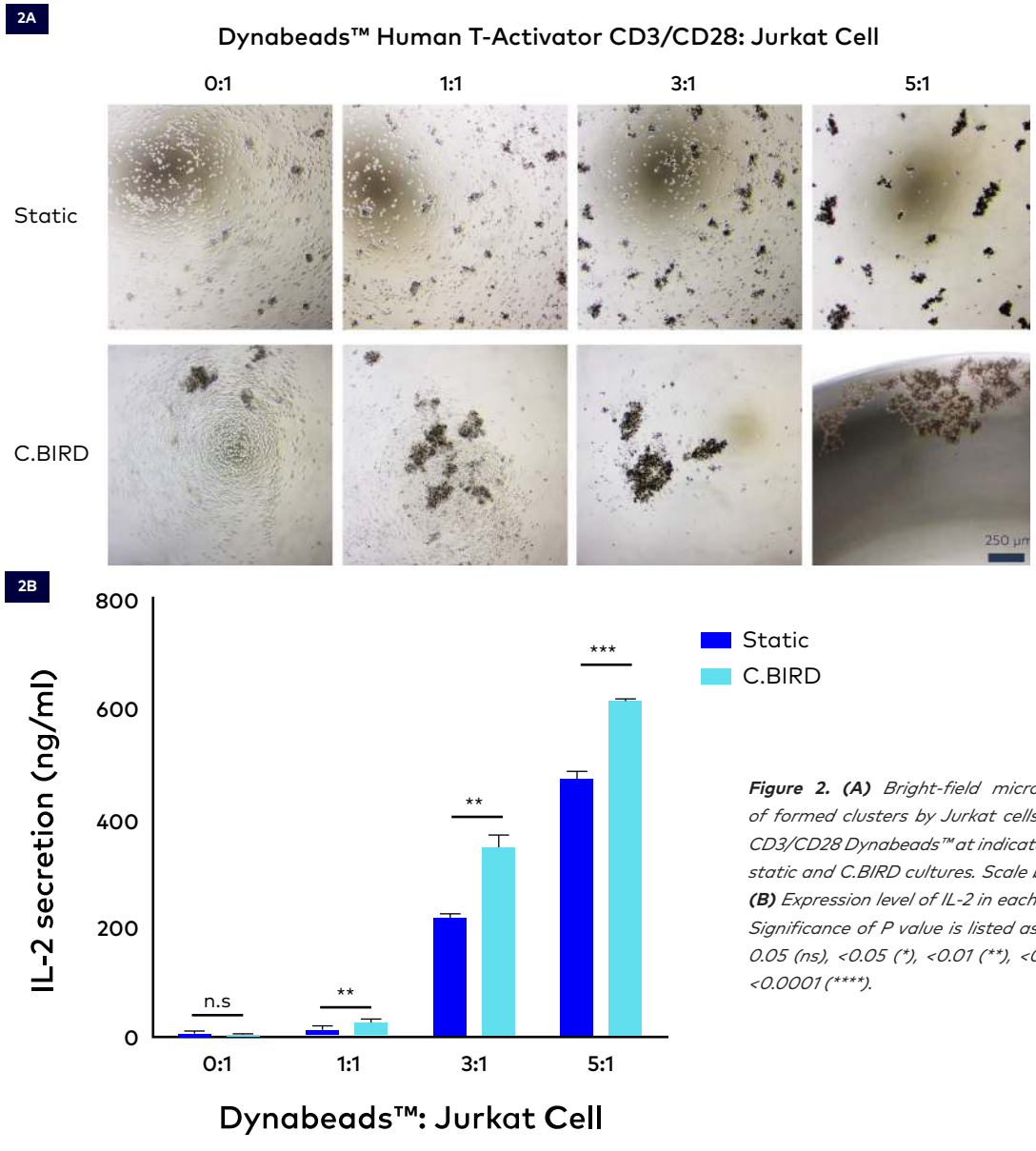

Figure 2. (A) *Bright-field microscopy images of formed clusters by Jurkat cells cultured with CD3/CD28 Dynabeads™ at indicated ratios under static and C.BIRD cultures. Scale bars = 250 µm.* ***(B)*** *Expression level of IL-2 in each culture group. Significance of P value is listed as the following: 0.05 (ns), <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****).*

Conclusion and future direction

T cell activation is an important procedure in the development or manufacturing process of T cell-based cell therapies. This study showed that the C.BIRD culture method augmented the activation level of the human T cell line in a 96-well culture scale. This feature can integrate with another C.BIRD function, improving human T cells growth. These features can help researchers shorten the timeline for process development of T cell-based cell therapies by using the C.BIRD culture method.

References

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2. Poltorak MP, Graef P, Tschulik C, et al. Expamers: a new technology to control T cell activation. Scientific Reports. 2020; 10, 17832. DOI:10.1038/s41598-020-74595-8

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