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CHO Cell Culture in Eppendorf BioBLU® 10c Single-Use Vessels

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Abstract

Substituting traditional glass bioreactors with single-use equipment can greatly simplify the bioprocess workflow. Single-use vessels eliminate the need for cleaning and autoclaving. This reduces the time needed to prepare the bioprocess run, and lowers the contamination risk. Another advantage, especially at larger bench scales, is the lower weight of plastic vessels compared to glass, reducing occupational hazards associated with overweight handling.

We tested the BioBLU 10c Single-Use Vessel in a Chinese Hamster Ovary (CHO) batch bioprocess. We achieved a peak cell density of approximately 12 million cells/mL, with a viability above 95 %. The results demonstrate that the BioBLU 10c Single-Use Vessel can substitute for 10 L glass vessels for larger capacity bench-scale cell culture bioprocesses.

Introduction

The BioBLU c Single-Use Vessel portfolio for cell culture covers working volumes from 100 mL to 40 L. With a working volume of 3.5 L to 10 L, the BioBLU 10c Single-Use Vessel provides an important link for bioprocess scale-up from small scale to large-capacity bench scale (Figure 1A).

Traditional 10 L glass bioreactors are bulky and heavy, making bench scale operations difficult. When filled with medium and assembled with an exhaust condenser, 10 L glass vessels weigh approximately 36 kg (80 lbs.), vastly exceeding the one-man lifting threshold of 24 kg (50 lbs.) recommended by the Occupational Safety and Health Administration (OSHA) [1]. In contrast, a BioBLU 10c Single-Use Vessel, filled with medium and assembled with an exhaust condenser, weighs only half, more or less. The single-use vessel is also much easier to handle, because it does not need to be autoclaved, whereas the sterilization of 10 L glass vessels requires a large-capacity autoclave.

In this study, we cultivated CHO cells in BioBLU 10c Single-Use Vessels controlled with a BioFlo® 320 bioprocess control station (Figure 1B). To evaluate process performance, we monitored cell growth and viability, the metabolic profile, and the production of an IgG antibody. We also compared the time it takes to prepare a bioprocess run using a single-use vessel and a glass vessel.

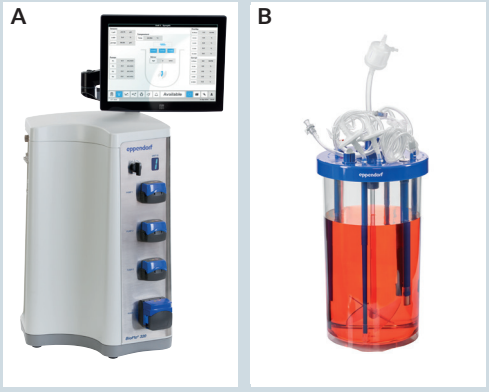


Fig. 1:
A: BioFlo 320 bioprocess control station
B: BioBLU 10c Single-Use Vessel

Material and Methods

Cell line and medium

We used a proprietary suspension CHO cell line producing a human monoclonal antibody (hMAb) from TPG Biologics, Inc. We cultured the cells in CD-FortiCHO™ medium (Thermo Fisher Scientific®, USA), which we supplemented with 1x Antibiotic-Antimycotic (Thermo Fisher Scientific, USA), 1x Anti clumping agent (Thermo Fisher Scientific, USA) and 8 mM L-glutamine (complete medium).

Bioreactor inoculum preparation and inoculation

We thawed a 2 mL cryopreservation vial containing 1 mL of CHO cells at 1 x 10^7 cells/mL, from a previously prepared cell bank, using the ThawSTAR® CFT2 Instrument (MedCision®, USA). We inoculated the cells into a 125 mL shake flask containing 30 mL (24 % of the total volume) of pre-warmed complete medium. We cultured the CHO cells in a New Brunswick™ S41i CO_2 Incubator Shaker (Eppendorf, Germany) at 37 °C, in an atmosphere of 8 % CO_2 and at an agitation speed of 125 rpm. We passaged the cells, without expansion, every other day for a week to allow enough time after thawing. We then expanded the culture first to three 250 mL flasks and then to six 1 L flasks. During expansion, we kept the inoculation density, percentage fill of the shake flasks, and all other culturing parameters constant. We combined the cultures from all 1 L flasks into a 2 L sterilized addition bottle (Eppendorf, Germany). The cell density was around 4.0 x 10^6 cells/mL. 99.2 % of the cells were viable. We used 729 mL of the combined culture to inoculate the BioBLU 10c Single-Use Vessel, containing 10 L of medium, at a targeted inoculation density of ~0.3 x 10^6 cells/mL. The workflow is shown in Figure 2.

