Fundamental Principles of Stem Cell Banking

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Abstract

Stem cells are highly promising resources for application in cell therapy, regenerative medicine, drug discovery, toxicology and developmental biology research. Stem cell banks have been increasingly established all over the world in order to preserve their cellular characteristics, prevent contamination and deterioration, and facilitate their effective use in basic and translational research, as well as current and future clinical application. Standardization and quality control during banking procedures are essential to allow researchers from different labs to compare their results and to develop safe and effective new therapies. Furthermore, many stem cells come from once-in-a-life time tissues. Cord blood for example, thrown away in the past, can be used to treat many diseases such as blood cancers nowadays. Meanwhile, these cells stored and often banked for long periods can be immediately available for treatment when needed and early treatment can minimize disease progression. This paper provides an overview of the fundamental principles of stem cell banking, including: (i) a general introduction of the construction and architecture commonly used for stem cell banks; (ii) a detailed section on current quality management practices; (iii) a summary of questions we should consider for long-term storage, such as how long stem cells can be stored stably, how to prevent contamination during long term storage, etc.; (iv) the prospects for stem cell banking.

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Keywords
Stem cells • Biobanks • Architecture • Management • Standardization • Quality management • File management • Long-term maintenance • Application • GMP

Abbreviations

AMI	Acute myocardial infarction
CFC	Colony-forming cells
CGMP	Current good manufacturing practice
CJD	Creutzfeldt-Jakob disease
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EMA	European Medicines Agency
ESCs	Embryonic stem cells
FDA	Food and Drug Administration
GMP	Good manufacturing practice
GvHD	Graft versus host disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSCs	Hematopoietic stem cells
HTLV	Human T-cell leukemia virus
ICH	International conference on harmonization
iPSCs	Induced pluripotent stem cells
LAL	Limulus amebocyte lysate test method
MCB	Master cell bank
MSCs	Mesenchymal stem cells
NRA	National regulatory authority
PCR	Polymerase chain reaction
QA	Quality assurance
QC	Quality control
SNP	Single nucleotide polymorphism
SPCs	Spermatogonial stem cells
STR	Short tandem repeat
UCB	Umbilical cord blood
WCB	Working cell bank
WHO	World Health Organization

3.1 Introduction

Stem cells are a class of cells that have the capability to indefinitely self-renew and differentiate to special functional cell types under the right conditions or given the right signals [1]. According to their potency, i.e. the range of cell types to which they can differentiate, stem cells commonly can be classified into totipotent stem cells, pluripotent stem cells, multipotent stem cells and unipotent stem cells, such as zygote, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), and spermatogonial stem cells (SPCs) respectively. Stem cells with properties of self-renewal and potency to generate different cell types are proving to be very promising resources for application in cell therapy, regenerative medicine, drug discovery, toxicology and developmental biology research [2–6]. They hold great potential to revolutionize healthcare and to supply new methods and tools for effective treatment of many diseases [7–10]. In the process, stem cell banks play a pivotal role in stem cell basic and translational research, as well as current and future clinical applications.

Banking stem cell is an extension of biobanking. The concept of biobanking has varied in history and there is no consensus [11]. The practice of "biobanking" has been broadly construed to involve storing health and genetic information and/or various types of biological materials in banks, repositories, or collections [12]. The practice of banking somatic cells, as well as primary

cell lines and immortalized cancer lines, has been a vital research resource for many years [12, 20]. In 1988, the first successful related umbilical cord blood (UCB) transplant was conducted in Paris, France on a 6-year-old male patient suffering from a blood disorder called Fanconi's Anemia [3]. In 1998, hESC isolation and culture methods were developed by James Thomson and his research group [5]. In recent years, many kinds of MSCs derived from a large number of different tissues show promising therapy potential [13–16]. In 2007, human iPSCs derived from human somatic cells were shown to have similar properties to hESCs and hold significant potential in disease modeling, drug discovery and selection [4, 6]. All these important events in the field of stem cells have sparked considerable public interest in stem cells banking.

Stem cell banks have been established in many countries in order to preserve stem cells and facilitate their research and clinical application [17, 18]. Many benefits accrue from stem cell banking, but standardization and quality control during banking procedures are essential to allow researchers from different labs to compare their results and to develop safe and effective new therapies. Furthermore, many stem cells come from once-in-a-life time tissues, UCB for example, thrown away in the past, can be used to treat many diseases such as blood cancers nowadays. Over the last several decades, the field of UCB banking and transplantation has grown exponentially. Over 600,000 UCB units have been stored for transplantation worldwide, and more than 30,000 UCB transplantations have been performed [19]. Meanwhile, these stem cells stored in banks are immediately available for treatment when needed and early treatment can minimize disease progression [20].

