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C.NEST™ | Investigation of a multi-scale microplate-based culture system for static culture

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Abstract

To increase throughput and efficiency in the pharmaceutical industry, microbioreactors that culture cells at a smaller scale have been developed. Such an advanced scale-down methodology could be applied in many fields, including in the early stage of cell line development (CLD), optimization of process development and drug screening. To reduce costs, increase efficiency and optimize industrial workflows, we established an advanced microplate cultivation system called the C.NEST. The C.NEST not only functions as a microbioreactor for mixing culture, but also offers a stable environment for static culture. In this study, we demonstrated that the C.NEST was compatible with different scales of microplate culture and had similar performance in cell growth and viability to a standard incubator.

Introduction

In recent years, the need for increased throughput for host or protein candidate evaluations in the pharmaceutical industry has given rise to the development of microbioreactors. Microbioreactor technology offers great potential to develop miniaturized versions of bioreactors. These scaled-down systems provide a variety of advantages such as reducing costs and the use of reagents, saving space for parallel operation and minimizing set-up time between experiments [1, 2].

Our best known culture system, the C.BIRD, was developed as a microbioreactor for 96-well and 24-well plate culture. The C.BIRD system is compact and readily fits in a standard incubator. It not only improves oxygen transfer, but also homogenizes media through gentle mixing. Our previous application notes have demonstrated that C.BIRD culture enhances cell growth, protein production, spheroid quantity and quality, accelerates single-cell cloning and has great potential to shorten CLD workflows. Considering how more and more applications could be executed on a microbioreactor, we integrated the characteristics of the C.BIRD and standard incubators to create a whole new culture system called the C.NEST, which not only serves as a microbioreactor for mixing culture, but can also be used as a regular culture system for static culture.

Compared to standard incubators, the C.NEST contains four independent chambers for cell culture (**Figure 1A**). Each chamber contains UV lights for sterilization, CO_2 and humidity sensors for environmental monitoring, and heating modules on the top and bottom of the chambers for temperature adjustment (**Figure 1B**). The conditions in each chamber, including temperature and CO_2 concentration, are not impacted when other chambers are opened or closed.

The standard microplate with a standard lid or a C.NEST lid can undergo static or mixing culture in the system (**Figure 1C**). The plate holder can also accommodate many scales of microplate simultaneously; in addition to 24- or 96-well plates with a C.NEST lid for mixing culture, the device can also fit two 384- and 96-well plates in a vertical way and one 24-well plate with standard lid for static culture. Furthermore, the operation for C.NEST culture is quite simple. All the culture conditions can be set through the C.NEST's software, which is capable of controlling up to four-system customized C.NEST (**Figure 1D**). As such, unlike a regular incubator, it's possible to operate 16 chambers with different temperatures and CO_2 controls at the same time.

In this application note, static culture of Chinese Hamster Ovary (CHO-S) cells in different scales of microplates was performed in a standard incubator and the C.NEST (**Figure 2**). We demonstrated that the C.NEST offers a stable environment for multi-scale static culture and can produce similar results on cell growth and viability as a standard incubator.

