Guidelines for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs)

Directorate General of Drug Administration
Ministry of Health and Family Welfare
Government of the People's Republic of Bangladesh

Message from the Director General, Directorate General of Drug Administration (DGDA)

It is my pleasure that "Marketing Authorization" related guidelines are now regularly published from DGDA for different classes of drugs. On this aspect "**Guidelines for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs)"** is one of the very important guidelines which will help Regenerative Medicines Manufacturers, Biotech Industry, Researchers, Academicians as well as Regulators to ensure quality, efficacy, and safety of Stem cell derived products registered in Bangladesh.

Since the 1960s, the stem cells have been extensively studied including embryonic stem cells, neural stem cells, bone marrow hematopoietic stem cells, and mesenchymal stem cells. In the recent years, several stem cells have been initially used in the treatment of diseases, such as in bone marrow transplant. Application of Stem Cell and Cell Based Products (SCCPs) in medical research and in the prevention or treatment of human diseases have become a significant area of interest during the last decade. With the rapid growth of the stem cell field and advances in regenerative medicine, it is critically important that SCCPs should maintain the product quality, safety, efficacy and also earn the public trust. To ensure these, DGDA releases the "Guidelines for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs)" which is an internationally agreed-upon set of principles harmonized with and mostly adapted from similar type of regulatory guidelines published by the different regulatory authorities.

The aim of this guidelines is to define the role of DGDA in regulating, licensing and approval of manufacturing/intervention premises of SCCPs. These also cover specific aspects related to Marketing Authorization (MA) and usage of SCCPs in Bangladesh. This multidisciplinary guideline addresses processing, manufacturing, quality control, stability study, pre-clinical & clinical studies, packaging, storage, transport, tracking, transplantation & post transplantation surveillance, advertisement, *etc.* of SCCPs in Bangladesh. The guideline is relevant to all medicinal products using stem cells as starting material. The final products may consist of stem cells as well as terminally differentiated cells, even a mixture of cells with varying differentiation profile.

As this is the country's first regulatory guidelines for SCCPs, therefore, it has not "reinvent the wheel" rather adapt the regulatory guidance and guidelines from other regulatory authorities. This guideline is prepared by a 14 members committee of experts of different medical specialization related to stem cell use and manufacture. Among these members, a working committee consists of 8 members worked hard compiling this guidance document. The draft copy is distributed to experts, medical professionals and uploaded to DGDA web site for opinion. Having edited by a 6 members editorial committee and approved by "Stem Cell Formulation Committee", this Guidance document has been finalized.

I would like to thank the members of working committee, editorial board and Stem Cell Formulation Committee" for their wonderful job which they performed.

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Contents

1. Abbreviations

AABB: American Association of Blood Banks

BM: Bone Marrow

CB: Cord Blood

CBB: Cord Blood Banking

cGMP: Current Good Manufacturing Practice

CMV: Cytomegalovirus

COA: Certificate of Analysis

COPP: Certificate of Pharmaceutical Product

DCB: Donor Cell Bank

DGDA: Directorate General of Drug Administration

DMF: Drug Master File

DMSO: Dimethyl Sulfoxide

ELISA: Enzyme Linked Immunosorbent Assay

EMA: European Medicine Agency

ESC: Embryonic Stem Cells

FACS: Fluorescence Activated Cell Sorting

FACT: Foundation for the Accreditation of Cellular Therapy

FBS: Fetal Bovine Serum

GCP: Good Clinical Practice

GLP: Good Laboratory Practice

GMP: Good Manufacturing Practice

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

HCT/P: Human Cells, Tissues, and cellular and tissue-based Product

HEPA: High Efficiency Particulate Air

hESCs: Human Embryonic Stem Cells

HLA: Human Leukocyte Antigen

HPC: Hematopoietic Progenitor Cell

HSCs: Hematopoietic Stem Cells

HSV: Herpes Simplex Virus

ID: Identity Document

ISO: International Organization for Standardization

iPSCs: Induced Pluripotent Stem Cells

LED: Light Emitting Diode

MA: Marketing Authorization

MAA: Marketing Authorization Application

MAH: Marketing Authorization Holder

MCB: Master Cell Bank

MSCs: Mesenchymal Stromal/Stem Cells

MWCB: Manufacturer's Working Cell Bank

NOAEL: No Observed Adverse Effect Level

NOC: No Objection Certificate

PCR: Polymerase Chain Reaction

PD: Pharmacodynamics

PMDA: Pharmaceuticals and Medical Devices Agency

PMF: Plasma Master File

POC: Proof of Concept

PPE: Personal Protective Equipment

PRP: Platelet Rich Plasma

QA: Quality Assurance

QC: Quality Control

rDNA: Recombinant Deoxyribonucleic Acid

SC: Stem Cells

SCCPs: Stem Cell and Cell Based Products

SOP: Standard Operating Procedure

TGA: Therapeutic Goods Administration

UCB: Umbilical Cord Blood

USFDA: Food and Drug Administration

WCB: Working Cell Bank

WHO: World Health Organization

2. General Introduction

Rapid development in the fields of biology, biotechnology, and medicine has led to the development of new treatments and highly innovative medicinal products, including medicinal products containing viable cells. Development of biological medicines has been extremely rapid and the potential of such products for improving health care on a global scale is immense. Regenerative medicines and cell-based therapies continue to engender bold prognostications about how they could revolutionize healthcare. These new cell-based medicinal products have a high potential in the treatment of various diseases where there is a previously unmet medical need.

Human cell-based medicinal products are heterogeneous with regard to the origin and type of the cells and to the complexity of the product. Cells may be self-renewing stem cells, more committed progenitor cells or terminally differentiated cells exerting a specific defined physiological function. Cells may be of autologous or allogeneic origin. In addition, the cells may also be genetically modified. The cells may be used alone, associated with biomolecules or other chemical substances or combined with structural materials that alone might be classified as medical devices (combined advanced therapy medicinal products).

Application of Stem Cell and Cell Based Products (SCCPs) in medical research and in the prevention or treatment of human diseases have become a significant area of interest during the last decade. This application could possibly lead to novel methods for treatments and potential cures for Critical Limb Ischemia, Osteoarthritis, Cancer, Diabetes, Parkinson’s disease and many other diseases. Stem cells are unspecialized cells that can self-renew indefinitely and also differentiate into more mature cells with specialized functions. Significant advances suggest that the use of cord blood and stem cells in human with public awareness will continue to expand with the inclusion of more diseases, treatment options and the emerging field of regenerative medicine, however safety and efficacy of these cells is yet to be established.

Although stem cells share the same principal characteristics of self-renewal potential and differentiation, stem-cell-based medicinal products do not constitute a homogeneous class. Instead, they represent a spectrum of different cell-based products for which there is a variable degree of scientific knowledge and clinical experience available. For example, while mesenchymal/stromal stem cells (MSCs) or hematopoietic stem cells (HSCs) have been more extensively used for therapeutic purposes, this is not the case for human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs).

In addition, varying levels of risks can be associated with specific types of stem cells. For example, the risk profile associated with pluripotent stem cells is expected to be different from those of adult stem cells (*e.g.* MSCs or HSCs) for which a substantial amount of clinical experience has already been gained.

Besides, Umbilical cord blood can be used as a source of primitive hematopoietic stem and pluripotent progenitor cells in clinical application to reconstitute the hematopoietic system and/or to restore immunological function in vivo. It has been used successfully as an alternative to bone marrow or peripheral blood progenitor cells for transplantation purposes and developing into a new field of study in medicine for treating diseases. Cord blood is considered a treatment option in pediatric and adult patients with hematologic malignancies and disorders (leukemia, thalassemia, sickle cell disease, *etc.*), bone marrow failures, inherited metabolic disorders, immunological defects and other genetic diseases. There is a continual need for suitable donors for one third of all patients in need of bone marrow transplants in which no available human leukocyte antigen (HLA) matched donors are found. Thus, umbilical cord blood as single- or double-units transplants, provide a potentially vast source of hematopoietic stem cells, and represent a valuable alternative for stem cell transplantation needs worldwide and with the added potential of decreased chronic graft versus host disease (GvHD) compared with bone marrow and peripheral blood progenitor cell donors.

Stem cells are abundant in the human body, easily accessible for regenerative medicine, and can be acquired from different types of adult and fetal tissues. An easily accessible source of adult stem cells is adipose tissue. These cells could be utilized clinically in areas such as plastic surgery, orthopedic surgery, neurological disorders, myocardial infarction, and many other rare diseases.

The aim of this guideline is to cover specific aspects related to stem cell and cell based medicinal products for Marketing Authorization (MA) as well as approval of SCCPs manufacturing/intervention premises in Bangladesh. In case of genetic modification of cells, the other "regulators' guidelines" (*e.g.* USFDA, EMA, TGA, PMDA *etc.*) for genetically modified cells should also be consulted. This regulatory guideline shall apply to all types of SCCPs regardless of their differentiation status at time of administration. This also provide standard practices for cord blood (CB), bone marrow (BM) and other cell and tissue banking.

This guideline is relevant to all medicinal products using stem cells as starting material. The final products may consist of stem cells as well as terminally differentiated cells, even a mixture of cells with varying differentiation profile.

3. Regulatory framework

3.1 Legal basis

As "Guidelines for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs)" having properties for treating or preventing diseases in human beings, or that they may be used in or administered to human beings with a view of restoring, correcting or modifying physiological functions by exerting principally pharmacological, immunological or metabolic action. Therefore, the SCCPs are classified as medicinal products and fit within the scope of medicinal products under the Drug Act 1940 section 3 (b) (i), further SCCPs would be classified as biological products in accordance to the statutory provisions. Thus, the essential aim of this regulatory guideline is to safeguard public health by ensuring of quality, safety and efficacy of SCCPs.

This document is consistent and integrated with the existing legislative framework. Hence, it should be read in conjunction with the relevant sections of the Drug Act 1940, Drug Rules 1945, the Bengal Drug Rules 1946, Drug (Control) ordinance 1982 and amendments there under.

3.2 About this framework

The regulatory framework aims to provide a clear and predictable pathway for SCCPs production and marketing/intervention based on internationally benchmarked regulations. The DGDA strives to emulate the examples set by better established regulatory authorities (*e.g.* USFDA, EMA, TGA, PMDA *etc.*). Therefore, it has deemed that it shall not "reinvent the wheel", rather adapt the regulatory guidance and guidelines from above agencies as appropriate for local use.

This document provides information for manufacturers, MA applicants, healthcare professionals and the general public to adhere to legal arrangements in Bangladesh for the registration of SCCPs productions and marketing/intervention.

This framework lays down specific regulation on registration and its data requirements (Chemistry, Manufacturing, Control (CMC), nonclinical and clinical), supervision, risk management plan (RMP) and pharmacovigilance of SCCPs. Other regulatory procedures and guidelines in new areas such as current Good Tissue Practice (cGTP) may be included.

The cross-boundary nature SCCPs involves a multidisciplinary approach; therefore, its full control will also be subject to various other regulations (authorities), hence an integrated oversight is imperative, as follows:

- The clinical use/medical procedure of the product will be under the ambit of The Medical Practice and Private Clinics and Laboratories (Regulation) ordinance, 1982, Human organ transplant act 2018. Directorate General of Drug Administration (DGDA) will ensure the medicinal product's quality, safety and efficacy.

The framework is based on a risk-management system approach, *i.e.* different levels of regulations are applied to SCCPs where the risks are associated with their use. The risk management should be applied throughout all stages of a product's lifecycle. The procedure of tissue selection, collection, processing, its release and distribution are essential for ensuring optimum product quality, safety and efficacy.

3.3 Guiding principles

The primary objective of SCCPs regulation is to safeguard public health and patient safety. SCCPs should meet the same stringent standards on quality, safety and efficacy, as of other biological products.

4. Scope

This multidisciplinary guideline will address manufacturing and quality control as well as nonclinical and clinical development of SCCPs which includes somatic cell therapy, tissue engineering products as defined in this document. This will also address the standard practices for cord blood (CB), bone marrow (BM) and other cell and tissue banking.

This guideline is intended for products entering into the registration process at DGDA. However, the principle laid down in the guideline should be considered by applicants for premises approval as well as entering into clinical trials.

Cellular-based medicinal products discussed in this document have the following characteristics:

- They contain viable human cells of allogeneic or autologous origin undergoing a manufacturing process
- They may be combined with non-cellular components.
- The cells may be genetically modified

The following are **included** in the framework

- Human stem cells (HSC, MSC, *etc.*)
- Genetically modified cellular products.
- Cell-based cancer vaccines and cell-based immunotherapies
- Dendritic cells, lymphocyte-based therapies, cell-based therapies for cancer, peptides, and proteins.
- Cord Blood (CB), bone marrow (BM) and other cell and tissue banking.
- Stromal Vascular Fraction (SVF).
- Conditioning medium
- Xenogeneic stem cell

The following are **not included** in the framework:

- Fresh viable human organs or parts of human organs, for direct donor-to-host transplantation.
- Labile (fresh) blood and blood components (*e.g.* fresh frozen plasma)
- Unprocessed reproductive tissues (*e.g.* sperm, eggs, embryos for *in vitro* fertilization (IVF) and other assisted reproductive technology procedures)
- Secreted or extracted human products (*e.g.* milk, collagen)
- Samples of human cells or tissues that are solely for diagnostic purposes in the same individual
- *In vitro* diagnostic devices

The inclusion and exclusion lists are not self-contained. The lists may be amended as required.

