CELLULAR RESPONSE OF CHO CELLS FOLLOWING EXPOSURE TO EXTRACTABLES AND LEACHABLES FROM SINGLE-USE BIOREACTORS

Sara Carillo, NIBRT, Foster Avenue, Mount Merrion, Blackrock, co. Dublin. Ireland
sara.carillo@nibrt.ie
Noemí Dorival Garcia, NIBRT, Foster Avenue, Mount Merrion, Blackrock, co. Dublin. Ireland
Christine Ta, NIBRT, Foster Avenue, Mount Merrion, Blackrock, co. Dublin. Ireland
Paul S Kelly, National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
Sam Pare, National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
Niall Barron, National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
Jonathan Bones, NIBRT, Foster Avenue, Mount Merrion, Blackrock, co. Dublin. Ireland

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The implementation of single-use disposable bioreactors as alternatives to the dominant stainless steel predecessor used in the manufacturing of recombinant therapeutic proteins introduces a variety of new materials into the bioprocessing pipeline. These plastics, when used under normal culture conditions, can degrade and leach breakdown products into the culture media such as bis(2,4-di-tert-butylphenyl) phosphate (bDtBPP).¹,²

In the present work the effects of bDtBPP on CHO cell growth was evaluated, using both a parental cell line (CHO-K1) and a mAb1 producing cell line (CHO-DP12). Results showed the effect of bDtBPP at concentrations commonly leached from single-use bioreactors. In particular, the growth curve of the two systems revealed a decrease around 50% of viable cell density, while viability seemed not to be affected, in agreement with previously published observations by Amgen.¹,² Critical quality attributes (CQAs) for mAb1 produced by CHO-DP12 cell line were analysed, including N-glycan analysis, peptide mapping, aggregation profile, charge and oxidized variants analysis. These analyses showed no differences between produced IgG1 when cells are spiked with bDtBPP.³

In order to understand the molecular mechanisms responsible for bDtBPP effects, a multi-omic study (including glycomic, proteomic and metabolomics) is under investigation.

Following these initial experiments, incubation of media in single-use bioreactors to obtain a preconditioned media containing leachables extracted under normal operational conditions (37°C for 7 days in rocking incubator) was performed. The media was used to evaluate the growth curve of CHO-K1 and CHO-DP12 cell lines; preliminary results showed a detrimental effect on cells growth.

Future experiments will focus on the comparison of media incubated in single-use bioreactors from several brands and models, followed by multi-omic analysis (glycomic, proteomic, metabolomic) of cells cultured with these preconditioned media. This will lead to a better understanding of the molecular mechanisms involving not only bDtBPP but the whole panel of leachables present in single-use bioreactors plastic films.