Gene therapy

Benefits of AGT culture media in gene therapy applications

Summary

Advancements in gene therapies over the past decade offer a great promise to treat life-threatening diseases. To that end, the biotechnology industry continues to focus on enabling a more seamless transition from early discovery work through process development and ultimately to commercial production of viral vectors. With Thermo Fisher Scientific, you have the support you need to push your therapeutics development strategies a step further. We offer a suite of scalable and robust solutions for all stages of the gene therapy workflow to meet regulatory standards with confidence. For production platforms that support gene therapy applications, it is often not considered how that molecule or cell line might be used in a commercial scale-up if the therapy was successful, which could be up to the 2,000 L bioreactor scale, thus requiring a large amount of cell culture media.

Here we summarize the specific benefits that media formats may provide, based on drug development stages and facilities. We present here examples of biopharmaceutical industry end-user experiences that influenced decision-making and ultimately led to choosing Gibco™ Advanced Granulation Technology™ (AGT™) dry media.

Introduction to media format

Culture media are the most complex of bioprocessing essentials. Some include more than 50 different components. The most basic factor differentiating culture media is its physical state, also called media format. Media formats, e.g., dry powder media (DPM) and prepared bulk liquid media, are usually changed over the course of a biologic's development. For example, R&D and process development at small scales typically use liquid culture media in sizes such as 1 L bottles. But most facilities switch to using DPM as bioprocessing scales increase, usually as a fully customized product, and particularly before the start of phase III and commercial manufacturing.

However, for gene therapy processes, as the field is more recent, liquid media are more commonly used, even at larger scales.

Liquid media have the benefit of being ready to use upon receipt. However, liquid media prepared by manufacturers are generally more expensive, with the end user having to pay for shipping of what is mostly water (e.g., water for injection (WFI)). Another limitation of prepared liquid media is their reduced shelf life, which may be limited to months or weeks, whereas a DPM can be stored much longer. Although prepared liquid media have some clear advantages when it comes to ease of use, many end users lack storage space for a large quantity of liquid media.

As such, most late-stage biopharmaceutical manufacturers currently purchase >90% of their culture media as DPM. This powdered form is then rehydrated by end users, in-house. Preparation of liquid culture media from powders being a core part of bioprocessing, most bioprocessing facilities are experienced with media and buffer preparation, and standard facilities are currently designed to handle powders in dedicated areas for storage, weighing, hydration, and storage after sterile filtration, prior to filling bioreactors with media.

AGT dry media for gene therapy

Trends in bioprocessing are being driven by the need for efficiency in manufacturing. Especially in gene therapy, better technologies are needed that cut down on time-to-market and streamline product development and eventually commercial manufacturing. End users also want flexibility in their bioprocessing supplies and increasingly prefer to adopt facility- or company-standardized platform technologies and products.

For these purposes, bulk liquids are indeed an attractive option for many users, particularly at precommercial scales. However, as noted above, bulk liquid media cost significantly more than comparable powders, effectively outsourcing the preparation and quality control tasks. Also, few liquid media can be stored as stocked items at very large scales.

In contrast, powders can be stored in large quantities to be prepared in-house for immediate use.

To facilitate gene therapy developers' standardization efforts, the latest products from the Gibco™ brand—the Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System and the Viral Vector HEK Media Panel—have been designed to provide developers with both liquid and AGT formats, with the Gibco™ AGT™ Viral Production Medium especially offering extended pack size options off the shelf.

The AGT dry media format refers to both the unique process used to manufacture highly dissolvable powder granules and to the resulting mammalian cell culture media products. The AGT dry media format offers significant benefits over other media formats, including conventional DPM, ready-to-use liquid media, liquid media, and liquid media concentrates. These help enable simpler, faster, and more consistent media preparation and use, making AGT dry media format an attractive in-house platform for mammalian cell culture media applications. The AGT technology was launched in 2004 and is a well-established media format with high quality, reliability, and robust supply chains. It is now being utilized by bioprocessing facilities at all scales, including those manufacturing multiple commercial products. In fact, according to comments from end users, the AGT dry media format is well suited for "facilities of the future" where users are engineering multiple biologics at different scales and the facilities are designed for exclusive use of single-use technologies, including widespread adoption of single-use mixers.

Overview: AGT dry media manufacturing

AGT dry media are manufactured using a unique process. Most culture media in this format are single-component, fully chemically defined, particulate dust–free, rapidly soluble powders for mammalian cell culture. The technology enables the manufacture of advanced dry media with complex formulations, including those developed by end users.

The AGT dry media are manufactured using fluidized bed granulation technology in cGMP facilities that are maintained animal origin–free (AOF). During granulation, dry nutrient powder components are first suspended using a continuous column of conditioned air flowing up from underneath. The free-flowing powder particles are sprayed with a fine mist of aqueous solutions, resulting in an even distribution of many trace components. As the water dries, through surface fusion of partially dissolved powders, larger particles form into porous, free-flowing, highly water-soluble granules ready for rapid hydration and use (Figure 1).

