CELL & GENE THERAPY INSIGHTS

EXPERT INSIGHT

Cell therapy post-production technologies: a select review of key innovations in the field

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The cell therapy industry continues to gain momentum as an increasing number of treatments move forward from clinical trials to commercialization. Recent changes in regulatory language and procedures specifically recognize the potential of cell-based therapeutics. The expected and observed boost in manufacturing of cellular therapies must be accompanied by innovations in the post-production processing of these products to accommodate the higher production volume and increased variety of cellular therapies. Novel post-production technologies facilitate and accelerate product delivery, allowing manufactured live cell therapies to reach patients more reliably and efficiently. This article reviews a select number of the latest innovations projected to drive successful commercialization of cell therapy products.

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Stem cells and other cellular products have generated enormous interest in the medical field owing to their potential to replace or repair defective or damaged cells, and, one day, treat almost any injury or disease. Regenerative medicine and cellular therapy are the two intertwined fields of biology that many hope will redefine our understanding of modern medicine. While regenerative medicine is a broader term that encompasses proteins, growth factors and other biological molecules, cell-based therapies are more concisely defined as living cells that are introduced into the body to achieve benefit in the recipient [1]. The process of efficiently delivering a live cell therapy to the patient presents many challenges. A recent review article focused on how technological developments in the downstream elements of cell therapy processing can help meet those challenges [2]. In this article, we present an update on new developments in post-production technologies that have taken hold since then, and examine how new regulatory oversight will impact the last mile of cell therapy commercialization.

THE NEW REGULATORY FRAMEWORK

Cell therapy, as a trade, has grown from humble beginnings to a multibillion dollar industry [3,4]. Over 20,000 cell therapy studies are currently listed on the federal clinical trials website for the USA alone [5]. The 21st Century Cures Act, which became part of US government policy in December 2016, introduces for the first time, new federal designations [6] and language specifically devised to recognize cell-based therapies and their incredible potential to treat or cure life-threatening diseases. The primary goal of the 21st Century Cures Act is to allow novel life-saving therapies to reach patients more quickly. While there are many safeguards in place to ensure that such therapies are both safe and effective, there are also specific clauses in effect that permit "compassionate use" of cell therapies in early stages of approval, in those cases where a patient's life is under imminent threat and no viable alternative treatment exists.

The US Food and Drug Administration (FDA) started accepting applications for the new 'Regenerative Medicine/Advanced Therapy' (RMAT) designation in January 2017. Approval under the RMAT designation allows a drug product to apply for a fast-tracked FDA approval process, as long as there is convincing clinical data verifying real-world safety and efficacy. Fast-tracked cell therapies will be subject to new FDA post-approval requirements, such as larger datasets and monitoring of all treated patients.

The RMAT designation will impact more than just cell-based products. It will also cover devices used in the isolation, recovery, or delivery of these products. RMAT labeling of such devices will be decided on a case-by-case basis, and decision criteria will include any limitations on use and any limitations on cell types the device will cover. While the specifics of the new RMAT designation are still being worked out, it is certain that implementation of the new regulatory language will have an impact on downstream technologies. Rollout of an expedited approval process means that novel cell therapies can reach commercialization faster. Consequently, post-production processes must urgently be streamlined, easily implemented and made highly scalable in order to keep up with FDA post-approval requirements.

SCALABLE MANUFACTURING

Once a cell therapy product has passed through clinical testing and the FDA approval process, the product needs to be scaled up for commercial manufacturing. Cell therapy manufacture generally falls under two broad categories: 'allogeneic' therapy and 'autologous' therapy. Allogeneic therapies, where cells from a single donor can be introduced into several recipients, require extensive compatibility testing before a patient can receive them. One might envision the scale-up of allogeneic therapies through the implementation of dedicated centralized manufacturing facilities producing millions of identical therapeutic doses. This model, however, presents challenges, for example in the adverse effects of prolonged cell culture [7]. For now, allogeneic therapies are produced on a smaller scale. Cells are collected from a healthy donor,

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and can be expanded, modified and cryopreserved until needed for a patient. These 'off-the-shelf' therapies may have an economic advantage over precision therapies, since they can potentially be used for multiple treatments in multiple patients. Several allogeneic cell therapy products are already on the market; exemplified by GINTUIT™ (Organogenesis, Inc; MA, USA), a treatment of oral gingivitis, and ALLOCORD (SSM Cardinal Glennon Children's Medical Center; MO, USA), a therapy against disorders affecting the hematopoietic system [8].