Before establishment of a stem cell bank, there are many factors that need to be taken into consideration including new technological developments and a deeper understanding of stem cells. For example, pluripotent stem cell culture has clearly improved in the past decade, from dependence on feeder cells to culture on synthetic matrices [21]. Thus, stem cell banks may need to change their SOPs and adopt new ones to increase productivity and stability etc. Although different stem banks may store different typed of stem cells, the major issues are common, such as the design of the bank, how to guarantee storage cell quality, and how do maintain and manage the bank over the long term? These are the main questions that will be addressed in this article, as well as the prospects for stem cell banking.

3.2 Construction and Design

3.2.1 Functional Areas and Equipment

Stem cell banking is a procedure wherein the cells isolated from various sources are collected, proliferated, stored and preserved for their future use [7]. Establishing a well-functioning biobank for stem cells provides the foundation for successful storage, quality control and assessment, and long-term maintenance and management.

The basic issues pertaining to the design and equipping of a stem cell biobank are very similar to those of a regular stem cell laboratory [22], but more functional areas and equipment should be considered and coordinated, as well as more rigorous manufacturing practices. The key considerations when setting up the bank include (1) defining the scope of the work, which includes the numbers and types of cell lines to be banked, and (2) determining the number of people who will work in the bank and their specific tasks. If more than one cell type will be stored in the same bank, what safeguards will be put into place to prevent cross-contamination? Do different cell types need separated areas to quarantine these cell types [22]? Furthermore, the processing of stem cells, including isolation, culture and collection, requires a physical environment in which air quality (i.e., the number of airborne particles) is controlled to minimize risk of contamination. The processing facility should be constructed and operated to minimize the introduction, generation and retention of particles and microorganisms. A formal program of environmental monitoring should be maintained in each stem cell bank to specify and assess key factors and

their influence on the microbiological quality of the process and product. This program should assure the manipulation of cells involved in the derivation of stem cell lines and their culture under established limits for airborne particles and for microbial contamination of the air and surfaces, especially for stem cell lines that will be applied to human therapy [23].

When designing and building a well-functioning stem cell bank, the design team usually includes architects, contractors, builders, tradespeople and a laboratory director or manager. One needs to consider budget, space, the biosafety level of the laboratory, major equipment, number of people, major functional areas, freezer rooms, storage areas in and adjacent to the laboratory, and databases for information storage and extraction etc. To avoid cross-contamination of different functional areas, carrying out cell isolation, culture and quality control in different laboratories is recommended. Figure 3.1 outlines the major functional areas commonly needed in a stem cell bank. The major function of the preparation areas focuses on cell isolation and expansion, while the function of the quality assessment area is to test the cell characteristics and insure the cells generated meet the requirement of good manufacturing practice (GMP) or good laboratory practice (GLP) or similar quality requirements according to the local government.

3.2.2 File Management

Figure 3.2 shows the workflow for stem cell banking under good manufacturing practice conditions, which can be partitioned into three components: manufacturing, quality control and file management. Following cell isolation, expansion, characterization, storage, and quality control and assessment, many record files will be formed and archived, including data files, informed consent, personal information from donors, SOPs for practice etc. All should be well managed to insure standardization, efficiency and privacy protection, and should guarantee cell quality. Ideally file management of stem cell banks should conform good manufacturing practice (GMP) or good laboratory practice (GLP) or similar quality requirements mandated by local government.

