APPLICATION NOTE No. 308 I

Leachable Studies on Mammalian Cell Culture in BioBLU® Single-Use Vessels

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Abstract

There is a growing awareness regarding the potential leaching of toxic or inhibitory chemicals from the plastic material of single-use bioreactors into cell culture medium. Based on a standardized cell culture test recommended by the German society for chemical engineering and biotechnology, DECHEMA®, we determined if there were no leachable chemicals from the Eppendorf BioBLU Single-Use Vessel material that affect cell culture performance. We did not observe any effect of leachables on CHO and Vero cell growth and viability, and the metabolic profile. The results suggest that the BioBLU Single-Use Vessels are safe for mammalian cell culture.

Introduction

Single-use bioreactors are routinely used in biopharmaceutical research and development and biologics manufacturing. While reducing turn-around times and contamination risk, one potential problem of single-use systems is the release of chemical compounds into the culture medium. These so-called leachables originate in the raw materials used for bioreactor fabrication, or are produced during irradiation and storage. They could potentially affect cell behavior, growth, and viability. Examples of the chemicals often used in plastic manufacturing are heavy metals (as catalysts for the polymerization process), UV-light stabilizers, antioxidants (to preserve the integrity of the consumable), plasticizers (to alter the mechanical properties), and slip or release agents (for easier and faster removal from the mold during manufacturing).

The scope of this study was to test whether leaching of any other chemicals from the Eppendorf BioBLU Single-Use Vessel material into the culture medium affects cell culture performance. Based on the standardized cell culture test developed by the DECHEMA working group, "Single-Use Technology in Biopharmaceutical Manufacturing" [1], we tested for effects of leachables from the BioBLU 0.3c, 1c, 5c, and 14c Single-Use Vessels (Fig. 1) on CHO and Vero cell growth, viability, and metabolic profile.

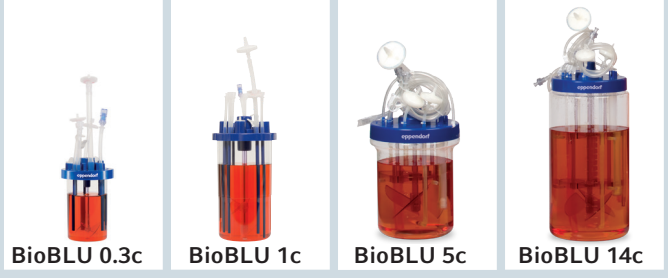

Fig. 1: The BioBLU Single-Use Vessels used in this study

Material and Methods

Cell lines

We used suspension FreeStyle™ CHO-S cells (Thermo Fisher Scientific®, USA) and Vero cells (ATCC®, CCL-81). The CHO cells were cultivated in CD CHO media (Gibco®, USA) supplemented with 8 mM L-glutamine (Gibco). Vero cells were cultured in VeroPlus SFM medium (ATCC, ACS-4001) supplemented with 4 mM L-glutamine.

Extraction

To extract potential leachables, we filled BioBLU 0.3c, 1c, 5c, and 14c Single-Use Vessels to 50 % of their maximum working volume with serum-free cell culture medium and incubated the vessels for three days at 37°C without agitation. As a control for the experiments with Vero cells we incubated the medium under the same conditions in a borosilicate-glass flask (Pyrex®; Corning®, USA), as recommended in the DECHEMA protocol. The control for the CHO cell experiments was incubated in a polycarbonate shake-flask for cell culture (VWR®, USA). Such flasks are widely used for the cultivation of suspension cell lines (Fig. 2A).

Toxicity study – CHO cells

We precultured CHO cells in CD CHO Medium in a shake flask in a New Brunswick™ S41i Incubator Shaker (Eppendorf) at 37°C, 125 rpm, and 5 % CO_2. We transferred 30 mL of the extraction and control media to separate shake flasks, inoculated the cultures with 0.25 x 10^6 cells/mL, and cultured the cells for two days at 37°C, 125 rpm, and 5 % CO_2. The cultivations were performed in triplicates (Fig. 2B). We took a sample from each culture twice daily, determined cell number and viability using a Vi-Cell® XR Cell Viability Analyzer (Beckman Coulter®, USA), and measured the concentrations of glucose, lactate and NH_3 using a Cedex® Bio Analyzer (Roche Diagnostics®, Switzerland) (Fig. 2D).