Autologous cell therapies are patient specific. Cells are collected from the patient, then modified such that they can help treat or cure an illness, and finally are re-introduced into the patient. Therapies based on autologous cells have a notable advantage in that they are generally safer for the patient since there is less chance of immunological rejection. Autologous cell therapy forms the basis of personalized, or "precision" medicine. Because such therapy is specifically targeted to each individual patient, it is more likely to be effective for that patient. Scalability is more challenging for autologous therapies, since the exact composition of cell types cannot be predicted beforehand, and the source of cells is limited. There is also the problem of donor-related variability, which can translate to variable efficacy between patients. For example, in an analysis of the reasons behind the failure of a mesenchymal stromal cell-based clinical trial, the authors cited inter-donor variability in immunoregulatory function as one of the primary reasons the trial failed [9]. In spite of this setback, several autologous cell therapies have already received FDA approval [10], and others have reached the clinical testing phase [11].

Scale-up of any cell-based product must ensure that quality and reliability are maintained from the point of collection of starting material, through manufacture, to the point of administration to the patient. Most of the current technology is focused on rapid expansion of a well-characterized, standardized cellular product. It is more difficult, however, to accommodate cell therapy products that are not easily standardized.

The key to improving scalability of these autologous therapies lies in innovative solutions for post-production processing technologies, particularly in the areas of automation, process integration and simplification, and product delivery. Technologies focused on post-production processes such as cryopreservation, therapy transport and shipping, and post-thaw cell revival are gearing up to meet the challenges involved in this process.

CRYOPRESERVATION

While a 'fresh' cell therapy product is often useful for pre-clinical or proof-of-concept studies, once a late-stage clinical or commercial product is under consideration, a cryopreserved final product is much preferred. Cryopreservation affords doctors and patients critical flexibility in scheduling treatment, and allows thousands of doses of a cellular product to be shipped to treatment centers all over the world. Cells are optimally cryopreserved at temperatures below -130°C for long-term storage. At such temperatures, all measurable biological activity stops, protecting cells from metabolic

and chemical damage. Studies have shown that a slow, constant freezing rate of -1°C/ min yields optimal post-thaw survival for cryopreserved cellular products [12,13].

Freezing of cellular therapies can reliably be achieved in controlled-rate freezers with variable cooling rates and storage volumes such as Thermo Scientific's CryoMed™ (MA, USA) or SP Scientific's Bio-Cool™ (PA, USA) freezers. -80°C freezers are often used in combination with passive freezing devices for short-term storage; a cost-effective alternative to a full-sized freezer is embodied in MedCision's BioT™ ULT Mini Freezer (CA, USA), an under-the-counter -80°C freezer with low power consumption, and a smaller footprint. Once cell samples reach -80°C, they can be transferred to liquid nitrogen storage containers and remain there almost indefinitely without degrading.

To ensure the safety and efficacy of the final product, standardized cryopreservation methods should be integrated as early as possible during clinical therapy development. One of the most important considerations in assuring a safe, reliable product is the composition of the freezing medium. Cryopreservation media should be optimized based on cell type, storage temperature, and freezing and thawing rate. Much thought has been put into the development of an ideal cryopreservation media over the years. Formulations usually include dimethyl sulfoxide (DMSO) at a certain percentage (5 or 10% are common). Where possible, lower concentrations of DMSO are preferred due to concerns about toxicity [14]. While some cellular therapy products undergo washing and concentration steps prior to patient administration, this is not always possible, or even desirable [15]. Often, minimal manipulation of the final product is preferred in order to avoid infection and handling accidents, and to increase the product's shelf-life. Because components of the cryopreservative media may be injected directly into patients, care must be taken to ensure the media is produced according to Good Manufacturing Practices (GMP) guidelines and optimizes cell health, while minimizing the chance of an adverse reaction.

CryoStor® media (BioLife Solutions; WA, USA) is a good example of a freezing media that is widely utilized in the cellular therapy field, and for good reason. This media encompasses several aspects listed as part of Biopreservation Best Practices. Scientifically, the media includes components that provide an intracellular-like balanced environment for the cells that is specific to low temperature conditions. This is in contrast to traditional isotonic-based home-brew freeze media cocktails, whose base formulations were designed for normothermic conditions. CryoStor® also includes components that scavenge free radicals, and provide pH buffering capabilities designed to act in concert with the intracellular-like salts and sugars to reduce cell damage and cell death due to apoptosis and necrosis. From a quality/regulatory risk perspective, CryoStor® is serum-free, protein-free and manufactured in accordance with GMP guidelines. Cell therapy products utilizing CryoStor® as an excipient material that is present as part of the final cell therapy product at point-of-care are tolerated similarly to traditional clinical home-brew

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freeze media, and consequently are frequently used in clinical trials and applications [16].

