Fraunhofer IPT

White Paper

Automation for Translation and Commercialization of Stem Cell-based Therapies

Abstract

Advanced therapeutics such as stem cell and gene therapies are expensive whether an autologous or allogeneic product is used for patients. There is an imperative to comply with good manufacturing practice for generation of approved products as well as extensive pre-clinical and clinical testing to validate the effectiveness of advanced therapy medicinal products (ATMPs) before use on patients. Minimising costs during the manufacturing process, without a loss of rigour, is a necessary step to enable expansion of the gene and stem cell fields for patient benefit. Manual production incurs significant costs associated with staffing of the facility for example. Even with the most stringent controls, there is also an increased risk of contamination events with human interventions. Closed automated systems where all steps, from receipt of the raw materials to cryopreservation of the final ATMP, are enclosed in a miniaturised Good Manufacturing Facility can minimise or even prevent contamination. Use of these can ultimately result in reduced manufacturing costs for patient benefit. One such system, the AutoCRAT platform is shown in Figure 1.

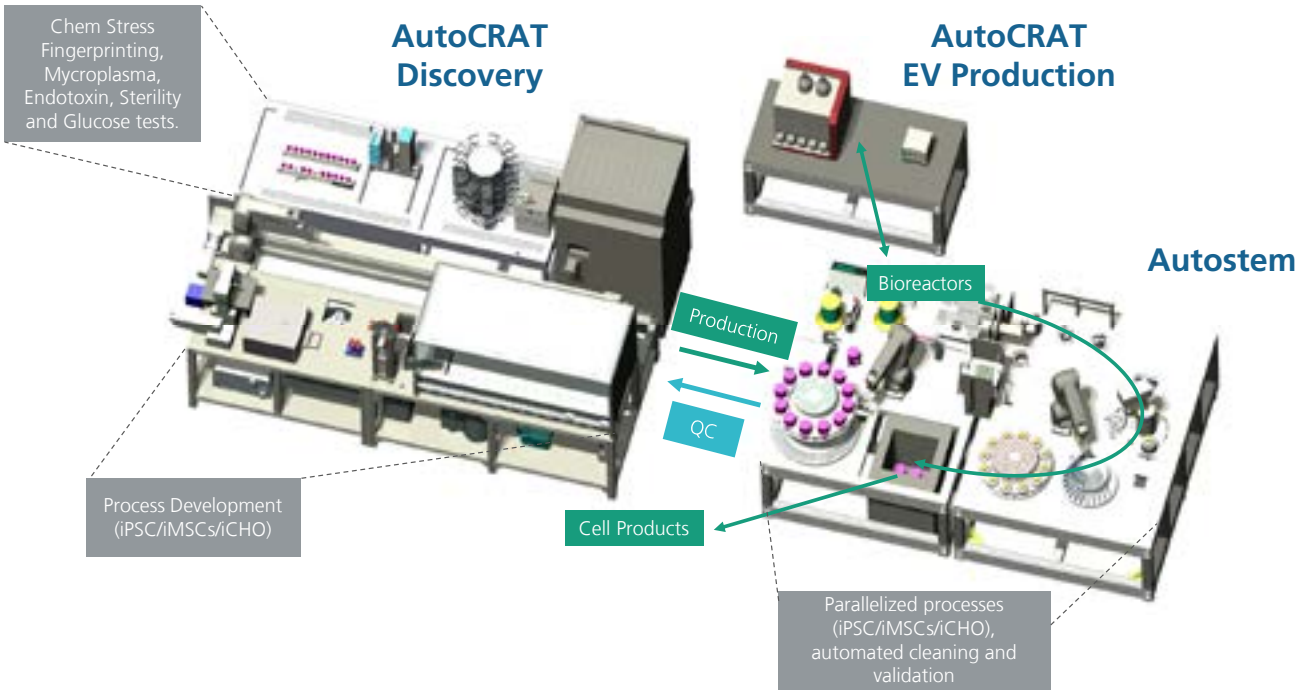

Figure 1: The AutoCRAT Regenerative Medicine Factory (ARM-F) represents a production platform that encompasses 1) a Stemcell-Discovery module to enable initial differentiation of hiPSCs to hiMSC and hiCHO and the implementation of several quality control inline test protocols, 2) the Autostem platform for bioreactor-based cell production or the "Production Module", 3) the EV separation module and 4) material storage for production and formulation, and cryopreservation of AutoCRAT cell and EV products

Introduction

Industry 4.0, also referred to as the Fourth Industrial Revolution, aims to make information available in a digital form for manufacturing processes. More importantly, it goes beyond this to use the digital information generated for an increased understanding of process controls and generation of output with improved product quality. As such the use of these tools will contribute to streamlining manufacturing and improve all aspects of industrial manufacturing, from the original concept to delivery of the final product. Implicit to achieving this aim for any manufacturing process is automation and the use of computer-based or online control systems [1], [2].