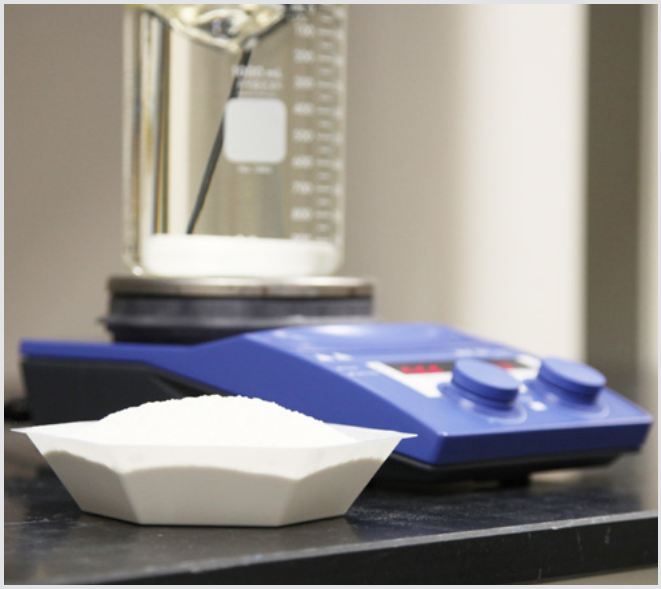

Figure 1. Advanced water-soluble granules ready for rapid hydration and use.

The gentle, controlled, minimal-shear environment of the fluidized bed does not adversely affect the various nutrient components being mixed and fused together. After granule formation, the granules are further milled and sized in a FitzMill™ comminuting device and blended in a drum ranging in capacity from 50 kg to 1,500 kg and in very large blenders handling up to 6,000 kg. The AGT dry media, as exemplified by AGT Viral Production Medium, are typically used at a concentration of around 20–25 g/L, with each kg providing 40–50 L of liquid media and 1,000 kg providing nearly 50,000 L, enough for manufacturing up to 15 AAV production lots. (This is required for high-dose systemic treatment, each using ~3,000 L of media.)

The process for manufacturing AGT dry media is fully scalable, producing consistent media regardless of lot and batch sizes. HPLC and other analyses of lots and batches show that biochemical constituents are homogeneously distributed throughout the granules, nutrient levels are comparable to those of conventional formats, and cell culture performance is equivalent to that of relevant reference media.

Converting to AGT dry media

Beyond off-the-shelf catalog offerings, we can also custom-manufacture and/or otherwise optimize AGT dry media. This includes Gibco™ media by design (development), Gibco™ Media Express™ manufacturing (rapid manufacture of small quantities in 10–15 days), and Gibco™ cGMP Media Custom Services. In contrast to standard DPM, AGT dry media are as complete as a single high-performance dry media formulation for mammalian culture can be, with at most a few supplements needed. Besides essential nutrients, AGT dry media may be formulated to contain recombinant insulin, other growth factors, or other supplements typically added separately.

The AGT dry media granules are so readily soluble that using separate mixing protocols does not result in inconsistencies in prepared liquid media. This is because upon mixing, even at large scale, no adjustment of pH or osmolality is required, due to the simplified media preparation process. Any mixer of suitable scale, whether single-use or stainless-steel, can be used for liquid media preparation with the AGT dry format, as confirmed through a mixing study utilizing Thermo Scientific™ single-use mixers and user feedback. The proven equivalence of AGT dry media and liquid media facilitates adoption and technology transfer across teams operating at different scales and sites using different equipment. For example, an AGT dry medium can be packaged as hydrated liquid, e.g., in 1 L bottles, for early comparability and validation studies in R&D, and then a user can seamlessly convert to using 1,000 L totes as pivot, as media preparation is scaled up alongside bioprocessing.

AGT dry media are manufactured at facilities in Grand Island, New York, and Inchinnan, Scotland. Between these redundant manufacturing facilities and the dry media format's compatibility with a diverse set of mixers and single-use packaging options for their hydrated media, users can increase the reliability of their supply chain when it comes to cell culture media sourcing and preparation. In addition, a variety of packaging options are available for the media themselves, all single-use, including bags, drums, and kegs with capacities up to 150 kg. Options include tapered bottle-shaped bags for easy transfer, tri-clover connectors and anti-static film to further improve AGT flow and transfer, and custom pre-weighed bags for simple attachment and addition to mixers.

Regulatory documentation can be made available for AGT dry media products, including Drug Master Files for most of the off-the-shelf catalog formulations. Regulatory agencies worldwide are familiar with and have approved products manufactured using AGT dry media—from investigational new drug through product approvals.

Conclusions

As discussed in this white paper, the AGT dry media format has been designed for simplicity and consistency. It can be adopted as a "platform" even though the format can be prepared by diverse facilities using different equipment. If desired, AGT dry media can be further improved with proprietary growth factors and other nutritional supplements that can be custom-manufactured for culture media optimized for specific processes.

In summary, compared to the standard DPM format, the AGT dry media format for cell culture offers significant benefits for gene therapy developers:

- AGT dry media are simpler, less expensive, faster, and more consistent to prepare and use than traditional powders.
- The AGT dry media format is an attractive in-house platform for mammalian cell culture media applications, including HEK293 cells.
- AGT dry media show lot-to-lot consistency at multiple lot/batch manufacturing scales.
- Users recognize the high quality of AGT dry media and their robust supply chains.

For further information, contact your Thermo Fisher Scientific media sales representative or visit **thermofisher.com/agt**