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Medicine Maker

Freezing Down Time in Bioprocessing

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Biotechnology, the industrial application of biological organisms, has fascinated me since school, so after obtaining my diploma, biopharma research and development was an obvious next step. For my PhD thesis, I focused on the response of mammalian cells to shear and other stresses. Subsequently, I worked as an industry post-doc on perfusion processes, including process development, medium development and high-density cryopreservation technology (HD Cryo). Today at MilliporeSigma, I am the head of the Cell Culture Media R&D Laboratory – my team works to improve HD Cryo in order to make bioprocessing more efficient and flexible.

At present, conventional upstream processes begin with 1 ml of banked (frozen) cells, which are then expanded to 15,000 liters for a classical fed-batch process. This takes weeks, during which time a part of the manufacturing site is blocked and unproductive. After thawing, cells typically go through a "crisis" with viability temporarily decreasing – sensitivity to this is very cell line dependent. Also, like many biological processes, cell growth after thaw isn't exactly predictable and there is some uncertainty as to when the final culture will be ready. So if you need to run different processes in a given plant, the facility will never be optimally used, due to bottleneck effects.

From my point of view, it seems odd to start with 1 ml in order to make several thousand liters. Compressing this phase would dramatically increase the flexibility and capacity of a manufacturing plant. Being highly conservative, however, the pharma industry has invested few resources in investigating methods for improving the efficiency of the expansion phase. That is why Millipore Sigma has turned its attention to this field. In particular, we believe that HD Cryo has the potential to significantly enhance bioprocessing capacity.

Bioprocessing in HD

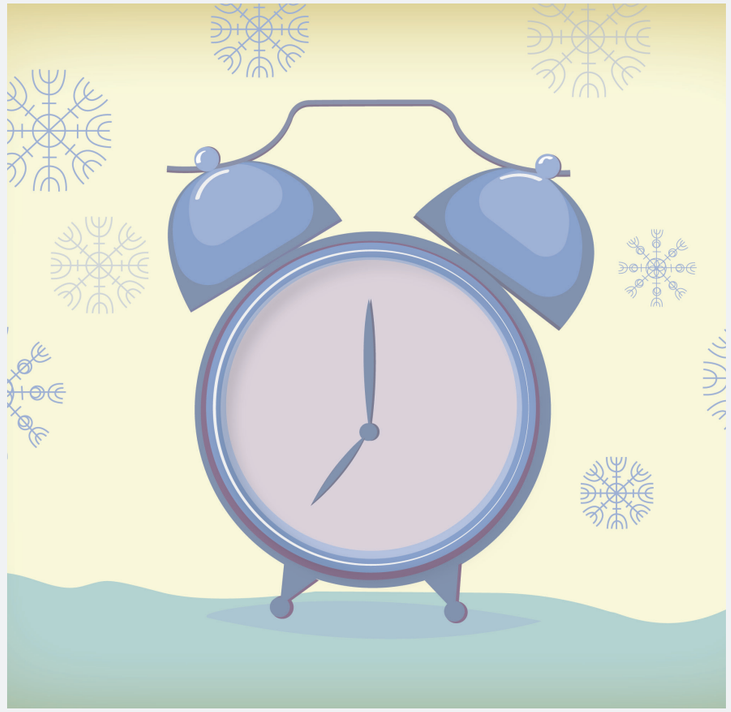
Briefly, HD cryo involves expanding cells of interest and preparing frozen seed train intermediates of them, not in 1 ml vials, but in larger vials, or in bags of up to 100 ml volume. Freezing culture aliquots in high volume and high density, dramatically shortens subsequent expansion processes because when you thaw one of the bags, you can start the expansion process at a later time point. In effect, you are freezing down time!

At present, there is no industry standard for HD Cryo technology. We have undertaken several internal case studies, focusing on different aspects, but pulling all the data together in a comparable way remains difficult. We are working to change this, and our overall aim is to look at the bigger picture and make HD Cryo simple, reproducible and effective.

Currently, we are investigating the criticality of the different components of HD Cryo processing – namely the freezing media, the bag assembly, and the filling and freezing process, all of which need to be performed without stressing the cells. We are examining different families of CHO cells and ensuring that we understand which aspects of the process they are sensitive to and why – and we are confident that our systematic approach will result in process technology suitable for all customer needs.

HD Cryo media have to protect the cells from stress during the freezing process, which is a significant factor in the viability drop-off after freeze-thaw. The idea is that the post-thaw cell population will be of very high viability and will start growing immediately, without any crisis/recovery lag phase. Development of the medium has required us to work backwards (upstream) from the medium we recently developed for intensified processing in production stage bioreactors (1), as it is critical that both media are compatible with each other. If they are too different then cells might go into a lag phase when the medium is changed, and the time advantage of HD Cryo would partially erode.

Our vision is of a seamless suite of mutually compatible bioprocessing products. Thus, having developed an intensified perfusion medium to boost productivity at the main stage bioreactor, we are now advancing HD Cryo to intensify processes upstream,



while remaining cognizant of the need for both sets of products to work together effectively. Essentially, we are giving our customers the tools to intensify all steps up to the main stage reactor. HD Cryo is a key component of the toolkit, making expansion processes, including N-1 bioreactor perfusion processes, faster, more reproducible and more flexible.

Reaping the rewards

I truly believe that the benefits of HD Cryo technology are very significant; for example, it can cut three weeks from the upstream process, enabling manufacturers to start the process two weeks prior to the main stage bioreactor, instead of five weeks prior. In fact, we've seen customers presenting at conferences who have increased the capacity of stainless steel manufacturing plants by two or three-fold through de-bottlenecking using HD Cryo. The technology is also advantageous in the context of disposable bioreactors in smaller plants, where it increases flexibility by enabling intensification of the seed train expansion process. It also enhances the capacity of processes run in small-scale bioreactors, which is very important for disposable systems with a maximum volume of only 2,000 litres. If you're replacing stainless steel plants with single use systems, you must be creative and intensify your process as much as possible, and HD Cryo can play an important role in this regard.

HD Cryo can play a role in R&D too. Freezing down 20 HD Cryo bags gives you 20 identical starting points (i.e., cell populations with exactly the same expansion history) for the process under development. The technology allows users to remove much of the variability associated with the manual steps currently used in expanding cultures from the first 1 ml vial through to shake flasks, and makes bioprocess R&D becomes much more reproducible.

Looking ahead, I foresee a continued trend for smaller and more flexible manufacturing plants based on single-use systems. This is partly a consequence of market fragmentation – blockbuster drugs are being replaced by drugs for smaller populations, and this implies smaller manufacturing volumes. Similarly, the advent of biosimilars puts downward pressure on manufacturing volumes because the innovator has to share the market with biosimilar competitors. Personalized medicine and the pursuit of niche indications also suggest relatively small product volumes. All this, together with a surge in innovative biopharmaceutical drug formats, indicates that manufacturers need to be ready to supply a greater number of products at lower volumes. This will require more flexibility – and that is one of the key advantages of HD Cryo. HD Cryo provides increased speed, flexibility and capacity, and for a given process these benefits are achieved without any detrimental effects on cost of goods, quality or yield. The pre-culture expansion can be done at any point and in any place; frozen HD bags can be shipped worldwide to carry out main stage production wherever and whenever it is appropriate. In effect, HD Cryo uncouples expansion from production in both time and space.

Jochen B. Sieck is Head of Cell Culture at MilliporeSigma.

Reference

1. *D Lyons "An Intense Focus on Perfusion", The Medicine Maker, 27 (2017). Available at: http://bit.ly/2mF5Dqp*