In the context of stem cell and gene therapy manufacturing, where significant personnel and infrastructural costs are associated with manual production systems as well as the increased risk of contamination events, it is clear that automated production with integrated quality control and quality assurance aspects will

1. Enable significant cost reductions for production campaigns
2. Reduce manufacturing campaign failures
3. Increase uptake of these therapies by many patient cohorts resulting in
4. Significant benefit for patients living with chronic diseases that have no effective, disease-altering therapies currently such as osteoarthritis (OA) through the use of cell and dell-derived therapies such as extracellular vesicles (EVs) and
5. Enable delivery of engineered gene therapies for haematopoietic malignancies, solid tumours and other indications.

Haematopoietic cell transplantation

Natural killer (NK) and CAR T-cell therapies were initially used to treat haematological malignancies as well as solid tumours with a focus on autologous, patient-targeted treatments for tumours in patients refractive to the standard-of-care [2]. With issues such as cell quality, cell number, exposure to chemotherapy and radiation, and CAR expression variability between patient batches, relatively high manufacturing failure rates added to the considerable costs associated with these treatments. However, significant effort has focused on the development of automated hospital-based systems for cell and gene therapy indications targeted to treat individual patients that should result in increased success rates [3].

For example, efforts towards enabling smart, automated manufacturing of autologous, chimeric antigen receptor (CAR-T) cell therapies have led to the emergence of strategies that have incorporated systems with a structure that enables the use of industry 4.0 for integration of artificial intelligence (AI) [4].

A trend towards the use of allogeneic treatments has also increased the availability and uptake of haematopoietic cell transplantation. Indeed 2019 figures indicate that 41 % of 43,581 patients treated in Europe and collaborating countries were spread over 700 centres and 51 countries [5].

Mesenchymal Stem / Stromal Cell-based Therapeutics

In terms of the development of other cell therapies, mesenchymal stem / stromal cells (MSCs) were first described by Friedenstein et al. in 1966 with Caplan and co-workers describing the capacity of the cells to promote repair of full thickness cartilage defects in rabbit knees in the early nineties [6]–[8]. One of the first successful MSC treatments in humans addressed large bone defects (4 to 7 cm). In this autologous study, three patients who were treated with bone marrow-derived cells loaded on a macroporous hydroxyapatite scaffold regained limb function [9]. The ability of the cells to modulate development of development of osteoarthritis (OA) was first shown in a goat model of knee OA with disease generated unilaterally by a combination of anterior cruciate ligament resection and complete excision of the medial meniscus [10]. Green fluorescent protein (GFP) labelled autologous cells were injected into injured knees six weeks post-injury with the contralateral, uninjured joint receiving the equivalent volume of the hyaluronan diluent. Analysis of treated joints at 12 weeks showed the presence of a medial meniscal structure with the labelled cells detected in the regenerated tissue; other phenomena associated with OA such as articular cartilage degeneration. Osteophytic remodelling and subchondral bone sclerosis were also reduced compared to control joints at this time point. However, there was no evidence of ligament repair [10]. This and other subsequent studies indicating positive effects of the cells in pre-clinical models for many other indications led to efforts to translate the therapies to human use.

Indeed, many MSC clinical trials have been performed for numerous different indications. A search for the term "mesenchymal stem cells" using Clinical Trials.gov (March 11 2024) highlighted completion of 1,794 trials in total comprising of 43 early Phase 1,860 Phase 1,772 Phase 2 and 103 Phase 3 trials. However, only 16 Phase 4 trials assessing side effects and persistence of treatment effects over time by an approved marketed treatment were found. The term "valley of death" has been used to highlight the difficulties for translation of drug development with reproducibility, clinical relevance, regulatory issues significant contributors [11].

These issues also impact delivery of ATMPs such as genes, tissues, cells or genetically modified cells. Although potentially ground breaking for patient care and the treatment of diseases that lack effective alternatives, translation of ATMPs to clinical practice has been slow [12]. More recently, MSCs derived from induced pluripotent stem cells (iPSC) or iMSCs have become the focus of considerable research in the area with the capacity for increased cell yields and a more sustainable source [13].

