Chapter 10
Stem Cell Production: Scale Up, GMP Production, Bioreactor

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Abstract Stem Cell production for therapeutic applications is increasingly gaining attention both for the great potential this therapy presents and the complexity of such therapies. Safety and efficacy have improved dramatically during the past decade, while technology improvement facilitated the research and development phase of cell therapy journey as well as the clinical production phase. All this led to innovative therapies that for the first time are treating diseases that once were considered untreatable; bringing hope to a wide sector of patients and their families. In this chapter we focus on stem cell production for therapeutic applications, then discuss their advantages and limitations. We briefly discuss the stem cell therapeutic applications before we list specific good manufacturing practices requirements and how this impact the cell therapy field as a whole.

Keywords Stem cell · Bioreactor · GMP · Quality assurance · Regulations · Viral vector

Stem Cell Production

Historical Overview

2019 marked the anniversaries of two milestones. BM transplant turned 40 this year, and patients are taking advantage of this therapy that became the treatment of choice for many diseases as indicated by the uniformity of the clinical care around the globe. This therapy though had a difficult start. BM transplant experiments started nearly 10 years earlier around the mid of 1950s, but most of the patient died. The failure of those experiments led many professionals to leave the field convinced the barrier between individuals cannot be crossed.

Though, those who persisted, like Thomas ED (the father of BM transplant as he became to be known) made the history. In 1979, Fred Hutch reported the 1st

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successful unrelated BM transplant case; it was a priceless breakthrough. But this successful case was not the group's 1st case, it was the 2nd. The Fred Hutch group reported that their 1st patient died soon after the transplant. The Hutch group reported their 1st case got infection that caused the death. The 1979 case infection was a CMV infection. The second anniversary that 2019 marks is the 30th anniversary of Gene Therapy. Steven Rosenberg reported their successful 1989 study where they inserted a gene to determine the traffic of the tumor infiltrating lymphocytes. As we will discuss later in this chapter, gene therapy is returning after a hard beginning.

Challenges—Difficulties and Shortcomings that Faced Researchers

"*Pluripotent stem cells can potentially switch from pluripotency to uncontrolled differentiation*" (Kropp et al., 2017). The use of stem cells for therapy was not without challenges; the self-renewal and differentiation capabilities that characterize stem cells and present them as advantageous, can be a source of failure if not controlled properly. The challenges that face this field concern both the safety and efficacy of the products. As safety is concerned, the carried risk is either product-related or process related. For the product-related risk, this risk stems from the nature of the therapeutic product. Specifically, not controlling the differentiation potential of stem cells can lead to either their differentiation too soon or losing their potency, therefore, not providing the right potent dose. Alternatively, stem cells may differentiate to a cell lineage that is harmful or not desired, which present a significant risk of delivering the wrong product, or failure of the entire process. For the right cell dose to be generated, cells would need to be manipulated for extended period which increases the risk of contamination due to extended time and more material use. The contamination risk is not limited to microbial or fungal, contaminants that originate from the material such as plastics and chemical toxicities can fail the entire process.

On the efficacy side, it becomes more important to determine some parameters that impact the efficacy of stem cell products. For instance, delivering the right dose of cells at the right site can improve the therapeutic effects by utilizing the product at its maximum capacity. For stem cell therapy to be effective, the required cell dose for a specific disease in a specific patient is important. Further, if not delivered at the impacted tissue soon enough, the therapeutic cells may lose viability, potency, or differentiation capability, therefore, becoming ineffective by the time they reach the desired site. Additionally, identifying the characteristics that improve the cellular function is important. Cells tend to upregulate/downregulate their gene expression at variable phases of their growth and maturation. Identifying the phenotypic and functional characteristics that are unique to potent cells, and then testing for these characteristics to ensure the product suitability becomes favorable.

Advances—Improvements in Stem Cell Production Over the Years

Cell therapy had made remarkable advances since its rough beginning more than 50 years ago. The initial scope of many of these advances was to improve the cell-based therapeutics safety. As the safety improved the scope started to include efficacy to deliver more potent products. The classical bone marrow transplant stands now in a very strong position, thanks to the improvements that were made over the years.

Scientists have been focusing lately on expanding the reach of cell therapy to include cells that were never used for therapeutic applications before such as the immune cells. Additionally, expanding the cell therapy reach resulted in treating diseases that were once considered untreatable, such as tumor. While the list of advances is long, we will only list few that concern stem cells as the therapeutic product.

Off-the-shelf cancer treatment

The cost of producing cell-based product is often front and center when such therapy is being considered. One way that the cost may be reduced is to move from patient-specific product to a condition-specific product. By utilizing this option, production facilities can produce a large batch that may be used to treat multiple patients as an off-the-shelf drug. In 2019 two induced pluripotent stem cells (iPSC) immune therapy products were unveiled by Century Therapeutics and Fate Therapeutics paving the way for more products to come.

Widening of gene therapy scope

Of the 9 approved gene therapies so far, two were approved during 2019. These two included treatments for rare disease (spinal muscular atrophy) by Novartis and for sickle cell disease by Bluebird bio. Although the start of gene therapy was tough, it is now becoming a reality that changes patients' lives.

Standardization of cell and gene therapy

As the field continue to expand, it becomes more important to standardize the entire supply chain from collection throughout infusion. For this reason, stakeholders started to discuss/collaborate to generate services and platforms that facilitate any future development.

Scale Up of Stem Cell Production

As cellular therapeutics became more popular, some challenges became evident and needed to be addressed. On one hand, after the completion of proof of concepts studies, it became necessary to generate stem cells at quantities that are enough to treat a patient. Those quantities varied from one patient to the other, from one

condition to the other, and from one protocol to the other. Additionally, the push for off-the-shelf therapies was gaining momentum, therefore, requiring even higher quantities of stem cells to be produced.