The aim and scope of this document is to:

- To define the role of DGDA in regulating the manufacturing, licensing and usage of SCCPs
- To describe the various categories of licensing/approvals for facilities, clinical trials and protocols for SCCPs usage for specific indications.
- To provide guidance to the applicants for isolation, characterization, culturing, processing, manufacturing, quality control, labeling and distribution of SCCPs.
- To provide guidance for compliance, with all the regulatory requirements, to carry out clinical trials with SCCPs

- To provide for compliance with the regulatory requirements of SCCPs banking procedure including Cord Blood (CB), bone marrow (BM) and other cell and tissue banking.
- To provide standard practices for Cord Blood (CB), Cord Tissue, Placenta, bone marrow (BM) and other cell and tissue banking and release for administration to achieve consistent production of quality placental and umbilical cord blood units for administration.

5. Definitions

Adult stem cell: (also known as somatic stem cell): A relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self-renewal (in the laboratory) and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

Adventitious agents: These are microorganisms that have been unintentionally introduced into the manufacturing process of a biological product. Include bacteria, fungi, mycoplasmas, rickettsia, protozoa, parasites, TSE agents, and viruses.

Aseptic technique: Practices designed to reduce the risk of microbial contamination of products, reagents, specimens, recipients, or donors.

Audit: Documented, systematic evaluation to determine whether approved policies, Standard Operating Procedures, or operations have been properly implemented and are being followed.

Autologous Use: The implantation, transplantation, infusion, or transfer of human cells or tissue back into the individual from whom the cells or tissue were recovered.

Bone Marrow: The soft, spongy tissue found in the center of most large bones that produces the cellular components of blood which is known as hematopoietic stem cells (white cells, red cells and platelets). It is also a source of mesenchymal and endothelial stem cells.

CD34: The 115 kd glycoprotein antigen, expressed by a small portion of cord blood cells, that is defined by a specific monoclonal antibody (anti-CD34) using the standardized cluster of differentiation (CD) terminology. Hematopoietic progenitor cells are largely contained within the CD34 cell population of cord blood units.

Cell line: A cell culture system consisting of identical cell population selected for uniformity from a usually homogeneous tissue source (as an organ).

Cell therapy: refers to the infusion of cellular and tissue products with the intent of providing effector functions in the treatment of disease or support of other therapy.

CFU (Colony forming unit): A clonogenic cell able to produce hematopoietic colonies in vitro under specific conditions in the presence of appropriate colony stimulating factors and defined by the type of mature progeny that develop.

Chimera: An organism, organ, or part consisting of two or more cell types of different genetic composition, produced as a result of organ transplant, grafting, or genetic engineering.

Clonal: Cells derived from a single parent cell.

Clone: A cell or organism derived from genetically identical to another cell or organism.

Cloning: The process of creating genetically identical copy of a biological unit (*e.g.* a DNA sequence, cell, or organism) from which it was derived, especially by way of bio-technological methods.

Competency: is the adequate ability to perform a specific procedure according to direction.

Consent: A process by which a subject voluntarily confirms his or her (or their next of kin/legal heir) willingness to participate in a particular study/clinical trial, after having been informed of the aims, methods, required data collection procedures and schedule, anticipated benefits and potential hazards of the study and the discomfort it may entail. Informed consent is documented by means of a written, signed and dated informed consent form. The consent besides being voluntary and informed has to be without any coercion or inducement. It can be withheld, or even withdrawn at any time, without giving any reason or prejudice to present or future treatment of the individual.

Cord Blood (CB): The infant's blood remaining in the placenta and umbilical cord after the umbilical cord has been clamped.

Cord Blood Bank (CBB): An integrated team under a single Cord Blood Bank Director responsible for donor management and the collection, processing, testing, cryopreservation, storage, listing, search, selection, reservation, release, and distribution of cord blood units.

Cord Blood Banking (CB banking): The processing, testing, cryopreservation, storage, listing, search, selection, reservation, release, and distribution of cord blood units intended for administration.

Cord Blood Collection: The procurement of cord blood for banking and administration before and/or after the placenta is delivered.

> **Ex utero:** The collection of cord blood cells from the placental or umbilical cord vessels after the placenta has been delivered.
>
> **In utero:** The collection of cord blood cells from the placental or umbilical cord vessels after the infant donor has been delivered and separated from the umbilical cord, but before the placenta has been delivered.

Cord Blood Processing Facility: The location where cord blood processing activities are performed in support of the Cord Blood Bank. A Cord Blood Processing Facility may be part of the same institution as the Cord Blood Bank or may be part of another institution and performs these functions through contractual agreement.

Cord blood stem cell: Stem cells isolated from the umbilical cord blood collected at the time of birth. Cord blood contains hematopoietic and mesenchymal (stromal) stem cells.

Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood related disorders.

Cryopreservation: The processing of viable cells or tissues that consists of cooling the product to a very low temperature where viability is maintained.

Donor eligibility: A donor-eligibility determination is a conclusion that a donor is either eligible or ineligible to donate cells or tissues to be used in stem cell-based product(s), based on the results of donor screening and testing.

Donor screening: The process of identifying risk factors for transmissible disease through review of a current donor medical history interview (to include high-risk behaviors), physical examination results, and other medical records.

High resolution typing: Determination of a set of alleles that encode the same protein sequence for the region of the HLA molecule called the antigen binding site and that excludes alleles that are not expressed as cell-surface proteins. The antigen binding site includes domain 1 and domain 2 of the class I α polypeptides, and domain 1 of the class II α and domain 1 of the class II β polypeptide chains.

Homologous (Allogeneic) Use: the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P (human cells, tissues, and cellular and tissue-based product) that performs the same basic function or functions in the recipient as in the donor, including when such cells or tissues are for autologous use.

In vitro and in vivo: outside and inside the body; in vitro (literally, in glass) generally means in the laboratory.

Labeling: This process includes steps taken to identify the original cell collection, any products, and any product modifications; to complete the required reviews; and to attach the appropriate labels.

Marketing Authorization: Marketing Authorization is the process of reviewing and assessing the evidence to support a medicinal product, such as a drug, in relation to its marketing, finalized by granting of a license to be sold.

Manipulated cell products: Manipulated cell products refer to cell products that have been functionally, quantitatively or genetically altered ex vivo, including ex vivo expanded cells.

Manufacturing: Manufacturing includes, but is not limited to, any or all steps in the recovery, processing, storage, labeling, packaging, or distribution of any human cellular or tissue-based product, and the screening and testing of a cell or tissue donor.

Minimal Manipulation: (minor processing including purification, centrifugation, washing, preservation, storage) – DGDA has the authority to regulate anything beyond minimal manipulation and homologous use:

- For structural tissue, processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement; and
- For cells or nonstructural tissues, processing that does not alter the relevant biological characteristics of cells or tissues.

Multipotent: Multipotent stem cells are those which are capable of giving rise to several different types of specialized cells constituting a specific tissue or organ.

Platelet Rich Plasma (PRP): PRP is defined as autologous blood with platelet concentrations above the physiological baseline. It is obtained by a centrifugation process which separates the liquid and solid components of blood.

Potency: is the therapeutic activity of a product as indicated by appropriate laboratory tests or adequately developed and controlled clinical data.

Processing: includes all aspects of manipulation, labeling, and infusion of products, regardless of source.

Somatic stem cell: an undifferentiated cell found among differentiated cells in a tissue or organ, which can renew itself and can differentiate to yield the major specialized cell types of the tissue or organ.

Stem cells: Stem cells (SC) are natural occurring cells in the body that have the ability to divide and produce a range of different cell types, pertinent to growth and repair after an injury.

Stem cell therapies: A stem cell therapy is any treatment that uses stem cells as the primary way of curing or reducing the severity of a disease or disorder. There are two main ways stem cells can be used: 1) as a transplant, where the desired stem cells are harvested either from the patient or a donor and refined or modified in some way before being injected or grafted into the patient, or 2) as a target for a drug or other biologic, where the drug or biologic is intended to activate a desired response from the stem cells that already exist in the patient's tissues or organs.

Tumorigenicity: Tumorigenicity is the process by which immortalized cells form tumors when inoculated into animals.

Validation: refers to establishment of documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. A process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use.

6. Classification of stem cells

Stem cells (SC) are natural occurring cells in the body that have the ability to divide and produce a range of different cell types, pertinent to growth and repair after an injury.

For the purpose of this guidelines, stem cells include the following:

a. Embryonic stem cells (ESC)
b. Adult or somatic stem cells
   i. Hematopoietic stem cells (HSCs)
   ii. Mesenchymal stromal/stem cells (MSCs)
   iii. Tissue-specific stem cells
c. Induced pluripotent stem cells (iPSCs)

6.1 Embryonic stem cells

Embryonic stem cells are pluripotent and have the capacity to differentiate to virtually every cell type found in the human body. Human embryonic stem cells (hESCs) can be characterized by a distinct set of cell surface markers, as well as marker genes for pluripotency. hESCs, when transplanted into a permissive host form teratomas, benign tumors consisting of various cell types derived from all three germ layers; endoderm, mesoderm and ectoderm. hESCs can be differentiated in vitro using either external factors in the culture medium, or by genetic modification. However, *in vitro* differentiation often generates cell populations with varying degree of heterogeneity.

6.2 Adult or somatic stem cells

6.2.1 Hematopoietic stem cells (HSCs)

Hematopoietic stem cells (HSCs) are a specific class of tissue-specific stem cells. They can give rise to differentiated cells of all hematopoietic lineages, myeloid and lymphoid, either in the hematopoietic bone marrow or in the thymus. These stem cells are also found in the placental and cord blood at birth in concentrations similar to levels found in adult bone marrow. In the adult body, HSCs are localized in the red bone marrow and found circulating at a lower frequency in the peripheral blood. They may also be found at low frequency in other tissues (liver, spleen and muscle) but their origin and relevance for normal hematopoiesis remains to be fully determined. HSCs are mobilized to the blood compartment after treatments with intensive chemotherapy and/or growth factors.

6.2.2 Mesenchymal stromal/stem cells (MSCs)

Mesenchymal stromal/stem cells (MSCs) are primarily derived from bone marrow, adipose tissue and umbilical cord. Additionally, MSCs have been isolated from numerous other tissues, such as retina, liver, gastric epithelium, tendons, synovial membrane, placenta, umbilical cord and blood. MSCs are defined by adherence to plastic, specific surface antigen expression and multipotent differentiation potential. They are lineage-committed cells as they can differentiate towards mesenchymal lineages, mainly adipogenic, osteogenic and chondrogenic cell lineages. Under appropriate culture conditions in vitro differentiation to tenocytes, skeletal myocytes, astrocytes and neurons has been also described.

6.2.3 Tissue-specific progenitor/stem cells

Tissue-specific progenitor/stem cells have a limited differentiation capacity and normally produce a single cell type or a few cell types that are specific to that tissue (*e.g.* tenocytes, myocytes, astrocytes).

6.3 Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) are artificially generated stem cells. They are reprogrammed from somatic adult cells such as skin fibroblasts to re-acquire both the stemness and differentiation capacity of self-renewing embryonic stem cells. iPSCs share many features of hESCs; they have self-renewing capacity, are pluripotent and form teratomas. Increasingly iPSCs are being produced from different adult cell types. Their differentiation capacity seems to be dependent on the cell type and age of the cells from which the iPSCs were reprogrammed. There is a current knowledge gap with respect to alterations of cell-specific regulatory pathways, differences in gene expression and in epigenetic control. These characteristics may result in tissues chimerism or malfunctioning of the cells.

7. Classification of stem cell-based products

Stem cell and cell-based products (SCCPs) used for prevention and treatment of human disease are classified as follows:

7.1 Autologous SCCPs

Autologous SCCPs shall include mononuclear cells, marker based enriched cells or mesenchymal stromal cells (MSCs) that have been isolated from hematopoietic or any other tissue of the recipient patient.

7.2 Allogeneic SCCPs

Allogeneic SCCPs shall include mononuclear cells, marker based enriched cells or mesenchymal stromal cells (MSCs) that have been isolated from hematopoietic or any other tissue of a matched live or cadaveric donor.

7.3 Xenogeneic cell-based products

There is a long and growing list of human disorders for which cell or organ transplantation promises more effective therapy. The problem is that the human tissue available for transplant, now or in the future, cannot begin to meet the need. Removing the barriers to xenogeneic (cross-species) transplantation could open the way to organ banks with an unlimited supply of replacement cells and organs.

Xenogeneic cell-based therapy is the use of viable animal somatic cell preparations, suitably adapted for: (a) implantation/ infusion into a human recipient or (b) extracorporeal treatment through bringing (non-human) animal cells into contact with human body fluids, tissues or organs.

7.4 Classification based on manipulation/processing

7.4.1 Minimal manipulation and homologous use

As defined by FDA in 21 CFR 1271.3(f), minimal manipulation means:

1) For structural tissue, processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement;

2) For cells or nonstructural tissues, processing that does not alter the relevant biological characteristics of cells or tissues.

Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function or functions. Similarly, relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function(s).

The homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with the human cell, tissue and cell tissue base products that performs the same basic function or functions in the recipient as in the donor.

If the processing of human cells, tissues, cell and tissue-based products (HCT/P) does not meet the criteria of minimal manipulation, then this considered as "more than minimal manipulation". SVF (Stromal Vascular Fraction), buffy coat, *etc.* are considered as minimal manipulated for homologous use.

