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EXPERT OPINION

Standards for the culture and quality control of umbilical cord mesenchymal stromal cells for neurorestorative clinical application (2017)

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Abstract: Formulating common standards for the culture and quality control of umbilical cord mesenchymal stromal cells (MSCs) is crucial for the standardization of clinical neurorestorative therapy. But to date, there have been no standardized guidelines for the culture and quality control of MSCs in neurorestorative clinical application. Based on a relatively comprehensive review of published clinical studies as well as the existing methods of MSC culture and quality control, the Chinese Association of Neurorestoratology has developed standards for the culture and quality control of umbilical cord MSCs which possess the potential in neurorestorative clinical application. These guidelines include standardized training and management procedures for laboratory operators; standardized use and management of materials and equipment; standardized collection, culture and proliferation of umbilical cord MSCs; standardized management for cell preservation, transport and related safeguard measures; as well as standardization of a clean environment, routine maintenance and related tests and examinations and so on. These guidelines represent the minimum required standards for the culture and quality control of umbilical cord MSCs for potential use in current neurorestorative clinical therapy, and will be further optimized according to the progress of preclinical and clinical studies.

Keywords: standardization, cell culture, quality control, mesenchymal stromal cells, clinical treatment, neurorestoratology, translational medicine

Introduction

Mesenchymal stromal cells (MSCs), originating in the early development of the mesoderm, are fibroblastic-like cells, which can be derived from autologous or allogeneic bone marrow, umbilical cord, adipose and other tissues in adults. Under distinctive induction conditions, the stem cells among MSCs have the capacity to differentiate into various types of tissues including bone, cartilage, fat and neurons. In 2006, the International Society for Cellular Therapy (ISCT) established the minimal identification requirements to define human MSC. These include: 1) plastic-adherent when maintained in standard culture conditions; 2) the positive expression of CD105, CD73 and CD90 and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules; and 3) the ability of the cells to differentiate into osteoblasts, adipocytes and chondroblasts in vitro.[1–3] The ISCT released a document again and highlighted the minimal identification criteria for MSCs in 2016.[4] However, many experts still mistake these criteria as the identification criteria of mesenchymal stem cells. The ISCT suggests that all fibroblast-like plastic-adherent cells, regardless of the tissue from which they are isolated, should be termed multipotent MSCs.

Investigators must clearly define the cell name used in their reports.[1] We have proposed the following guidelines in order to bring clarification to the cultivation processes for MSCs and avoid mistaking these for mesenchymal stem cells.

MSCs are derived from a wide range of sources.[5–11] The advantage of MSCs derived from umbilical cord is that the collection process is relatively simple and noninvasive; furthermore, the placental barrier protects the umbilical cord from contamination by viruses and bacteria.[12,13] A large number of preclinical and clinical repair studies have been performed on various systemic diseases, including neurologic disorders and damage.[14–24] But to date, there have been no standardized guidelines for the culture and quality control of MSCs in the neurorestorative clinical application. In order to ensure the safety and effectiveness of clinical application of this type of cells, it is important to standardize the cell culture and quality control processes. Based on a comprehensive review of the published clinical studies as well as the existing methods of MSC culture and quality control, the Chinese Association of Neurorestoratology has developed the following set of standards for the culture and quality control of umbilical cord MSCs in neurorestorative clinical application. These guidelines include standardized training and management procedures for laboratory operators; standardized use and management of materials and equipment; standardized collection, culture and proliferation of umbilical cord MSCs; standardized management for cell preservation, transport and related safeguard measures; as well as standardization of a clean environment, routine maintenance, related tests and examinations and so on. The purpose of these standards is to maximize the therapeutic efficacy, minimize the possible side effects and promote the worldwide replicability of utilizing umbilical cord MSCs for neurorestorative clinical application.

Standardized training and management for operators

There should be at least one senior scientist/manager who is in charge of overseeing the cell culture laboratory. The laboratory personnel should have an education background in cell biology and receive systematic and professional training in cell culture techniques and quality control management for umbilical cord MSCs.