Toxicity study – Vero cells

We inoculated nine 6-well plates with the same concentration of Vero cells from the same pool and let them grow to confluence. Initially we cultivated the cells in presence of 0.125 % serum to promote cell attachment. After the cells had attached we changed the medium to serum-free medium. When the cultures reached 100 % confluence, we exchanged the medium for the extraction and control media, respectively (Fig. 2C). Each day we determined cell number and viability using a Vi-Cell XR Cell Viability Analyzer and measured the concentrations of glucose, lactate, and NH_3 using Cedex Bio Analyzer (Fig. 2D). All analysis were carried out in triplicates using cells and supernatants from three wells.

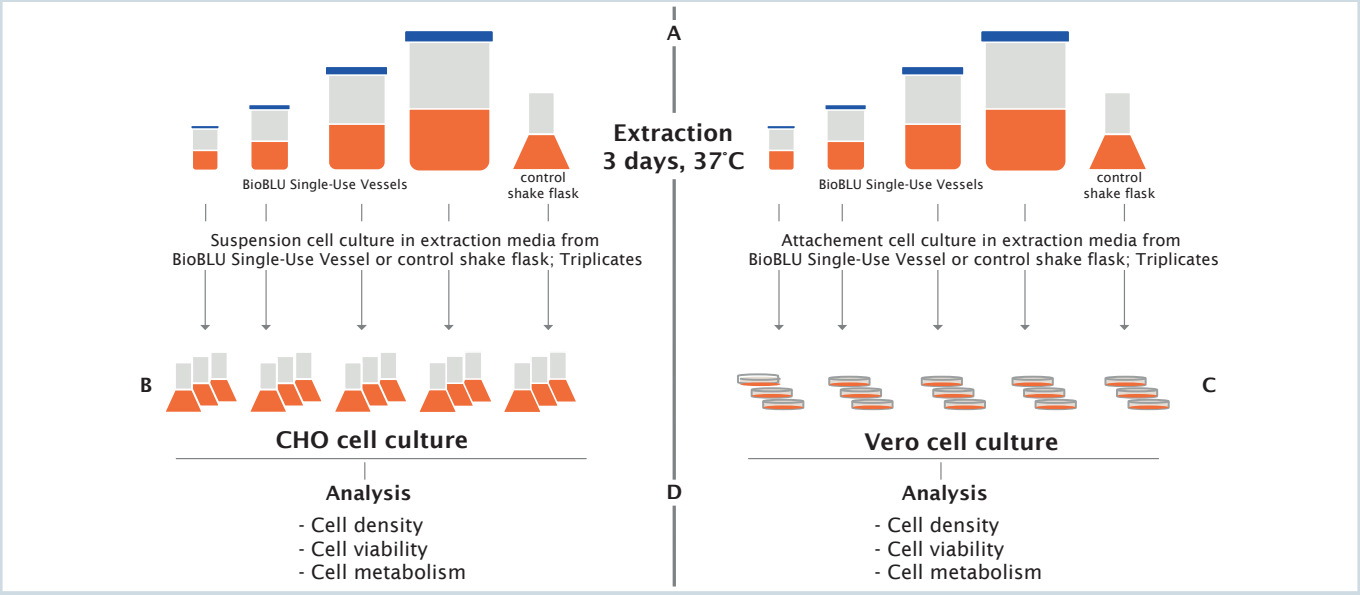

Fig. 2: Experimental setup. **A:** Extraction with medium. **B:** CHO-cell culture in extraction media. **C:** Vero cell culture in extraction media. **D:** Analysis of cell density, viability, and metabolism.