To apply the minimal manipulation criterion, first it needs to determine whether the HCT/P is structural or cellular/nonstructural. This determination is made based on the characteristics of the HCT/P in the donor, before recovery and before any processing that takes place. Then, the appropriate definition can be applied to determine whether the HCT/P has been minimally manipulated.

Structural tissue

Tissues that physically support or serve as a barrier or conduit, or connect, cover, or cushion in the donor are generally considered structural tissues for the purposes of determining the applicable regulatory definition.

Examples of structural tissues include:

- Bone;
- Skin;
- Amniotic membrane and umbilical cord;
- Blood vessel;
- Adipose tissue;
- Articular cartilage;
- Non-articular cartilage; and
- Tendon or ligament

Cells or nonstructural tissues

Cells or nonstructural tissues are generally those that serve predominantly metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions.

Examples of cells or nonstructural tissues include:

- Reproductive cells or tissues (*e.g.*, oocytes);
- Hematopoietic stem/progenitor cells (*e.g.*, cord blood);
- Lymph nodes and thymus;
- Parathyroid glands;
- Peripheral nerve; and
- Pancreatic tissue.

7.4.2 Extensive manipulation and homologous/heterologous use

If the human cells, tissue, cells and tissue-based product (HCT/P) are processed other than minimal manipulated, then these considered as extensive manipulation. Examples of extensive manipulated HCT/P are Hematopoietic stem cells (HSCs), Mesenchymal stromal/stem cells (MSCs), *etc.*

8. Structure of stem cell-based product manufacturing and banking organization

Each stem cell-based product manufacturing and banking organization must define its organizational structure. In defining the organizational structure, one must pay attention to the responsibility chain and define the intra- and inter-organizational relationships. Everyone, in or out of the organization, should has a clear insight about his/her duties in the organization, interaction with others. Organizational chart is an appropriate tool to describe this structure.

Minimum requirements for an organization are:

- ✓ A clearly defined goals, visions, and missions of the organization.
- ✓ An approved organogram of the organization.
- ✓ Each organization must comply with the national rules and regulations on registration, licensing and certification necessary for their activities.
- ✓ An established Quality Management System (QMS)
- ✓ Job descriptions, roles, and responsibilities of the employees.
- ✓ Human resources area of expertise and experiences.
- ✓ Laboratory facility and infrastructure requirements.

9. Legal and ethical considerations

Cell therapies possess advantages and potential therapeutic applications; however, there are some concerns about the possible side effects such as transmission of infectious diseases, unwanted differentiation, uncontrolled replication, and tumor formation.

Therefore, the clinical trials for any stem cell-based product should follow the "Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products Bangladesh".

10. Cell and tissue recovery and transport

10.1 Donor eligibility

Donor eligibility is determined by performing the donor screening and testing. When appropriate, the donor safety testing that is performed should be documented. The testing and acceptance procedures should be described, and any deviations should be justified.

10.1.1 Medical history and physical examination

Donor must be assessed for transmissible diseases through medical history taking, scrutinizing the patient's medical records and physical examination. History and physical examination must at list cover the items below:

- History of vaccination
- History of travel to high-risk areas
- History of transfusion of blood, blood products or biological products
- Familial history of genetic disorders
- History of malignancies
- History of acute and chronic infectious disease like syphilis, chlamydia, gonorrhea, tuberculosis *etc.*
- History of risky behavior (multiple sexual partners, addiction, incarceration) and evidence of non-therapeutic intravenous injections and tattoos
- Sepsis and fever of unknown origin

- Significant weight loss (more than 10% in 6 months)
- Metabolic and autoimmune diseases
- Jaundice with unknown etiology
- Wounds in the genital area
- Disseminated lymphadenopathies and spleen enlargement

10.2 Laboratory tests

Besides history taking and physical examination, laboratory tests are used for assessment of donor's health status. These tests must be performed at most 30 days (in case of lymphocyte-rich products, 7 days) before sampling.

In autologous transplantation, the following tests are necessary

- Human Immunodeficiency Virus (HIV) types-1 and type-2
- Hepatitis B Virus
- Hepatitis C Virus

In case of allogeneic transplantation, in addition to the tests mentioned, the following tests must be performed:

- Diagnostic tests to assess infection with *Treponema pallidum*(Syphilis)
- If necessary, laboratory tests for infection with following items must be performed: Parvovirus B19, Herpes Simplex Virus (HSV), Epstein Barr Virus, Human Papilloma Virus, Cytomegalovirus (CMV), *Toxoplasma gondii*

All donors volunteering to donate their tissue/cells for the purpose of making a SCCP has to undergo the following screening tests.

- Complete Blood Count
- Blood Glucose
- Renal Function Tests
- Liver function Tests

- HIV I & II (PCR method -Qualitative)
- HBV (PCR method -Qualitative)
- HCV (PCR method - Qualitative)
- Human T-lymphotropic virus Type I &2 HTLV-1&2
- CMV (IgM – Serology)
- RPR
- Urine Routine
- ECG
- ECHO
- Chest X ray
- Bleeding Time, Clotting Time, PT, APTT
- In allogeneic transplantation HLA-A, B, C, DR and cross-match with blood sample from recipient may be necessary
- If necessary, testing for malignancies

11. SCCP banking procedures

The creation of a regulatory-compliant licensed SCCP bank is essential for the production of uniform biological products. Their facility should be GMP compliant for clinical trials and GLP compliant for pre-clinical testing purpose. SCCP stocks should be handled by a formal SCCP banking system (often a two-tiered system as mentioned below). All the banking including cell, cord blood, cord tissue, placenta, bone marrow *etc.*, should follow the common principle as follows:

11.1 Cell line banking strategies and methods

SCCP bank system should consist of two tiers:

- ✓ A master cell bank (MCB) or donor cell bank (DCB);
- ✓ A working cell bank (WCB), sometimes called a manufacturer's working cell bank (MWCB).

The MCB represents a collection of cells of uniform composition derived from a single source prepared under defined culture conditions. The WCB is derived from one or more vials of cells from the MCB of one or more donors, which are expanded by serial subculture. The pooled cells are dispensed into individual vials and cryopreserved to form the WCB. One or more vials from the WCB are generally used for the production of stem cell product. If cells from more than one WCB vial are used, the cell suspensions can be pooled at the time of thawing. The population doubling level or passage level of cells used for production should not exceed an upper limit based on the cell substrate characterization, including at end-of-production level.

Where cell lines are used as SCCP, an appropriately characterized Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established in compliance with the regulatory and ICH guideline Q5D (quality of biotechnology product guideline) as applicable to SCCP's. Cell bank conditions, storage methods, and transport procedures are also described in the section No. 24.

The following information of the cell bank system used should be described:

11.1.1 Origin and history of cells

- A description of the origin and history of cells should be provided.

11.1.2 Procedures

- The procedure for freezing and for recovering the cells should be described.
- Components used (such as DMSO or other suitable excipients) should be specified.
- The number of vials preserved in a single lot.
- The storage conditions should be specified.

11.1.3 Characterization

- SCCP should be appropriately characterized.
- Karyotype of SCCP must be maintained after each passage

- The same characterization program shall be applied to each new cell lot.
- The identity of the cells should be confirmed by appropriate genotypic and/or phenotypic markers, and
- The fraction of the cell population having such identity markers measured as an indication of purity.

11.1.4 Testing for contaminating organisms

- MCBs should be free of contaminating biological agents, including bacteria, fungi, viruses, and mycoplasma.

11.1.5 Expiration dating

- Product development plans should include accumulation of data demonstrating how long and under what conditions the cells can remain frozen at appropriate temperature and still be acceptable by release criteria when thawed.

11.1.6 Tests on thawed cells

- Tests of viability, cell identity, and function should be repeated after thawing and/or expansion.
- The yield of viable cells and of quantitative functional equivalents should be compared to those values before freezing.
- Sterility should be confirmed using aliquots of the frozen cells.

Working cell bank (WCB)

If there is a two-tiered cell bank system in place (MCB and WCB), following test for WCB is recommended:

- In vitro adventitious viral agent testing
- Bacterial and fungal sterility
- Mycoplasma
- Limited identity testing (*e.g.*, Flow cytometry)
- Should undergo limited testing for identity by phenotypic or genotypic markers.

- Should also be shown to be free of microbial and viral contamination; when appropriate.

11.2 Cord blood banking strategy and method

A Cord Blood Bank (CBB) is a facility which stores umbilical cord blood for future use. Cord blood is one of three sources of blood-regenerating cells used in stem cell transplantation; the other two sources are bone marrow and peripheral blood stem cells. Cord blood banking system shall be established following the requirements of stem cell banking, whenever applicable. However, standard process with proper documentation is essential. The following activities are essential:

- Collection of cord blood cells, regardless of the methodology or site of collection;
- Screening, testing, and eligibility determination of the maternal and infant donor according to Applicable Law;
- All phases of processing, cryopreservation, and storage, including quarantine, testing, and characterization of the cord blood unit;
- Making the cord blood unit available for administration, either directly or through listing with a search registry;
- The search process for selection of specific cord blood units;
- Reservation and release of cord blood units for clinical use;
- All transport or shipment of cord blood units, whether fresh or cryopreserved.

To be compliant with the Standards, Cord Blood Banks must use validated methods; qualify equipment, supplies, and reagents; maintain a comprehensive, properly documented Quality Management (QM) Program; and track the clinical outcomes of patients who receive cord blood units from that bank.

Cord blood banking protocols and standards should be concerned the followings things

- The donor selection process for umbilical cord blood banks is carefully conducted and generally a family history is collected to minimize the potential risk of transmitting unrecognized hereditary disorders that could impact on the recipient.

- Cord blood may not be collected if there are known hereditary diseases specifically involving hematopoiesis in the family or if severe disabilities or diseases are identified in the donor fetus before birth.

- Additional exclusion criteria include infectious diseases (*e.g.*, HIV, hepatitis) in the mother, severe pregnancy complications or premature delivery with birth weight less than 1,500 g or if perinatal asphyxia is present in the fetus and cell counts.

- The mother must consent before thorough testing for infectious diseases, recording of medical or clinical information to be added to the dataset and for HLA typing to be completed before the collection and storage of cord blood in the bank for future transplantation purposes.

- The collection of cord blood, in general, must not affect the delivery of the baby and should only be performed by trained staff (*e.g.*, physicians and midwives) who are knowledgeable about cord blood collection, processing and handling for screening, transportation and storage.

- The recommended protocol for umbilical cord blood collection, after the birth of the infant and before the placenta is delivered, is to clamp the umbilical cord and thoroughly clean and disinfect to prevent contamination with maternal blood or by infectious agents.

- The blood cell counts for nucleated, mononucleated and CD34+ cells are recorded before the blood units are stored in liquid nitrogen or in the vapor phase of liquid nitrogen.

- The total number of nucleated cells that are transplanted strongly correlates with the clinical outcome. A minimum of 2.5×107 total nucleated cells per kilogram of recipient's weight is generally required.

- The average cord blood unit contains about 1×10^9 total nucleated cells. The use of two cord blood units is often requested to transplant adults which appear to lead to a better outcome.

- Even among siblings, cord blood samples should be HLA matched, if possible, before transplantation.

Minimum requirements for a cord blood banking system are mentioned in annexure section.

12. Requirements of facility for cell and tissue recovery

The organization shall have to follow standard guidelines and SOPs for recovery process, monitoring, cleaning, decontamination, and sampling of recovery environment

- The personnel conducting the sampling process must be free from transmissible diseases. Members of the sampling team must comply with all health and safety measures.
- It is necessary to predict any potential side effects and reactions related to the sampling process (such as shock, nausea, vomiting, respiratory alkalosis, anxiety-related muscle spasm, *etc.*) and have measures to deal with it.
- For risky sampling, like bone marrow aspiration, the sampling centers must be equipped with cardiopulmonary resuscitation equipment and have access to 24-hour emergency services.
- The sampling center must have an allocated space for history taking, examination, sampling and storage of harvested samples and consumables. Sample and consumables storage area must be separate from areas for other daily activities of the center. Working areas must be separated in a manner that minimizes the possibility of contamination, cross-contamination, and any errors in sampling. Also,

personnel trafficking in the sampling area must occur at the minimum level possible.

- For taking several samples during one working shift, all stages of sampling, labeling, and packaging for each donor, must be performed separately. Before sampling from the next donor, cleaning and decontamination of sampling room and gowning change must be done.
- Environmental conditions of the sampling place such as ventilation, air quality (degree of cleanness), temperature, moisture and contamination must be controlled carefully. In hot and humid areas, temperature and moisture must be decreased to a level which minimizes the growth of microorganisms in the space as well as temperature-related damages to the sample. Furthermore, the sampling environment must have enough light and access to wastewater disposal and wash basins. Sampling and storage area must not be exposed to harmful irradiations.

13. Tissue/sample harvesting method

Cells and cellular products could be obtained from multitudes of sources such as umbilical cord blood and tissue, bone marrow, peripheral blood, adipose tissue, skin, dental pulp, skeletal muscle, and peripheral nerves. Sampling method and the maximum harvestable sample mass must be determined for each case based on scientific evidence to minimize damage to the donor site.

- It is necessary to match the identity of the donor with labels on the container before sampling and after discharge of donor.
- Care should be taken that the donor site does not have symptoms of local infections like warmness, pain or redness.
- All sampling sites must be disinfected according to the guidelines applied to operating rooms (aseptic techniques). The physician in charge of sampling must wear appropriate clothing including sterile gown, face mask, surgical cap and sterile surgical glove.
- It is recommended to use closed systems for sampling; otherwise, the time of exposure of samples to the air must be minimized.

