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Chapter 13

Transportation of Mesenchymal Stem Cells for Clinical Applications

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Abstract

Cell-processing procedures are conducted in accordance with Good Manufacturing Practices, and clinical procedures are performed by highly optimised methods. A high-quality transportation system is essential for safe and effective handling of mesenchymal stem cells (MSCs) between cell-processing and transplantation stages. For MSC transportation, either frozen cell or non-frozen cell transportation is performed. There are many requirements for transporting a package by either type of transportation. In frozen cell transportation, some issues have yet to be resolved: the primary receptacle and cryoprotectant reagents. In non-frozen cell transportation, control of cell metabolism and protection from environmental changes are more serious problems. Stabilisation of temperature, shock resistance, gas control, and an ultraviolet radiation (UVR) shielding technology should be considered. The transportation system should be established in compliance with the guidelines. Both development of a high-quality transportation package and establishment of a high-quality transportation system are important for the effective use of MSCs in clinical applications.

Keywords: transport, mesenchymal stem cell, regenerative medicine, clinical use

1. Introduction

Cell-processing procedures are conducted in accordance with Good Manufacturing Practices, and clinical procedures such as transplantation and infusion are performed using highly optimised techniques [1]. Thus, a high-quality transportation system is necessary for safe and effective handling of materials and mesenchymal stem cell (MSC) products between cell-processing and transplantation stages. Frozen cell transportation is conventionally used in laboratories. Nonetheless, there may be some disadvantages of this method for clinical

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applications. For example, the tubes usually used for frozen transportation are not completely sealed receptacles, and the cryoprotection reagents have some effects on the human body. In non-frozen cell transportation, control of cell metabolism and protection from environmental changes are serious problems. A transportation system should be established to prevent man-made incidents. In this study, the elements required for transportation of MSCs are discussed.

2. Conditions required for MSC transportation

There are many possible scenarios of MSC transportation. When the MSCs are isolated immediately from materials in a hospital, establishment of a transportation system inside the hospital is needed (**Figure 1A**). If further manipulation of MSCs or mass culture is performed, MSC materials such as bone marrow, cord blood, and adipose tissue are transported from the

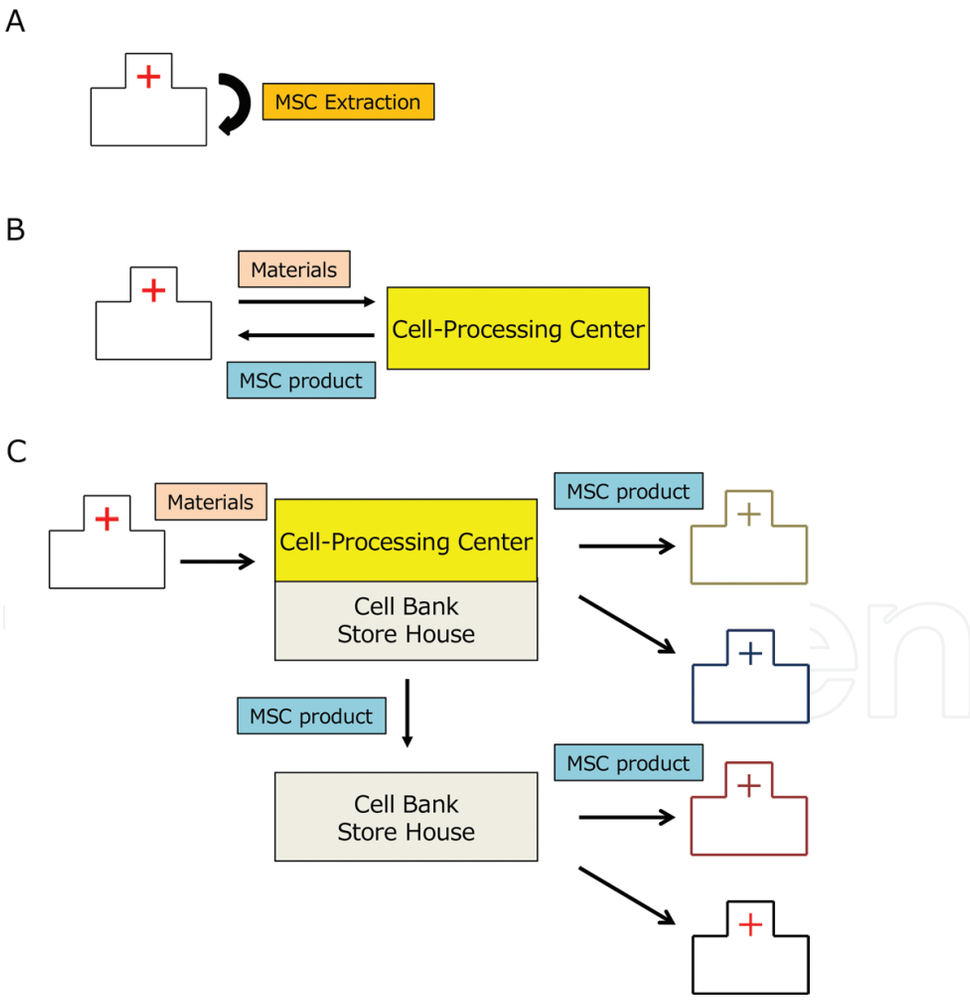

Figure 1. The scenarios of MSC transportation. (A) Extraction of materials in a hospital. (B) Extracted materials are transported to a cell-processing centre, and MSC products are transported to the hospital. (C) In some cases, MSCs are stored in a cell bank.