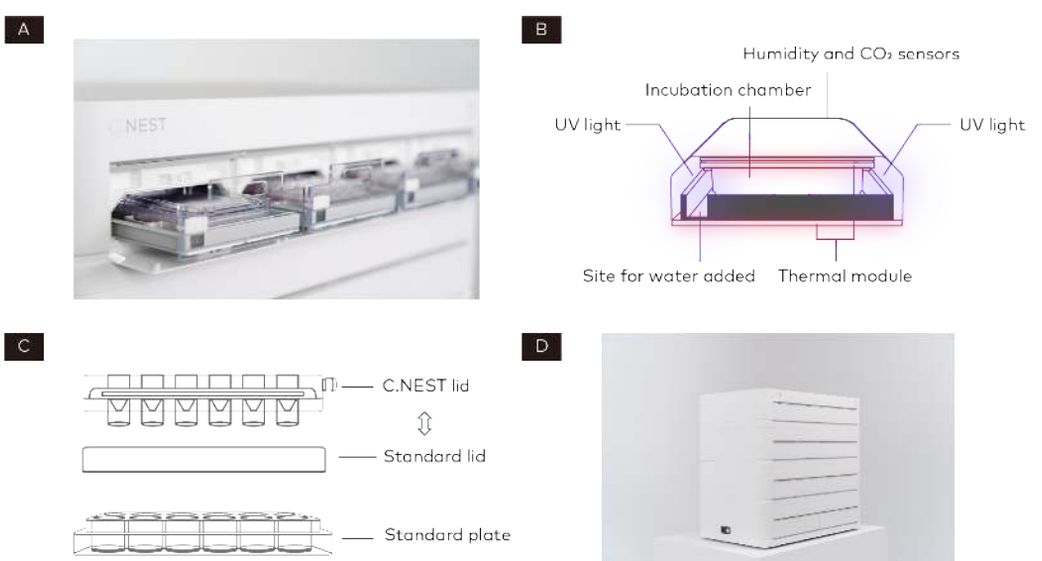


Figure 1. (A) *The C.NEST culture system contains 4 independent chambers.* ***(B)*** *A schematic diagram of environmental settings in the C.NEST.* ***(C)*** *The C.NEST supports static culture with a standard lid and mixing culture with the C.NEST lid.* ***(D)*** *The C.NEST software can control up to four-system setting of C.NEST instrument at the same time.*

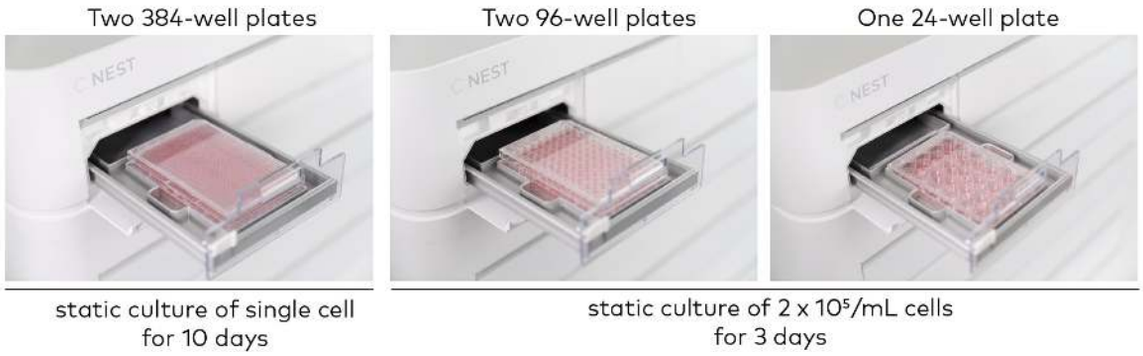


Figure 2. *Experimental designs of different scales of microplates with static culture in the C.NEST. Single cells were seeded in two 384-well plates with static culture for 10 days. 2 x 10⁵/mL of CHO-S cells were seeded in two 96-well plates and one 24-well plate with static culture for 3 days. Each culture group was compared with standard incubator.*

Materials and methods

Cell culture

A monoclonal antibody (mAb)-expressing CHO-S cell line was used in this study. CHO-S cells were cultured in CD Hybridoma Medium (Thermo Fisher Scientific, Gibco, #11279023) supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin (Thermo Fisher Scientific, Gibco, #5140122), 8 mM L-Glutamine (Corning, 25-005-CI), 0.2% Anti-Clumping Agent (Thermo Fisher Scientific, Gibco, #0010057DG) and 1X Cholesterol Lipid Concentrate (Thermo Fisher Scientific, Gibco, #12531018). The cells were cultured in either a humidified standard incubator or the C.NEST with 5% CO_2 at 37˚C.

Static culture of 384-well plates

Single-cell cloning (SCC) medium was prepared with 80% EX-CELL CHO Cloning Medium (Merck, #C6366) and 20% conditioned medium. 80 µL of SCC medium was added to each well. A dispensing cartridge was loaded with 5 x 10⁵/mL of CHO-S cells and attached to the F.SIGHT 2.0 to dispense single cells. The size of dispensed cells was set to 15-20 µm and the roundness was set to 0.7-1 (norm). The plates were then cultured in the C.NEST and standard incubator for 10 days. Distribution and confluency of clones were observed under microscopy. Cell growth and viability were analyzed with an automated cell counter (CURIOSIS, #FACSCOPE).