Other GMP-compliant DMSO-based solutions include CryoSolutions™ by Akron Biotech (FL, USA), and Syth-a-Freeze®, by Thermo Fisher. Still other companies offer freezing media specifically designed for stem cell applications, notably STEM-CELLBANKER® media (Amsbio, UK), HyClone™ HyCryo-STEM (GE Healthcare, IL, USA) and StemMACS™ (Miltenyi Biotec, Germany).

While optimized cryopreservation media are vital, choosing the correct vessel for cryopreservation of the therapy is equally important. Cell suspensions need to be stored in a way that safeguards against contamination and water entry during and after cryopreservation. Several different companies offer closed-system cryogenic vials that use aseptic filling technology that minimizes contamination risk. The Crystal® Closed Vial system (Aseptic Technologies, Belgium) is currently used for storage of both allogeneic and autologous cell therapy products. Cook/Regentec (IN,USA) market a similar product, the CellSeal® cryogenic vial. Both the Crystal® and CellSeal® vials meet particulate matter injection standards required by the US Pharmacopeial Convention's safety regulation USP 788.

Cryobags are held to similar safety standards to prevent leaking and contamination. Origen Biomedical manufactures two distinct types of cryopreservation bag. One, the Cryostore freezing bag, is marketed for blood, blood components, and cord blood storage, while the other, the PermaLife™ bag, is specialized for cell cryopreservation. Macopharma (France) have developed innovative cryobag processing technology for cord blood freezing and storage. Their cryobags are registered sterile medical devices (CE marked) intended for long-term storage of cells in liquid nitrogen at temperatures down to -196°C. KryoSure bags, offered by Saint Gobain Performance Plastic Group (UK) are another innovative product. Both these and Origen's PermaLife™ bags are made of fluoro ethylene propylene (FEP), a biologically, chemically and immunologically completely inert substance. The bags remain flexible when immersed in liquid nitrogen at temperatures down to -196°C, and are currently being used by numerous cell therapy companies.

TRANSPORT & SHIPPING

The nature, extent and character of the transport and shipping processes for cellular products are dependent on the necessary transport distance and speed. For autologous cell products, point-of-manufacture and point-of-care may be in proximate locations, even in the same clinic. However, no matter how short the travelling distance, cells need to be protected from temperature fluctuations.

There are many solutions currently available for easily portable, short-term shipping of cellular products. MedCision's line of BioT™ temperature stability systems accommodate both dry-ice and liquid nitrogen based transport needs. Traditional practices of shipping products on dry ice in a simple polystyrene container have been shown to lead to irregular temperature regulation [17] that can damage sensitive products. The BioT™ ULT

Transporter maintains highly reproducible temperatures below -50°C for more than 24 hours, while the BioT™ LN_2 Transporter maintains temperature at less than -180˚C to -150˚C, for 1.5 to 2 hours.

For longer transport needs, cryogenic containers are often regarded as the best solution, and liquid nitrogen dry vapor shippers are the most common method employed to keep temperature sensitive products safe. Cryoport (CA, USA), Taylor-Wharton Cryogenics (AL, USA) and Chart MVE (Luxembourg) all market liquid nitrogen dry vapor shippers capable of maintaining stable temperatures below -150°C for up to a week. Most of these cryogenic containers can be paired with data logging and temperature tracking devices that continuously record temperature readings during shipment.

THAWING

Thawing is the last procedure a cellular product is subjected to before being administered to a patient, and as such, is one of the most critical points in post-production processing. The thawing rate is just as important as the freezing rate in protecting cell viability, and should ideally be carefully controlled and optimized.

Historically, cryopreserved cells have been thawed using a 37°C water bath, but it is increasingly being recognized that this method exposes therapeutic cells to risk. Non-standardized thawing methods have repeatedly been shown to have deleterious effects on cellular products [18–20], including, but not limited to, lowered viability, lowered proliferation and altered cell subset recovery. In addition to these hazards, use of a waterbath carries the risk of water-borne contamination, a fact that restricts, and in some cases, prevents their use in GMP-regulated facilities.