On the other hand, as pharmaceutical and biotechnology companies started investing in cellular therapeutics, they developed their processes and standardized them in a fashion that mirrors the pharmaceuticals production lines. The standardization concept relies on removing as many variables as possible. The two major variables in cell therapy production are the starting product and the human operator. While the variability amongst the starting products is not possible to be rolled out, the human factor is. Because of these challenges, scientists started to explore production methodology that satisfies few but challenging criteria:

- Maintain the product safe, pure, and effective
- Meets the regulations
- Ease of maintenance.

Scale Up Methodology

Scale up methodology varied in capacity, in automation level, and therefore, in cost. At one end of the spectrum are production systems that increased the potential quantity of produced cells but relied on human operator. On the other end are production systems that increased the produced quantity while simultaneously reducing the human involvement. Between these two ends are some production systems that have variable degrees of high quantities and human involvement.

2D Production Systems

Stacks and Factories

Stacks presented an attractive production system because it maintained the flask methodology that most R&D laboratories utilizes while utilizing the vertical incubator space. This system made scaling up production of stem cells easier for the stacks needed no special requirements or specialized staff. But this ease of use comes with an expensive price; such production system requires more of staff time, significant risk of contamination, and extended footprint.

Despite this, stacks still provide a reasonable option for small production facilities and academic centers, who may have limited budget and facility and are only working on phase I/II clinical trials that enroll small number of participants. The cell factory resembles the stacks in several aspects such as being a 2D manual system with limited capacity. One advantage of the cell factory is its improved gas exchange that the ports provide. The ports make filling, venting, and harvest an easier process.

3D Production Systems

Bioreactor

The expanded use of cell therapy products made bioreactors even more attractive option of production. Unlike the manual production method, bioreactors ensure several benefits such as reduction of cost, control process, and quality of production. There are several bioreactor types that differ in their mode of operation or the types of cells they support, the main categories include stirred tank, fix bed, hollow fiber, and rocking platforms (Eaker et al., 2017). Majority of bioreactors have the capability of real-time monitoring of critical parameters such as dissolved oxygen and CO_2, pH level, and temperature. One important factor that impact the choice of bioreactor is the anchorage dependency of the culture. Therefore, based on the nature of the culture, some cultures require substrate to adhere to while others are suspended.

Over the years the design and engineering of the bioreactor has been adjusted to overcome obstacles and challenges related to upscale and diversity of culture applications. While most of the focus has been directed towards adjusting the design and operation of the bioreactor to match the nature of the microculture environment, some studies has been designed to adjust the nature of the culture to match or improve the scalability and applications of the bioreactor. Because of their scalability and cost effectiveness, bioreactors became a very good option for large scale productions (Oppermann et al., 2014). Similarly, patient specific production of therapeutic products became a common option for multiple disorders and the use of the bioreactor for these patients became one way to control the cost of the production and to improve efficient modality.

Stirred tank bioreactor

Is a widely used 3-dimenstional (3D) stem cell production system with two major production models, the immobilized and cell aggregates. Studies have showed that the optimum production results by optimizing and controlling individual factors to reach the optimal production model of each cell type. Factors such as seeding density, aggregate size, media type, and operation mode are all critical in determining the in-process and end-of-process quality of product. Types of cells that were successfully propagated using stirred tank bioreactor include human pluripotent stem cells (hPSC) (Kropp et al., 2017), this approach was facilitated by the use of a Rho-associated coiled-coil containing kinase (ROCK) inhibitor (Watanabe et al., 2007). Besides being commercially available, stirred tank bioreactor provides a flexible operating system and efficient gas exchange of culture. Although this system is robust, the need to detach the cells from the carrier remains a critical component of the process (Weber et al., 2010). One alternative is to utilize a system that operate as a carrier-free system such as the fixed bed bioreactor. A full list of advantages and disadvantages of suspended and immobilized bioreactor systems appear in Table 10.1 (Pörtner and Faschian, 2019).

Table 10.1 Summary of advantages and disadvantages of stirred tank and fixed-bed bioreactor (Pörtner and Faschian, 2019)

	Advantages	Disadvantages
Stirred tank/suspension	Known technology	Aeration difficult at high cell densities (relevant for aerobic cells)
	Good mass transfer	call damage by shear and aeration (e.g., Mammalian cells))
	Good mixing	Foaming (relevant for aerobic cells))
	Cell count possible	Low cell density and volumetric productivity cell retention required for perfusion culture, techniques insufficient for long-term culture
High potential for scale-up		
Fixed-bed/immobilized cells	High cell density and productivity per unit	Concentration gradients
	Easy exchange of medium	Nonhomogeneous
	High productivity over long periods of time	Cell count impossible
	Low-shear rates (relevant for mammalian cells))	

Fixed and packed bed bioreactors

Immobilized bioreactor technologies are being considered for the many advantages they provide such as reduced contamination susceptibility and protection against high shear environment (Fig. 10.1). In addition, the high productivity advantage is being attributed to the culture microenvironment provided by the carrier. In a two parts study, A and B, (Weber et al., 2010) successfully expanded Human mesenchymal stem cells (hMSC) using the fixed bed bioreactor. Although the fixed bed bioreactor offers several advantages, the low industrial models is reasoned in part to the lack of both process development tools and operation concepts (Pörtner and Faschian, 2019).

Rocking bioreactor

Rocking bioreactors utilize the rocking motion to distribute gas and nutrient via certain rocking speed and angel. Davis et al. (2018) reported the successful expansion of pluripotent stem cells using a rocking bioreactor.

Hollow fiber bioreactor

Described by Knazek (1972), the hollow fiber membrane bioreactor consists of hollow fiber membrane that separate the cells from the medium compartment.