Static culture of 96 and 24-well plates

2 x 10⁵/mL CHO-S cells were prepared and seeded in each 96-well and 24-well plate. The culture volume was 200 µL and 1.4 mL per well in 96- and 24-well plates, respectively. The inter-well space of the 24-well plate was filled with 18 mL Dulbecco's phosphate-buffered saline (DPBS) (GeneDireX, #CC702-0500) to prevent evaporation. The plates were then cultured in the C.NEST and standard incubator for 3 days. Cell density and viability were analyzed on day 3.

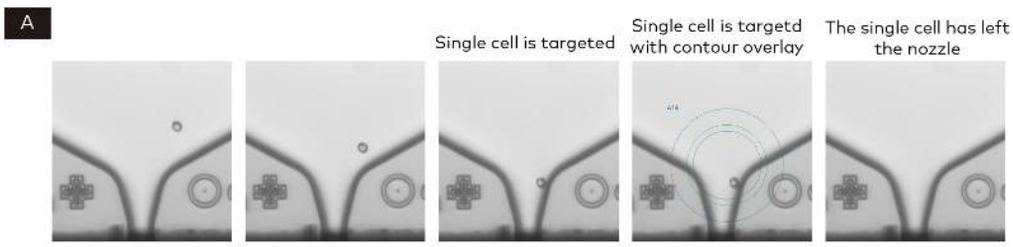
Results and discussion

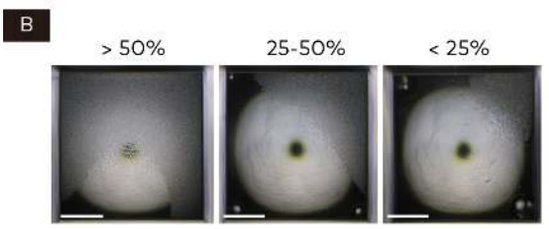
Static culture of 384-well plates

Our previous application note indicated that the C.BIRD culture system could improve single-cell cloning and optimize CLD workflows [3]. In this application note, we investigated the C.NEST's potential to optimize CLD workflows by testing its single-cell cloning abilities. Using the F.SIGHT 2.0, single cells were dispensed into 384-well plates with 80 µL of SCC medium in each well. Single-cell dispensing was verified by examining the chip in the dispensing cartridge with microscope objective and the nozzle images taken to assure clonality (**Figure 3A**). Two 384-well plates were placed in the C.NEST chamber and the other plate was cultured in a standard incubator as control. Confluency, numbers and distributions of clones as well as cell growth were all measured on day 10.

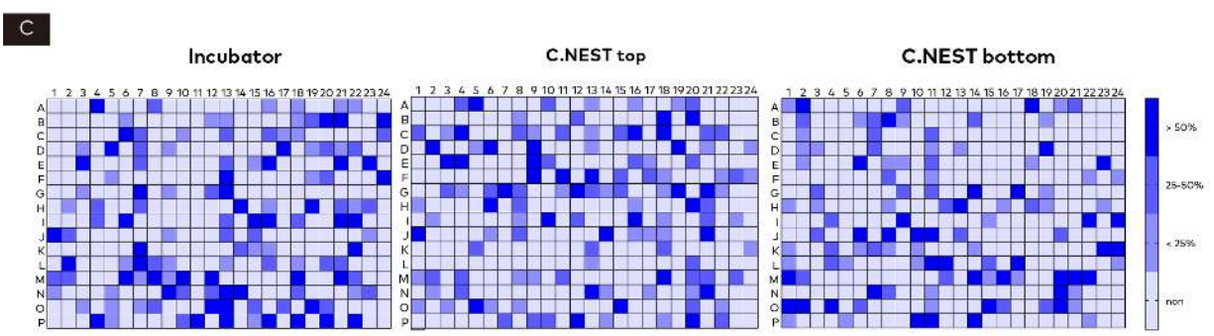
Confluency of clones was evaluated under microscopy and the criterion was divided into three grades of confluency: more than 50%, 25-50% and less than 25% (**Figure 3B**). 29%, 27% and 38% of clones were observed with more than 50% confluency in the incubator plate, the C.NEST top plate and the C.NEST bottom plate, respectively. 31%, 29% and 23% of clones were observed with 25-50% confluency in the incubator plate, the C.NEST top plate and the C.NEST bottom plate, respectively. 40%, 44% and 39% of clones were observed with less than 25% confluency in the incubator plate, the C.NEST top plate and the C.NEST bottom plate, respectively. The percentage of cell cloning in each grade of confluency was similar between different groups (**Figure 3B**). Moreover, the distributions of different grades of clones were even in each plate (**Figure 3C**).