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hospital to a cell-processing centre (CPC). At a CPC, the materials are cultured and manipulated and then transported to the hospital (**Figure 1B**). These transportation scenarios (**Figure 1A**, **B**) work effectively with autogenic transplantation. When allogeneic transplantation is performed, some temporary storage is used. In such cases, other facilities such as cell banks or stock houses are added [2] (**Figure 1C**).

Basically, when the transportation is performed within a hospital, there are not many requirements for the transportation package and transport system if a good environment is maintained in the hospital. Some packaging technology is needed when outside transportation is performed among a hospital, CPC, and cell bank (**Figure 1B**, **C**). In the outside environment, the transported materials and MSC products should be protected from some external environmental factors such as micro-organisms, temperature, shocks, humidity, ultraviolet radiation (UVR), and atmospheric pressure. The transportation package should withstand such external environmental stimuli.

3. Requirements for transportation and three-level packaging

There are some requirements for transportation of MSCs for clinical use:

- Leakproofness
- Sterility
- Temperature stabilisation
- Shock resistance
- Gas stability
- UV shielding
- Monitoring

Each requirement is important, but the degree of importance is different in each transportation scenario (**Figure 1**, **Table 1**). A single type of package cannot fulfil all the requirements. A three-level packaging system is recommended by the World Health Organisation (WHO) [3] (**Figure 2**). Each package satisfies each requirement. Tests required for compliance with the criteria include a 9-m drop test, a puncture test, and a stacking test for the packages [3].

3.1. The primary receptacle

The materials and the cell products are inserted into the first package. The primary receptacle should be completely sealed to prevent invasion of micro-organisms and leakage of contents. The materials constituting the primary receptacle should be chosen carefully to prevent elution of chemical materials. Packaging the contents into the primary receptacle is performed in the CPC area, and the primary receptacle should be sterilised.

	Sterility	Shock resistance	Temperature stabilisation	Monitoring
Inside hospital	✓✓	✓	✓	✓
From hospital to CPC (materials)	✓✓	✓✓	✓✓	✓✓
From CPC to hospital (products)				
Non-frozen	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Frozen	✓✓	✓	✓✓	✓
From cell bank to hospital (products)				
Non-frozen	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Frozen	✓✓	✓	✓✓	✓
From stockhouse to hospital (products)				
Non-frozen	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Frozen	✓✓	✓	✓✓	✓

MSC, mesenchymal stromal cell; CPC, cell-processing centre.

Table 1. Technologies needed for each processing scenario of MSC materials and products.

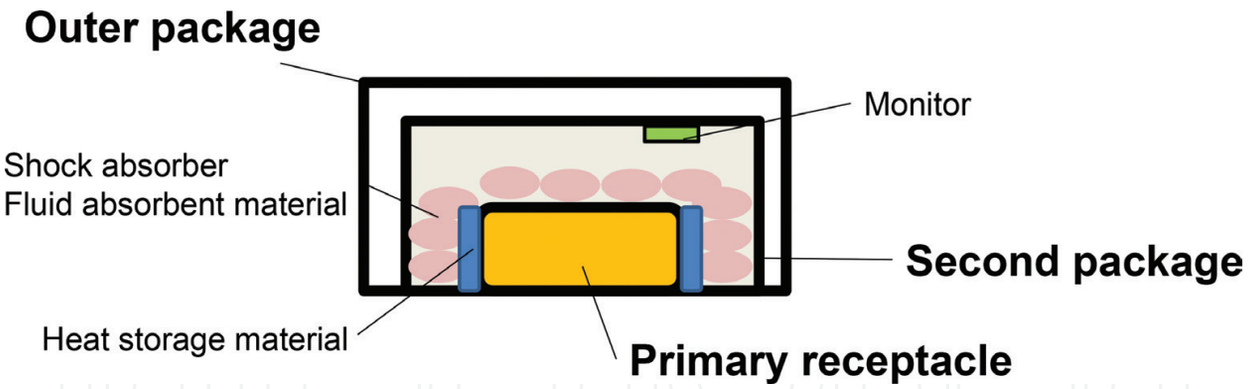

Figure 2. A three-level packaging system of a transportation box in regenerative medicine.

3.2. The second package

The primary receptacle is inserted into the second package (**Figure 2**). Sterilisation treatment of the second package is not necessary if the complete sterilisation and sealing are achieved for the primary receptacle. Some materials for absorbing liquids are needed if the first receptacle is broken and the contents leak out. A shock absorber and temperature control system such as materials for hot storage are included in the second package. For monitoring the temperature and shocks, a sensing device is also included.