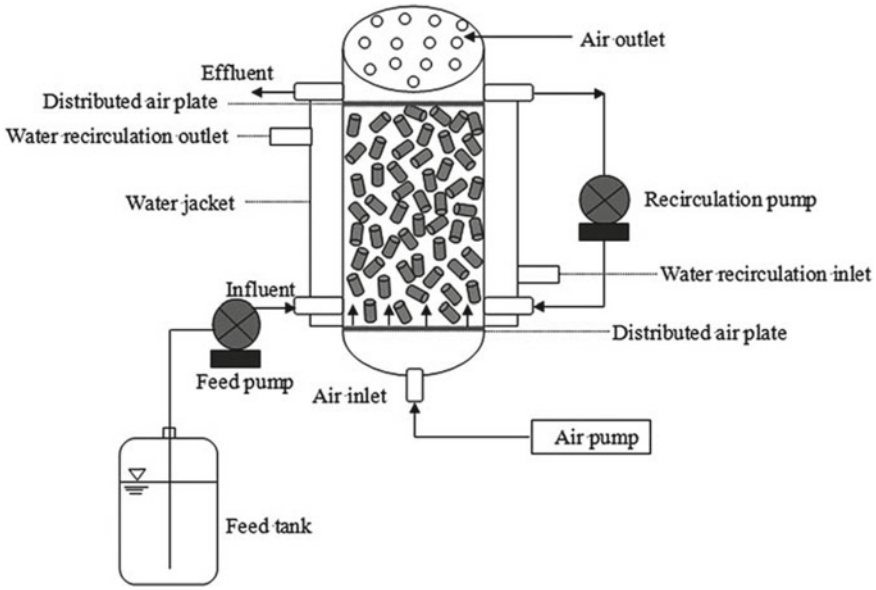

Fig. 10.1 Schematic diagram of packed-bed bioreactor

Medium flow and waste removal occurs through the membrane, which comprise a major disadvantage of this system due to the resistance of mas transfer. Several advantages are presented by this system, with the ability to grow high density cells and support of 3D growth being the most relevant to the field of cell therapy. Frank et al. (2019) in a recent study described the successful cultivation of MSC using this system, and utilizing different coating reagent. The final product met the standards as described by the international society for cell and gene therapy (ISCT).

Considerations for bioprocessing

Production facilities that are considering the use of bioreactor in their processes are utilizing a significant improvement that would lead to several advantages such as:

- Process standardization
- Eliminating /reducing man made errors
- Enhancing Product Safety /sterility
- Large scale production
- Cost reduction.

Complying with GMP requirements mandates taking reasonable measures to ensure product safety; production facilities are required to implement measures that eliminate the risks of mix-ups and cross-contamination between products. A major improvement that became available for bioprocessing utilizing facilities is the introduction of disposables bioreactor set. With this improvement, production facilities

are able to eliminate the cost, labor, and risk associated with re-using a bioreactor such as:

- Purified water utility
- Validation of cleaning procedure
- Integrated pre-sterilized pH and dO_2 sensors.

Several types of bioreactors are now being offered as disposable; this facilitates the selection process as more options are now available. However, selecting the right bioreactor depends on multiple factors such as:

- Cellular growth pattern
- Scale and engineering parameters of bioreactors (e.g. flow rate/time/volume, mixing/residence times)
- Biosafety/GMP compliance
- Capital/running costs.

After phase I and II of bioreactor development, phase III marked the launch of disposable bioreactors in 1990 (Eibl et al., 2010). Currently, the available disposable bioreactors include wave-mixed, orbitally shaken or stirred bioreactors. Stirred disposable bioreactors were introduced 2006 to the market, but have since gained a major share of the technology, and by 2010, have 10 different commercially available bioreactors; some models are flexible utilizing bags while others are rigid using plastic cylinders.

One time use bioreactors are emerging as a viable option to satisfy several requirements with safety and prevention of cross-contamination being the most relevant. Hähnel et al. (2011) group evaluated one of the available one-time bioreactor models and concluded that the setup is remarkably short.

There are, though, several aspects that need to be considered when a decision to use bioreactors is made. Often, choosing a production system is done long after building and qualifying the facility, this means that there potentially are modifications that need to be made to accommodate a specific production system for a specific process; below is a list of potential changes:

Gas lines

Some bioreactors utilized 2–4 types of gas to complement the bioreactor operation. For instance, N_2 is used to reduce /adjust the oxygen concentration in cultural media. CO_2 is used to adjust the media pH to reach /maintain a set point. Utilizing gases means that there need to be a network of pipelines that delivers the gas from the closet to the production suite. Such a change to install gas pipeline requires the facility to be re-qualified. In some situations where installing pipeline is not possible, the production facility need to install the gas cylinders inside the facility utilizing all required safety measures.

Large scale production

Scaling up production requires large quantities of media, and eventually generate large volumes of waste. Moving large quantities of liquid in and out of the facility

requires good planning to ensure the facility is kept in status at all times. Further, using large volumes of media requires appropriate controlled /monitored storage space for media before and during use. Similarly, using large volumes of media results in generating similar volumes of liquid waste that need to be disposed of appropriately. Additionally, facilities utilizing large volumes of media should be prepared for spill accidents with appropriate spill kits and trained staff.

Product segregation

For facilities that enroll multiple patients simultaneously, careful attention should be paid to ensure elimination of products mix up risk. Production facilities are required to make efforts and take measures that ensure proper product segregation at all stages of the production process. Particularly, stages where the product is being manipulated present the highest risk. For example, stage of bioreactor inoculation, cell transduction, expansion, and harvest.

Although the production system is expected to be closed which should lead to enhanced product safety, product mix up remains a potential risk; measures that may help reduce this risk are:

Physical segregation

Culturing different products in physically different locations, such as different suites, can significantly reduce the mix up risk. Although this model requires a proper planning during busy production times to ensure appropriate staffing in several locations, the safety enhancement is worth the cost.