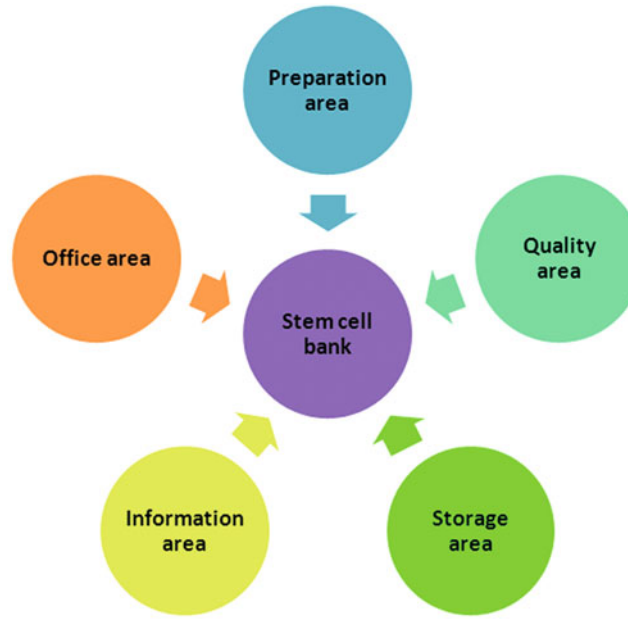

Fig. 3.1 Major functional area in a stem cell bank

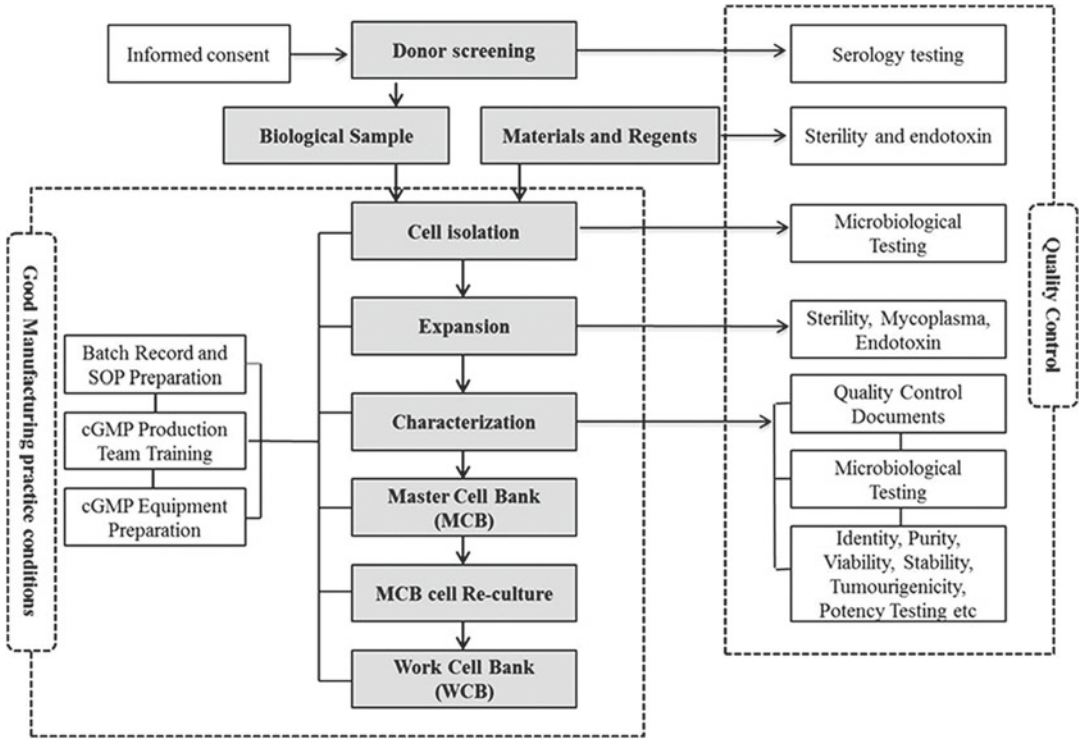

Fig. 3.2 Workflow for stem cells banking under GMP conditions

3.2.3 Master Cell Banks and Working Cell Banks

When a cell line is to be used over many manufacturing cycles and may be distributed to others, a two-tiered cell banking system consisting of a master cell bank (MCB) and a working cell bank (WCB) is recommended [24]. Upon arrival at a banking facility, tissue and newly acquired cells lines should be quarantined until shown to be clear of contamination (such as mycoplasma, HIV etc.), and then clean tissues and cells can be used to create the MCB [12]. This MCB must be characterized and extensively tested for contaminants such as bacteria, fungi, and mycoplasma after isolation and expansion before freezing. Most cell lines also require sterility and PCR testing for viruses. The analysis process starts with the isolation of a sufficient number of clones to adequately sample the population of cells in the culture. The characterization of single clone isolates insures that there are no significant numbers of cells with missing or mutated genetic elements present in the cell bank.