In addition to examining the C.NEST's single-cell cloning and clone distribution abilities, we measured viable cells and cell viability in each grade of confluency and found that the number of viable cells and viability were similar in each culture group (**Figure 3D and 3E**). Clones with more than 50% confluency were counted as 4.6×10^4, 5.4×10^4 and 4.6×10^4 viable cells in incubator, C.NEST top and C.NEST bottom groups, respectively; clones with 25% to 50% confluency were measured as 3×10^4, 3.3×10^4, 3.1×10^4 viable cells in each culture group; clones with less than 25% confluency were counted as 1×10^4, 1×10^4, 7×10^3 viable cells in each culture group (**Figure 3D**). Moreover, clones with more than 25% confluency maintained above 90% of cell viability in both the incubator and the C.NEST culture. Clones of each culture group with less than 25% confluency also kept above 85% viability (**Figure 3E**). Regarding higher evaporation effect in the outer wells for 10-day culture, viability of clones in the outer wells were examined. Cell viability in the C.NEST groups was similar to the incubator group. The viability was measured as 89.3%, 92.6% and 90.6% in the incubator, the C.NEST top and the C.NEST bottom groups, respectively (**Figure 3F**).





	>50%	25-50%	<25%	total
Incubator	29% (40)	31% (42)	40% (54)	100% (136)
C.NEST top	27% (35)	29% (37)	44% (56)	100% (128)
C.NEST bottom	38% (54)	23% (34)	39% (56)	100% (144)



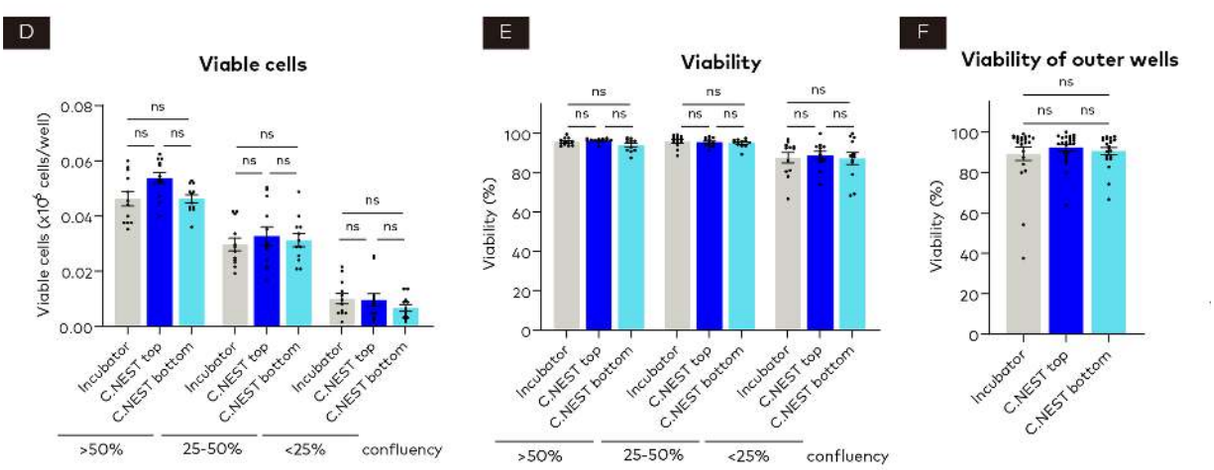


Figure 3. *Comparison of single-cell cloning ability in 384-well plates with the C.NEST and standard incubator culture.* ***(A)*** *Each single cell was monitored through a series of images to confirm every single cell was ejected from the F.SIGHT.* ***(B)*** *Clones were observed under microscopy (scale bar = 1mm). 3 grades of confluency were divided. The number and percentage of clones in each grade were evaluated.* ***(C)*** *A heat map of clone distributions in each group was shown.* ***(D and E)*** *Representative data of viable cells and viability in each grade of confluency were shown on day 10.* ***(F)*** *Due to the edge effect, the viability of outer wells was confirmed. Data were analyzed with one-way ANOVA test and shown as mean ± SEM. The significance of P value was listed as: P >= 0.05 (ns), P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), P < 0.0001 (****).*