Proper verification

Verification of critical steps is a GMP requirement, and should be built into the process; two technologists independently verifying the information should eliminate the mix up risk.

Identity testing

While proper labeling the product at all times of the production process is a GMP mandate, identity testing of the final product serves as the final assurance of product identity. One way for such testing is to run human leukocyte antigen (HLA) testing of the initial and final products; identical results indicate the product identity was maintained throughout the production process.

Stem Cells Types

Classification of stem cells gained a lot of attention and still does, for this field is still evolving. Comprehensive classification of stem cells has been addressed in chapter two of this book. Therefore, we will briefly classify stem cells to complement the clinical applications section of this chapter. One way of classifying stem cells is through the lens of origin and the lens of potency see Fig. 10.2. The ability of stem

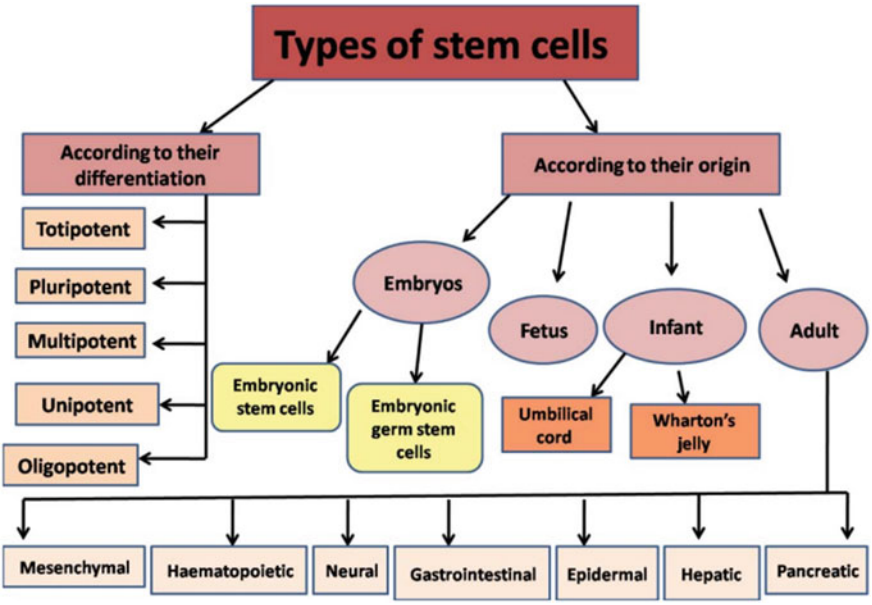

Fig. 10.2 Classification of stem cells

cells to reproduce themselves and to differentiate to any cell lineage is related to the stem cell level of potency, Good level of potency is largely dependent on the origin of stem cells. Due to this interdependency, it seems only logic to classify the stem cells based on origin and based on potency.

Stem cell classification based on origin

Embryonic stem cells ESC

Embryonic stem cells hold a unique capacity to differentiate to any cell type in the human body, which comprise a valuable source of potential therapies. The capacity to differentiate to any cell type depends on the timing of ESC isolation. ESC's isolated from embryos early after fertilization (4–5 days), or isolated primordial germline cells (PGCs) are thought to be the most potent. While a ESC isolated from the fetus organs are pluripotent and have the potential to differentiate into hematopoietic stem cells (Amira Ragab et al., 2017).

Infant stem cells

The umbilical cord has been shown to be a reliable source of potent stem cells. The most radially available source of umbilical cords stem cells (UCSC) is the umbilical cord blood containing multi potent stem cells. The other source of UCSC is umbilical cord matrix (Wharton's Jelly) Which is considered to be a source of mesenchymal stem cells.

Adult Stem Cells

Adult stem cells are isolated from mature tissues of child or adult body. Due to the stage of their development, unlike ESC's, adult stem cells have limited potential to develop into other cell types. Generally, adult stem cells are vital in repairing and regeneration of their tissue of origin, to which they are referred. Several adult stecm cells have been described, below is a brief listing:

Hematopoietic stem cells (HSC)

HSC have the potential of self-renewal and differentiation to all hematopoietic lineages. Hence, are used for transplantation, and hematologic and malignant diseases.

Neural stem cells (NSC)

NSC are established in the adult brain microenvironment and holds the potential to treat neural related disorders.

Gastrointestinal stem cells (GSC)

Residing in a niche and intestinal crypts and gastric glands, GSC nature and position is not fully established.

Epidermal stem cells

Epidermal stem cells have the capacity of self-renewal. They reside in the basal layer of the epidermis and are essential in maintaining homeostasis and wound healing.

Hepatic stem cells

Liver holds a strong regeneration capacity; therefore, liver injury gives rise of stem cell compartment who's sells later differentiate into hepatocytes.

Pancreatic stem cells

Isolated from islet cells, pancreatic stem cells are multi potent cells that can differentiate into pancreatic phenotypes.

Stem cell classification based on potency

Based on their differentiation potentials, stem cells may be classified to:

- Totipotent stem cells: have the total capacity to give rise to all self tribes and reproduce fertile offspring
- Multipotent stem cells: are capable to give rise to tissue from which they were isolated
- Unipotent stem cells: are adult stem cells that can give rise to a limited number of cell types
- Oligopotent potent stem cells: Are those cells that can differentiate into a few cell types.

Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSC) are adult stem cells that can be isolated from bone marrow or cord blood. MSC potency makes them capable of differentiating to several cell lineages in the body such as neural cell, bone cells, skin cells, muscle cells, and cornea cells. MSC are thought to be able to avoid rejection by immune system and are used to treat multiple disorders due to their potency.

iPSC Stem Cells

Induced Pluripotent Stem Cells are adult cells that were isolated from skin or blood and were re-programmed to function like pluripotent stem cells. This re-programming allows the iPSC to differentiate to any cell type for therapeutic applications provided the right signals and culture micro-environment

Stem Cells Applications

The applications of stem cell therapy grew over the years to cover a wide range of conditions. While some applications have been approved by the FDA, other applications are still in their infancy. In between are applications that are progressing in clinical studies towards gaining approval.