A WCB can be prepared from live cultures or a single frozen vial from the MCB. Cells are grown for several passages, allowing for minimal necessary characterization and scale-up prior to aliquoting into vials and freezing. Cells from the WCB are normally used for distribution to researchers. Thus, creation of a two-tiered frozen cell bank enables a supply of reliable quality-controlled cells at the same passage over a long period of time, which is the principal requirement for biomedical research and product development [12, 24]. Furthermore, WCBs are best maintained at low passage to avoid phenotypic and genetic variation. In order to minimize the risk of complete loss of a cell line due to, for example, contamination or equipment or facility failure, it is critical that frozen stocks are also stored at a second site [25].

3.3 Quality Management

The application of stem cells in cell therapy has obviously increased in the past few years. The risk posed by the administration of a cell-based medicinal product is highly dependent on the origin of the cells, the manufacturing process and quality control program. In order to facilitate the effective use and prevent contamination and deterioration of stem cells in clinical applications, stem cell banks should establish rigorous quality assurance (QA) and quality control (QC) processes, which cover the entire procedure of ex vivo expansion, and apply quality control tests for identity, sterility, viability, and other important characteristics such as potency, genetic stability, etc.

This section aims to provide a thorough understanding of the characteristics of stem cell banks and the development of standardized methods and common strategies to meet current good manufacturing practice (cGMP) compliant conditions, including tissue sourcing, manufacturing, testing, and storage.

3.3.1 Current Good Manufacturing Practices

The goal of banking stem cells is to preserve their characteristics, prevent contamination and deterioration, and facilitate their effective use in research and clinical applications. National and international regulatory authorities such as the World Health Organization (WHO), European Medicines Agency (EMA) and Food and Drug Administration (FDA) recommend that the general principles of GMP should be applied from the first stage of cell banking onwards [26–28]. Many important guidelines on good cell culture practice [29] and cell banking [30, 31] have been developed. Briefly, the fundamental regulatory principles of stem cell banking include the creation of quality controls for master cell bank and working cell banks, supported by traceability of procured cell lines and thorough documentation of the whole stem cell banking process. Ideally banks should operate minimally in "the spirit" of GMP that complies with national and international quality systems standards [32, 33].

3.3.2 Principles of Good Cell Culture Practice

In all aspects of sourcing, banking and preparing cell cultures, the principles of good cell culture practice should be observed [29, 30]. Fundamental features to be considered in the development of stem cell bank are: (a) authenticity, including identity, provenance and genotypic/phenotypic characteristics; (b) absence of contamination with another cell line; (c) absence of microbiological contamination; (d) stability and functional integrity over extended in vitro passage [28].

In order to avoid catastrophic failure of the stem cell bank, minimizing the opportunities for contamination of cell cultures is very important. Taking into consideration the specifics of the manufacturing process, the steps of cell manipulation and open processing should be minimized. It is critical to employ a rigorous aseptic technique and appropriate environmental controls for cell culture processing and the preparation of growth media [28]. Meanwhile, the variation of physical culture parameters such as pH, temperature, humidity, gas composition also can significantly affect the viability of cells and should be specified within established standard operating procedures. In summary, a consistent process should be demonstrated.

Although antibiotic and antifungal agents may be required for primary cell cultures, the using of antibiotics in a stem cell bank is discouraged. When antibiotics have been used, the potential inhibitory effects of the antibiotics on contaminating organisms should be considered in sterility-testing.

3.3.3 Selection of Donors, Materials and Reagents

All materials should be subjected to risk assessment and testing when necessary because raw

materials can be a primary source for the introduction of adventitious agents into the stem cell bank [28]. Careful attention should be paid to sourcing, production, handling, testing and quality control. According to international guidelines [28, 34, 35], the sections below detail the information on manufacturing components and materials that should be considered:

- **Donor screening and testing**: Donor screening and testing is performed to determine donor eligibility. Details of the requirements for screening and testing donors of human cells and tissues are described in FDA and EMA documents [26, 28, 34, 35]. Briefly, the donors of stem cells must be screened and tested for HIV-1, HIV-2, hepatitis B virus (HBV), hepatitis C virus (HCV), Treponema pallidum (syphilis), and CJD. Donors of viable leukocyte-rich cells or tissues should be screened and tested for human T-lymphotropic virus types 1 and 2 (HTLV-1, HTLV-2) and CMV.
- **Quality control of reagents**: Reagents which are used for cellular growth, differentiation, selection, purification, or other critical manufacturing process can affect the safety, potency, and purity of the stem cells, especially by introducing adventitious agents such as bacteria, fungi, mycoplasmas and viruses [26, 28]. For example, trypsin and bovine serum have been known to contain mycoplasma species and sometimes more than one viral contaminant. The common reagents include fetal bovine serum, trypsin, digestion enzymes, growth factors, cytokines, monoclonal antibodies, antibiotics, cell separation devices, media components and so on. Documentation should be gathered on their manufacturing history, production, quality control in order to ensure the reagents' quality and safety [26–28]. The reagents should be subjected to strict tests for quality and freedom from contamination by adventitious agents to assure their acceptability for use in cell culture. Meanwhile, the reduction and elimination from the manufacturing process of reagents and materials derived from humans and animals is encouraged, where feasible.