Static culture of 96 and 24-well plates

In addition to testing the C.NEST's single-cell cloning ability at a 384-well plate scale, cells were also cultured and examined at a larger scale. Two 96-well plates with 2 x 10^5/mL of CHO-S cells were cultured in one chamber of the C.NEST and compared with the other 96-well plate in the standard incubator as control. Total cell density (TCD), viable cell density (VCD) and viability were measured on day 3. There was no significant difference in cell density between the incubator and C.NEST culture. The TCD was measured as 8.6 x 10^5, 9.8 x 10^5 and 8.8 x 10^5 cells/mL and the VCD was 8.3 x 10^5, 9.4 x 10^5 and 8.4 x 10^5 cells/mL in incubator, C.NEST top and C.NEST bottom groups, respectively (**Figure 4A and 4B**). Moreover, cells were maintained above 95% in all groups (**Figure 4C**). For the 24-well plate culture, one 24-well plate with 2 x 10^5/mL cells was cultured in one chamber of the C.NEST and the incubator for 3 days. The TCD and VCD of the C.NEST culture group reached 1.08 x 10^6 and 1.04 x 10^6 cells/mL, respectively, which were similar to the incubator culture group (**Figure 4D and 4E**). Both of the incubator and C.NEST culture groups also maintained cell viability above 95% (**Figure 4F**).

