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S/C.NEST™ | Investigation of seeding density and mixing speed for optimal CHO cell culture in 24-well scale mixing culture

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Abstract

C.NEST and S.NEST are 24-well scale microbioreactors that offer the patented reciprocal mixing conditions for cell cultures. To obtain optimal mammalian cell growth in C/S.NEST, new users need to determine appropriate initial seeding density and mixing speed. In this study, we evaluated six initial seeding densities and three mixing speeds for CHO cell 24-well culture in C/S.NEST. Our findings offer guidance for researchers looking to incorporate the NEST system into their cell culture workflows.

Introduction

Chinese Hamster Ovary (CHO) cells are the workhorse of the biopharmaceutical industry, playing a pivotal role in the production of therapeutic proteins and monoclonal antibodies [1]. Over the past few decades, as the demands for biopharmaceuticals have surged, there has been a concomitant emphasis on refining cell culture methodologies to achieve maximal product yields [2]. One of the foremost parameters under investigation in this quest for optimization is seeding density.

Seeding density sets the trajectory for nutrient consumption, metabolic waste accumulation, and overall cell health in the bioreactor environment [3]. An improper seeding density can lead to reduced growth rates, sub-optimal protein production, and even apoptosis. Furthermore, studies have demonstrated that cell-seeding density is linked to the production yield of monoclonal antibodies in CHO cells, underscoring the necessity to control this parameter [4].

In this context, we performed this study to explore how initial seeding densities and mixing culture speeds may influence CHO-S cell growth in a NEST microbioreactor, a system that replicates bioreactor conditions at a compact 24-well scale.

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 S.NEST with 5% CO_2 at 37˚C.

Mixing culture with different cell seeding density

CHO-S cells were prepared in $2x10^4$, $5x10^4$, $1 x10^5$, $2 x10^5$, $5x10^5$ and $1x10^6$ cells/mL concentrations for seeding into four 24-well plates (Greiner, #662160). 1.4 mL of cell suspensions at each cell density was dispensed into 4 wells in each 24-well plate. 18 mL of Dulbecco's phosphate-buffered saline (DPBS) (Corning, #21-031-CM) was added to the inter-well space to avoid high evaporation. Three of the four 24-well plates were cultured in S.NEST at 10, 25 and 50 sec/cycle mixing rate for 4 days. The one remaining plate was cultured in a humidified standard incubator as control. Viable cell density and viability were measured with an automated cell counter (CURIOSIS, #FACSCOPE) on day 3 and day 4.

Statistical analysis

The data points were expressed as the mean±standard deviation (SD). Statistical analyses were performed using two-way ANOVA followed by Tukey's multiple comparisons test. A p value of less than 0.05 was considered a statistically significant difference. The P values are indicated listed as the following: $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) and $P \leq 0.0001$ (****).

Results and discussion

In our study, CHO-S cells were seeded at various densities ranging from $2x10^4$ to $1x10^6$ cells/ml within four 24-well plates, as detailed in the "Materials and Methods" section. Three 24-well plates were employed in mixing culture at 10, 25, and 50 sec/cycle mixing rates in the NEST incubator. One plate was cultured in a humidified standard incubator as a control.