3.4 Quality Control Testing of the Stem Cell Bank

The recommendations for characterization testing for cells in the stem cell bank include, but are not limited to, identity, purity, viability, microbiological testing (including sterility, mycoplasma, and adventitious viral agent testing), tumorigenicity, stability (including stability during cryostorage and genetic stability), potency etc.

3.4.1 Identity

Cell line misidentification, contamination and poor annotation affect scientific reproducibility [36–38]. Cell banks should be authenticated by a cell identification method approved by the NRA in order to confirm that no switching or cross-contamination has occurred [28]. We recommend that the identity of the cells in the MCB and WCB should be characterized by phenotypic and/or genotypic profiles including cell morphology, genetic tests (STR, SNP), specific surface markers and gene expression products.

For adherent cells, morphology may be a useful method in conjunction with other tests. For human stem cells, genetic tests such as short tandem repeat (STR) analysis are most commonly used to confirm cell line identity [39–42], and single nucleotide polymorphism (SNP) genotyping is also used for identification of cells as well as for monitoring their genomic stability. We also recommend additional identification based on cell surface markers or genetic polymorphisms to distinguish between the multiple cell lines.

In summary, the combined use of variety methods for cell identification depending on the different cell types and tissues can achieve more rigorous identification.

3.4.2 Purity

The cellular population of interest usual contains other cells, which belong to different lineages or differentiation stages that may be unrelated to the intended population. The unwanted cells should

be defined and their amount in the final cell bank should be controlled by appropriate specifications, Acceptance criteria for the amounts of contaminating cells should be set. Cell surface markers, genetic polymorphisms and specific biological activities are recommended methods to test the cell purity [43].

3.4.3 Viability

A high level of viability of cell lines derived from stem cell banks is important for efficient and reliable cell-based products [28]. Documents of the China FDA recommend that different testing methods (such as cell counting, cell doubling time, cell cycle, cloning efficiency, telomerase activity) can be used to determine cell viability and growth status [43]. The recommendations of WHO state that thawed cells should typically have viability levels in excess of 80 %, though this is not always achieved and may depend on the cell line [28]. Lower viabilities may still result in suitable growth recovery and in acceptable product qualities. Meanwhile, for somatic cellular therapies, the minimum acceptable viability specification recommended by the FDA is generally set at 70 % [27].

Cell viability can be easily assessed in culture by employing widely applied assays. However, in some cases, such as pre-apoptotic cells excluding trypan blue, viability assays may give misleading results. So we suggest that when a membrane-integrity test is used for cell viability, additional cell markers such as indicators of apoptosis should be studied in order to avoid significant overestimation of viability.

3.4.4 Microbiological Testing

3.4.4.1 Sterility Tests

It is important in risk evaluation for the manufacturer to bear in mind that standard compendial tests for "sterility" are intended to give an indication of the effectiveness of aseptic processing in preventing general bacterial or fungal contamination. For suitable sterility tests one should refer to certified documentation and Pharmacopoeias. If antibiotics were used in manufacturing, documentation should be provided indicating that the antibiotics were removed prior to sterility testing.

3.4.4.2 Mycoplasma

Animal serum products used in culture and the culture facility environment are the two main sources of mycoplasma contamination. Testing for mycoplasma contamination should be conducted on both cells and supernatant. Due to the limited dating period of many cellular products, it is frequently not feasible for a sponsor to perform the recommended culture-based assay. The EMA and FDA recommend the use of polymerase chain reaction (PCR)-based mycoplasma assays or another rapid detection assay during cell manufacturing [26, 44].

3.4.4.3 Pyrogenicity/Endotoxins

The rabbit pyrogen test is the currently required method for testing biological products for pyrogenic substances. Although the pyrogenicity test is required, there may be specific cases where this test method cannot be performed due to properties of the cellular product. Another test such as the Limulus Amebocyte Lysate test (LAL) may be used as an alternative method [27]. For any parenteral drug, the FDA recommends that the upper limit of acceptance criterion for endotoxin be 5 EU/kg body weight/hour. For intrathecally-administered drugs, an upper limit of acceptance criterion is 0.2 EU/kg body weight/hour [27].

