Production of Umbilical Cord Mesenchymal Stem Cell Preparations and Related Requirements (Postprint)

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Date: 2018-08-06T00:00:00+00:00

Abstract

The U.S. Food and Drug Administration has approved Prochymal, the world' s first stem cell drug. Umbilical cord mesenchymal stem cell drugs have been approved for marketing by the Korean Food and Drug Administration. Umbilical cord mesenchymal stem cell preparations exhibit promising clinical application prospects and can be utilized to treat diseases that are refractory to or exhibit poor response to conventional therapeutic interventions, including systemic lupus erythematosus, diabetes, pediatric autism, craniocerebral injury, degenerative arthritis, and knee joint cartilage damage, among others. Also known as umbilical cord mesenchymal stem cell injections, these preparations should be manufactured in accordance with the fundamental principles, relevant regulations, and normative documentation of Good Manufacturing Practice (GMP) for pharmaceutical products, alongside other requirements, with stringent controls applied to each stage of the production workflow. The production process for umbilical cord mesenchymal stem cell preparations encompasses a series of steps including umbilical cord collection, isolation and purification, stem cell characterization, large-scale expansion, injection formulation, filling, packaging, and labeling; the final product must be stored in liquid nitrogen at -196°C and reconstituted prior to use.

Full Text

Preamble

Production and Related Requirements of Umbilical Cord Mesenchymal Stem Cell Preparations –Serial Part Three of "Umbilical Cord Mesenchymal Stem Cells"

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Abstract

The United States Food and Drug Administration has approved Prochymal, the world's first stem cell drug, and umbilical cord mesenchymal stem cell drugs have been approved by Korean regulatory authorities. Umbilical cord mesenchymal stem cell preparations exhibit promising clinical potential for treating diseases refractory to conventional therapies, including systemic lupus erythematosus, diabetes, childhood autism, craniocerebral injury, degenerative arthritis, and knee cartilage damage. Also known as umbilical cord mesenchymal stem cell injections, their production must comply with Good Manufacturing Practice (GMP) principles, regulations, and guidelines, with stringent controls at each production stage. The manufacturing workflow encompasses umbilical cord collection, isolation and purification, stem cell identification, large-scale expansion, injection formulation, filling, packaging, and labeling. Final products require storage in liquid nitrogen at -196°C and resuscitation before use.

Keywords: Stem cells; Umbilical cord mesenchymal stem cells; Stem cell preparations; Stem cell injections; Stem cell drugs; Good Manufacturing Practice

Introduction

In December 2009, the United States Food and Drug Administration (FDA) approved Prochymal, an allogeneic bone marrow mesenchymal stem cell product manufactured by Osiris Therapeutics, marking the world' s first approved stem cell drug [1]. Subsequently, stem cell drugs have been launched or approved in Australia, Canada, South Korea, Italy, New Zealand, and Japan (marketed as TemCell) for treating pediatric acute graft-versus-host disease (GVHD), complex Crohn' s disease with anal fistulas, acute myocardial infarction (AMI), degenerative arthritis, bone injuries, knee cartilage damage, and moderate to severe limbal stem cell deficiency (LSCD) in adults caused by physical or chemical burns. Notably, in January 2012, the Korean Food and Drug Administration (KFDA) approved Cartistem from Medi-post, a stem cell product derived from neonatal umbilical cord blood mesenchymal stem cells, primarily for treating degenerative arthritis and knee cartilage injuries.

Umbilical cord mesenchymal stem cell preparations utilize qualified umbilical cord mesenchymal stem cells—obtained through in vitro isolation, purification, expansion, and testing from neonatal umbilical cord tissue—as the active ingredient. These cells are formulated and packaged with balanced solutions and cryoprotectants to meet biological preparation standards and viable cell product requirements for direct clinical transplantation. As cell suspensions typically prepared for injection use, these preparations are also termed "umbilical cord mesenchymal stem cell injections" [3-5]. The production process involves two main stages: umbilical cord mesenchymal stem cell preparation and injection formulation.

1.1 General Requirements

Stem cell preparation must adhere to the fundamental principles, regulations, and normative documents of Good Manufacturing Practice (GMP). Preparation facilities should establish a complete GMP-compliant quality management system with an independent quality management department responsible for quality assurance and control. Risk assessments based on the characteristics and preparation processes of each stem cell preparation should inform appropriate quality management strategies. Facility design should ensure rational layout of work areas, with functional zones being relatively independent and equipped with adequate space, facilities, equipment, and cleanliness levels to meet operational requirements. The quality control area must be physically isolated from the preparation area, while administrative, living, and auxiliary zones should not interfere with preparation activities.

Both internal and external preparation environments must satisfy quality assurance and intended use requirements, with stringent control of contamination risks from microorganisms, particulates, and pyrogens. The preparation management director, quality management director, and quality authorized person should possess relevant professional knowledge in cell biology, microbiology, biochemistry, or pharmaceutical sciences, along with at least five years of relevant experience or appropriate professional training. The preparation management director cannot concurrently serve as the quality management director or quality authorized person.