Therapeutic Stem Cell Applications

Classical Cell Therapies

Following their success with advanced diseases, Hematopoietic Cell Transplantation (HCT) was ethically justified for malignant diseases. Transplants for patients with diseases in first remission or at early signs of relapse were largely successful. Soon after, the trials to treat non-malignant disorders were initiated. The results were not satisfying in the beginning, but had improved dramatically with changes of transplant timing, and preparations of patients. Generally, classical cell therapy includes all processes of which the product is minimally manipulated. These processes have limited impact on the product, therefore, are covered by a specific set of regulations. Despite the progress that was made in the field of cell therapy, most of these processes remained unchanged which indicate how robust these processes are. Below is a brief list of these processes:

- Volume reduction

- Red Blood Cells depletion
- Cryopreservation.

Gene Therapy

Gene therapy is a rapidly evolving field that is gaining attention from all stakeholders such as scientists, biotechnology, big pharma and patients. The field started with the intention to correct genetic diseases and was faced by several obstacles. Throughout the years, the technology has improved and the tools are now more advanced which led scientists to utilize this technology to treat diseases other than those of genetic origin. Cell-based gene therapy requires the transfer of the genetic material into the therapeutic cells. Several approaches exist to accomplish this task with each of them having advantages and disadvantages. Therefore, scientists are utilizing the mode of transfer based on the cellular nature and the disease being treated.

Viral vectors

The use of viral vectors experienced major setbacks that halted the field progress, decreased its funding, and raised a wide skepticism among the scientific community. But the persistence of gene therapist led to improvement of vector design, delivery, and safety, and eventually, regaining the trust of clinicians and scientists. Viral vectors vary with some being utilized for transient gene expression, while others being used for permanent expression. Further, the nature of cells being modified dictates the types of vectors that may be used. Some vectors can only adhere to dividing cells, while other vectors can adhere to both resting and dividing cells. Another variable is the capacity of the vector with regard to the size of the gene being inserted (Lundstrom et al., 2018).

Adenovirus

Is widely used vector that lead to temporary expression of the transferred genetic material. The 7.5 kb capacity vector initially led to strong immune reaction, but the following generations were modified to be less immunogenic.

Adeno Associated Virus (AAV)

Is a limited capacity, 4 kb, viral vector that results in long term genetic expression. Although considered of low pathogenicity and toxicity, AAV use resulted in immune response in subsequent administration, an issue that was addressed by utilizing different serotypes for subsequent administrations. Further, the limited vector capacity was increased by engineering the vector to a dual vector.

Herpes Simplix

Is considered a low toxicity long term transgenic vector, with a capacity of >30 kb of foreign DNA.

Retrovirus

Is generally a long term transgenic vector with low capacity of foreign insert; 8 kb. A major disadvantage of this vector is its inability to transduce non-dividing cells.

Lentivirus

Belongs to the retrovirus family but is capable of infecting both dividing and non-dividing cells. Lentivirus also has the integration and packaging capacity of retrovirus and can provide a long term expression. Therefore, Lentivirus vectors gained a lot of interest for cell therapy applications.

Vector Production

For cell-based therapeutic products, process components are of significant importance particularly when those components become part of the final product. Viral vectors that are used to transfer genetic material to, and permanently modify a cell product, are required to have stringent criteria for them to be approved for clinical use. Similar to clinical cell production, viral vector production for clinical applications is required to be GMP compliant. Regulatory bodies acknowledge that viral vector use in production renders the clinical product as significantly manipulated, or more than minimally manipulated per FDA's definition, and therefore apply regulations that match the category of drugs. Besides the GMP requirements, viral vector release follows a long and stringent list of criteria to demonstrate purity and safety, below is a brief list of release criteria.

- Replication competency: viral vectors are usually created from pathogenic viruses. Therefore, it is important to test the vector intended for clinical use for replication competency before final release.
- Sterility: vector products should be tested for bacterial and fungal burden, as well as for endotoxin and mycoplasma presence.
- Purity: vector product should be tested for impurities derived from the host cell system that was used to generate the vectors.
- Infectious unit/viral particles: There need to show the ratios of infectious and particle titers; this facilitate in standardizing the production process.
- Stability: vector product should be tested in temperature, volume, and container used for clinical applications to demonstrate maintenance of quality and infectivity over the expected storage time.

Regenerative Medicine

Described first by Kaiser et al. (1992), regenerative medicine (RM) implies the utilization of cells to regenerate tissues or organs and/or restore their function. The demand for unusual approaches to treat diseases has been around for decades, but being deficient in tools such as the right material, comprehensive knowledge, and appropriate facility, patients continued their efforts to manage the symptoms of untreatable

diseases rather than treating them. The advances in technological tools as well as in research methodology, resulted in a shift of scientist' perception towards the possibility to treating what one day were considered untreatable diseases. These advances touched the three approaches of RM as described by Sampogna et al. (2015):

- Cell Based Therapies
- Scaffolds
- Scaffolds with cells.

Tissue or organ regeneration requires a large number of specialized cells that were differentiated to perform the physiological functions of the specific tissue or organ. In theory, the involved organ is the best source of such cells for they provide the right function, but there are rarely enough cells to aspirate especially if the organ is damaged or is malfunctioning. Therefore, there need to be innovative approaches to provide such cells in large enough quantities. To accomplish this task, the intended cells need to be capable of differentiating to the desired lineage, and capable of expanding ex-vivo to reach the required cell dose.