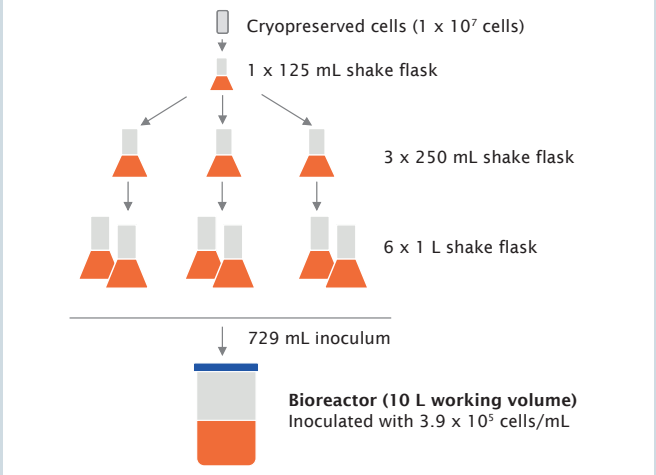


Fig. 2: Inoculum preparation for the bioprocess run.

Bioreactor control and process parameters

The BioBLU 10c Single-Use Vessel was controlled with a BioFlo 320 bioprocess control station. The process parameters are summarized in Table 1.
We cultivated the CHO cells at 37 °C. The temperature was controlled using a heat blanket.

We monitored the dissolved oxygen (DO) in the culture using an optical ISM® DO sensor (Mettler Toledo®, Switzerland), and controlled it at 50 % in 3-Gas Auto mode. The BioFlo 320 that we used in this study had a 4 TMFC, high-flow sparge drawer with a gas flow range of 0.04 – 20 SLPM. Since higher gas flow can cause excessive DO fluctuation in the beginning stage and excessive foaming towards the end stage of culture, we proactively limited the oxygen flow to 0 – 3.0 SLPM and the air flow to 0.04 – 3.0 SLPM in the controller setup screen. In addition to gas flow limiting, we also added Antifoam C Emulsion (Sigma-Aldrich®, USA) as needed.

We used an optical ISM pH sensor (Mettler Toledo, Switzerland) to control the pH during the run at 7.0 (deadband = 0.2), using a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base). We took a sample from the vessel daily and measured the cell density and viability, the pH, and the concentrations of various metabolites offline.

The built-in DO and pH sleeves in the BioBLU 10c Single-Use Vessel allowed the use of optical DO and pH sensors non-invasively without the need for sensor sterilization. The non-invasive design increases sensor lifespan while reducing the workload, as well as eliminating the

Table 1: Process parameters and setpoints.

Parameter	Sensor/device	Setpoint
Temperature	Heat blanket	37 °C
Gassing	Macrosparger; 3-Gas Auto Gas Mixing	Air flow: 0.04 – 3.0 SLPM; O_2 flow: 0 – 3.0 SLPM
Dissolved oxygen (DO)	Optical ISM sensor	50 %
Agitation	Pitched-blade impeller	100 rpm
pH	Optical ISM sensor	7.0 (deadband = 0.2) Cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base)

incremental sensor damage caused by repeated sterilization at 121 °C.

Analytics
We measured the cell density and viability once a day using a Vi-Cell® XR viability analyzer (Beckman Coulter®, USA). This cell counter uses a Trypan blue exclusion method to determine the values. We also measured the pH offline using an Orion Star A211 pH meter (Thermo Fisher Scientific, USA), which we calibrated daily using buffers of known pH. We used these values to standardize the controller's optical pH calibration and reduce discrepancies between online and offline pH measurements.

Results

Preparation time
Figure 3 compares the time needed for preparation and tear-down of glass and single-use bioreactors. The use of the BioBLU 10c Single-Use Vessels saved time, because the vessel did not need to be autoclaved before the run and cleaned after.

Bioprocess data
In the bioprocess run we reached a peak in viable cell density on day 7 at 12.05 x 10^6 cells/mL. After that, cell density and viability declined as anticipated (Figure 4A). The cell growth curve in the single-use bioreactor was comparable to growth of this cell line in a batch process in a traditional glass vessel [2].

By day 7, the cells had consumed the initially supplied glucose. The ammonia concentration gradually increased every day up to a toxic level of 10.52 mmol/L on day 8 (Figure 4B). Both the depletion of glucose and the rise of the ammonia concentration probably contributed to the decrease of the viable cell density, starting from day 7.
In the eight days of the batch culture, the cells had produced 212 mg/L of hMAb (Figure 4C).
We terminated our culture by the end of day 8.