Results

To evaluate the cytotoxicity of leachables, the DECHEMA recommends to evaluate three criteria, namely cell density, viability, and metabolism. We extracted BioBLU 0.3c, 1c, 5c, and 14c Single-Use Vessels and used the extraction medium for the cultivation of Vero cells and FreeStyle CHO-S cells.

CHO cell culture

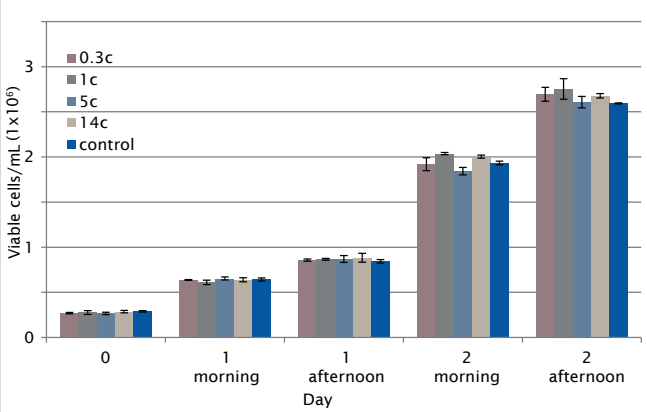

Fig. 3: Viable cell density. CHO cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control). Sampling was done twice daily.

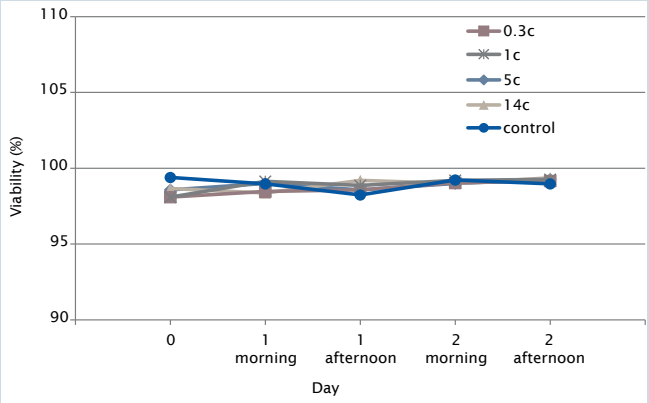

Fig. 4: Viability. CHO cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control). Sampling was done twice daily.

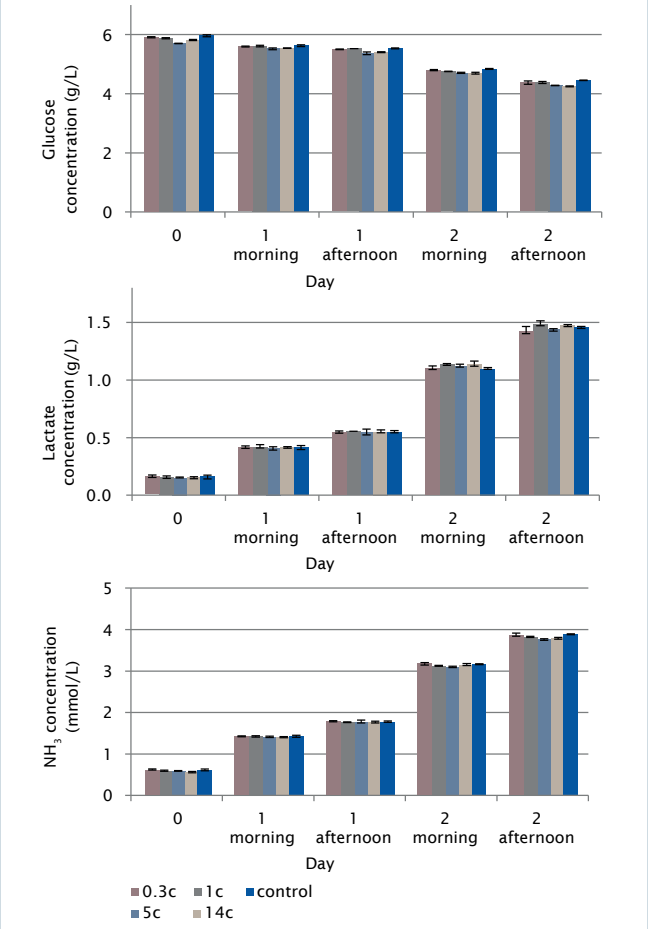

Fig. 5: Cell metabolism. CHO cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control) and substrate (glucose) and metabolite (lactate, NH_3) concentrations were measured twice daily.