- While using anticoagulants for bone marrow and peripheral blood samples, they should be mixed appropriately. Tissue samples must be stored in sterile transfusable isotonic solutions. If necessary, antibiotics / antimycotics could be added to the transfer solution.

A report on sampling process must be prepared in written format and a copy sent to the processing center along with the samples. This report must contain at least information about the type and volume of the sample, sampling condition, side effects and reactions, donor identity, and identity and signature of person who performed sampling. The organization must have appropriate forms for gathering and registration of this information.

14. Processing and production of cellular products

14.1 Materials used during manufacturing

A general rule in case of cell processing is that minimally manipulated cells are safer than culture expanded. Materials used during *in vitro* manipulation procedures, for example cytokines, serum, antibiotics, other chemicals or solid supports such as beads, can affect the safety, purity and potency of the final SCCPs. These components should be clearly identified and a qualification program with set specifications should be established for each component to determine its acceptability for use during the manufacturing process.

Using animal-derived materials for production and processing of cellular products is a challenging cause for concern and it is recommended to refrain from using animal-derived products for development of pharmaceutical and cellular products for human use. When suitable alternatives are not accessible, the safest preparation of these products must be used.

- Materials and reagents which are used for cell manufacturing must be of pharmacopoeial grade or GMP complied and cell therapy certified whenever available.

- Appropriate references should be given when the raw materials, reagents and/or excipients have a marketing authorization or mentioned in a pharmacopoeia.
- As much as possible, use of animal-derived products should be avoided in all processing steps. However, sometimes when replacement of these products is not possible, the following measures must be considered:
  - ✓ The source of the animal-derived products must be from countries which are free from infectious disease according to the "World Animal Health Reports on the animal health status".
  - ✓ Source of products certified with authorities of the animal health (like USDA or AAALA).
  - ✓ Chemical or physical methods should be used for improving the safety and reduction of potential residual contamination.
  - ✓ Production process of the product must comply with GMP.
  - ✓ Necessary tests should be performed to detect potential microbial, mycoplasma or viral infections of the materials.
  - ✓ The quality of biologically active additives in culture media such as growth factors and cytokines should be documented with respect to identity, purity, sterility and biological activity and absence of adventitious agents. It is recommended to keep the use of such materials to a minimal and to avoid the use of reagents with sensitization potential *e.g.* β-lactam antibiotics.
- In case of fetal bovine serum (FBS), if possible, alternatives such as autologous serum, serum free chemically defined media, pooled human AB serum, or platelet lysate should be used. With these alternatives, the risk of developing immunologic reactions is lower but despite assessment and testing of the donor samples, considering the window period and not using the viral clearance methods for production of human sera, the risk of infectious diseases transmission is not completely eradicated. If using these alternatives is not feasible, besides taking the measures mentioned in previous paragraph, for further safety the source of FBS must be from the USA, Australia, Mexico, Central America, Japan, New Zealand and EU countries (according to the changes in the disease pattern). The

next recommendation is to use gamma irradiated FBS. Animal derived materials should be tested or confirmed for adventitious agents as appropriate.

14.2 Cell manufacturing facility considerations

14.2.1 Environmental conditions

- Processing environment must have enough space, and appropriate design and location for desired activity. It must be divided into areas with enough space to prevent labeling mistakes, mixing, contamination or cross-contamination in the processing, quarantine, storage, release or distribution. Also, processes must be established and implemented for control and monitoring of the mentioned items.
- Design and location of the cell processing facility and cell bank are of paramount importance. Intended space must be far enough for waste storage and disposal place as well as animal laboratory.
- Entrance to the production space must be two-step and contain enough facilities for changing clothes and slippers proportionate to the number of staff or visitors. Different parts of the production space must be isolated.
- Walls must be smooth without any vent or opening from ceiling to the floor. Walls must be scratch resistant, must not shed any particle and must be washable with water and antiseptics. Angles between walls, ceilings and floors must be curved.
- The floor must be washable with water and detergents and resistant to particle shedding and scratch. Floor must be void of pores and made of a material which does not absorb particles and dusts.
- Windows are only allowable for passage of light; they must be made of double-walled glasses. Windows must form a uniform surface with the walls.
- The lighting system in the ceiling must be washable and form a uniform surface with the ceiling. The height of the false ceiling must enable washing, repairing, and exchange of filters and bulbs from outside the clean room. Break room must be separated from other areas. Installations, locker rooms and WC must be

easily accessible and proportionate to the number of staff. These areas must be spacious and not have direct access to the production area.

- Quality control labs, especially those in which biological and microbiological tests are performed, must be separated from production area.
- Trafficking into or out of the processing area must be controlled to prevent entry of unauthorized people, including unauthorized staff members.
- Clean room shall be designed by Heating, ventilation, and air conditioning (HVAC) system with maintaining differential air pressure in different classes (*e.g.* D, C, B, A classes). Having required air change through HEPA filter ensuring clean room class by regular environmental monitoring system. The clean room class for cell handling required clean room class B or C under HEPA filter (class A).
- Except for the processes performed in closed system, all open procedures must be performed under at least class II microbiological safety cabinet which is placed in a clean room. It is noteworthy that environmental monitoring and the tests for performance of clean room and laminar air flow hoods should be conducted and documented in defined time intervals according to the manufacturer's instructions and regulatory guidelines.
- There must be a SOP for maintenance, cleaning and decontamination of clean rooms and its equipment on daily, weekly, and monthly basis. Also, efficacy of this process must be monitored through periodic quality control programs and documentation. A 70% solution of ethanol in distilled water or other appropriate disinfectants could be used for cleaning the surfaces.
- Tests for clean room monitoring include measuring temperature, moisture, air pressure, and the number of particles. Particle count comprises viable (microorganisms) and non-viable(dust) particles. Monitoring viable particles must be conducted by processing center using standard microbial sampling methods periodically. Other parameters should be usually checked in 6-month to one-year time intervals (preferably by companies involved in controlling clean areas) and documented.

- Adequate equipment, materials and facilities must be prepared according to the ongoing processes in the processing center. Equipment effective on the quality of the product must be maintained and calibrated periodically.
- Processing environment and the staff involved in cell processing must meet the minimum requirements recommended for hygiene and cleanness.

14.2.2 Safety considerations

- Processing facility must be designed and implemented so as to reduce the potential risks for health and safety of the staff, patients, donors and recipients to the possible minimum level.
- Processing facility must have documented safety guide on actions on exposure to agents of communicable disease, chemicals or biological.
- Biological wastes of the center must be disposed using a defined and controlled method to reduce any risk of harm to staff and processing environment. These methods must comply with the current guidelines.
- While working with biological samples, one should use gloves, suits and other coverings (PPEs). The protective equipment must not be used out of the processing environment.

14.2.3 Production processes

To reduce the risk of contamination and harm to the product, these points should be considered:

- Using disposable sterile ware and closed systems for collection and processing of stem cells, as much as possible.
- Reducing the time interval between receiving the sample to processing and storing the sample at 2-8^{o}C. It is noteworthy that the appropriate time for storage of the samples before processing is different according to the type of the tissue and cell, and must be validated by processing center.
- Besides complying with aseptic practice rules, it is recommended to wash the harvested tissue and cells using antibiotics/antimycotics before starting

processing. The type and concentration of the antibiotics used depend on the tissue or cells used or the method of harvesting.

- Trafficking in the processing room must be minimized and fast movements (like running, walking rapidly, shutting the doors fast, *etc.*), unnecessary talking especially in the area around laminar flow hood and near the incubator must be seriously abstained.
- Biological safety cabinet must be located away from the entrance and located in a way that does not interfere with air circulation in clean room. It is recommended to install it with enough space from back wall to facilitate the cleaning of that area. The space of 30 cm height between the top of the hood and the ceiling is appropriate.
- For water bath, incubator and other equipment, sterile water must be used with appropriate disinfectants, like copper sulfate, added to it. Also, for preventing contamination, the water in the equipment must be changed in defined intervals.
- Labeling of culture wares, tubes and other related objects must be resistant to scratching and fading. Labeling on the cap of containers must be avoided and labeling must be done on the body.
- Refraining from simultaneous processing of samples from different donors under the same safety cabinet. It is preferred that all stages of donor's sample processing should be performed by the same staff from beginning to end, instead of other staff members continuing the same processing activity.
- Using independent incubators or different areas under the same incubator for cultured cells from different donors, as much as possible.
- Processing the animal samples and working with viral vectors in the clean rooms which are dedicated to clinical grade cell processing are prohibited. It is recommended not to perform research activities related to preclinical studies in rooms considered for clinical cell preparation.

14.2.4 Aseptic techniques

- All stages of cell processing must comply with the principles of aseptic techniques. All working surfaces used in processes must be disinfected with appropriate disinfectants (such as 70% ethanol or isopropanol solution) before and after practice.

- The number of the objects under laminar flow hood should be minimized and disinfected with 70% ethanol before placement in the work space. In case it is necessary to process a sample from another donor, the working area of the hood must be disinfected.
- Laminar flow hood must be switched on 10 minutes in advance to experiment to raise the air pressure to the desired level and five minutes after end of the practice and disinfection to eradicate potential pollution from the environment.
- If the clean room is planned not to be used for a short time, it is better to keep the air-conditioning system working. If air-conditioning system is turned off, enough time from the reoperation of the air-conditioning system must be considered according to the manufacturer's guide to let the system reach the minimum degree of desired cleanness of the room. Actions must be prompted to eradicate other potential pollutions.
- It is recommended that the tissue and cell processing is performed hands free, as much as possible. In cases direct touching of samples is inevitable, appropriate covering and aseptic surgical techniques must be used. All materials, solutions and tools which are in contact with the sample, must be sterile.

14.2.5 Gowning procedure

- Before entering the clean room, all clothes must be exchanged by special suits. Type of the covering and the material of the clothing vary depending on the cleaning degree of the room but as a general rule, clothes must cover the whole body (coverall clothing). These garments are usually one-piece. Moreover, suits must offer the greatest protection possible against pollution leakage from samples.
- Clothing could be single-use or re-usable. In both forms, they must be sterilized before use. Based on the activities, one could use the clothes for several times but in case of any exposure to contamination or fluids and any suspicion of contamination, the clothes must be changed quickly.

- Reusable clothes must be washed separately from other garments (like gowns, bed sheets, and patients clothing) and sterilized with proper methods (according to the material of the fabrics) after packaging.
- Using 3-layered surgical masks is sufficient for covering the face. In cases there is possibility of harm or splash of the solutions to the eyes, using protection glasses and/or face shields is necessary. Gloves used in the clean room must be sterile and powder-free.

14.3 Manufacturing process

A detailed description of all the manufacturing process of SCCPs should be carefully designed and validated. The requirements should be defined and justified. A detailed description of the manufacture of the active substance and of the finished product should be provided.

A flow diagram depicting the entire process starting from biological specimen or from cell banks indicating critical steps and intermediate products (*e.g.* intermediate cell batches), as well as operating parameters, in-process controls and acceptance criteria helps to provide this information more critically.

- The type of manipulation(s) required for SCCPs processing and the physiological function shall be described. Minimally manipulated products refer to SCCPs that have not been subjected to an *ex vivo* procedure that functionally or genetically alters specific nucleated cell populations.
- Information on procedures used to transport material during the manufacturing process of the product, including transportation and storage conditions and holding times, should be provided.

- Attention should be paid to biodegradable materials, which may possess the potential for environmental changes (*e.g.* raising pH) for the SCCP during the manufacture or after administration. The manufacturing area should be physically separated from the procurement area. If SCCPs are processed and stored in the same manufacturing area there is an increased risk of cross contamination during each step of the procedure, *e.g.* via processing equipment or in storage containers such a liquid nitrogen tanks, and therefore, adequate control measures to prevent cross-contamination should be put into place.

- Equipment and premises used for manufacturing of SCCPs should be suitable and qualified for aseptic production. It is recommended that dedicated, product-specific or single-use equipment are used in the production, whenever possible.

14.3.1 Preparation of autologous and/or allogeneic SCCPs

All cell preparation procedures should be justified in terms of their intended purpose.

- Inappropriate handling and improper processing of biological specimen must be avoided as they can impair or destroy the integrity and/or function of the SCCP and thus result in therapeutic failure.
- Microbiological control is a pivotal aspect of process control and quality evaluation of all cell preparations.
- Monitoring of *in vitro* culturing at selected stages of the production should be performed where feasible.
- The culture should be examined for any microbial contamination in accordance with the culturing procedure and growth characteristics of the cells.

The following procedural description is recommended:

14.3.1.1 Cell and tissue collection/processing/culture conditions

Method of Cell Collection/Processing/Culture Conditions should be described as follows:

- The number and volume of cells and tissue collected.
- The procedure to obtain the cells from the organ/tissue has to be described (with respect to the type of enzyme, media, *etc.*) and validated.
- Any procedure used to isolate and/or purify the cell population of interest should be described. Its effectiveness should be addressed in relation to the intended use and the method(s) should be validated.

- Use of any cell selection device or separation device, including density gradients, magnetic beads.
- SCCP cultures must be handled aseptically under Grade B or C clean air background using Grade A clean air equipment and air systems.
- In-process testing that will be performed during these procedures.
- Consideration should be given to the degree of disruption applied to the tissue in order to preserve the intended functional integrity of the cellular preparation and to minimize cell-derived impurities in the product (cell debris, cross contamination with other cell types).