All personnel involved in stem cell preparation, quality control, and related functions—including cleaning, maintenance, and material storage staff—must receive training in professional knowledge, safety protection, and emergency protocols, plus continuing education based on their job responsibilities. Personnel files, including health and hygiene records, should be maintained, with files for departed employees who directly participated in preparation or quality control operations retained for at least 30 years. Preparation, quality control, and packaging personnel must promptly document and report any potential contamination situations, including type and extent. Strict measures should prevent personnel with open wounds, infectious diseases, or other contamination risks from engaging in preparation, quality control, or packaging operations.

Management archives should be established for all equipment, instruments, and facilities, with a unique coding identification system ensuring usage traceability. Comprehensive use and maintenance management systems should be developed according to manufacturer instructions. Instruments and equipment directly related to cell preparation and quality control—such as sterilizers, clean benches, biosafety cabinets, air purification systems, and process water systems—must be validated or qualified and approved by the quality management department before use, with regular planned calibration and maintenance. When electronic information systems are employed, management procedures for system design, operation, use, upgrades, and modifications should be established, with regular

verification of system accuracy and integrity.

1.2 Umbilical Cord Collection Requirements

Collection of all human-derived materials must obtain informed consent from donors or their legal representatives/guardians. Collectors should be medical institutions possessing a Medical Institution Practice License and donor screening capabilities. Embryonic stem cell providers must be professional institutions approved by relevant national authorities. Preparation facilities should verify and regularly assess the qualifications of collection institutions or providers, which should establish standard operating procedures and emergency response plans for collection. The collection process must strictly follow these procedures with accurate records, and all collection information should be double-checked.

Collection should be performed by medical personnel holding physician or nurse practice certificates who have received appropriate training. Preparation facilities must clearly communicate quality standards for collected materials, requirements for collection information and records, temporary storage conditions before shipment, and packaging and shipping requirements to collection institutions and personnel, providing training and guidance when necessary.

The collection process must protect donor health and safety while minimizing contamination, infection, and pathogen transmission risks through aseptic techniques. Reagents and materials contacting collected materials must be sterile, meet clinical safety standards, and be within their validity period. Sterile reagents and materials provided by the preparation facility must be validated and qualified by its quality control department.

Donor requirements specify that umbilical cords should be obtained from healthy full-term pregnant women. Prior to collection, donors must be tested for human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), *Treponema pallidum* (TPPA), Epstein-Barr virus (EBV), cytomegalovirus (CMV), alanine aminotransferase (ALT), and mycoplasma. Only donors passing all tests may be selected. Critical cell preparation information must include stem cell source and acquisition method plus relevant clinical data, including donor general information, medical history, and family history. Family and medical histories should thoroughly document genetic disease information (monogenic and polygenic diseases, including cardiovascular diseases and tumors). When necessary, donor ABO blood type and HLA Class I and II typing data should be collected for retrospective tracing. Donors with severe infectious diseases in their medical history or clear hereditary diseases in their family history must not serve as allogeneic stem cell sources. Collection requires informed consent or authorization from the umbilical cord owner and ethics committee approval.

After fetal delivery, physicians or nurses should perform routine umbilical cord ligation, then disinfect and cut the cord 20–40 cm from the severed end, gently squeeze out blood from the vessels, rinse thoroughly with normal saline, place

it in a sealed single-use container prefilled with preservation solution, and affix a special label detailing relevant clinical information. Collection and preparation institutions should establish donor privacy protection mechanisms to ensure personal information security. A unique identification system should be established for collected materials to ensure traceability of stem cell preparations throughout subsequent processes.

1.3 Culture Media and Related Materials Requirements

All materials used for stem cell preparation must be evaluated and qualified by the quality management department. Before receipt, completeness of supplier documentation should be verified for compliance with quality management requirements, including product manuals, certificates of compliance, composition descriptions, certificates of analysis, and chemical safety data sheets. When necessary, professional testing institutions should conduct quality inspections and issue reports.

Materials directly contacting stem cells should be clinical application products approved by relevant national authorities, with a sampling inspection system established. Materials may only be released for use by the quality management department after meeting specified quality standards. If no nationally approved clinical application products are available, they must comply with relevant national management regulations.

Culture media used for in vitro stem cell expansion should be sterile, virus-free, mycoplasma-free, and low in endotoxins, with residual culture media components in stem cell preparations having no adverse effects on recipients. Culture media must not affect stem cell biological activity while supporting normal growth. Human- or animal-derived materials should be avoided whenever possible. If animal serum is required, it must be free from specific animal-derived viral contamination. If porcine trypsin is needed, it must be free from porcine parvovirus contamination. Bovine serum from spongiform encephalopathy epidemic areas is strictly prohibited.

If culture media contain human blood components such as albumin or transferrin, nationally approved clinical application products should be used whenever possible, with clear documentation of source, lot number, manufacturer, and manufacturer' s quality inspection certificate. Antibiotics should not be used during stem cell culture, particularly in the final culture stage, unless absolutely necessary. All culture liquids used in stem cell preparation—including salt solutions, digestive enzymes, buffers, and water—must meet required purity levels (e.g., water should meet standards for water for injection) and be sterile.

Note: Figure translations are in progress. See original paper for figures.