Basic standardized training schemes include knowledge learning, skill development and familiarity with applicable rules and regulations. Training methods include centralized teaching, teacher demonstration, workshops, small seminars or symposiums and so on. Training objectives include mastering professional knowledge, cell culture techniques and quality control processes for umbilical cord MSCs. A standardized management system includes filling and organizing original work records and data, complying with the laboratory maintenance standard operating procedures and current Good Manufacturing Practices and completing operating records.

Standardized use and management of materials and equipment

Cell culture laboratories must be managed by a designated person who is responsible for the equipment maintenance, regular calibration and personnel training. The designee should maintain a normal operational status of clean rooms and also establish and manage the equipment quality certification file records.

1. CO_2 incubator: The temperature should be kept at a consistent 37°C and the concentration of CO_2 at 5%.
2. Centrifuge: Be sure to balance the centrifugal object before the centrifuge works.
3. Water bath box: Distilled water should be changed and disinfected at least once a month.
4. Inverted microscope: The lens should be cleaned after using an oil mirror. The power should be turned off and the microscope should be covered after each use.
5. Super-clean bench or biologic safety cabinets: Operation guidance for procedures should be strictly followed.

After finishing each program or procedure, the designated person should determine whether all instruments are working appropriately. The power should be turned off for equipment not in use. Using records for equipment should be updated timely.

Umbilical cord MSC culture process

Donor selection

Female donors should have no history of infectious diseases such as HIV, hepatitis B, hepatitis C or syphilis. Umbilical cord should be derived from the fetal umbilical cord of normal, cesarean birth or induced abortion without brain abnormalities. The cell procurement team should carry out the informed consent process with potential donors and their families. After understanding and consenting to the donation, the female donor should sign the informed consent form. The process must be approved by the relevant hospital ethics committees. Samples of the umbilical cord should be kept in a sterile bag, then placed in the freezer and delivered to the laboratory as soon as possible.

Culture medium

The culture medium of MSCs should consist of basal medium and serum substitute. The basal medium components include

combination of DMEM and F12 with 1:1 ratio (DF12). The other components of the culture medium include glutamine, fetal bovine serum and serum replacement. All materials must meet the quality standards of sterile, nonpathogenic microorganisms and endotoxins.

Umbilical cord MSC preparation procedures

1. The sample tissue of umbilical cord should be put into saline containing antibiotics. After being transferred to the clean bench, the sample tissue should be placed on a sterile culture dish. A stripping forceps is used to strip off the outer membrane and blood vessels from the surface of the umbilical cord carefully with naked eye, and the integrity of the matrix layer should be maintained.
2. The tissue should be cut into granular tissue with sterile scissors. The broken down pieces of tissue should be taken into a 50 mL sterile centrifuge tube and centrifuged under 1000 rpm for 5 minutes. The supernatant should be discarded; saline containing antibiotics should be added, the precipitated tissue blocks thoroughly mixed to make them loose, and then they should be centrifuged under 1000 rpm for 5 minutes.
3. Tissue blocks are suspended with DF12 containing 15% fetal bovine serum, fully mixed and then centrifuged under 1000 rpm for 5 minutes. The tissue blocks are precipitated evenly, grown on culture plastic flasks with poly-lysine–coated surface and then placed in 5% CO_2 incubator. After the tissue has completely adhered on the flasks, the DF12 medium containing 15% fetal bovine serum is added.
4. After culturing for about 14 days, on finding cell adhesion, spindle cells or polygonal cells around the tissue blocks under inverted microscope, suck out and discarded all tissue blocks, then add DF12 containing 15% fetal bovine serum in the original flask, and continue to culture cells in the 5% CO_2 incubator.
5. In the next 1 week, cells grow rapidly and become relatively uniform in the shape of a long spindle with swirling growth. When the cells cover 80% of the bottom of flask, they should be digested with 0.25% trypsin-EDTA. The cultured cells in one flask should be divided and taken in two flasks in serum-free medium (with serum replacement) for passage. When the MSCs are passaged to the fifth generation, they can be used for clinical application or cryopreservation. In this step, antibiotic residues of the cells need to be detected.