As we described earlier in this chapter, several stem cell types have been shown to be Pluripotent. Therefore, are capable to differentiate to the desired cell lineage. Similarly, as we described in bioreactor section, the technology has improved to allow for expansion of stem cells ex-vivo while maintaining their self-renewal and differentiation capabilities. Scientists are leveraging the late advances to guide the stem cell differentiation at the right time of the production process. Although this has been accomplished in research setting, generating a large number of appropriately differentiated cells in a GMP manner may be challenging at times. There is a need to form multidisciplinary teams of researchers and clinicians to participate in early stages of process development as well as in fine tuning late stages of clinical production.

Organoids

Organoids are three dimensional invitro tissue constructs that mimics the structure, function, and aspects of the intended organ (Natalie de Souza et al., 2018). Organoids provided the scientists with the ability to firsthand monitor the impact of therapeutic drugs on the organ tissues without affecting the patient. This ability resulted in speeding up drug discoveries while protecting humans from drug effects for the entire period of the drug development.

As the organoid technology became more robust providing the ability to generate larger and more complex organoids, attempts have started to widen the scope of organoids from only diagnostics and development to include therapeutic applications. In short, the idea of developing mini organs that can reduce the impact of a failed organ started to gain a lot of attention due to the dramatic impact of this idea on the patients and the medical field alike. Organoids generation approaches vary, hence, result in different outcomes. It is worth mentioning that the complexity of the organoid

being generated is related to the potentials of the stem cells that were used to begin with; pluripotent stem cells are capable of generating complex organoids, while less capable stem cells can only differentiate into specific cell lineages.

One approach is to utilize the potentials of stem cells to differentiate to different cell lineage; this method relies on the intrinsic capabilities of stem cells to regenerate the entire organ. By providing culture environment and some development signals, scientists rely on the stem cells to do the rest of the organ generation. Another approach is to generate specific cell lineages and then fusing these cells together in an environment that cultivates the organoid generation. Unlike the first approach, this approach provides developmental signals to stem cells to ensure proper differentiation of cell stem cells to the desired cell lineages.

Organoid generation is still evolving with several obstacles that need to be addressed. Unlike the full organ, organoids lack some essential tissue components. Therefore, mimicking full organ function and structure is deficient. For example, organoid lacks vasculature, therefore, can only grow to a limited size before losing the ability to expand due to lack of nutrition. Further, lack of immune cells limits how these organoids recapitulate the organ physiological response.

Another area that scientists are working on is the maturity of organoids. Generally, organoids maturation matches the level of fetal tissue. To address this issue, scientists have used different culture environment where more control over developmental signals in stem cell differentiation is granted. This change is intended to reduce the variability between organoids being generated by unifying the level of maturity. In their recent paper, Blackford et al. (2019) reported successfully generating iPSCs-derived hepatocytes in a GMP-compliant manner. Using FDA approved scaffold material, they report generating current GMP-constructs from human pluripotent stem cells that remained viable and functional long enough after transplant for the recipient to recover from acute liver failure if product was used.

Spheroids

Spheroids are self-assembling aggregates of cells in an environment that supports 3D culture. They are generally utilized for diagnostic and experimental purposes but are also gaining increased attention in regenerative medicine for therapeutic purposes. Spheroids possess several advantages over the 2D culture such as cell–cell contact and mimicking tissue microenvironment. Therefore, are increasingly considered for applications such as tissue and organ reconstruction. For example, MSC spheroids transplant had been shown to provide advantages for organ reconstruction as well as for tissue formation (Ryu, 2019). Further, spheroids injection had shown to improve engraftment, while transplanting genetically modified spheroids led to longer periods of expression of the gene of interest.

GMP Production

The range of stem cell applications is constantly expanding, bringing hope to wider patient sectors like never before. After the proof of concept phase, scientists are constantly working to develop production methodology that simplify the process and reduce the cost while meeting the regulatory requirements. Regulations, as defined by the European medicines agency (EMA) and the Food and Drug administration (FDA), require the advanced therapy medicinal product (ATMP) and more than minimally manipulated (MMM) products to satisfy good manufacturing practice GMP requirements.

ATMP and MMM Products

Per the FDA, the minimal manipulation is "*processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement*". To grant an ATMP designation, EMA considers several factors including the level of manipulation. EMA defines the substantial product manipulations as "*resulting in a change of their biological characteristics, physiological functions or structural properties*". Therefore, products that do not qualify as minimally manipulated and those that qualify for the substantial manipulation designation are required to meet the GMP requirements.

GMP Regulations

GMP regulations cover the entire production process, environment, and personnel. To keep the focus on stem cell-related applications, we will briefly describe the requirements.

A. **Organization and personnel**
Quality control
The organization is required to define an entity that is tasked by approving or rejecting process related parts including product containers, in process materials at labeling. This entity shall also have the authority to review production records to ensure full compliance.
Personnel qualifications
Staff involved in any component of the production process need to have adequate education and training. The current GMP training shall be conducted on a continuous basis by qualified person. The number of the production staff should be adequate to perform and supervise the entire process. Personnel engaged in production shall wear clothing and protective apparel appropriate for the duties and necessary to protect the product from contamination. Personnel are required to practice good sanitation; their authorization to enter the restricted production areas is provided by supervisory personnel, and is contingent on being healthy and competent to protect the quality of the product.

B. **Building and facilities**
Design and construction

The facility should be designed to facilitate proper cleaning, maintenance, and operation. Therefore, the facility size need to be appropriate for the intended operations and equipment placement. Likewise, the flow of material and personnel need to be designed to prevent mix up and contamination. Several processes need to be considered during design such as:

- Receipt/holding/storage of components
- Manufacturing and processing operations
- Quarantine/release of products
- Aseptic processing including cleanable surfaces, temperature and humidity control, - HEPA filtered air supply, environmental monitoring ventilation, air filtration, heating, and cooling.
- Adequate use of equipment is required to ensure proper ventilation and proper control over temperature, humidity, dust, pressure, and microorganisms.