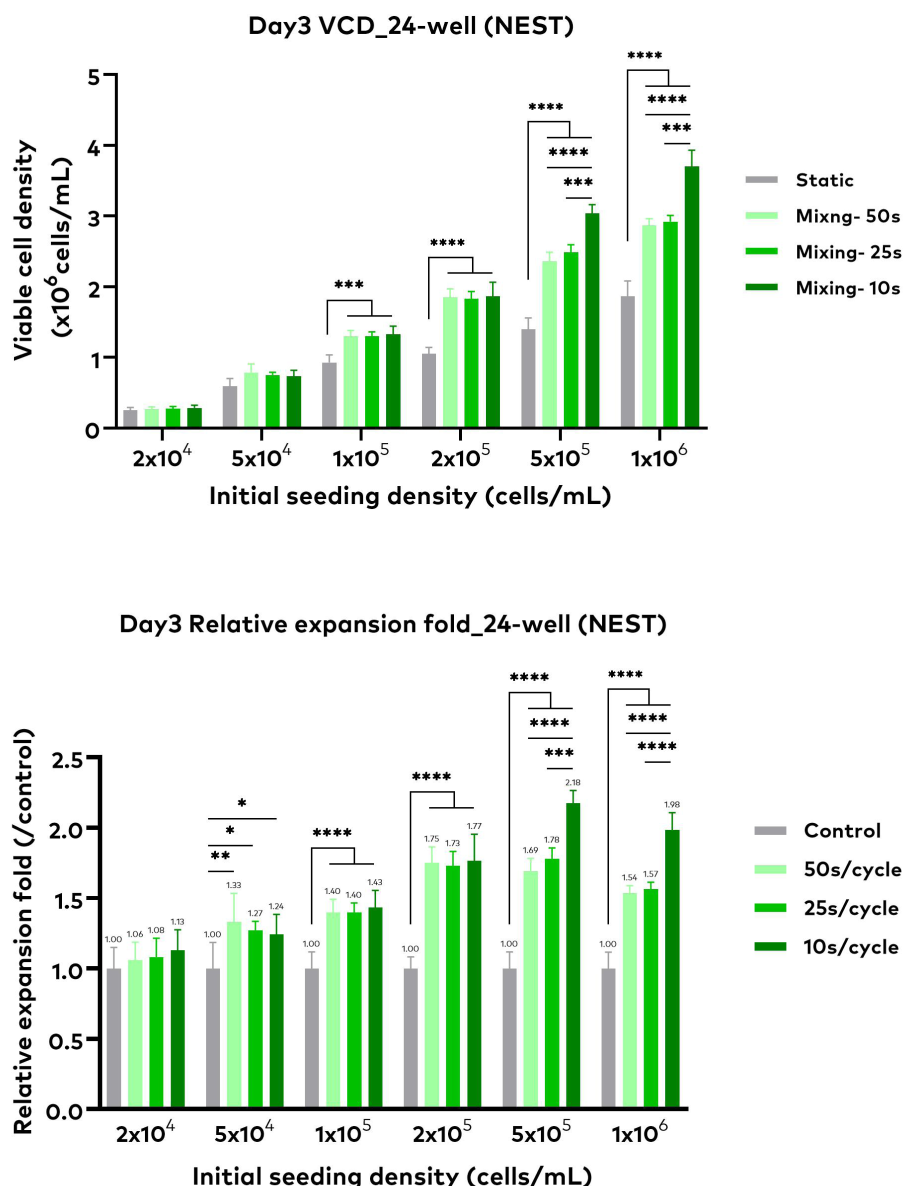
On day 3, the cell growth profile of the CHO-S cells was quantified by measuring viable cell density (VCD), relative expansion fold, and viability (**Fig 1**). As illustrated in **Fig 1A**, mixing groups with seeding densities at or exceeding $1x10^5$ cells/ml had significantly elevated VCD relative to the static control. Notably, for seeding densities at or higher than $5x10^5$ cells/ml, the 10 sec/cycle mixing speed significantly accelerated CHO-S cell proliferation compared to slower mixing speeds. Moreover, analyzing the relative expansion fold (**Fig 1B**) revealed that for seeding densities at or greater than $5x10^4$ cells/ml, dynamic mixing yielded superior expansion folds than static conditions.

These observations are consistent with our prior findings, underscoring the advantages of the NEST patented mixing culture, which provides homogenous nutrient and cell distribution uniformity and increases dissolved oxygen levels for cells. Nonetheless, viability measurements (**Fig 1C**) indicated that when cells were seed at the highest initial seeding density tested ($1x10^6$ cells/ml), a faster mixing speed of 10 sec/cycle might have the drawback of lowering viability.

