Preserving cell integrity and viability to drive the discovery of cell therapies

Cell therapies are poised to play a pivotal role in the development of precision and personalised medicines that will transform healthcare for millions of patients worldwide.

By Mary Kay Bates

Involving the direct use of whole cells or cellular material in patients, cell therapy is an umbrella term for many subdivisions of biological research, encompassing fields such as stem cell therapy, gene therapy and immunotherapy. These powerful techniques are deeply reliant on high-purity environments for culturing cells. As such, preserving the integrity of sensitive samples is a daily challenge for cell culture laboratories in their search for innovative new therapies.

The cells used in transplantation can either be derived from an individual patient (autologous cell therapy) or from another donor (allogeneic cell therapy). Allogeneic methods are more akin to the pharmaceutical model of drug manufacturing as products can be produced on a larger scale and can be prepared for ‘off the shelf’ distribution, provided the recipients have an immunological profile matching the cells or cell products. For research purposes, autologous cell therapies are often favoured because they do not require immunological matching and are personalised to the patient.

One area in which autologous therapies have shown particular potential is in the treatment of cancer. Recent advances in immuno-oncology research, for example, have resulted in a paradigm shift for treatment of some cancers. Immuno-oncology approaches have been found to be more effective at long-lasting tumour regression than alternatives such as surgery, radiotherapy, chemotherapy and targeted therapy\(^1,2\). Immuno-oncology therapies typically use genetically modified T-cells to combat and kill cancerous tissue by identifying the cancer cells as a foreign body in need of attack. T-cell therapies based on chimeric antigen receptor (CAR) T-cells are widely employed for this purpose, as they have the capacity to proliferate within the patient after infusion and offer sustained functional immunity. This efficacy has been proven in a wide range of cancers\(^3\) and has shown encouraging clinical data from Phase I trials in solid tumours, such as neuroblastoma.

These innovative therapies rely on the use of techniques and technologies that minimise the risk of contamination and achieve the strictly-controlled, secure environment necessary for growing healthy, viable cells. This article discusses the challenges facing cell therapy research, and how the latest cell culture technologies are delivering enhanced safety capabilities, improved laboratory performance and, ultimately, optimised cell therapy processes.
The modern challenges of cell therapy

While cell therapies show great promise in bringing innovative and much needed treatments to patients, there are a number of key issues currently limiting their broader adoption. Safety concerns over the administration of live cells to patients, uncertainties over the way in which these therapies should be regulated, unknown downstream effects and the need for sufficient investment to fully commercialise research are all challenges that must be overcome in order to accelerate further development of cell therapies.

Because of the huge potential for variability within treatments, an additional challenge in cell therapy is the need for robust manufacturing processes that consistently deliver safe and effective products. Cell therapy manufacturing processes can range from the simple expansion of autologous cells that will be administered back to patients, to the complex genetic manipulation of allogeneic cells that could be stored and banked for the treatment of multiple patients. The regulations surrounding cell therapies must therefore be sufficiently broad to cover this wide range of therapeutic processes, while still safeguarding product safety and quality.

From a manufacturing point of view, autologous cell therapies can be more challenging to produce than allogeneic cell therapies, as each dose is a single batch and is derived from a different source. This presents problems for cell culture, as cells from different patients will behave in distinct ways and source material will contain a variety of cell types with various growth and differentiation capabilities. Additionally, if the cells are stored together there is an increased risk of contamination across batches.

In contrast to autologous cell cultures, allogeneic cell therapy manufacturing processes are less prone to cross-contamination as they are usually derived from a single large batch. However, other manufacturing challenges remain. Maintaining product consistency throughout the different stages of development is often a difficult task as there are many variables that can affect cell growth. As such, developers of cell therapy products must consistently monitor their processes and adopt the best cell culture methods to ensure that products are produced with the desired critical quality attributes.

Avoiding contamination in cell cultures

Underpinning the major advances in cell therapy research is the need for robust cell culture conditions. Contamination of cell culture experiments is one of the most common problems encountered in
laboratories, and can have far-reaching consequences. Cell culture processes are highly sensitive to contamination from bacteria, viruses, fungi and other biological contaminants. For cell therapy development, which demands the highest levels of sample purity, it is vital that contamination is minimised. Given this importance, a wide range of innovative technologies have been developed to ensure that these strict levels are met.

The latest biosafety cabinets (BSCs), for example, offer very robust protection from contamination. BSCs are enclosed, ventilated workspaces that serve to protect laboratory workers and the surrounding environment from pathogens by filtering workspace air to remove harmful bacteria and viruses. Improvements in BSC design mean that cabinet inflow and downflow air velocities can now be monitored in real-time and automatically balanced to ensure optimal conditions. Should out-of-spec conditions be reached, alarm signals alert the user to take remedial action. This is incredibly important for cell cultures as maintaining optimum purity is the key to successful, reproducible results.