C. **Equipment**

Equipment used in production areas need to be of adequate size and construction to protect the product. Likewise, equipment need to be maintained and cleaned properly, and inspected immediately before use. A routine inspection or calibration of equipment used in production is required. Computers need to be controlled, backed up, and limit their change to authorized personnel with appropriate record keeping of any change.

D. **Components, containers, and closures**

Upon receipt, components, containers, and closures (CCC) need to be inspected for damage, and where appropriate, need to be tested before being released for use for production. Each shipment of CCC needs to be identified with a unique code, quarantined until tested. CCC need to meet all approved specifications; and only those that meet all approved specifications may be released for use, otherwise, should be rejected.

E. **Production and process control**

There need to be written procedures that cover all aspects of production, such procedures should be reviewed for change and followed at all times. All components and equipment used in production need to be identified. End of process sample testing procedures need to be written and followed, approved end of process specifications need to be met.

F. **Packaging and labeling**
Similar to components, labeling and packaging materials need to meet approved specifications before being released for use, and each shipment shall be identified with a unique code. Control over label issuance is required, with an approved system to reconcile used, returned or damaged labels. Packaged and labeled products need to be examined and verified for accuracy.

G. **Laboratory control**
A written program is needed to assess the stability of products. Results of such program should be used to identify the appropriate storage conditions and expiration dates. A representative reserve sample need to be retained and stored in conditions consistent with the final product storage.

H. **Records and reports**
Production records need to be maintained. Similarly, records of components containers and closures need to be retained. Equipment use and cleaning log are needed. For each product, a master and batch production record should be described in a written procedure. Such records need to include the product name, strength, component, equipment, manufacturing instructions, specifications, sampling, in process results, identification of persons performing the process, and end dates. Production, control, and labeling records need to be reviewed by quality control unit to ensure compliance with written procedures before being approved and released. Any discrepancy or failure to meet approved specifications need to be fully investigated.

I. **Returned product**
Product that were returned after proper distribution need to be identified. If the reason for return implicate the whole batch, then the batch needs to be investigated. Return products may be used if the return condition did not impose any potential risk, provided the product was tested and met the approved release criteria. Similarly, a product may be reprocessed provided the new product meets the approved release criteria.

Cost of Clinical Production

Production of minimally manipulated cell-based therapeutics is largely standardized and the main reason being the collective experience that both, the clinical team and production teams, had built over the years. Another reason that participated in standardizing these therapeutics is the improvements of tools and materials that collectively improved the entire process. Since the first successful cases, bone marrow transplantation made wide leaps on all of its sides, transplant clinic, collection, and processing. The transplant clinic had made major improvements to the transplant process and disease management. Changes such as the timing of intervention, intensity of preparation regimen, and management of medications had improved the overall outcomes of the transplantation process. Similarly, the bone marrow collection process had improved to maximize the quality of the collected bone marrow

while reducing the impact on the donor. In many cases, stem cell collection source had shifted from bone marrow to peripheral blood. This shift provided the transplant and collection teams with increased control over the quality of the product being collected and dramatically improved the donor experience. Due to this shift, the collection can now be completed in in a donor center setting instead of operating rooms. Further, product processing had utilized the higher quality material, the closed production system setting, and improved product storage and monitoring technology.

All these changes /improvements lead to shorter hospital stays, more successful collection rounds, and faster and safer processes. The improvements on the three sides of the cell therapy was reflected on the cost of the entire clinical care. The health care cost of transplant unit became largely predictable, as a result, the insurance companies started to cover the cost of the transplantation process, making a huge difference by providing this option to a wide sector of the patients.

Scientists have utilized the long experience and knowledge generated by transplantation programs around the globe to expand the scope of transplantation to include diseases that once were considered untreatable, and to include therapeutic cellular products that showed promising results in preclinical studies. Widening the scope of cell therapy have led to new set of regulations to ensure patient safety and wellbeing. Regulatory bodies then started to mandate clinical programs and production facilities to comply with these regulations.

Often, scientific breakthroughs stem from institutions of academic setting; these institutions encourage innovations and provide a supportive learning and experimenting environment. Scientists of academic centers usually provide the proof of concept, then with the help of teams who specialize in translational research, move the product from research bench to clinic. Most of the academic institutions include a teaching hospital which makes such translation from research to clinic an easier process.

Running cell-based therapeutic facility incurs fixed expenses whether the facility is being used or not (ten Ham et al., 2020). The level of this cost is related to the size of the facility, but is nonetheless a significant portion that should be considered when estimating the cost of production. Unlike the fixed cost of running the facility, operational cost varies according to the facility and to the volume of services offered. For example, utilizing platforms, such as CliniMACS, requires the use of expensive materials, but is also thought to result in reducing the cost of personnel and facility.

The major drivers of the operational cost are the specialized materials/equipment, and personnel. But this cost may be reduced by developing production modular or sharing of facility/equipment. It has been shown that such sharing can provide small-scale developers an opportunity to develop innovative therapeutics without having to make a substantial investment upfront. Considering the elevated cost of clinical GMP production, the fact that academic centers are the source of innovative therapeutic products, and due to the limited funding of academic centers research studies that depends mainly on grants from sponsors, it is essential to adapt a model that protects the patient safety while supporting academic centers to continue to produce such

therapies. There is no magic solution that can create this model, but the following approaches should lay the stage for the improved model.

Scale relevant regulations

Phase I or II Clinical studies that are usually led by an academic institution tend to be of small scale with a small number of patients. Therefore, are well controlled and monitored. Applying a full-scale regulations on such studies can result in a multi-faceted burden. On one side, the GMP facility is often not available in academic institutions, and when available, would require skilled personnel to maintain it in status. On the other side, the mandated process validation (media fills) can be exhaustive to staff and management if applied in its entirety. There needs to be a set of regulation that takes the scale of production in consideration. Clinical studies that enroll few patients per year such as phase I trial, should have a relevant regulation, while phase III/IV clinical studies that enroll hundreds of patients in multicenter studies should be regulated differently.