Manual versus Automated Production Systems

Another issue that has to be taken into account to enable and promote widespread availability of cell and gene therapeutics are the costs associated with manual production of the developed therapies [14]. Whether manual or automated systems are used for production of cell and gene therapeutics, core costs associated with annual facility licensing, clean room certification and the retention of a "qualified / authorized person" to certify that all manufacturing process are in compliance with current Good Manufacturing Practices (cGMP) and regulatory requirements will not change. Additionally, critical personnel required include the leaders for both the production facility and personnel covering quality control and quality assurance for production campaigns.

In the context of manual processes, a minimum of two operatives are required for the implementation of all steps in an approved process including:

1. Cleaning of the general GMP production area and the sterile hoods used, as well as validation that this has been achieved through the collection of swabs for QC analysis
2. Implementation of approved processes and
3. Validation that all steps required by the relevant standard operating procedure (SOP) are adhered to.

This is a laborious process and as the field developed with increasing numbers of cell and gene therapies and increasing numbers of regulatory approvals being obtained, there is an emerging and pressing need for industrial translation. Indeed, process efficiency, associated cost drivers and regulatory requirements are issues that need to be addressed before industrialisation of cell and gene therapies can be established. Automation has the potential to address these issues and pave the way towards commercialisation and mass production as has been the case for 'classical' production industries. As such the potential to reach a point where fully compliant and GMP qualified closed and automated production systems with no need for manual intervention once the process has begun has been the focus of extensive research. The development of early, first and second generation automated systems responded to the growth of cell and gene therapies and the increase in associated regulatory approvals [12]. Subsequently, different modes of automation were developed to address

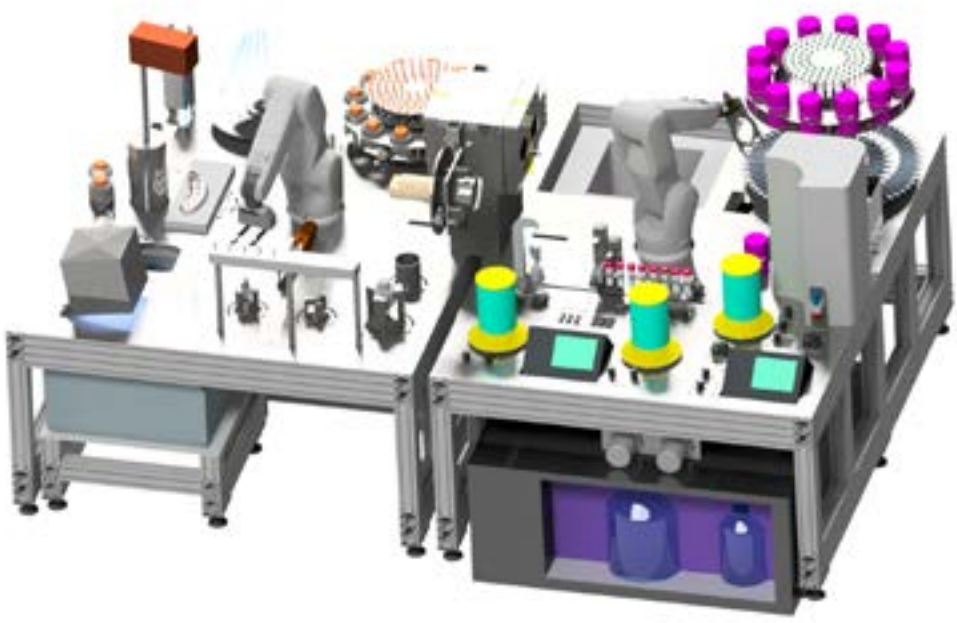

Figure 2: A more detailed view of the Autostem platform for bioreactor-based cell production or the "Production Module" as a CAD model

1. Robotic automation (i.e., plates) with open manufacturing, well suited to research, process development and high levels of parallelisation and high-throughput screening (Stemcellfactory [15], Jointpromise [16] and StemcellDiscovery)
2. StemcellDiscovery [17]. Fully Automated Cultivation of Adipose-Derived Stem Cells in the StemcellDiscovery- A Robotic Laboratory for Small-Scale, High-Throughput Cell Production Including Deep Learning-Based Confluence Estimation
3. Fluidic automation in closed manufacturing works well for scale-up of allogeneic or even autologous therapies such as in the research project AIDPATH addressing development of an autologous CAR-T Cell Therapy using artificial Intelligence and automation for build-up of a Smart Manufacturing system [18].
4. Autostem [19] where a fully automated, robot-assisted platform using multi-litre stirred-tank bioreactors addressed the large-scale production of MSCs. This platform enabled "needle-to-needle" closed processes from the collection of bone marrow through cell isolation and expansion and collection into cryovials for patient delivery. This modular fully-closed system ensured that there was no direct operator interactions with biological material with a graphic interface providing all robotic commands. The Autostem platform CAD model is shown in Figure 2 and the implemented platform in Figure 3.
5. Combine with 3. addressed genetically modified autologous CAR-T cells where a modified receptor enabled recognition of and subsequent destruction of the tumour cells [20].
6. Magnetic / X planar automation with open manufacturing is well suited to tissue manufacturing and highly parallelised sequential manufacturing such as the BellaFactum project addressing a fully automated production system for resorbable, 3D-printed breast implants [21]. These systems implemented the transition from descriptive to reactive and on to predictive process control. In the context of descriptive systems, automation focuses on the implementation of automated systems that were carried out according to pre-programmed steps described in SOPs [22].