3.4.4.4 Adventitious Agent Testing

As appropriate, adventitious agent testing should include in vitro viral testing, in vivo viral testing and selected species-specific testing for adventitious viruses.

In vitro viral testing should be conducted by inoculating the test sample (MCB) onto various susceptible indicator cell lines such as the human cell line MRC-5 or Vero cells. We recommend that in vivo viral assays be carried out by inoculating the test sample (MCB) into animals such as adult and suckling mice and embryonated hen

eggs. Finally, assays for the presence of species-specific viruses also should be performed especially when the cell lines are used as a therapeutic product. We recommend that testing for human pathogens (CMV, HIV-1 & 2, HTLV-1 & 2, EBV, HBV, HCV, B19), and other human viral agents, for which PCR-based assays are available, should be performed [44, 45].

3.4.5 Tumorigenicity

Testing for tumorigenicity of cells derived from cell culture and banked will be required with a test approved by the NRA. Several in vitro systems, such as cell growth in soft agar [46] and muscle organ culture [47], have been explored as alternatives to in vivo tests for tumorigenicity. The test should involve a comparison between the cell line and a suitable positive reference preparation (e.g. HeLa cells) and a standardized procedure for evaluating results.

Other assays can also be considered, such as proliferative capacity, dependence on exogenous stimuli, response to apoptosis stimuli and genomic modification, in order to evaluate the risk of cellular transformation and subsequent potential for tumorigenicity.

3.4.6 Stability

The stability of cell banks during cryostorage, and the genetic stability of cell lines are key elements in a successful cell bank programme.

3.4.6.1 Stability During Cryostorage

Data should be generated to support the stability or suitability of the cryopreserved cell banks during storage. Continuous monitoring records and successful periodic production runs could be useful strategies to monitor the stability of the cell bank.

3.4.6.2 Genetic Stability

Cell genome stability is the key quality and safety indicator of stem cell banks. Genomic instability refers to an increased tendency to generate alterations in the genome during the life cycle of cells. It is main driving force for tumorigenesis [40]. The WHO has pointed out that any features of the cell lines that might affect quality should be discussed with the NRA to ensure that tests used by the manufacturer to monitor genetic stability are adequate [28].

However, there is no standard method to evaluate the genetic stability of cell lines in MCB and WCB at present. The common methods such as doubling time of cell lines and karyotyping already cannot meet the comprehensive assessment of genomic stability. Here we recommend high-throughput sequencing which has better resolution and accuracy to assess genetic stability. Other approaches include examining telomerase activity, chromosomal integrity, expression of proto oncogenes, etc.

3.4.7 Potency

According to ICH guideline 6QB, potency is the quantitative measure of biological activity [48], but in addition, qualitative biological assay may be included.

Basically, potency assays can be performed in vitro using cell systems and in vivo using animal models [48, 49]. As stem cells have a tendency to spontaneously differentiate in culture, the stem cell bank should ensure that pluripotency is not lost during in vitro expansion and cryopreservation. As the function of cells such as viability, self-renewal, differentiation and apoptosis are critical to their quality, potency assays may need to be evaluated during manufacturing using appropriate markers and technology, such as gene expression profiles, flow cytometry, cell cloning, PCR, etc. [50]. In vivo assays for potency may also be useful especially using available animal models. As described in the referred guideline, the combination of multiple methods may be better to adequately evaluate the potency of the cells in the stem cell bank.

3.5 Long -Term Maintenance and Management

Stem cells have been applied in research and clinical use for many years, and appropriate cryopreservation protocols for long-term storage is essential [51]. Standardization of processes and the implementation of quality control programmes should be established during the banking process in order to prevent contamination and deterioration and maintain post-thaw quality, pluripotency and genetic stability of recovered cells [17, 24]. Moreover, viability tests, functional assays, stability measurements, authentication and documentation have to be carried out [52, 53].

3.5.1 Quality Control During Stem Cell Cryopreservation Process

3.5.1.1 Storage Temperature

Storage temperature is a key factor for long-term storage of stem cells in a stable state. Lower temperatures can reduce metabolism of cells and efficiently reduce the rate of degradation [53]. In general, the lower the storage temperature, the more stable the cells remain and the longer they can be preserved [54, 55]. The traditional method for long-term cryopreservation of stem cell is storage in liquid nitrogen, which can reach a terminal temperature of −196 °C [52].