Measurements on day 4 echoed the results and trends observed on day 3 in VCD, expansion fold, and viability (**Fig 2**). Nonetheless, by comparing

results from day 3 and day 4, we can observe that high mixing speed may be beneficial at lower seeding densities when the culture is extended in duration. Specifically, on day 3, the 10 sec/cycle mixing speed conditions outperformed the 50 sec/cycle conditions at seeding densities at or higher than $5x10^5$ cells/ml. However, on day 4, this outperformance occurred for seeding densities at or higher than $2x10^5$ cells/ml. However, caution is warranted: although rapid mixing amplified the expansion fold for seeding densities at or higher than $2x10^5$ cells/ml on day 4, decreased viability was also observed at higher seeding densities and mixing rates.

An overview of the cell growth improvement across different seeding density and mixing speed conditions is shown in **figure 3** and compared against the static culture controls. The chart shows that a NEST dynamic mixing culture could significantly accelerate cell growth at seeding densities exceeding $5x10^4$ cells/ml in a 24-well plate. Collectively, these findings demonstrates that beyond the NEST mixing speed, initial cell seeding density also profoundly impacts cell growth, viability, and culture duration, warranting careful calibration.



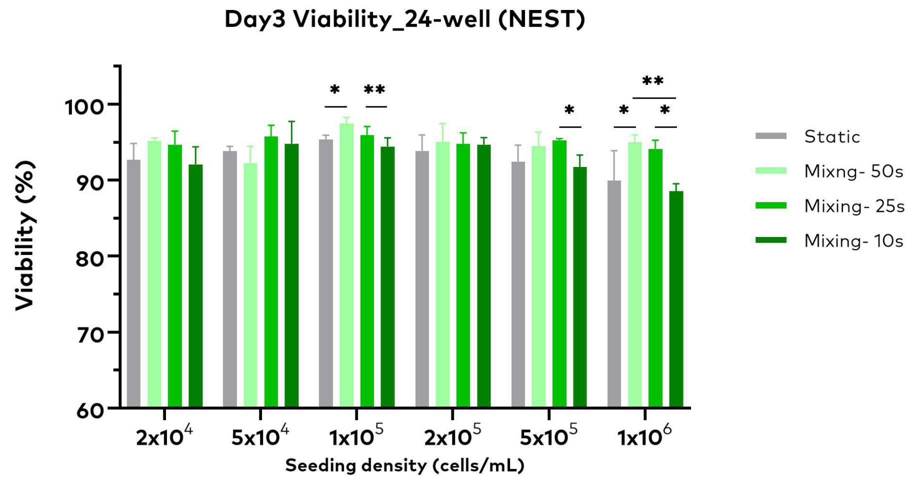
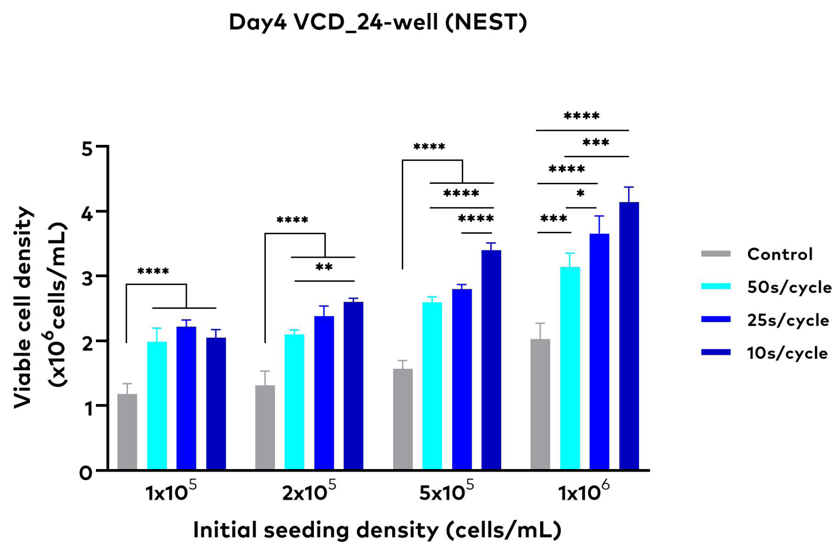
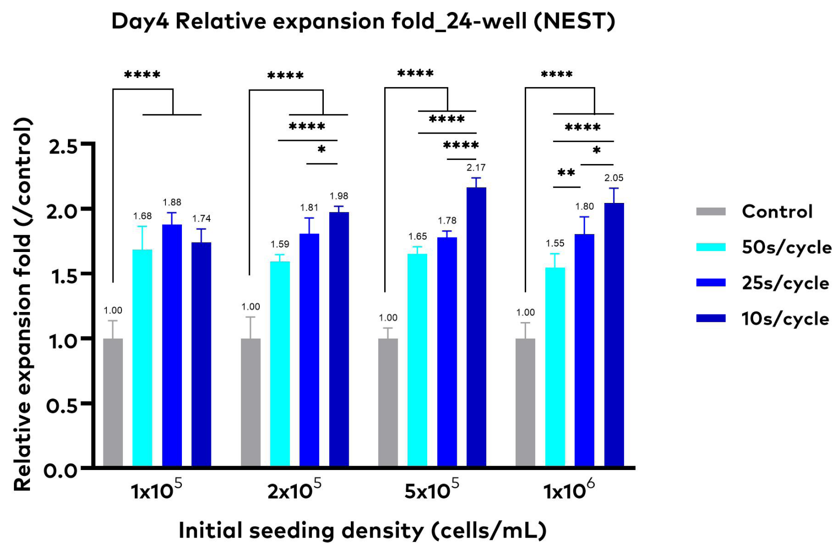