Reactive automated systems enabled measurement of process/product parameters but also enabled a response when needed. For example, cell passaging was guided by assessment of confluency, whereby medium exchange was enabled until the cells reached 80 % confluency as the signal to consider passaging [17]. The use of predictive control in this system enabling optimised system utilisation is an important parameter in production engineering. Predictive models for process control use artificial intelligence (AI) garnered from historical data to provide the capacity to anticipate what might happen during production campaigns. In essence, these models can use defined process limitations to identify and adjust conditions where necessary during production. The main advantage of Model Predictive Control (MPC) is that it is able to anticipate the future using knowledge gathered on what the limitations of the defined processes are (safety limits, operating limits, and quality specifications). The model can then calculate adjustments for the process ensuring optimal working of automated systems. MPC designed control systems can also operate for extended periods without operator intervention adding to optimal operation and associated cost effectiveness. However, this system is relatively short-term due to the time required for the generation of a cost control measure algorithm.

Figure 3: The Autostem platform in the laboratory environment.

MPC is based on iterative, finite-horizon optimization of a plant model. At time the current plant state is sampled and a cost minimizing control strategy is computed (via a numerical minimization algorithm) for a relatively short time horizon in the future. Moreover, the ongoing development of required software, as well as an incrementally trained model, is necessary to ensure the required implementation of operations on the relevant platform. Updates for control systems are available to ensure up-to-date software to comply with industry standards, enable integration of new device drivers, improve user interfaces as needed and ensure the generation of GAMP and CFR part 11 compliant data management. CFR-compliance will ensure that electronic records are validated and that all production steps are operating correctly and consistently and archived.

Traditionally, GMP manufacturing has required the availability of an approved Good Manufacturing Facility (GMP) with the presence of a unit lead and two operators at a minimum required for implementation of each step in any process. Additional personnel for required quality control and quality assurance assessments throughout production campaigns are also necessary. An analysis comparing manual versus automated production was performed to highlight some of the costs here. With respect to manual production systems, costs for facility maintenance, annual cleanroom certification and required clean room garb for staff implementing the processes with two operators required at all times were taken into account. Based on data obtained from the Centre for Cell Manufacturing Ireland (CCMI), Galway, Ireland [23], the requirements for manual cleaning and the necessity to have a back-up operator in place at all times, led to the calculation that a manual facility could be used for manufacturing campaigns for approximately 250 days per annum. By comparison, this "working" time for the automated facility (AutoCRAT) was calculated as ~330 days annually (Appendices 1 and 2, respectively).

Cost of Goods

With respect to costs assessed, the following categories were covered: Facility, Staffing, Equipment (incl. depreciation over 10 years) and consumables (Appendices 1 and 2). Possible manual campaigns were capped at 10 production runs for iMSCs (480 units) whereas generation of 750 units was possible using the automated system from 14 production runs. Ultimately, the calculated cost per unit/vial of cells produced using automation was € 1291, whereas this was increased to €2039 with manual production. Furthermore, if EVs derived from the cells are included as a cell product in these calculations the cost per unit can be reduced further to € 646. Finally, the AutoCRAT has additional functionality in that there is a capacity to increase production through the use of three bioreactors in parallel.

Summary

Production of ATMPs, and in particular the generation of product for stem cell and gene therapy indications, using manual processes can incur significant and ongoing financing. In particular, staffing as well as infrastructural costs can be prohibitive for small units in particular. Additionally, manual processing, even with ongoing and comprehensive training of operators, is associated with an increased risk of contamination events requiring a production shutdown and a complete de-contamination of the areas affected. Closed, automated systems where there is no exposure to human intervention during the production process will result in reduced production costs, reduced process failures and the ability to control the cost of goods for patients in need of the relevant therapeutics. Widespread use of these automated mini-factories for production of therapeutic cell or cell-derived products such as EVs, once validated clinically through controlled trials, will result in significant patient benefit.

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