14.3.1.2 Cell/tissue culture and manipulation

During *in vitro* cell/tissue culture, consideration should be given to use all reagents and culture media of tissue culture grade and ensure acceptable kinetic growth and manipulation of the isolated cells. Various treatments (physical, chemical or genetic) can be applied to SCCP. The method used to modify the SCCP should be fully described.

- The processing steps should be properly designed to preserve the integrity and control the function of the cells.
- The procedures for any manipulation should be documented in detail and closely monitored according to specific process controls.
- The duration of cell culture and maximum number of cell passages should be clearly specified and validated.
- The relevant genotypic and phenotypic characteristics of the primary cell cultures, of the established cell lines and the derived cell clones should be defined and their stability with respect to culture longevity determined.
- Consistency/repeatability of the cell culture process should be demonstrated and the culture conditions including the media and the duration should be optimized with respect to the intended clinical function of the cells.
- Special consideration should be given to the growth potential of cells in response to growth factors since cell subpopulations may gain a growth advantage under defined *in vitro* culturing conditions.

14.3.1.3 Final harvest

A detailed description of the final harvest should be provided.

- If the final SCCP harvest is centrifuged prior to final formulation, description of the wash conditions and media used should be provided.
- If the SCCPs are cryopreserved after formulation or formulated immediately and given to the patient should be provided.
- If the final SCCP harvest is stored, description of the storage conditions, the length of storage, and appropriate supporting data should be provided.

14.3.1.4 Process timing and intermediate storage

The approximate time elapsed for each step from cell/tissue collection to final harvest should be reported

- Time limit of each step-in production should be noted to determine what, if any, in-process testing should be performed.
- If SCCP is cryopreserved before injection into patients, this information should be included along with any stability studies if performed.
- The time and conditions of storage prior to patient administration should be described.

14.3.1.5 Final formulation

Description of the formulation of the final product, including excipients such as growth factors or human serum albumin should be provided. An excipient is any component that is intended to be part of the final product, such as human serum albumin or Dimethyl Sulfoxide (DMSO).

List of all excipients used during manufacture of the product that are intended to be present in the final product should be provided.

- State the source of these components.
- Identify the vendor and final concentration of excipients and describe the cell density or cell concentration used in the final product.

- If the final product is delivered to the clinical site frozen, a description of how the product will be shipped and data to show that the product can be thawed with consistent results should be included.
- If the product is shipped, data should be provided on product stability during shipping.

14.4 Characterization

Identification and characterization of SCCPs can be a challenging and often an evolving process. The characterization of SCCPs should encompass all the components present in the finished product obtained throughout the development and/or manufacturing process.

The expected biological function of SCCPs encompasses complex interactions that may range from a biochemical, metabolic or immunological action to the structural replacement of damaged tissue or organ. Therefore, when considering the extent of characterization, the following issues should be taken into account:

- Autologous cells vs. Allogeneic cells
- Cell type
- Extensively or minimally manipulated *in vitro*
- Immunologically active or neutral
- Proliferative capacity of the cells
- Dynamic interactions amongst cells
- Intended use
- Karyotype

N.B. the above information should also be provided for SCCP cell banks

When biologically active molecules (*e.g.*, growth factors, cytokines *etc.*) are present as components of SCCPs, they should be described adequately and their interaction with the other components characterized.

Non-cellular components should be characterized in the context of their required function in the finished product. This includes structural components designed to support the cellular components such as scaffolds or membranes which should be identified and characterized in chemical and physical terms such as porosity, density, microscopic structure and particular size according to the type of substances and intended use.

The characterization should be designed to allow setting up the routine controls that will be applied for release of the active substance and finished product as well as those to be performed at several steps of the process to guarantee batch consistency. An extensive characterization of the cellular component should be established in terms of the following.

14.4.1 Cell identity

Quantitative testing by phenotypic, biochemical, and cytogenetic analysis should be used to confirm cell identity and assess heterogeneity.

- Cell identity should be assessed quantitatively (for example, by monitoring cell surface antigens or biochemical markers). The method of identification chosen should also be able to detect contamination or replacement by other cells in use in the facility.
- Acceptable limits for each culture component should be defined.
- Quantitative assays of functional potency may sometimes provide a method for population phenotyping.
- The desired SCCP functions should be monitored when the cells are subjected to manipulation, and the necessary tests to be carried out periodically to assure that the desired trait is retained.
- Identity testing should in some cases include verification of donor-recipient matching and immunological phenotyping.

14.4.1.1 Cellular component

The identity of the cellular/tissue components, depending on the cell population and origin, should be characterized in terms of phenotypic and/or genotypic profiles.

- Relevant markers should be used when addressing the phenotype of the cells; these markers may be based on gene expression, antigen presentation, biochemical activity, response to exogenous stimuli, capability to produce biologically active or otherwise measurable molecules, *etc.*
- For adherent cells, morphological analysis may be a useful tool in conjunction with other tests. Where applicable, a description of the procedures which could lead to a modification of the characteristic of the product, including adhesion, absorption, degradation, presentation of components of the culture media, should be provided.
- For cellular components of Allogeneic origin, identity should include histocompatibility markers, where applicable, and identification of genetic polymorphisms with specific reference to the intended use.
- If the intended therapeutic effect is based on a particular molecular species synthesized by the cells, enough structural and biological information should be provided to show that an appropriate and biologically active form is present.
- The essential characteristics of the cultured cell population (phenotypic markers such as cell surface antigens, functional properties, activity in bioassays, as appropriate) should be defined, and the stability of these characteristics established with respect to time in culture. This profile should be used to define the limits of the culture period.

14.4.1.2 Non-cellular components of the active substance

All non-cellular components should be appropriately characterized as such and identity parameters established.

- If the finished product contains a distinct active substance in addition to the cellular component, then that active substance should be characterized with respect to identity in accordance to relevant guidelines, depending on the nature of the active substance, whether it is of chemical or biological origin.

- Structural components designed to support the cellular components such as scaffolds or membranes should be identified and characterized with respect to their composition and structural characteristics.

14.4.2 Cell purity

Product purity is defined as relative freedom from extraneous material in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity testing includes assays for pyrogenicity/endotoxin, residual proteins or peptides used to stimulate or pulse cells, reagents/components used during manufacture, such as cytokines, growth factors, antibodies and serum and unintended cellular phenotypes.

SCCP of interest should not contain other cells that are of different lineages and/or differentiation stage or that may be unrelated to the intended population. However, if other cell types are present in the final SSCP they should adequately characterized (immuno-phenotype, percent present, lineage specificity *etc.*). The acceptable limits based on clinical trials of the other cell types in the final SCCP should be provided.

- In cases, where the desired biological activity and efficacy of the SCCP requires a complex mixture of cells, the quantities of each cell component in final SCCP needs to be established based on safety and efficacy data.
- Irrespective of the cell type, the SCCP can be contaminated with non-viable cells. Since SCCP viability is an important parameter for product integrity and directly correlated to the biologic activity, the ratio between non-viable and viable cells should be determined and specifications should be provided.

14.4.3 Impurities

The appropriate purity testing should include assays for residual peptides, and proteins used during production and purification, and reagents used during manufacture, such as cytokines, growth factors, antibodies, beads and serum. Appropriate purity testing should include a measurement of contaminating cell types or cell debris.

14.4.3.1 Product or process-related

During the production of SCCPs, variable amounts of impurities, product and process-related, may be introduced into the final product. Any reagents known to be harmful in humans should be analyzed in the final product (or in individual components if otherwise not possible) and acceptance criteria should be set. The specification limits should be justified by levels detected in batches used for toxicological and/or clinical studies. Any material capable to introduce degradation products into the product during the production, *e.g.* biodegradable materials, should be thoroughly characterized in this respect and the impact of the degradation products to the cell component(s) should be addressed.

14.4.3.2 Adventitious agents

A critical aspect is to establish that SCCPs are free from adventitious microbial agents (viruses, mycoplasma, bacteria and fungi). The contamination could originate from the starting or raw materials or adventitiously introduced during the manufacturing process.

- A risk assessment should be performed to evaluate the possibility of reactivation of cryptic (integrated, quiescent) forms of adventitious agents.
- A thorough testing for the absence of bacteria, fungi and mycoplasma shall be performed at the level of finished product.
- In cases where the short shelf life of the SCCPs is prohibitive for the testing of absence of bacteria alternative validated testing methods may be acceptable, if justified.

14.4.3.3 Pyrogenicity/endotoxin

The pyrogenicity/endotoxin testing must be conducted as per the existing pharmacopoeial procedures for sterile dosage form, and the acceptance limits for release should be described.

14.4.4 Potency

Potency is the quantitative measure of biological activity based on the attribute of the product, which is linked to the relevant biological properties. The assay demonstrating the biological activity should be based on the intended biological effect which should ideally be related to the clinical response. If development of a quantitative biological assay is not possible, then a quantitative physical assay which correlates with and is used in conjunction with a qualitative biological assay can be used.

- A suitable assay for SCCP potency should be in place and validated based on clinical trial data.
- Lot release and shelf life specifications for potency should be determined and amended during product development, if appropriate.
- Major cellular functions as viability, self-renewal, death, and differentiation are pivotal to the quality, function and sustainability of the SCCPs. They need to be monitored during production and at release using surrogate markers and appropriate technology (*e.g.* gene expression profiles by microarrays, flow cytometry immune fluorescent analysis, cell cloning, PCR and many others).
- Markers for purity and potency should not be mixed in the same assay.
- A combination of multiple methods may be needed to adequately define the potency of cell-based products during the development. Certain assays may be needed to control process changes, whereas others are more suitable for release testing.

14.4.4.1 Tissue repair and regeneration assays for SCCPs potency

- An *in vivo* test can either be performed in an animal model mimicking the intended clinical tissue repair/regeneration or can be based on a clinical trial data.
- An *in vitro* assay can be based on the expression of markers that have been demonstrated to be directly or indirectly (surrogate markers) correlated to the intended biological activity, such as cell surface markers, activation markers, expression pattern of specific genes.
- A physiological response under defined conditions such as differentiation in specific cell types and/or secretion of tissue specific proteins (*e.g.* extracellular matrix components) can be used as a basic principle for a potency test. The manufacturers should, however, ensure that the method of characterization is relevant for the intended biological effect in clinical trial data.

14.4.4.2 Metabolic or pharmacological activity

The potency assay should be based on the detection of the active molecule(s) produced and the biological activity expected, if the intended biological function of the SCCPs is mainly based on the capacity of cells to secrete specific molecule(s) *e.g.* to repair a metabolic disorder, to promote growth, to release a metabolite.

14.4.5 Tumorigenicity

The tumorigenicity of SCCPs differs from the classical pharmaceutics. The transformation can also happen due to chromosomal instability of SCCP and not due to host factors.

Therefore, testing of chromosomal integrity (*e.g.* karyotyping, mutation analysis *etc.*) and tumorigenicity of SCCP is necessary before final product release.

15. Quality control and release tests

The final product is the final formulated product used for administration to human subjects. The final SCCPs to be administered, as well as the production process and materials used, should be subjected to quality control testing. The specifications to be applied to the final product and to other elements of the production process, along with the range of acceptable values for each, should be specified.

For proper quality control, the active substance and/or the final product should be subjected to release testing, whenever possible. All release testing should be performed using methods validated at the latest at the time of submission of an application.

Final product release criteria testing should be performed on each lot of product manufactured. In some situations, each dose could be considered a single lot, depending on the manufacturing process. The results from final product release criteria testing should be available prior to administration to a human subject.

The release specifications of the active substance and finished product should be selected on the basis of parameters defined during the characterization studies. Selection of tests is product-specific and has to be defined by the manufacturer. In case the primary function of the MSCs is the excretion of specific proteins, specifications regarding these excreted proteins should be set.

The amount of available product is limited to the clinically necessary dose (*e.g.* due to very limited cell numbers at collection or low proliferation rates). The release of the product should be justified by the validation of the cell manipulation process and the in-process controls.

Before releasing the final product for transplantation or storage in the cell bank, the following release tests must be performed:

- ✓ Cell viability and count
- ✓ Monitoring of contaminations using microbial culture, gram staining, endotoxin test, and mycoplasma tests. Microbial culture must include searching for aerobic and anaerobic bacteria, as well as fungi.
- ✓ Assessment of identity and purity of the cells using appropriate methods according to the cell type. It should be noted that performing these tests is not necessary for all samples and it is enough to do these tests for a small number of samples which have been isolated and processed according to the standard protocol. This is to validate each protocol and verify if it is working appropriately. Also, according to the cell type, assessment of the function and potency of the cells may be necessary for each batch.

Considering the possibility of development of chromosomal aberrations during processing of the cells, it is recommended to examine the samples cytogenetically before release. Otherwise, at least a few samples which have been processed with the same protocol must be assessed.

Certain release tests can be performed only on key intermediates and/or as in-process tests. In these cases, an adequate quality control has to rise from the manufacturing process, supported by the results of the clinical studies.

16. Stability testing

Stability testing should be performed during early phases of the clinical trial to establish that the product is sufficiently stable for the time period required by the study. Stability testing should be conducted in all phases, to demonstrate that the product is within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation.

