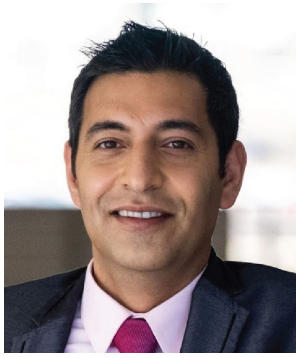
CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL & GENE THERAPY MANUFACTURING

SPOTLIGHT

INTERVIEW

Happy medium: considerations in scaling cell culture media strategies



While attractive from an initial cost perspective, performing early development in a cell culture substrate that does not match a therapy's final manufacturing platform can have critical implications on development timelines. In this podcast, **Charlotte Barker,** Editor, BioInsights, speaks to **Dalip Sethi,** Director of Scientific Affairs, Terumo BCT, about best practices for scaling up cell culture substrate.

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Q Cell culture media is a big consideration for therapy developers—does Terumo Blood and Cell Technologies (BCT) have a media offering?

DS: Media is critical to developers because is the vehicle by which cells obtain nutrients, gases, and growth factors, and secrete waste products. Terumo BCT does not have a media offering but our equipment and devices are designed to be cell-type and media-agnostic.

When we are considering protocol development experiments in-house, we consider different media formulations available on the market, as often as possible. We share those results in various methods or channels through our peer-reviewed publications, webinars, posters, and seminars.

Q What are some of the main considerations in choosing a cell culture medium?

DS: One of the main considerations is whether the media can be manufactured at a GMP grade to fully move a process into the clinical scale. Due to cost considerations, a researcher may choose to use the research-grade media to start with, but as they move into clinical and later stages of development, they will need to move to GMP grade. The ideal solution would be either a commercially available formulation that comes in research grade for early development work and GMP grade for manufacturing, or a so-called 'home brew' media formulation that has been tech-transferred to a CDMO for both grades of production.

Another consideration is the type of media that you are using. Does your media contain serum or is it serum free? Is it xeno free? You should consider these factors before moving on to manufacturing. Try to have the best media composition for all cell types that you are growing and avoid animal-derived components. If you cannot go completely xeno-free, there are some further considerations to be made. How will one use a serum substitute without impacting the quality of the cells, including the phenotype of the cells, as one moves from the research to the manufacturing stage? A lot of media companies now provide chemically defined media (CDM) so it is worth considering if there is a CDM that can be utilized to give the best quality cells.

There are many more factors to consider too. For example, in a T-cell culture media, there are multiple components, including proteins, glucose, vitamins, amino acids, trace elements, and inorganic salts. When you are culturing T cells, you should ensure that they maintain the right physiological pH. When cells are growing, they will consume glucose and produce lactate, which can change the pH of the media. To maintain that pH, you need a buffering system. A simple bicarbonate buffering system can maintain pH for a certain concentration of hydrogen ions and lactate, but when you are working with very fast-growing cells, you may need to consider media containing different buffers, such as HEPES.

In addition to the above factors, when we consider T-cell cultures, we cannot forget about interleukins. What kind of interleukins will you be using in your complete media, what will be their sources, and what will be the grade of those interleukins? Again, a researcher may choose to use a research-grade interleukin in the early stages. It will be important to consider if the supplier provides the same cytokines in GMP grade. If the GMP grade is not available, what are the qualifications required as one continues with the research grade?

As you go from a small-scale research development grade to a manufacturing grade, the scale of production will also change. You should have the right supply chain in place so that you can get the media that you need.

There are a lot of questions to think about, and I am sure it keeps researchers up at night!

Q What about media-related considerations when you are using a closed automated bioreactor system—including the in-process analytical components?

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DS: With closed automated bioreactor systems—and I am thinking here about perfusion-based systems—the first thing you must consider is that media have different protein concentrations. In a bioreactor system, you want to make sure that the bioreactor has enough flexibility to accommodate media with different protein concentrations. If you have a low protein concentration medium or a high protein concentration medium (which is sometimes needed to get the right phenotype and culture conditions for a particular cell type), the automated bioreactor systems should be able to handle that without getting clogged or blocked. Having a large membrane surface area helps with that, and it allows you to culture cells with a high-protein medium.

> "Try to have the best media composition for all cell types that you are growing and avoid animal-derived components."