Figure 1: Cell growth in different initial seeding density and mixing speed conditions after 3 days of 24-well plate culture. (A) Viable cell density. (B) Relative cell expression fold. (C) Cell viability.





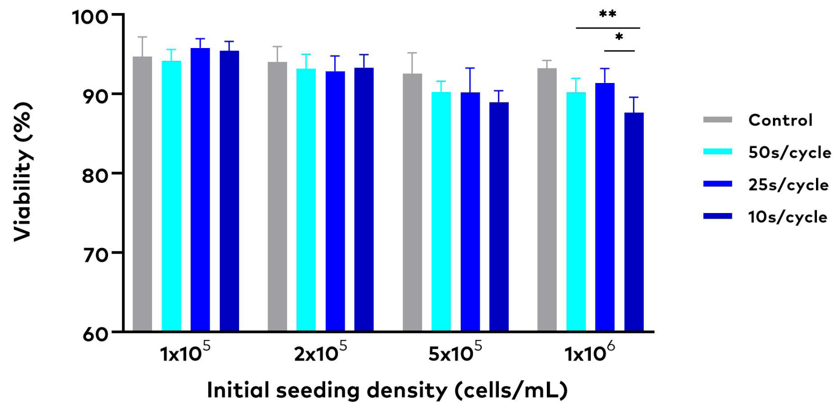


Figure 2: *Cell growth in different initial seeding density and mixing speed conditions after 4 days of 24-well plate culture. (A) Viable cell density. (B) Relative cell expression fold. (C) Cell viability.*

	Seeding density (cells/mL)	$2x10^4$	$5x10^4$	$1x10^5$	$2x10^5$	$5x10^5$	$1x10^6$
Day 3	50s	-	+	+	+	+	+
	25s	-	+	+	+	+	+
	10s	-	+	+	+	++	++ Viability ↓

	Seeding density (cells/mL)	$1x10^5$	$2x10^5$	$5x10^5$	$1x10^6$
Day 4	50s	+	+	+	+
	25s	+	+	+	+
	10s	+	++	++	++ Viability ↓

Figure 3: *Overview of cell growth in different initial seeding density and mixing rate conditions in 24-well mixing culture. [-] no benefit over the static culture. [+] significantly improved cell expansion relative to static culture. [++] significantly improved cell expansion relative to mixing culture at lower mixing speed.*

Conclusion

CHO cell culture optimization is pivotal in biopharmaceutical workflow. Utilizing the NEST microbioreactor, we explored the influence of initial cell seeding density and dynamic mixing speed on cell growth kinetics and viability in 24-well cell culture. Our data shows that high seeding densities, combined with high mixing speeds, enabled by the patented NEST microbioreactors, could provide optimal viable cell density (VCD). This observation is relevant to scaling bioprocesses where maximizing yield is imperative.

References

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