Quality control methods should be applied to keep a stable temperature environment in long-term storage vessels as stored cells may be subjected to temperature changes during the maintenance of storage vessels. For example, storage vessels are opened frequently to gain access to stored vials, intermittent warming may occur to cryovials. Furthermore, some storage racking material can act as good heat conductors and may promote warming cycles and temperature gradients within the storage vessel. Finally, there may be failures in the liquid nitrogen filling process [25]. A number of straightforward procedures can be taken to keep a stable temperature environment in stem cell storage vessels during the long-term storage process: (1) choose a suitable terminal temperature for the long-term storage of stem cells; (2) use appropriate facilities to cryopreserve samples and manual or automatic continuous temperature monitoring to detect and provide useful monitoring data of temperatures; (3) set up an automatic liquid nitrogen filling system; (4) keep vessel vacuum checks, electrical testing, and other maintenance procedures regularly and periodically audited [25, 53].

3.5.1.2 Contamination

It is crucial to prevent microbial/cross contamination during the long-term storage of stem cells. The risk of stem cell products is mainly associated with contaminated liquid nitrogen during long-term storage [51] as microbial flora can easily accumulate within ice sludge debris [25]. Quality control methods should be taken to prevent contamination of cell samples in these long-term storage vessels [56]. It has been shown that vapour phase storage can largely overcome sample contamination as it can prevent the liquid nitrogen permeating into cryovials thereby avoiding contamination by contaminated liquid [57]. However, all long-term storage samples run the risk of cross contamination risk when they are stored in the same cryotank, rewarmed in the same water bath, and because of other potential environmental sources.

Quality control methods can be taken as follows to reduce the contamination of stem cells during long-term storage: (1) choose a vapour phase liquid nitrogen containment vessel; (2) set security caps when sample have to be submersed in liquid nitrogen; (3) employ internal threads and double bagging to store cell samples; (4) maintain the storage container closure integrity and within the product shelf life; (5) set up a quarantined storage area to control the spread of pathogens and infectious agents; (6) keep storage containers in a clean and controllable environment to reduce the contamination risk from the surrounding area; (7) make sure the source of liquid nitrogen is clean [53].

3.5.2 Quality Validation for Recovered Stem Cell

Stem cells should have the ability to survive long-term storage and keep their functions after recovery. Post-thaw cell viability testing is vital to evaluate the effects of cryopreservation on stem cells. Quality assessment including viability tests, functional assays, stability measurements and authentication should be applied to analyze the post-thaw stem cells metabolic functions, genetic integrity and totipotency after recovery [25, 58].

These quality control measures have been shown to be necessary in various studies. For example, the numbers of colony-forming cells (CFC) and CD34 cells, which can effectively predict engraftment kinetics and hematopoietic recovery, were measured to evaluate the function of 9.5-year cryopreserved hematopoietic stem cells [59], and the differentiation potential of long-term cryopreserved human adipose-derived stem cells was measured by using different induction medium to test totipotency after rewarming [60].

Trypan Blue exclusion assay is used to determine the post-thaw cell viability of stem cells, and a light microscope is used to count total number of dead cells and live cells [60]. Stability assessment for stem cells includes: evaluation of totipotency by cell differentiation potential assays and morphological characteristics; studies of phenotypes; cytological and karyotype testing; confirmation of functional biochemistry (metabolites/proteins, proteins/enzymes); omics analyses; biomarker studies;, investigations of genome structure, DNA damage and repair, chromatin analysis, analysis of DNA adducts (methylation, oxidation) and confirmation of genetic modifications [61]. Furthermore, since cells that have been held in long-term storage may have been cross-contaminated by other samples, authentication is particularly important and quality control needs to be performed to insure sample identity following the cells retrieval from storage [53].

3.6 Prospects for Stem Cell Banking

Many countries have now established stem cell banks. Table 3.1 shows the major stem cell banks in the world. Stem cells stored in banks play an important role in promoting biological development and providing medical science with a powerful tool. Several types of conditions are currently being treated with stem cell-based therapies, including autoimmune diseases, neurological disorders, cancers, and infertility [62]. To date, several stem cell therapeutic products have been approved in different countries (Table 3.2). Especially worthy of mention is umbilical cord blood. Since the first successful transplantation, the field of UCB banking and transplantation has grown exponentially. Over 600,000 UCB units have been stored for transplantation worldwide, and more than 30,000 UCB transplantations have been performed [19].