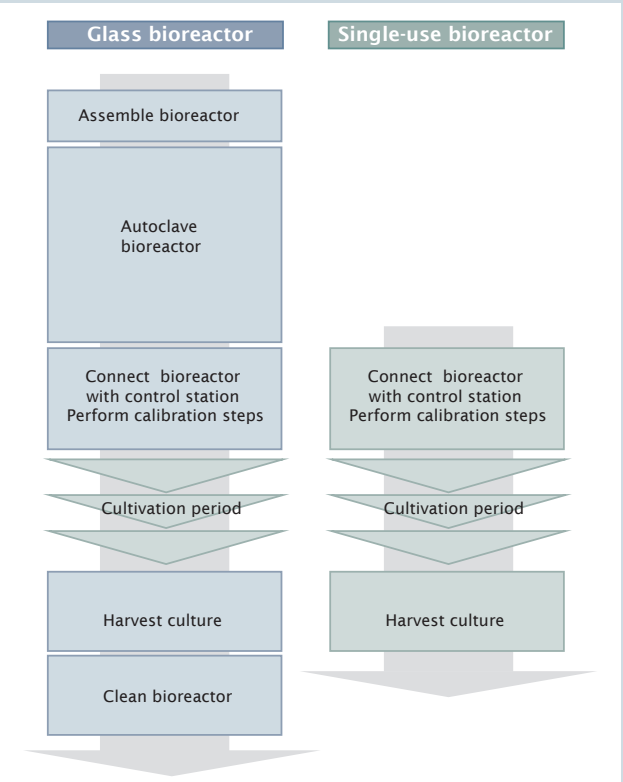
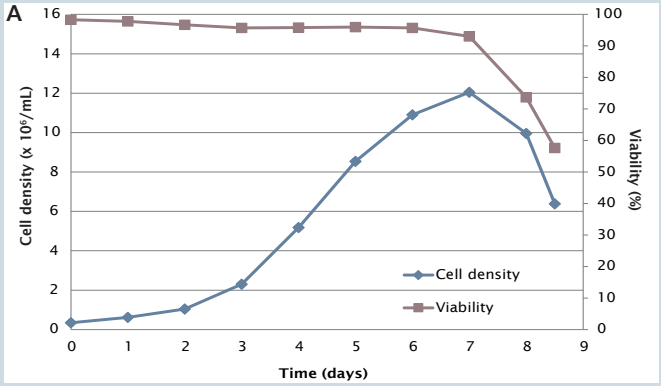
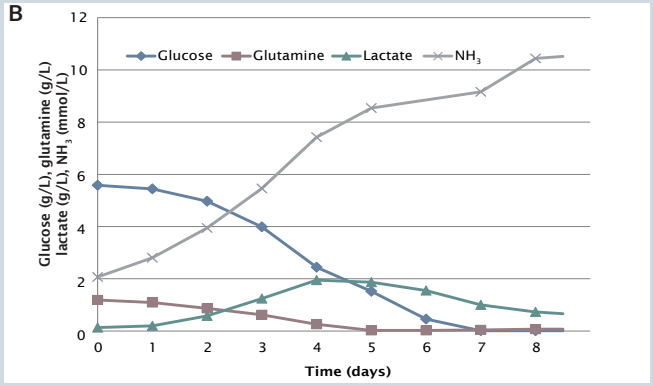


Fig. 3: Sequence of events in bioprocess runs using glass and single-use bioreactors.





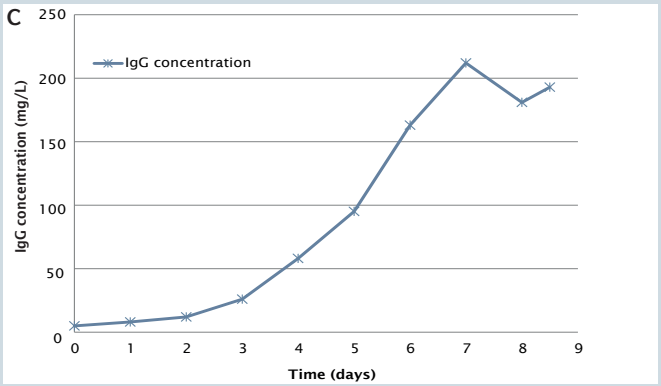


Fig. 4: Bioprocess data, measured during the CHO cell culture batch process
A: Cell density and cell viability
B: Metabolic profile
C: Antibody production

Conclusion

Using the BioBLU 10c Single-Use Vessel we obtained more than 12 million cells/mL in a CHO batch bioprocess. This is comparable to results obtained previously in a traditional glass vessel [2].
Using the BioBLU 10c saved a significant amount of time on preparation, vessel tear-down, and cleaning as compared to typical glass vessels. Furthermore, operational safety risks were reduced, due to the much lower weight of the single-use vessel. The versatility of the BioFlo 320 bioprocess control station, able to operate both glass and single-use bioreactors, and the comparable rigid-wall design of Eppendorf glass and single-use vessels, make it easy to switch between single-use and autoclavable equipment.

Literature

[1] https://www.osha.gov/SLTC/etools/electricalcontractors/materials/heavy.html. Accessed on January 31, 2018.
[2] Willard, S., Suttle, A., Han, K., Dorceus, M., Cheng, P-J., Sha, M. Comparing culture methods for monoclonal antibody production. BioProcess International. 2017.

Ordering information

Description	Order no.
BioFlo® 320 bioprocess control station	Please inquire
BioBLU® 10c Single-Use Vessel, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile, 1 piece	1386141000
New Brunswick™ S41i, 170 L, CO_2 incubator shaker with inner shelf and touch screen control, 1 (2. optional) shelves, orbit diameter 2.5 cm (1 in)	S41I-120-0100
Addition/Harvest Bottle Kit, for aerobic processes, 2 L	M1362-9902

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