Maximising the reproducibility of results allows for more standardised processes, which streamlines the production of therapeutic products, and can accelerate the commercialisation of cell therapies.

Once cultures are no longer actively manipulated in BSC workspaces, they are typically placed into incubation for cell expansion. But ensuring that cultures remain contaminant-free when incubated for long periods of time can be a challenge. An innovative approach, used by many researchers, involves securing cultures inside specially-designed chambers or cell lockers to ensure maximum protection against cross-contamination. These chambers include membrane filters that permit air circulation, but exclude microbial contaminants, minimising cross-contamination and allowing lots to be quarantined individually. As such, these storage solutions can be very useful in larger labs, and are especially useful for autologous cell therapy research where several different batches of cells may need to be incubated at the same time.

Furthermore, another way that the cell therapy industry is benefitting from the advancements made in modern equipment design is with innovative CO₂ incubators. A CO₂ incubator is one of the most important pieces of technology for effective cell culture and subsequent cell therapy applications as it mimics in vivo conditions and reduces experimental variability, as much as possible. The latest incubators now allow adjustments to be made to O₂ levels to simulate hypoxic environments, and employ proven, on-demand high temperature sterilisation cycles designed to eliminate microbial contaminants and simplify cleaning procedures. On this latter point, modern incubators often now feature a polished stainless-steel interior with curved corners for easy cleaning or, alternatively, systems with surfaces made from pure, natural copper, which are also very easy to clean. Moreover, copper surfaces provide a long service life, are safe for cultured cells and are recyclable, making for a sustainable choice.

Available on a few state-of-the-art incubators, high efficiency particulate air (HEPA) filters ensure airborne particulates are removed and cell cultures are protected. Filter technologies have advanced to such an extent that conditions can be restored to the highest levels of cleanliness within just five minutes following a 30-second incubator door opening. Additionally, features present in the most effective incubators, such as the ability to directly connect external water supplies, allows refilling without opening the chamber, minimising water-based contaminants. Modern, easy-to-use humidity controls and water-level indicators ensure that sample desiccation does not occur.

Minimising cell contamination in these ways can help researchers achieve the highest levels of sample integrity and maximise the reliability of results. These equipment innovations are also allowing researchers to reduce costs and save time by avoiding the need for repeat experiments. This improved efficiency is enabling more in-depth research to be performed, with far-reaching benefits for the entire cell therapy field.

Ensuring cell viability
Maintaining cell viability is critical for cell therapy applications, but the complexities of manufacturing can make this a difficult task. The parameters that define cell viability in an experiment can be as diverse as the redox potential of the cell population, the integrity of cell membranes or the activity of cellular enzymes. However, recent innovations in equipment design are helping to overcome this multifaceted challenge and ensuring greater cell viability.

Specially-designed cell viability indicators have been developed that can provide a visual readout of cell health using a fluorescence microscope, microplate reader or flow cytometer. These can be incredibly useful tools for the cell therapy industry as they reduce the risk of experimental failure and increase the reliability of cell culturing techniques.

Maintaining a homogenous environment is key to the success of cell culture experiments, but a major challenge in the way of achieving consistent
cell culture conditions is the so-called ‘edge effect’. The edge effect refers to a pair of related problems that affect the consistency of results achieved when using cell culture plates – the differential temperature distribution across the plate and differential evaporation between wells.

Temperature gradients across well plates can pose a significant problem when culturing cells. The evaporation of culture media can change the pH and osmolality, affecting cell development and reducing cell viability. Because evaporation effects tend to be greater on the outermost wells of the plate, this effect is not uniform, prompting uneven cell seeding. Traditionally, this problem is typically overcome by leaving the outer 36 wells empty, or by filling them with sterile water. However, while these approaches mitigate the edge effect, they can reduce the size of a typical 96-well plate by more than a third.

To combat this problem, plates with a perimeter moat filled with sterile water or agarose have been recently developed. These innovative culture vessel solutions reduce the evaporation and increase cell viability, while providing use of a full-size well plate so that efficiency and productivity are maintained. Elegant solutions to cell viability problems, such as these, are benefitting the cell therapy industry by increasing throughput without altering workflows.

Creating realistic in vivo conditions

The development of effective therapies relies upon consistent cell culture methods and the ability to recreate realistic in vivo conditions. Traditional two-dimensional (2D) monolayer cell cultures can offer highly reproducible environments that are suitable for applications, such as drug screening. However, to recreate the complex environment found in cancer tissues, more elaborate structures are typically required. Here, the additional dimensionality of 3D cultures can lead to more predictive cellular responses, not only influencing the spatial organisation of cell surface receptors engaged in interactions with surrounding cells, but also inducing physical cell constraints.