- If a very short-term clinical investigation is proposed, the stability data submitted may be correspondingly limited.
- If the product is planned to be used post the duration of the clinical trial (*e.g.* for a separate trial being conducted after the initial trial), testing should establish stability throughout the relevant time period.
- A proposed stability protocol should include a measure of product sterility, identity, purity, quality, and potency. For each test conducted, the test method, sampling time points (there should be a zero-time point), testing temperature, and other appropriate information, including justification of the assays used to indicate product stability, measuring those parameters for the duration of storage required by the clinical protocol should be described.
- Sterility testing to be performed at zero-time, end of stability study, and at an intermediate point during the study is recommended.
- A stability protocol and data for both in-process material and the final cellular based pharmaceutical product should be determined and submitted.

16.1 In-process stability testing

If stem cell and cell-based products are cryopreserved, the stability protocol that will be used to ensure that the product is stable during the period of cryopreservation should be described. A comparison is often made of analyses carried out pre-freeze and post-thaw. Any stability testing performed on the product during the holding steps, such as cryopreservation of SCCPs and storage of bulk product needs to be described.

16.2 Final product stability testing

Any data that demonstrate that the product is stable between the time of product formulation and infusion to subjects to aid in establishing an expiration-dating period should be included. Conduction of the testing at the appropriate temperatures and at time points consistent with predicted storage times is recommended. If the product is shipped from the manufacturing site to the clinical site, the time and shipping conditions (*e.g.* packaging, temperature) should be described. The stability protocol should be adequate to demonstrate that product integrity, sterility and potency are maintained under the proposed shipping conditions.

A shelf life for the cells under specified storage conditions should be determined for the following materials:

- All intermediate components subject to storage, if applicable
- Components of the combined SCCPs
- The active substance
- The finished product

Furthermore, a valid in-use shelf life (after opening from the transport container) should be assigned to the SCCPs. Also, all storage conditions including temperature range should be defined. Transportation and storage conditions should be supported by experimental data with regard to the maintenance of cell integrity and product stability during the defined period of validity. If relevant, appropriate methods for freezing and thawing should be documented.

Due to the complex nature of the active substance of SCCPs, requirements for stability should be defined on a case-by-case basis. Whenever possible, stability should be assessed for both the cellular as well as the non-cellular component prior to combination and together as a finished product in the final packaging.

17. Packaging, labeling, shipment and transport

Packaging, labeling, shipment and transport of stem cell and cell-based product should strictly follow the standard requirements for cryogenic or temperature sensitive products as per label claim during marketing authorization.

In addition to the general guidelines of packaging, some additional considerations should be taken especially for stem cell-based products

- All materials and preservatives which are in contact with samples must be sterile, and should not shed any toxic materials. They should be certified for clinical use.
- Size and the shape of container must be such that the sample can be placed in it readily and in sterile conditions. The cap must be closed easily after placement of the sample. Each container must be checked for physical appearance, contamination, and damage before and after packaging and also controlled on time of delivery.
- Container must be labeled before placement of the sample and checked again after sample placement. This label must be resistant to conditions like moisture, cold, abrasion and so on. It must contain complete legible information to enable identification and tracking of the samples.
- Samples which are sent to other centers for processing must be transferred to an outer protective container. The outer protective container must be resistant to leakage, temperature changes, perforation, severe shaking and other common adverse conditions.

- Sample must be transported in insulated container in appropriate temperature either cryogenic temperature or temperature between 2 to 8 °C. To maintain this temperature, dry shipper or ice packs should be used without direct contact with samples.
- Date and time of the sending and receipt of each sample, in every stage of the transportation, must be recorded using data logger.

17.1 Container and closure system

A description of the container closure system should be provided.

- Compatibility with the product should be demonstrated before commercial launch.
- Information on the sterilization procedures of the container and the closure should be provided.
- The choice of packaging materials should be addressed as part of the development pharmaceutics.
- Additional data may be required if packaging components are used in the transport and/or application procedure.

17.2 Labelling, coding and traceability during product processing

Labeling procedure should be done in a way to prevent incorrect labeling of SCCPs products. The following points must be considered:

- Label of raw materials, intermediates and the final product must contain:
    - Donor identity (or donor code), time and date of the process, date of expiration (if applicable), name and description of the content (for example, the name of the tissue or cell and the additives) and storage conditions. It is noted that additional necessary information must be recorded in details in the accompanying forms.
- The organization must have SOPs for labeling, coding and registration of the necessary information.

    - Labels for different products must be kept under controlled conditions before use to prevent any mistakes.
    - All extra or unusable labels which have not been used for any raw material, intermediate or processed material must be discarded.
- In case of using printing systems, a SOP must be written for control, validation and verification of the system to ensure appropriate performance of these systems and their conformity with workflow of the organization.
- Double checking is an effective solution to prevent labeling and information registration errors.
- As cellular product manufacturing consists of numerous pieces of processing, samples' container may be changed in each step. Therefore, labeling of the new container must be done prior to removal of samples from the previous container.
- Durability of the labels in different storage conditions must be validated.
- Raw materials (tissue, body fluids or cells) must be coded with a numeric or alphabetical indicator or a combination of them. Coding must be unique to enable tracking of the donor and all related documents to the recipient and vice versa. For example, primary tissue, intermediate product, finished product, blood sample of the donor, quality control samples of the product and the production process documentation must be defined with a unique code.
- If various cellular products are taken from the same donor, coding of each batch of the product must be done in such a way as to enable the tracking of each product batch.
- The label for an investigational product must contain the following statement: "Caution: New Drug – Only for Investigational Use."

18. Quality assurance and quality management system

An efficient quality management system must be implemented in the organization. The organization must have a documented program for risk management, especially in critical processes.

Recommended international standards and guidelines exist for implementation of quality management systems in organizations which provide a good framework for cell processing and cell therapy centers. ISO 9001-2015, ISO 13485-2016, Pharmaceutical quality management system ICH Q10, Product life cycle management ICH Q12, Foundation for the Accreditation of Cellular Therapy (FACT), and American Association of Blood Banks (AABB) are amongst the most widely used standards for implementation of quality management systems in the centers manufacturing medical equipment and tissue and cellular products. Other standards like GMP have specifically defined the quality requirements of this area and should be observed together with the previously mentioned standards.

QA system needs to be established and demonstrated from source of cell to final SCCPs. Since SCCPs are under new drug category, based on specific SCCPs Quality Assurance need to be established for delivering consistent quality product in all respect and establishing Quality by final testing of SCCPs is not sufficient. Few points need to be considered are listed below but they are not limited to:

18.1 In-process controls

Several in-process controls at the level of critical steps or intermediate products are required to control the manufacturing process. Intermediate cell products are products that can be isolated during the process.

- Specifications of these products should be established in order to assure the reproducibility of the process and the consistency of the final product.
- Tests and acceptance criteria should be described.
- If storage occurs, it is necessary to validate the storage conditions (*e.g.* time, temperature).

18.2 Batch numbering system

There shall be SOPs describing details of batch (Lot), numbering set up with the objective of ensuring that each batch of intermediate, bulk or finished product is identified with specific batch number. Batch numbering SOP's applied to a processing stage and to the packing stages shall be same or traceable to demonstrate that they belong to one homogenous mix. Batch numbering allocation shall be immediately recorded in a log book or by electronic data processing system. The record shall include date of allocation product identity and size of batch.

A clear definition of a production batch from cell sourcing to labeling of final container should be provided (i.e. size, number of cell passages/cell duplications, pooling strategies, batch numbering system). In the autologous setting, the manufactured product should be viewed as a batch. The purpose of the batch definition is to ensure consistency and traceability.

18.3 Validation of the manufacturing process

The manufacturing process for SCCPs entails the use of reagents and source materials of differing complexity, variability and risk for introduction of adventitious agents. Validation of reagents and source materials, as well as ensuring that appropriate controls are in place for monitoring manufacturing consistency and product quality, are key elements in ensuring that subjects receive a product that is consistently safe, pure, and potent.

The critical manufacturing process steps which can impact on product quality and yield should be validated. This includes

- ✓ Cell/tissue collection
- ✓ Cell seeding
- ✓ Cell harvesting
- ✓ Cell manipulation processes
- ✓ Maximum number of cell passages
- ✓ Combination with other components of the product

- ✓ Filling, packaging, transport and storage

- Each step of the manufacturing process of the active substance, supportive components and final product should be demonstrated to be well controlled.
- The selection and acceptance criteria of the operational parameters and the in-process controls should be justified.
- Accepted variability, related to starting materials and biological processes, should be taken into account in the validation.
- The critical points of the manufacturing process should be defined and validated, especially the aseptic processing. Any preservation steps, holding periods and/or transportations of the active substance, final product, supportive structures or intermediate products during the manufacturing process should be validated.
- It is recommended that validation of such a manufacturing process is performed depending on the product characteristics, for adventitious agents, identity, potency, viability, purity/impurities and other product specific parameters.
- Establishment and implementation of written procedures to ensure proper manufacturing oversight are recommended prior to production of clinical lots and initiation of clinical studies. This includes the responsibilities and procedures applicable to the quality control unit.
- Establishment of a quality control (QC) plan in writing must be provided. The QC plan should be summarized to prevent, detect, and correct deficiencies that may compromise product integrity or function, or that may lead to the possible transmission of adventitious infectious agents.
- The QC responsibilities must be performed independently from production responsibilities by dedicated QC personnel who are familiar with QC principles. Internal audits at planned intervals should be conducted to evaluate effective implementation of the quality plan and to determine if processes and products meet established parameters. Internal audits of the manufacturing operations and those of contract testing, vendors, or other parties should be documented properly.

- The changeover procedures that are followed to ensure that no cross-contamination occurs among cells intended for an individual subject and other products stored or produced in the same facility should be described.
- The assays for detecting area clearance, cleaning and decontamination reagents and segregation of activities, and the qualification of aseptic processing steps should be described.

19. Product tracking

The system must enable the tracking of all products from the donor to the recipient or final disposition and from recipient or final disposition to donor.

20. Pre-clinical studies

The studies should be conducted in GLP certified facilities. Preclinical study requirements to assess the safety and efficacy of the cell and regenerative products are given below.

20.1 Pharmacodynamics

Assess the pharmacodynamics (PD) or proof of concept (POC) in the appropriate animal diseases model based on the ultimate clinical indication. The POC should optimize route of administration and possibly highlight potential mechanism of action. The study should also yield dose levels for optimum therapeutic efficacy. The selection of animal model and duration of the study should adequately justify the persistence and functionality of the administered cell products.

20.2 Toxicology

Safety and toxicology of stem cell and cell-based product should be conducted in healthy animals, preferably in rodents. The study must address route of administration as per intended clinical indication, dosage (justify based on the intended clinical use), migration, survival, engraftment, differentiation, and proliferation if any. Standard toxicology end points and no observed adverse effect level (NOAEL) needs to be determined.

20.2.1 Systemic toxicity

Single dose studies in rodents: Single dose toxicity study should be carried out at least in one rodent species. The route of administration should be the intended clinical application or should be as close as possible to that proposed for clinical use. An extended single dose toxicity study could be justified, to mimic the single dose clinical protocol. In the extended single dose toxicology study, in addition to single standard toxicology observations, detailed observations should be conducted as necessary. Dosage levels should be selected to provide information on a dose-response relationship, including a toxic dose and a no observed adverse effect level (NOAEL).

20.2.2 Tumorigenicity study in Nude/SCID mice

Tumorigenicity potential should be assessed in immunodeficient mice to understand inappropriate cell proliferation and differentiation if any.

21. Clinical studies

The clinical trial guidelines of DGDA "**Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products Bangladesh**" should be followed during clinical studies. Some important aspects of clinical trial SCCPs are highlighted below.

In general, the same requirements as for other medicinal products apply when SCCPs enter the clinical development phase. The clinical development plan should include

- Pharmacodynamics studies
- Pharmacokinetic studies
- Mechanism of action studies
- Dose finding studies
- Randomized clinical trials

- Due to specific biologic characteristics of SCCPs, alternative approaches to Phase I to Phase III clinical trials might be required and acceptable for clinical development, if justified.
- For demonstration of the “proof of principle” and the choice of clinically meaningful endpoints for safety and efficacy evaluation the relevant nonclinical studies, previous clinical experience of the treated pathology and initial clinical studies could be applied.

- SCCPs might require administration through specific surgical procedures, method of administration or the presence of concomitant treatments to obtain the intended therapeutic effect.

21.1 Pharmacodynamics

The main effects of the SCCPs should be known, even if the mechanism of action is not understood in detail. When the purpose of the SCCPs is to correct the function of deficient or destroyed cell/tissue, then functional tests should be implemented. If the intended use of the SCCPs is to restore/replace deficient or destroyed cell/tissues, with an expected lifelong functionality, structural/histological assays may be potential pharmacodynamics markers.

21.2 Pharmacokinetics

- Study requirements, possible methodologies and their feasibility shall be discussed, attention being paid to monitoring of viability, proliferation/differentiation, body distribution / migration and functionality during the intended viability of the products.
- If multiple (repeated) administrations of the SCCP’s are considered, the schedule should be discussed in view of the expected in vivo life span of the SCCP’s.

21.3 Dose determination

- The selection of the dose should be based on the findings obtained in the non-clinical development of the product and it should be linked with the potency of product.