When it comes to process analytical technologies, there are a number of different classes. Off-line analysis involves taking a sample out of your bioreactor system and passing it to a separate QC lab, close by, to test for metabolites, cell phenotype, cell viability, etc. At-line analysis is when the analytical technology is right next to the bioreactor system. In-line analysis is when the process analytical technology is connected in line to the bioreactor system.

It is important to consider how the samples are taken from the bioreactor system and if they are coming with or without a filter. Particularly in the case of high-protein media, you want to make sure that the filter can handle those sampling considerations.

Q Where do equipment and consumables factor into this conversation?

DS: One of the most important factors that any manufacturing equipment provider has to consider is extractables and leachables (E&L). Typically, any surface that encounters the cells—and by extension their media—must go through extensive E&L testing. This ensures that no substances can be drawn out from the substrate into the media to the detriment of cell expansion.

We must also consider interactions between complete cell culture medium and substrate surfaces that, while not toxic, may drastically affect cell expansion. For example, certain materials may have a high affinity for binding to proteins such as cytokines or other growth factors. In this instance, these proteins may bind to and be sequestered from the cells, which may 'starve' them or delay growth curves.

We have also already talked about having the right surface area so that your filter does not get foul or clogged. Filter fouling, wherein the pores of a filter membrane may become clogged with protein over time during media perfusion, is an especially critical consideration for protein-rich formulations such as serum-completed media and even some basal media with high

protein concentrations. Oftentimes this isn't an issue in small-scale testing but can reveal itself upon scaling up.

Q That's very interesting to note... Not the 'first line' issues that you mentioned earlier, but certainly something that can affect therapy development timelines. What does Terumo BCT see as a mitigation strategy?

DS: These are unwelcome surprises to any researcher, to be sure. One of the best ways to avoid them is to use like-for-like substrates from early research to the manufacturing level. The same materials of contact, the same environmental controls, and ideally the same platform should be used. Bioreactors with small volumes and low seeding density requirements that have robust, scaled-up counterparts should be a top consideration for researchers.

Our Quantum Flex is a good example of this: it is one device with two sizes of consumables offered, a small and a standard bioreactor. The small bioreactor is roughly 1/10th of the size of the standard bioreactor and can produce 1B suspension cells or 100M adherent cells under the right culture conditions. This sizing option is great because there is no need to perform a manual pre-culture step in a T-flask or gas-permeable bag—so no substrate changing is required.

The standard bioreactor uses the exact same hollow fibers and materials of contact but can produce 10B suspension cells or 1B adherent cells—again, under the right culture conditions.

There is also data in the literature showing different media types used in Quantum System that resulted in the successful generation of T cells, including Miltenyi's TexMACS™ medium, Irvine Scientific's PRIME-XV T-cell expansion XSFM medium, and Lonza's X-VIVO-15 medium.

Q How would you say your customers' needs are changing as the industry matures?

DS: Customers are increasingly considering xeno-free or serum-free media for potentially smoother regulatory and CMC processes. Manufacturers are also thinking about how to control lot-to-lot variability, CDM is also being discussed in the literature.

Ultimately, the 'happy medium' will come from the combination of the bioreactor and media composition. Getting the right phenotype and number of cells, allowing a cell therapy manufacturer to be able to dose a particular patient or clinical trial participant in the right way, is key.

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BIOGRAPHY

DALIP SETHI PhD currently serves as the scientific lead for Terumo BCT's Cell Therapy Technologies portfolio. He holds a doctorate and conducted post-doctoral studies at Thomas Jefferson University, School of Medicine. In his post-doctoral research, Dalip focused on the development of cancer gene-specific RNA and DNA analogs targeted against cancer genes in the signal transduction pathway for use as cancer diagnostics and therapeutics. Throughout his career in the industry, Dalip has been engaged in developing technologies & methods for use in cell therapy applications. Dalip has authored multiple scientific publications and is a co-inventor on several patents & patent applications. He recently co-authored publications on modular automated systems for $CD3^+$ T-cell manufacturing and monoculture of cord-blood derived $CD34^+$ using an automated, membrane-based dynamic perfusion system. The articles highlighted the benefits of modular automation in cell therapy manufacturing. Dalip is also an ISCT member and participates in committees focused on cold chain, particulates, and process analytical technologies.

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AUTHORSHIP & CONFLICT OF INTEREST

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