Unsurprisingly, efforts are now being undertaken to standardize and de-risk the cell thawing process. Several dry cell thawing devices have been developed in the last few years. Plasmatherm (Genesis BPS; NJ, USA), Sahara-III (Sarstedt, Germany), SmartMax (BioSafe, Switzerland) and Cyto-Therm's CT-D4 (NJ, USA) are all plasma thawers designed to thaw or warm standard-sized cryobags. A fully automated and customizable system, known as the ThawSTAR® automated thawing platform, is offered by MedCision Inc. This automated platform is a water-free system designed to handle both cryovials and cryobags [21]. MedCision's line of automated cell thawers are predicated on the understanding that factors such as cell type, cell size and choice of cryopreservative all affect the optimal thawing rate [22] and their technology incorporates a thawing algorithm that can be pre-customized for a given cellular therapy. Studies show that use of this automated thawing system improves both cell subset recovery, and post-rest cell recovery when compared to a traditional water bath [19,23].

Other companies entering the cell thawing market with their own water-free thawing devices include CPSI Biotech's SmartThaw system, and more recently, the CellSeal® Thaw System introduced by Cook Regentec and Asymptote Ltd (UK). Recent studies have shown that thawing can affect not only short-term outcomes such as post-thaw

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viability, but also longer-term outcomes such as cell recovery, and thus potentially impact the long-term efficacy of live therapeutics [23]. As the full impact that thawing can have on highly temperature sensitive cell-based therapeutics becomes clear, the role that automation plays in cell therapy commercialization is rapidly growing, and automated post-production solutions are becoming more common. We predict that this current trend will gain momentum as more cell therapies are fast tracked into the market place.

DATA TRACKING/ CONNECTIVITY

A key requirement of regulators when approving cell therapy products or granting RMAT designation is for developers to demonstrate quality control throughout each product's chain of custody. Products are usually tested at several points during their production, as well as immediately prior to the release of the product for clinical use. While it's obviously important to assess parameters such as potency, safety and consistency of a cellular product, such testing is complicated by a number of factors. For example, it is often not possible to test the raw materials or consumables used during manufacturing, but these materials can have a significant impact on product safety and efficacy. FDA regulations allow for a good deal of flexibility when determining product potency, so it can be difficult to compare product quality across different types of therapies. And while the evaluation of cryopreserved final products (in terms of purity, potency and tumorigenicity) is very important, it is not always correlated with clinical response. These and other challenges are still being worked out as cellular products approach commercialization.

Standardization and documentation go a long way toward ensuring product quality and consistency. However, as the number of cell therapies grows, and automated equipment increases the number of sites capable of supporting cell therapy products, maintenance of quality processes is becoming more challenging. TrakCel (CA, USA) provides a software platform designed specifically to manage the cell therapy supply chain, and is helping cell therapy developers demonstrate compliance [24]. Workflows built into TrakCel ensure that each step of the process, regardless of where it is happening, is performed in a consistent manner and according to the developer's SOPs. Integration of data captured in TrakCel with that from their participant's systems and equipment provides full visibility across the supply chain in a single data report. In the case of autologous therapies, TrakCel provides chain-of-custody functionality that ensures that starting material is infused back into the original donor, guaranteeing patient safety and regulatory compliance.

TRANSLATIONAL INSIGHT

The cell therapy industry is developing rapidly, and automation is a key component of that development. The dream of fully automated stem cell line production may soon be realized. Projects such as Europe's AUTOSTEM [25] are working to transform the industry

with 'stem cell factories' capable of delivering cell therapies to thousands or even millions of people. New, fully synthetic growth substrates are making it possible to produce billions of stem cells within a short time period [26]. The specific recognition by the FDA of the enormous potential of cellular therapies has prompted the rollout of a fast-tracked regulatory approval pathway designed to accelerate the cell therapy industry within the USA. The manufacturing process for cell therapies is speeding up, and if we are to avoid a bottleneck, post-production technologies related to product scale-up and delivery must keep pace with it.

The primary concern of any cell therapy process is delivery of a safe, reliable product to the patient. For this reason, the importance of post-production requirements of therapeutic cells cannot be overstated. Innovation can ensure that each step in the process of bringing a finished cell therapy product to the patient is carried out in a way that limits variability and prevents contamination risk. In practice, automated technology can go a long way toward achieving that goal, because it supports both process integration and data-driven accountability. Innovations in the packaging and storage of cellular suspensions can limit contamination risk, while novel transport and shipping solutions can track or prevent any damage due to non-optimal temperatures. Automating cell thawing protects the safety and efficacy of live cell therapeutics by reducing contamination risk and removing subjectivity. Incorporating data connectivity into these processes ensures regulatory compliance, chain-of-custody, and predictable patient outcomes.

Ultimately, the goal for post-production technologies should be process integration; to streamline and improve last mile procedures so that they dovetail neatly into upstream processes. Many serious diseases have no known cure. Regenerative medicine and cell therapy offer a new hope for many people, and we will continue working toward making that hope a reality.

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