- Even though the dosage for SCCPs might be determined by individual characteristics of the intended patients (i.e. cell mass density per body weight/ volume of missing tissue/ missing surface), the dose to be tested in the confirmatory trial should be supported by the evidence provided by the Phase I/II studies.
- Phase I/II studies should be designed to identify
    - Minimal effective dose, defined as the lowest dose to obtain the intended effect.
    - Optimal effective dose range, defined as the largest dose range required to obtain the intended effect based on the clinical results for efficacy and tolerability.
    - If possible, the sponsor should identify the safe maximal dose, defined as the maximal dose which could be administered on the basis of clinical safety studies with adverse effects that are compatible with the benefit-risk expectations of the product.

21.4 Clinical efficacy

- Clinical efficacy studies should be adequate to,
    - Demonstrate efficacy in the target patient population using clinically meaningful endpoints
    - Demonstrate an appropriate dose-schedule that results in the optimal therapeutic effect
    - Evaluate the duration of therapeutic effect of the administered product
    - Allow a benefit – risk assessment taking into account the existing therapeutic alternatives for the target population
- Deviations from these will need a justification. For example, the fact that the nature and the mechanism of action of the SCCPs may be entirely novel does not mean necessarily that the therapeutic benefit should be measured by different endpoints from those recommended in the current disease-specific guidelines (*e.g.* medicines vs. cell implants for Parkinson’s disease).

- For new therapeutic applications of stem cell and cell-based products where limited guidance exists, consultation of regulatory authorities on the clinical development plan, is highly recommended.
- The use of previously validated or generally accepted surrogate endpoints is possible provided that a correlation-between clinical meaningfully endpoints and efficacy can be established. Sometimes, the desired clinical endpoint, such as prevention of arthritis, can be observed only after a long follow up. In such cases, the marketing authorization can be based on surrogate markers, if applicable. If the efficacy is dependent on the long-term persistence of the product, a long-term follow up plan of the patients should be provided. Thus, the use of novel meaningful endpoints, clinical or other, is acceptable if justified.

21.5 Clinical safety

- The safety database should be able to detect common adverse events. The size of the database might be decided also in the light of previous clinical experience with similar products.
- The risk of the therapeutic procedure as a whole, *e.g.* the required surgical procedures to administer the stem cell and cell-based products shall be evaluated and used to justify the clinical studies and the choice of the target patient population.
- All safety issues arising from the preclinical development should be addressed, especially in the absence of an animal model of the treated disease or in the presence of physiologic differences limiting the predictive value of homologous animal model.

21.6 Pharmacovigilance and risk management plan

- The routine pharmacovigilance and traceability of the product should be described. Stem Cell and Cell Based Products may need special long-term studies to monitor specific safety issues, including lack of efficacy. The long-term safety issues, such as infections, immunogenicity/immunosuppression, teratogenic effects and malignant transformation as well as the *in vivo* durability of the associated medical device/biomaterial component should be addressed. Special pharmacoepidemiologic studies may be needed. The specific requirements are linked to the biologic characteristics of the cell-based product. Traceability in the donor-product-recipient axis is required in all circumstances as described.

- A data monitoring plan, which involves an independent data safety and monitoring process, is required for all clinical studies, and aggregate updates should be provided to peer review committees on demand, complete with adverse event reporting and ongoing statistical analysis.

22. Storage and transfer of samples for transplantation

Stem cell and cell-based products shall be stored in cryopreservation method and frozen sample be transferred for transplantation in liquid nitrogen container or on dry ice. Unfrozen samples be transferred in temperature 2-8°C in proper container with data logger support.

22.1 Cell bank conditions and storage methods

- In some cases, long-term storage of cells for future applications may be needed. A variety of methods exist for cell banking, almost all of which rely on using cryoprotectants and controlled rate freezing. The control rate freezer (CRF) and cryogenic vessels should be used.
- Regardless of the cryopreservation method, cells must be stored in liquid nitrogen tanks or freezer that can maintain temperature lower than -130°C.
- Considering that nitrogen could diffuse into the packing of the samples, it is recommended to store the samples in the vapor phase or under the liquid nitrogen.
- Each bank should validate the freezing and storage protocols according to the cell type and parameters such as post-thaw viability, replication and function.
- Materials used for cell freezing must have the same conditions as processing materials and the freezing solution (mostly DMSO) must be prepared according to the GMP, and should be sterile and endotoxin-free.

- Cell/tissue in the stock must be documented for each cell bank and updated continuously.
- For labeling the samples, the materials and methods used must not be sensitive to exposure to alcohol or nitrogen.

22.2 Carriers (buffers, gels, scaffolds, devices *etc.*)

- For preparing cell suspensions, buffers certified for clinical applications must be used.
- If carriers or scaffolds are being used for cell transplantation, these products should be certified for clinical use or manufactured in controlled conditions complying with GMP.

23. Cell transplantation and post-transplant surveillance

It is necessary for the cell processing center to attach a data sheet to each sample which clearly shows sample code, cell count, documentation of the quality control testing, buffer properties, materials or carriers associated with the cell and the instruction for preparation of the cells for transplantation.

- In case of need for short term storage of the cells, all conditions recommended in the associated guideline, including temperature, must be met.
- If possible, cells should be transplanted in conditions identical to the operating room conditions. Otherwise, the area considered for transplantation must be controlled for cleanliness of surfaces and equipment, trafficking, and air ventilation. Also, emergency medical services with enough skilled staff must be prepared to cope with potential unwanted side effects.
- In case of need for patient hospitalization after transplantation, the conditions must be ready for hospitalization of the patient proportionate to the disease and the cares necessary for the patient.
- Transplantation must be performed by a skilled team under supervision of a specialist in the respective disease having specialized adequate training on stem cell therapy. The physician in charge of transplantation is liable for predicting and managing potential unwanted side effects of the transplantation.

- Number of cells for transplantation, intervals of transplantation, sample size, and the route of administration must be based on recent scientific evidences.
- All processes related to cell transplantation including identity of the recipient, identity of the transplantation team, date and time of transplantation, number of transplanted cells, method of transplantation and any side effects must be documented and a copy must be sent to the processing center.
- A series of examinations and follow-up must be conducted to assess the potential side effects and efficacy of the transplantation, depending on the disease, conditions of the patient, type of transplanted cells and the method of transplantation. The follow-up program must be determined according to the latest scientific evidences and the principal investigator (PI) is responsible for this.
- In case of any unwanted side effects during or after transplantation, all units involved in acquisition, processing, distribution and transplantation of the product must be informed quickly. A written report of probation of side effects and a mandatory act must be sent out to the competent authorities.
- In case of incidence of adverse effects, protective and compensatory acts such as reviewing processes, retraining of the staff, justification of the quality control tests and if applicable, recall of the products must be done.

24. Documentation and management of documents

24.1 General recommendations

- All processes applied in the organization must be documented.
- Documents must be clear, easily identifiable and retrievable.
- All registered information must be confidential, precise, comprehensive, clear and non-erasable.
- Documents must clearly express who should perform the process, and how and when it should be performed.
- Organization must define the level of access and facilitate the access of authorized personnel to related documents.

- Any suggestion for changes in documents must be done in written by handwriting of an authorized person. Final changes will be effective after confirmation by technical committee assigned by the organization (for example: medical director, quality manager and the executive manager).

24.2 Control of documents

- Documentation control system must define the new version of documents and distribute the related documents to the related staff. Also, archiving and preservation of original documentations and old copies is the responsibility of this system. The following criteria must be considered in archiving the documents:
    - Documents are valid and up-to-date and are revised in defined intervals
    - Copies are under control and the distribution list is clear.
    - Outdated documents are collected to prevent mistaken use.
    - Every new change must be applied quickly and the applied changes are done by an authorized person and are notified by signature and date officially.

24.3 Record keeping

- History of the documented processes and completed forms must be defined and stored to be retrievable for future follow-up. Original versions of outdated documents must be archived in a secure place for 7 years (as papers or electronic form).
- Information related to donation, processing, storage and distribution processes must be stored (in paper or electronic format) for at least 7 years after transplantation. This information must show all stages of the processing and quality control of the product.

24.4 List of required documents

Each organization must define the list of required documents according to the type of activity and the quality requirements. These documents must at least contain the following:

- Organization chart, job descriptions, accountability chain of the organization, eligibility criteria for job positions

- Documentation of the cooperating organizations: when two or more organizations are cooperating on sample acquisition, processing, testing, storage and distribution, the level of their cooperation and the responsibilities of each organization must be defined and documented clearly and precisely. These documents must comply with the professional standards and regulations of each unit.
- Standard operating procedure (SOP): Organization must document and store SOPs of the processes in detail. Operational procedures must be documented to comply with the minimum standards recommended by professional associations and national or regional regulations. SOPs modifications and documentation of validation of processes related to these procedures must be reviewed and verified by the medical director. A copy of related SOPs must be available to all staff in charge and inspectors of regulatory bodies. These documents must be in written and reviewed and, if necessary, updated in defined interims. All outdated SOPs must be archived for at least 10 years. SOPs must at least cover the following:
    - Donor eligibility criteria, obtaining informed consent, sampling, processing, banking, laboratory tests, storage and distribution
    - Properties of the materials used in different stages of product processing
    - Repairing, maintenance and calibration of equipment
    - Environmental conditions and methods applied for quality control, testing and analyzing the product
    - Short-term and long-term storage conditions of the products
    - Defining the contents on the instructions for use of the products and the labeling
    - Process of issuance of release under concession for the product
    - Process for reporting side effects and corrective actions
    - Traceability, recall of nonconforming products, and recipient follow-up
- Tracking system: Cell processing centers must use a defined coding system to enable identification and tracking of the sample in any stage of acquisition, processing, distribution and use. Specific code of each donor must be indicative of

tests, registered information and processing details of the product and the recipient.

- Registered information must contain information on identity and assessment of the donor, blood tests, microbial assessments, harvesting and processing conditions of the cell and storage of the final product. Using standard coding systems like that of International Society of Blood Transfusion (ISBT), in addition to facilitating and increasing the accuracy of the processes, also results in unifying the coding system in different centers. This, in turn, facilitates tracking and follow-up for organizations and authorities.
- List of products in stock: List of processed, quarantined, banked and distributed products must be available and updated regularly.
- Adverse effects and nonconformities: Unwanted side effects and nonconformities must be monitored and all related information and actions must be documented. Corrective actions (if necessary, preventive actions) must be documented.

24.5 Electronically registered information

If computerized registry systems are used, the systems must be able to keep information precise and secure and make printed copies of the registered information. Properties, functioning and the requirements of this system must be documented. The staff in charge of information registration must be qualified. Any changes in the system must be documented and re-validated by written SOPs. It is preferred that an alternative system should be available in spare to be used when the computerized system is incapable of operating.

25. Environmental health and safety

25.1 General considerations

Organization must prepare a safe working environment with application of surveillance systems. Environmental surveillance systems must be defined as a SOP to be capable of proving a safe working space in compliance with current standards and regulations. Surveillance systems must at least include the following:

- Instructions of fire prevention and control
- Disposal of sharp objects
- Prevention of work injury damages and prevention of contact with harmful biological materials
- Safe methods of transportation, storage, handling and disposal of toxic and potentially hazardous materials
- Defining the method of cleaning for potential leakage of toxic materials
- Vaccination of the staff exposed to blood borne contagious diseases, history of vaccination or refusal for vaccination must be recorded in the staff records.
- Appropriate gowning for each work area
- Modalities for dealing with disasters and precautionary acts for reducing the damages due to these events
- Complying with the rule of prohibition of smoking in the organization

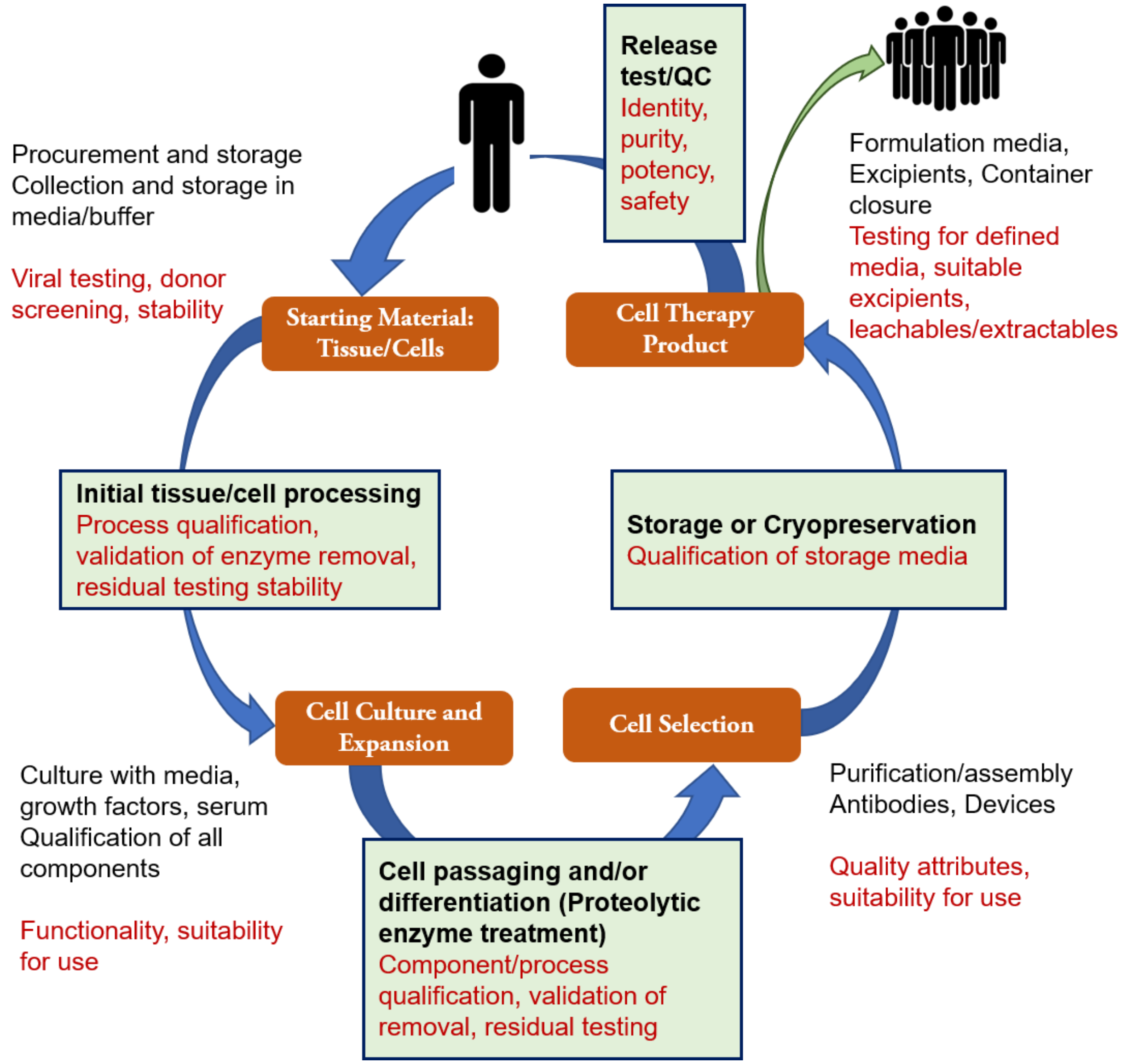
25.2 Safe disposal of biological materials

Waste from tissue and hazardous materials must be disposed in a way not to damage staff and environment. There must be a defined SOP for safe disposal of waste in the organization and all processes related to waste disposal should be done in compliance with this SOP. This process must be in compliance with national regulations of medical waste disposal.