Recent cultureware innovations are addressing this challenge by making it possible to culture cells in 3D without a scaffold, allowing cells to spontaneously aggregate and produce more physiologically relevant environments. Cells cultured in this manner form spherical clusters called spheroids, which have been successfully implemented in many aspects of cell therapy. In stem cell research, for example, 3D aggregates of pluripotent stem cells, known as embryoid bodies, provide the physiological signals that prompt the differentiation to different cell lineages. They are, therefore, highly useful for regenerative medicine applications as they may be used to repair damaged or diseased tissue, and could also be used for in vitro testing in the pharmaceutical industry or as a model of embryonic development.

Spheroids and other such 3D cell cultures provide a much closer representation of an in vivo environment. However, their biological complexity means they can be difficult to culture consistently in vitro. To deliver more reproducible results, many researchers are employing round-bottomed microwell plates for spheroid production. Round-bottom plates make it possible for cells to aggregate to a much more uniform size and shape than is typically possible using traditional flat-bottomed culture vessels.

In addition, the vessel surface can be treated with a low binding polymer coating which encourages the cells to bind to each other rather than the surface, minimising variability and supporting the formation of consistent spheroids. By creating realistic cell culture environments in this way, more reliable results can be achieved, ultimately accelerating the development of safe and effective cell therapies.

Maximising lab space

Ongoing advances in equipment design are not only helping to improve the quality of results, they are increasing productivity too. Thanks to simple design improvements, the latest solutions are helping laboratories operate more efficiently by maximising the space available for research.

Take the design of orbital shakers, for example. These important pieces of equipment are needed to aerate and agitate suspension cell cultures effectively, ensure that oxygen and nutrients are available throughout the mixture, and avoid any cell settlement on the bottom of the flask. Traditional orbital shakers are not robust enough to be used inside a CO₂ incubator, since the heat, humidity and weak carbonic acid atmosphere are very damaging to the electronics. However, recent innovations have led to the development of orbital shakers with magnetic drives to dramatically reduce vibration, and sealed electronics that significantly extend the life of the equipment used in the CO₂ incubator. Remote monitoring systems that can be mounted on the incubator door serve to reduce disturbance to cultures, and allow at-a-glance observation of settings and conditions.

Capable of being used either within or outside the CO₂ incubator, CO₂-resistant orbital shakers
can help to maximise lab space and ensure that different cell types can be cultured simultaneously. As a result, these innovative technologies are helping researchers achieve high-quality results, while optimising speed and efficiency.

**Conclusion**

Cell therapies have the potential to improve healthcare for millions of patients worldwide. However, to fully realise this goal, they require robust cell culture methods that afford high sample purity and cell viability. Alongside ongoing advances in the capabilities offered by cell therapies, the equipment used for cell culture has also evolved. These innovative technologies are helping to maximise the consistency of results by maintaining optimal conditions and minimising the risk of cell contamination.

**Mary Kay Bates** is a Senior Global Cell Culture Specialist with Thermo Fisher Scientific. Her knowledge is based on 20 years of experience in academic and industrial cell and molecular biology labs, focusing on cancer and gene therapy. Mary Kay holds an MS in microbiology from the University of Wisconsin-Madison.

---

**ADVERTISEMENT INDEX**

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Cell Diagnostics</td>
<td>15,17</td>
</tr>
<tr>
<td>Agilent Technologies, Inc</td>
<td>23,33</td>
</tr>
<tr>
<td>Biostrata Ltd</td>
<td>39</td>
</tr>
<tr>
<td>BioTek Instruments, Inc</td>
<td>25</td>
</tr>
<tr>
<td>BMG Labtech GmbH</td>
<td>8</td>
</tr>
<tr>
<td>Charles River Laboratories, Inc</td>
<td>40-41,54</td>
</tr>
<tr>
<td>ELRIG</td>
<td>36</td>
</tr>
<tr>
<td>IntelliCyt Corporation</td>
<td>3</td>
</tr>
<tr>
<td>Labcyte, Inc</td>
<td>6</td>
</tr>
<tr>
<td>PerkinElmer, Inc</td>
<td></td>
</tr>
<tr>
<td>Quanterix Corporation</td>
<td></td>
</tr>
<tr>
<td>Select Biosciences Ltd</td>
<td></td>
</tr>
<tr>
<td>Source Bioscience</td>
<td>4</td>
</tr>
<tr>
<td>Taconic Biosciences, Inc</td>
<td>45</td>
</tr>
<tr>
<td>Thermo Fisher Scientific</td>
<td>60</td>
</tr>
</tbody>
</table>

DDW