Vero cell culture

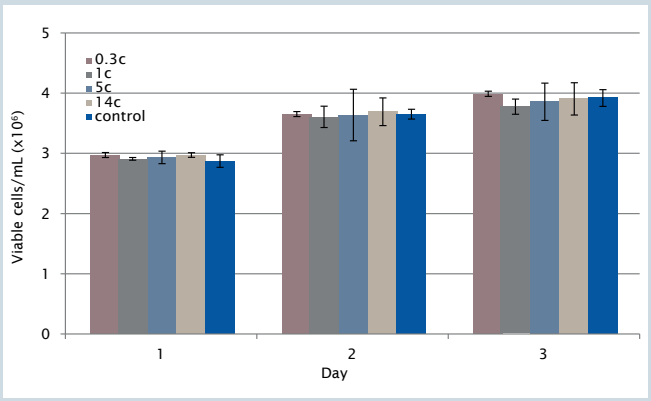

Fig. 6: The viable cell density was determined daily. Vero cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control).

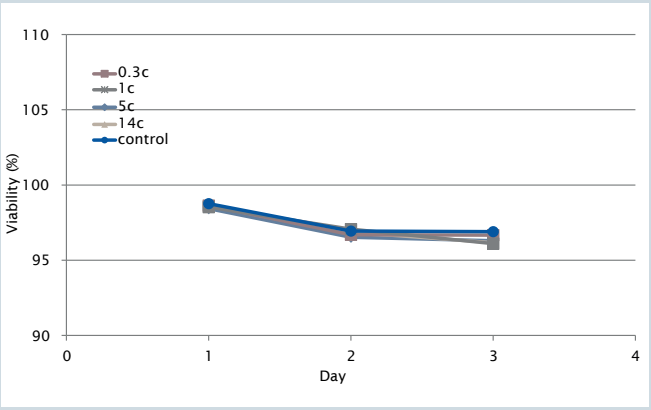

Fig. 7: Viability was analyzed daily. Vero cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control).