Treatments in regular clinical use are limited to adult stem cells. Many stem cell therapies are based on the regenerative capacities of stem cells to produce a variety of tissues, either in the patient's body or in vitro [62]. And other therapies depend on the transplanted stem cells to provide signals that regulate the activities of nearby cells [63]. Although unanticipated challenges in safety or efficacy still exist, we can optimistically believe that products and medicine based on stem cells will be widely used in the future.

3.7 Summary

Since significant progress has been made in stem cell research and application over the last few decades, stem cell banks have become very important platforms to preserve their characteristics, prevent contamination and deterioration and facilitate their effective use in baicl and translational research, as well as clinical application all over the world [17, 18]. A number of stem cell banks have been established in many countries

Table 3.1 Example of stem cell banks in the word

Name of bank	Stem cell type	Country/region	Website and/or reference
UK Stem Cell Bank	Comprehensive	UK	http://www.nibsc.org
National Centre for Cell Science Cell Repository	Comprehensive	US	http://www.nccs.res.in
WiCell International Stem Cell Bank	Comprehensive	US	http://www.nationalstemcellbank.org
Cord Blood Registry	Cord blood/cord tissue stem cells	US	http://www.cordblood.com/benefits-cord-blood
Australian cord blood bank	Cord blood	Australian	http://www.stemlife.com.au
BioEden	Child milk tooth stem cells	US	https://us.bioeden.com
Human Pluripotent Stem Cell Registry	Human pluripotent stem cells	Global registry	http://hpscreg.eu
Swiss Stem Cell Bank	Cord blood	Swiss	http://parentsguidecordblood.org/en/bank/swiss-stem-cell-bank
Stem Med	Haemopoietic stem cells/ mesenchymal stem cells	Singapore	http://stem-med.sg/about-stem-med
Dental stem cell banking	Dental stem cells	US	http://www.store-a-tooth.com
Cell Center of China National Genebank	Comprehensive	China	http://stemcell.cngb.org
The International Placenta Stem Cell Society	Placenta stem cells	International	http://www.iplass.net/ii

Table 3.2 Example of stem cell therapeutic products approved in the world

Country	Year	Product/company	Source	Adaptation disease
Europe – EMA	2009	Chondro Celect/TiGenix	Autologous chondrocytes	Knee cartilage defects
USA – Food and Drug Administration (FDA)	2009	Prochymal/Osiris	Allogeneic bone marrow mesenchymal stem cells	GvHD and Crohn disease
Australia-Therapeutic Goods Administration (TGA)	2010	Mesenchymal Preursor/ Mesoblast	Autologous mesenchymal progenitor cells	Bone repair
Korea – Food and Drug Administration (FDA)	2011	Hearticellgram-AMI/ FCB-Pharmicell	Autologous bone marrow mesenchymal stem cells	Acute myocardial infarction(AMI)
USA – FDA biologics license	2011	Hemacord/New York Blood Centre	Umbilical cord blood hematopoietic progenitor cells	Genetic acquired hematopoietic system disease
Korea – Food and Drug Administration (FDA)	2012	Cartistem/Medi-post	Mesenchymal stem cells from umbilical cord blood	Degenerative arthritis and knee joint cartilage injury
Korea – Food and Drug Administration (FDA)	2012	Cuepistem/Anterogen	Autologous fat source of mesenchymal stem cells	Complexity Crohn's disease complicated by anal fistula
Canada	2012	Prochymal/Osiris	Bone marrow stem cell	Children graft versus host disease(GvHD)

and regions, and the number will increase. There are many factors that need to be carefully considered before establishing a stem cell bank. New technology development and knowledge will undoubtedly lead to improvements in stem cell bank maintenance and management. For example, at present most banks banking MSCs adhere to the minimal criteria proposed by the International Society for Cellular Therapy (ISCT), i.e. (1) MSCs must be plastic-adherent when maintained in standard culture conditions; (2) MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. (3) MSCs must differentiate to osteoblasts, adipocytes and chondroblasts in vitro [64]. However, many investigations reported recently have shown that MSCs isolated utilizing these criteria are heterogeneous and different MSCs subtypes may have discordant functional properties, implying their applications need further elaboration [65, 66], and as a result banking SOPs need corresponding modification. Thus, the practice of stem cell banking is a dynamic process, which will advance with the times.

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