Often, phase I/II clinical studies are led by academic institutions, then are handed over to big pharma or biotechnology companies once the feasibility studies are completed by demonstrating safety, and possibly efficacy, via Phase I/II studies. It makes logic for the proof of concept phase to be regulated accordingly. Such approach requires a close collaboration between scientists and regulators to create a set of regulations that ensure patient safety while facilitating the scientific innovations.

Semi closed system

During the process development phase, the production system is usually open such as tissue culture flasks. Proof of concept using this type of culture ware includes safety such as sterility, in addition to the efficacy. As the process progresses to be suitable for clinic, production systems are switched from open to closed, materials are changed from research level to higher quality, and production from research laboratory to stem cell laboratory. Sometimes closed production systems do not exist for a specific product purpose. In situations like this, the clinical production is carried out using open culture wear, but in a GMP facility. In other times, which is more often, a semi closed production system exists but the GMP facility does not. For a small scale clinical study, there needs to be some tolerance of facility level in exchange for an as much closed system as possible. This approach requires scientists and manufacturers working side-by-side to invent production systems that protect the product safety while being used in a clean room setting.

We have witnessed several inventions in the last decade, such as the Gas Permeable Rapid Expansion (G-Rex) that in many cases had improved the quality and quantity of the produced cells while enhancing the product safety at the same time by being semi closed (Fig. 10.3).

Process validation

Regulations require the drug manufacturer to demonstrate process safety by a simulating the production process, also known as aseptic process validation, but using

Fig. 10.3 G-Rex 500M

culture media. Following this mandate in its entirety means running simulation processes that simulate all aspects of production including:

- Number of involved staff
- Process duration
- Process interruptions
- Process materials.

While it is understandable how simulation would demonstrate the process safety in its most comprehensive manner, mandating such simulation from small scale production facility can be determinantal. Therefore, there needs to be consideration for the limited capacity of small cell therapy programs.

An ongoing process validation rather than aseptic process simulation might be the required adjustment that small production facility need to thrive while maintaining the minimum safety level. Such ongoing process validation utilizes the production data as they are being generated to demonstrate the process safety/efficacy. The results of process validation should be shared with regulators, and should be used to decide if the process need any type of adjustments. Acknowledging that small scale production facility need an appropriate set of regulations, and clarifying the mandates in terms of process material / process validation should lead to more innovative production systems. Taking all this together, the clinical production cost of cell based therapeutics should not be a hurdle that prevent cell therapy programs from offering new therapies, and should not prevent patients from taking advantage of such therapies.

Quality Assurance

To meet the regulatory standards, and one's own commitment, organizations need to develop a quality plan that serves as a road map to demonstrate how this organization will achieve the committed quality. Such a plan need to be written and controlled document, need to be accepted and supported by management, and need to be unique for specific organization.

Translating the quality plan into actionable procedures is usually achieved via developing standard operating procedures to ensure the quality plan is fully integrated in the day to day operations. Organizations are required to have a quality control unit that have adequate facilities available to them for the testing and approval of all process components such as containers, processing and packaging materials, and final products. Such unit need to have the authority to undertake their responsibility to approve or reject a product or any of its components, procedures or specifications. The quality control responsibilities need to be clearly identified in writing. Accordingly, procedures need to be developed to ensure quality control responsibilities are attained.

Facility

Cell therapy facility and process depends on each other; your process defines the facility specifications that you may utilize, similarly, your facility dictates the type of processes that you may do in that facility.

Minimally manipulated products

Because the product processing of this category is limited, the risk of contamination and mix up is therefore limited. For such products, a small dedicated, or even shared, lab space is sufficient (Leemhuis et al., 2014). However, several factors need to be considered with design and location. Starting a cell processing lab (CPL) is usually intended to support a small autologous cell therapy program at an academic center. Hence, the CPL is expected to be located on campus with close proximity to patients.

For example, sharing lab space or equipment with microbiology or radioactive isotope utilizing laboratory need to be avoided. Standard electrical supply is sufficient, but it is better to connect product storage equipment to uninterrupted power source. Access to the lab need to be restricted, and all biohazardous waste should be handled according to the appropriate hospital procedures.

Lab cleaning procedure should be clearly described and validated. Further, measures should be taken to limit the introduction of contaminants to the processing areas. For example, restrict access to processing staff, limit the delivery of materials directly to processing areas, and control of temperature and humidity to improve the material storage conditions and limit growth of contaminants. CPL requirements

of equipment is limited; critical equipment need to regularly be maintained and calibrated. Further, backup for critical equipment should be identified.

Substantially manipulated products

ATMP and MMM product are considered sterile drug product that need to be processed in a GMP compliant manner. GMP facility need to be designed, constructed and maintained to provide protection against cross contamination, buildup of dirt, and any adverse effect to product quality. GMP facility need to be qualified before it may be used for production. Similarly, the cleaning protocol used at these facilities need to be validated and approved. Access to GMP facility need to be limited to qualified personnel members who work in those facilities. Likewise, personnel are required to have a periodic GMP training. Environmental monitoring (EM) is required for the facility, such monitoring is based on a previous classification of the clean areas; it need to be validated and trended. EM is expected to monitor viable and non-viable particles during both at rest and an operation status. Further, air pressure between GMP areas need to be maintained to ensure maintenance of classification.

Equipment that come in contact with products are required to not impact the product quality. Such equipment need to be installed, cleaned, validated, and maintained. Cleaning of GMP facility is a critical part of the facility maintenance. Cleaning materials need to be validated, and measures to prevent development of resistant strains such as use of more than one decent factor should be considered.

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