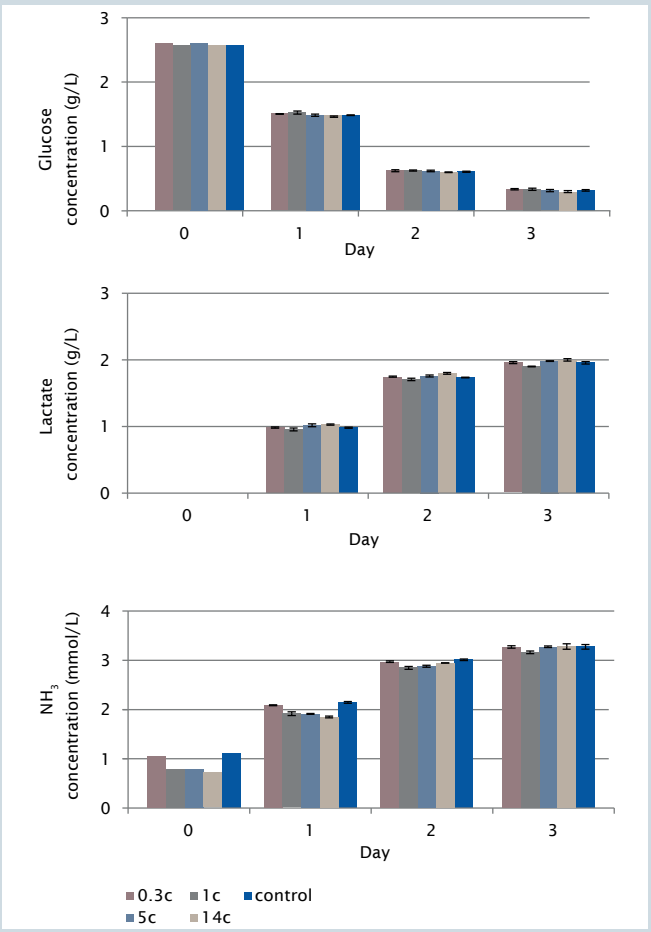

Fig. 8: Cell metabolism. Vero cells were cultivated in extraction media from BioBLU Single-Use Vessels or a shake flask (control) and substrate (glucose) and metabolite (lactate, NH_3) concentrations were measured daily.

For both cell lines, cell growth in the extraction media and the control medium was comparable (Fig. 3, 6). Under all conditions close to 100 % of the CHO and Vero cells were viable (Fig. 4, 7). The concentrations of glucose, lactate, and ammonium developed very similar in the different cultures (Fig. 5, 8).

Conclusion

The materials used for the fabrication of BioBLU Single-Use Vessels were chosen to mitigate issues with leachables. The vessel body and head plate of Eppendorf BioBLU Single-Use Vessels are made of single-layer injection-molded plastic. No additives such as softeners are used for its fabrication. Only virgin raw materials are used to eliminate uncertainties arising from the use of recycled materials.

While it should be noted that the sensitivity to leachables can vary between cell lines, we did not observe any negative effects caused by leachables from the BioBLU vessel material on the growth, viability, and metabolic profile of the two cell lines tested. Hence, the vessel material of the BioBLU vessels can be considered "non-critical" with regard to cytotoxic leachables.

Literature

[1] DECHEMA Expert Group Single-Use Technology; Recommendations for leachables studies; 2014. http://dechema.de/en/papers-path-1,123212.html.

Ordering information

Description	Order no.
BioBLU® c Single-Use Vessels	
BioBLU® 0.3c Single-Use Vessel, cell culture, 1 pitched-blade impeller, sterile, 4-pack	78903508
BioBLU® 1c Single-Use Vessel, cell culture, 1 pitched-blade impeller, sterile, 4-pack	78903511
BioBLU® 1c Single-Use Vessel, cell culture, 2 pitched-blade impeller, sterile, 4-pack	78903506
BioBLU® 3c Single-Use Vessel, cell culture, microsparger, 1 pitched-blade impeller, sterile, 1-pack	1386000100
BioBLU® 3c Single-Use Vessel, cell culture, macrosparger, 1 pitched-blade impeller, sterile, 1-pack	1386000300
BioBLU® 5c Single-Use Vessel, cell culture, microsparger, 1 pitched-blade impeller, sterile, 1-pack	Contact us for details
BioBLU® 5c Single-Use Vessel, cell culture, macrosparger, 1 pitched-blade impeller, sterile, 1-pack	Contact us for details
BioBLU® 5p Single-Use Vessel, cell culture, microsparger, packed-bed impeller, sterile, 1-pack	M1363-0119
BioBLU® 5p Single-Use Vessel, cell culture, macrosparger, packed-bed impeller, sterile, 1-pack	M1363-0133
BioBLU® 14c Single-Use Vessel, cell culture, microsparger, 1 pitched-blade impeller, sterile, 1-pack	M1363-0126
BioBLU® 14c Single-Use Vessel, cell culture, macrosparger, 1 pitched-blade impeller, sterile, 1-pack	M1363-0122
BioBLU® 50c Single-Use Vessel, cell culture, microsparger, 1 pitched blade impeller, sterile, 1-pack	M1363-0131
BioBLU® 50c Single-Use Vessel, cell culture, macrosparger, 1 pitched blade impeller, sterile, 1-pack	M1363-0129

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