MSC cryopreservation and recovery procedures

Once the cells are vigorously growing and have covered the culture flask (generally when they have fully filled the flasks) at the fifth passage, they may be cryopreserved if they are not to be used immediately for clinical application. Suck out the medium from the flasks and add 2 mL of DF12 to each flask to wash the cells. Add 2 mL of trypsin-EDTA to each flask. After 2 minutes, add 2 mL of termination solution to stop digestion. Fully blow and wash out the cells with a thick elbow suction tube from one side to the other in order to avoid creating foam. After centrifugation under 1000 rpm for 5 minutes, the cells should be collected and then added to the cell cryopreservation solution according to the amount of MSCs. The cells are lightly blown with a thick elbow tube to make them distribute evenly and then moved into the cryopreservation tube. The number of the cells is marked and the identification number of the sample recorded in order to maintain good storage records.

Cryopreservation process

Cells should be processed at 4°C for 45 minutes, −20°C for 1 hour and then −80°C overnight. Finally, the processed cells are transferred into a liquid nitrogen tank.

To resuscitate the sample for use in clinical application, remove the frozen tube from the liquid nitrogen tank and quickly immerse it into a hot 38.5°C water bath with continuous shaking to induce thawing as soon as possible. Use sterile gauze to dry the surface of any excess water and use a straw to suck out the cell suspension to a sterile 15 mL centrifuge tube. Then add 10 mL of DF12 culture medium, blow the cell suspension, and centrifuge for 5 minutes (1000 rpm) and discard the supernatant. Then rinse the cells with DF12 medium for three times, and then put them into a culture flask with DF12 medium without serum.

MSC quality control standards

Detecting exogenous factors in cell products

Follow the current version of "China Pharmacopoeia" related to biologic products, in order to detect any exogenous bacteria, fungi, mycoplasma and endotoxins.

Cell activity

Using trypan blue staining, cell activity should reach more than 95%.

Cellular immunophenotype

1. Morphologic identification: The morphologic characteristics of MSCs should be fibroblastic-like, spindle-shaped and with a swirling growth under an inverted microscope.
2. Phenotype detection: Over 95% of the cells exhibited positive surface antigenicity for CD73, CD90 and CD105, and >99% of the cells were negative for CD34, CD45 and HLA-DR, as measured by flow cytometry.[2,25]

Tumorigenicity

There have been no tumor formation reports after the transplantation of umbilical cord MSCs.

Cell quality control and management

Cell products must be double reviewed before using them in any clinical transplant treatment. Laboratory personnel are responsible for performing the double quality review and testing, and should sign a standardized form indicating that all checks have been satisfactorily carried out.[26]

Clinical cell dosage

The appropriate cell dosage should follow the Chinese clinical cell therapy guidelines for neurorestoration.[27,28]

Temperature and time control for clinical cell transportation

Cell products should be kept at 4°C in a biosafety transport box and be used within 2 hours after preparation.[28]

Management of clinical application cell bank

Files of clinical therapeutic application and MSC cell bank management system should be established according to cell sources, the number of culture passage, location in the liquid nitrogen tank, the date of cryopreservation and other relevant details.

Summary

Formulating common standards for the culture and quality control of umbilical cord MSCs is crucial for the standardization of clinical neurorestorative therapy. Our objective in publishing this set of standards is to promote the worldwide safety, effectiveness and replicability of utilizing umbilical cord MSCs for neurorestorative clinical application. These guidelines represent the minimum required standards for the culture and quality control of umbilical cord MSCs for use in current neurorestorative clinical therapy. These guidelines will be further optimized and improved according to the progress of preclinical and clinical studies.

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Disclosure

The authors report no conflicts of interest in this work.

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