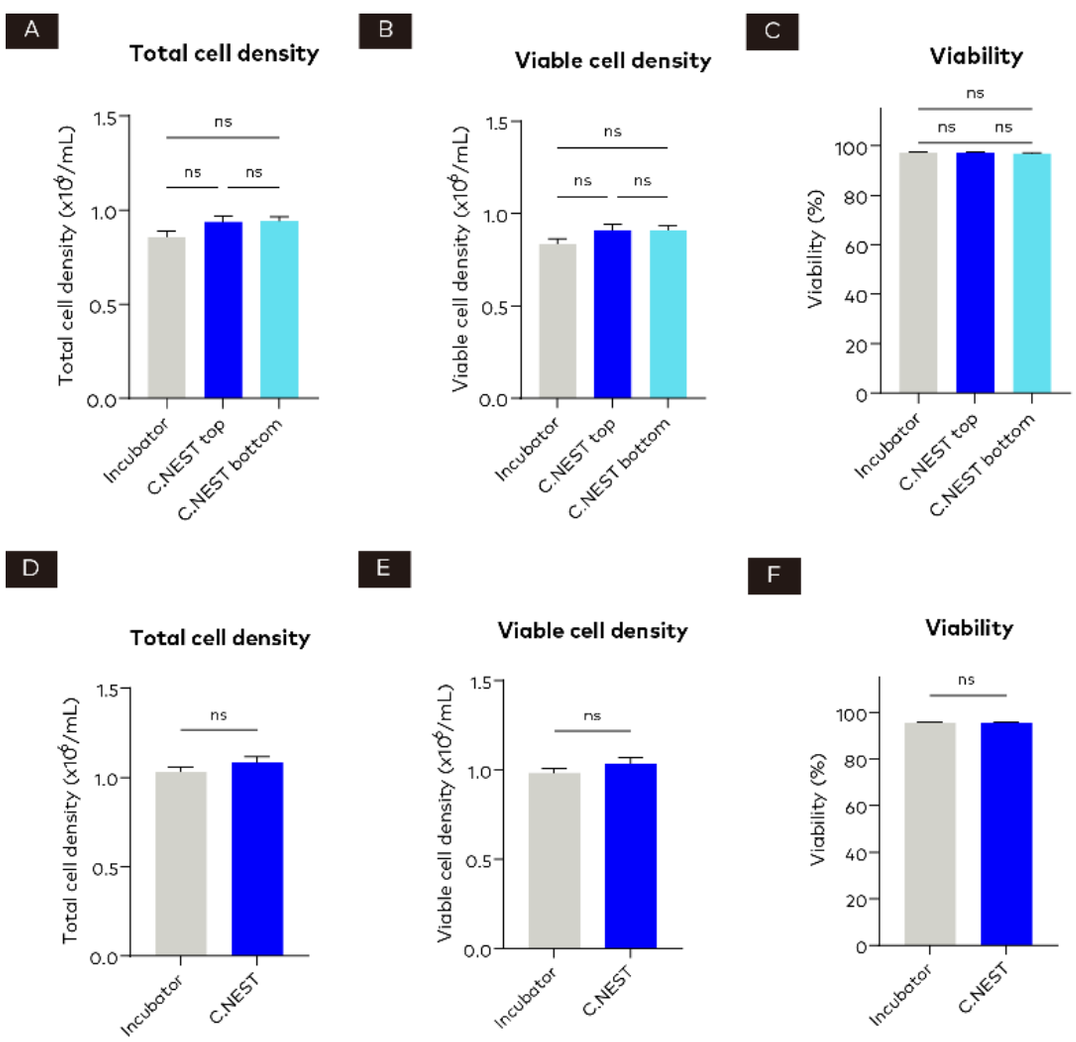


Figure 4. *Comparison of cells cultured in 96-well and 24-well plates with the C.NEST and incubator cultivation.* ***(A-C)*** *Total cell density, viable cell density and viability of 96-well plate culture were analyzed on day 3. Data were analyzed with one-way ANOVA test.* ***(D-F)*** *Total cell density, viable cell density and viability of 24-well plate culture were measured on day 3. Data were analyzed with unpaired two-tailed t-test. All data were shown as mean ± SEM and the significance of P value was listed as P >= 0.05 (ns), P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), P < 0.0001 (****).*

Conclusion

Mammalian cell lines, such as CHO-S, are usually used to produce therapeutic proteins in the biopharmaceutical industry. In general, therapeutic proteins are secreted by cells with bioreactors up to 2000 L. Before producing such a large quantity of proteins, highly productive cell lines need to be selected and confirmed in a complex process. Shake flasks are typically applied in process development, but tend to take up a lot of space and are resource intensive. Instead of using a shake flask, microplates could address these limitations and be executed in parallelized experiments for process development [2,4].

In this study, the C.NEST culture system shows its compatibility of different cell culture configurations (**Figure 3 and 4**). Therefore, it is a great opportunity to apply CLD and process development on the C.NEST culture system. We have confirmed that single cells can be sorted and cultured in 384-well plates with the C.NEST culture (**Figure 3**). Since the C.NEST could also provide a consistent environment for 96- and 24-well culture (**Figure 4**), clones could also be scaled up to 96- and 24-well plates for static or mixing culture in the same C.NEST chamber. Furthermore, multi-scale of microplates could be applied in various fields, such as 384-well plate for screening of virus-cell fusion assay, 96-well plate for spheroid formation in drug discovery, and 24-well plate for media screening in process development. Miniaturized assay could reduce cost of consumables for large scale campaign and is believed to be a trending application in the fields of medicine, biotechnology and pharmacology.

References

1. Markert S, Joeris K. Establishment of a fully automated microtiter plate-based system for suspension cell culture and its application for enhanced process optimization. Biotechnology and Bioengineering. 2017; 114(1): 113-121. DOI: 10.1002/bit.26044.
2. Hemmerich J, Noack S, Wiechert W, Oldiges M. Microbioreactor systems for accelerated bioprocess development. Biotechnology Journal. 2017; 13: 1700141. DOI: 10.1002/biot.201700141.
3. Yu N, Chiu S, Lin S. C.BIRD: improving the single-cell cloning workflow through increasing cell growth rate by C.BIRD mixing culture. www.cytena-bps.com. 2022. CBSAPP13.
4. Fink M, Cserjan-Puschmann M, Reinisch D, Striedner G. High-throughput microbioreactor provides a capable tool for early stage bioprocess development. Scientific Reports. 2021; 11: 2056. DOI: 10.1038/s41598-021-81633-6.

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