26. Annexures

26.1 General overview of different stages of product development and manufacturing of SCCPs

The figure shows a general overview of the different stages of product development and manufacturing of SCCPs, in which different measures can be taken to ensure the quality.



Courtesy: Atouf F. *et al.* BioProcess International 11(8) September 2013

26.2 General equipment list to start a cell processing lab and manufacturing of SCCPs

Tissue Culture Laboratory

Class II Biosafety Cabinet (BSC)

CO_2 incubator

Pipettes

Vacuum flask/aspiration device

Water bath (37^0C)

Low-speed centrifuge (clinical grade, for spinning cells)

Microscopy

Phase-contrast microscope

Dissecting microscope

Storage

Cabinets and shelves for the storage of tissue culture supplies

Refrigerator (4^0C)

Low-temperature freezer (-70^0C to -85^0C)

Cryogenic freezer (storage below -130^0C) or Liquid Nitrogen tank storage.

Cryo-transporter (-80^0C) or liquid nitrogen dry shipper

Molecular Biology Laboratory / Quality Control Laboratory

RT-PCR

Flow cytometer (might be in a Core facility)

Fluorescence microscope (might be located in a Microscopy Core)

Confocal microscope (might be located in a Microscopy Core)

Microbiology lab for bacterial and fungal culture

Quarantine Laboratory

Class II Biosafety Cabinet (BSC)

CO_2 incubator

Phase-contrast microscope

Water bath (37^0C)

Low-speed centrifuge (clinical grade, for spinning cells)

Pipettes

Aspiration/vacuum flask

Sink

Additional Access to Common Equipment or Core Facilities

Microscopy

Flow cytometer

Microarray gene expression

Genomics

Proteomics

Bioreactor (if required)

Water purification system

Autoclave system

Effluent Treatment Plant (ETP)

Cleanroom facility

26.3 Human resources area of expertise

Haematology, Orthopedics, Gynecology, Plastic surgery, Neuro surgery, Cardiology, Dermatology and Aesthetic Medicine *etc.*

Nursing

Biotechnology

Genetic Engineering

Biochemistry

Molecular and Cellular Biology

Developmental Biology

Microbiology

Immunology

Mechanical / Chemical Engineering.

Pharmacy

Chemistry

Applied Chemistry

Physiotherapy

Degree/diploma in relevant fields

26.4 List of documents for donor eligibility

- Written consent form
- Statement that the donor has been determined to be either eligible or ineligible, based upon results of donor screening and testing
- Summary of records used to make the donor eligibility determination
- Name and address of the establishment that made the donor-eligibility determination
- Listing and interpretation of the results of all communicable disease testing performed

26.5 Active pharmacovigilance report

SCCPs (allogeneic, autologous and xenogeneic) practicing physician/ hospital/medical center shall maintain each case record form both electronically and manually. To keep contact with patients and in case of any adverse reaction occurs, to record. To keep record for further any intervention and for individual outcome. For any adverse event, shall be reported to DGDA.

26.6 Advertisement

The physician or the medical center or hospital needs prior permission for publishing any advertisement related to this therapy by any means.

26.7 License / approval

To manufacture allogeneic or xenogeneic SCCPs needs license of premises and product registration from DGDA. For minimum cell manipulation of autologous cell needs approval of premises, having adequate cell manipulation facilities.

26.8 Requirement for sending samples to overseas

For sending Stem cell and cell based products, cord blood, bone marrow and other biological samples to overseas for testing or commercial purpose needs DGDA prior permission / NOC / export license.

26.9 Requirements for Cord Blood Banking system

The Cord Blood Bank (CBB) system shall ensure the donor management; collection, processing, testing, cryopreservation, storage, listing, search, selection, reservation, release, and distribution of cord blood (CB) units, and recipient follow-up.

26.9.1 Minimum requirements for a Cord Blood Banking system

- ✓ The CBB (Cord Blood Bank), each CB (Cord Blood) collection site, and each CB processing facility shall operate in compliance with applicable law and standards.
- ✓ The CBB premises shall be licensed with DGDA for the activities performed.
- ✓ The CBB shall have an adequate number of qualified staffs for its operations.
- ✓ There shall be a Quality Unit that has responsibility for ensuring that the Quality Management System (QMS) is established and maintained effectively. The CBB shall establish and maintain a written Quality Management (QM) plan that describes the QMS. The QM Plan shall summarize and reference policies and Standard Operating Procedures (SOPs) addressing errors, accidents, biological product deviations, adverse events, variances, complaints, and other relevant things.

- ✓ There shall be a defined process that includes policies or procedures for the detection, documentation, tracking, evaluation, acceptance criteria, quality control, and quality assurance.
- ✓ The CBB shall establish and maintain policies or Standard Operating Procedures addressing critical aspects of operations and management. These documents shall include but are not limited to:

    - ❖ Donor requirement; Informed consent; Suitability assessment of maternal and infant donor; Donor eligibility criteria and determination; Interaction between the CB collection site and the CBB; Documentation of infant donor health at birth; Collection of CB units, associated samples, and maternal samples; Completion of records at the CB collection site; Storage of CB units, associated samples, maternal samples, and documentation at the CB collection site; Transport and/or shipping of the CB unit, associated samples, maternal samples, and completed records to the CB processing facility; Labeling of the CB unit, samples, and records at the CB Collection Site, at the CB processing facility, and at release for administration; Acceptance criteria for CB unit receipt, processing, cryopreservation, and storage; Process control, including product specifications and management of nonconforming products and processes; Storage information including sample location and storage temperature of associated, representative, reference , retention, and maternal samples for testing; Acceptable levels of hemodilution of maternal samples used for communicable disease testing; Communicable disease testing, microbial cultures, hemoglobinopathy testing, and other testing. Acceptance criteria for test results shall be defined; Criteria for release of CB units from quarantine, including nonconforming CB units; For allogeneic use, verification that the infant donor and recipient are different individuals in the case of complete HLA matches; Collection and analysis of transplant outcome data.

26.9.2 Minimum testing criteria for both cord blood and maternal blood samples

	CB (Cord Blood) samples						**Maternal Samples**
Test	Pre-processing	Post-processing prior to cryopreservation	Any time prior to cryopreservation	On an appropriate sample type at any time prior to listing	Thawed segment or thawed representative sample prior to release to the Clinical Program	On an appropriate sample type at any time prior to	Obtained within seven (7) days before or after CB collection
Cell Count							
CBC with differential	X						
Total nucleated cell count		X			Should be performed		
Nucleated red blood cell count		X					
Total CD34		X					
Total Viable CD34		X			Should be performed		
Viability							
% Viability of total nucleated cell or % Viability of CD45		X					
% Viability of CD45					X		
% Viability of CD34		X			X		
CFU or other validated potency assay		Should be performed					
HLA Tissue Typing							
Low Resolution: HLA-A, HLA-B, HLA-DRB1				X			
Low Resolution HLA-C				Should be performed			
High Resolution: HLA-A, HLA-B, HLA-DRB1						X	
High Resolution HLA-C						♦	
Verification Typing					X		
Infectious Disease							
HIV 1				X			X
HIV 2				X			X
Hepatitis B				X			X
Hepatitis C				X			X
HTLV I				X			X
HTLV II				X			X
Syphilis				X			X
CMV				X			X
Additional other tests							

Microbial culture		X					
ABO/Rh blood group			X				
Hemoglobinopathy						♦	
X – All CB units regardless of intended use; ♦ - CB units for unrelated use only.							

Ref. NetCord-FACT, Sixth Edition, July 2016

26.9.3 Specification requirements for Cord Blood (CB) stored for clinical administration

Test	Unrelated Specification		Related Specification	
	Fresh Post-Processing Sample	Post-Thaw Attached Segment or Representative Sample Prior to Release	Fresh Post-Processing Sample	Post-Thaw Attached Segment or Representative Sample Prior to Release
Total uncleated cell count	≥ 5.0 x 10^8		Enumerated	
Total uncleated cell recovery	Should b ≥ 60%		Should b ≥ 60%	
Total viability	≥ 85%		≥ 70%	
Viable CD34 count	≥ 1.25 x 10^6			
Viability of CD34 cells	≥ 85%	≥ 70%	≥ 85%	≥ 70%
Viability of CD45 cells		≥ 40%		≥ 40%
CFU (or other validated potency assay)		Growth (or positive result for potency)		Growth (or positive result for potency)
Sterility	Negative for aerobes, anaerobes, fungus		Negative for aerobic and anaerobic bacteria and fungi – OR – identify and provide results of antibiotic sensitivities	
Donor screening and testing	Acceptable as defined by Applicable Law		Acceptable as defined by Applicable Law	
Identity		Verified		Verified

Ref. NetCord-FACT, Sixth Edition, July 2016

26.10 Registration process flow for stem cell manufacturing / intervention site

1. Application to DGDA for permission of stem cell manufacturing / intervention site
2. Layout plan for the premises
3. R&D facilities
4. Organogram
5. List of qualified human resources
6. List of equipment and machineries for production / intervention of SCCPs
7. List of equipment for quality control
8. List of ancillary services
9. Job description of technical personnel
10. SOPs for different activities

26.11 Registration process flow for locally developed SCCPs

1. Application to DGDA to start preclinical study

    1.1 Information about product development

    a) Preparation / establish of cell bank.
    b) Procedure to prepare working cell bank.
    c) Data generated from development of R& D batch, manufacturing flow chart, preliminary characterization and manufacturing process in brief.
    d) Analytical specifications.

    1.2 Information about preclinical study

    a) Protocol for pre-clinical study for local study or NOC to send sample overseas.

2. Information about production of clinical lot.

    a) Pre-clinical study report.
    b) Analytical methods.
    c) Stability studies of developmental batch.

3. Application to DGDA for permission to start clinical trials

   3.1 If clinical trials be conducted in overseas, application to DGDA for NOC to send sample.

   3.2 If clinical trials be conducted in Bangladesh, application to DGDA for permission to start clinical trials with following documents:

   a) DGDA approval certificate for CRO.
   b) Approved Protocol by BMRC (within 3 months) /IRB/IEC (after 3 months)
   c) Investigator's Brochure.
   d) Informed Consent Form.
   e) Copy of signed agreement with sponsor.
   f) CV of Principal Investigator & his/her Team Members
   g) Whether they have GCP training.
   h) List of SOPs.
   i) GMP certificate of the manufacturing plant.
   j) Test Samples, COA, Summary Protocol.

4. Application for Marketing Authorization (Annexure Approval)

   a) DGDA format CTD dossier for all 5 modules / ICH M4 (Module 1, 2, 3, 4 and 5).
   b) Clinical trial full report

5. After approval of annexure, subsequently approval of Packaging materials, Price and then MAH certificate is issued.

6. Post marketing documents:

   a) Real time stability data up to shelf-life
   b) Post marketing observational study report on reasonable number of subjects within 6 months to 1 year.

c) Any change should be submitted as per ICH Q5E guideline.

26.12 Registration process flow for imported SCCPs

Steps for registration:

1. COPP from one of the 7 developed countries like USA, France, Germany, UK, Switzerland, Japan, Australia or EMA certificate.

2. Certificate of Analysis (COA).

3. Temperature monitoring record, such as data logger report, shipment validation report.

4. Local agent must submit proof of availability of cold chain storage and supply facilities together with expert human support having adequate training on stem cell and cell based products storage and distribution.

5. Samples and packaging material submission.

27. References

These guidelines mostly adapted and some parts are completely reproduced from similar type of regulatory guidelines published by the different regulatory authorities, and also from related scientific articles.

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