3.3. The outer package

The second package is placed into the outer package (**Figure 2**). The outer level is the packaging most exposed to external environmental stimuli. The characteristics of heat insulation, toughness, waterproofness, and UV shielding are necessary. For the handling, slings and belts are attached to the outer package. A notice, security lock, and invoice are attached as acquired.

4. Requirements for the packages

4.1. Leakproofness

Tight sealing is required from the standpoint of leakproofness and sterility. According to the 'Guidance on Regulations for the Transport of Infectious Substances', leakproofness is required in the primary receptacle and second package [3]. The guidance itself is used for transportation of 'infectious substances', but it defines the 'patient specimens' and 'biological products' as 'infectious substances'. Thus, this guidance is to be used as a reference. If the primary receptacle loses leakproofness and springs a leak, some absorbent materials in the second package should absorb all fluid [3]. Whatever the intended temperature of the consignment, the primary receptacle and secondary package should withstand (without leakage) the internal pressure no less than 95 kPa in the range of temperatures from -40 to +55°C [3].

4.2. Sterility

The importance of sterility does not need to be explained. To guarantee sterility, the primary receptacle should be completely sealed. Therefore, the semi-closed cap-type receptacle typically used for frozen transportation is not recommended as a primary receptacle. Besides, the primary receptacle itself should be sterilised.

4.3. Temperature stabilisation

Stabilisation of temperature is employed to control cell metabolism and to prevent degradation of cells and products. There are two categories for temperature stabilisation: frozen cell or non-frozen cell transportation.

In the field of frozen cell transportation, research and development on the freezing procedure are conducted for the purpose of storage. The success of frozen cell transportation and storage depends on the temperature control and cryoprotective agents [4]. According to Hubel, understanding the mobility of water is paramount for good insight into biochemical reactions during freezing [4]. The temperature dependence of protein activity obeys the Arrhenius equation [4, 5]:

$$K = A \cdot e^{-Ea/(RT)} \quad (1)$$

where k is the rate constant, T is the absolute temperature (Kelvins), A is the pre-exponential factor, E_a is the activity energy for the reaction (Joules), and R is the universal gas constant. The optimal temperature for transportation and storage should be selected below the threshold temperature to manage activity of a protein [4]. Distinct changes are observed near -53°C [6, 7]. A substrate fails to bind to ribonuclease A at -58°C [8]. Some authors reported that activity of β-glucosidase is observed even at -70°C [9, 10]. These results suggest that a trace protein activity may persist even at a low temperature, below -80°C [4]. To stabilise proteins and minimise cell dehydration, pharmaceutical agents are used for cryoprotection. Cryoprotectants are classified into permeating and non-permeating agents depending on their ability to cross the cell membrane [11, 12]. The permeating cryoprotectants, such as dimethyl sulphoxide (DMSO), are used for clinical preservation and banking of MSCs for the treatment of graft-versus-host disease (GVHD) [13]. The International Stem Cell Initiative (ISCI) recommends the use of dry shippers involving liquid nitrogen (-196°C) for frozen stem cell transportation [2]. Nonetheless, some issues should be resolved regarding cryopreservation and frozen cell transportation. A significant loss of living cells after recovery occurs after cryopreservation. The proportion of apoptotic and senescent cells increases during cryopreservation in comparison with fresh live culture [14–16]. Not only cell viability but also alteration of MSC characteristics is to be considered. Immunosuppressive properties of MSCs are altered after cryopreservation although these alterations may be favourable for the treatment of GVHD [17, 18]. DMSO is the most widely used cryoprotectant in the world, but it has some adverse effects on patients such as nausea, headache, hypotension, hypertension, and diarrhoea [19]. Further research and development on cryoprotectants, freezing and thawing technologies, cell containers, the primary receptacle, and administration method are needed for frozen cell transportation.

In non-frozen cell transportation, control of cell metabolism is compromised. There is little information about the non-frozen cell transportation of MSCs. Information on the storage of platelets serves as a useful reference. Platelets are stored between 20 and 24°C with continuous agitation, suspended in plasma with a citrate-based anticoagulant [20]. Platelets at 4°C undergo a shape change, α-granule release, aggregation, apoptosis, and a significant reduction of the lifespan. When MSCs that are detached from a culture dish are incubated at 4, 20, or 37°C in a culture medium for 24 h, the living cell numbers after 37°C incubation are higher. Nevertheless, dead cell numbers increase after 37°C incubation (**Figure 3**). This result shows that cell metabolism is not sufficiently controlled during 37°C incubation. The development of a preservation solution and verification of suitable temperature are necessary for non-frozen-MSC transportation.

4.4. Shock and vibration resistance

There is little or no information about the bioresponse of MSCs to shocks and vibration during transportation. One study evaluated the shocks and vibration associated with truck and railway transportation [21]. Peak shock acceleration for the longitudinal axis was 2.8g, for the transverse axis 2.3g, and for vertical axis 7.0g on a truck, and all axes were 4.7g on a train when a 22-ton cargo was transported [22]. Peak vibration acceleration for the longitudinal axis was 0.27g; for the transverse axis, it was 0.27g; and for the vertical axis, 0.52g on a truck.

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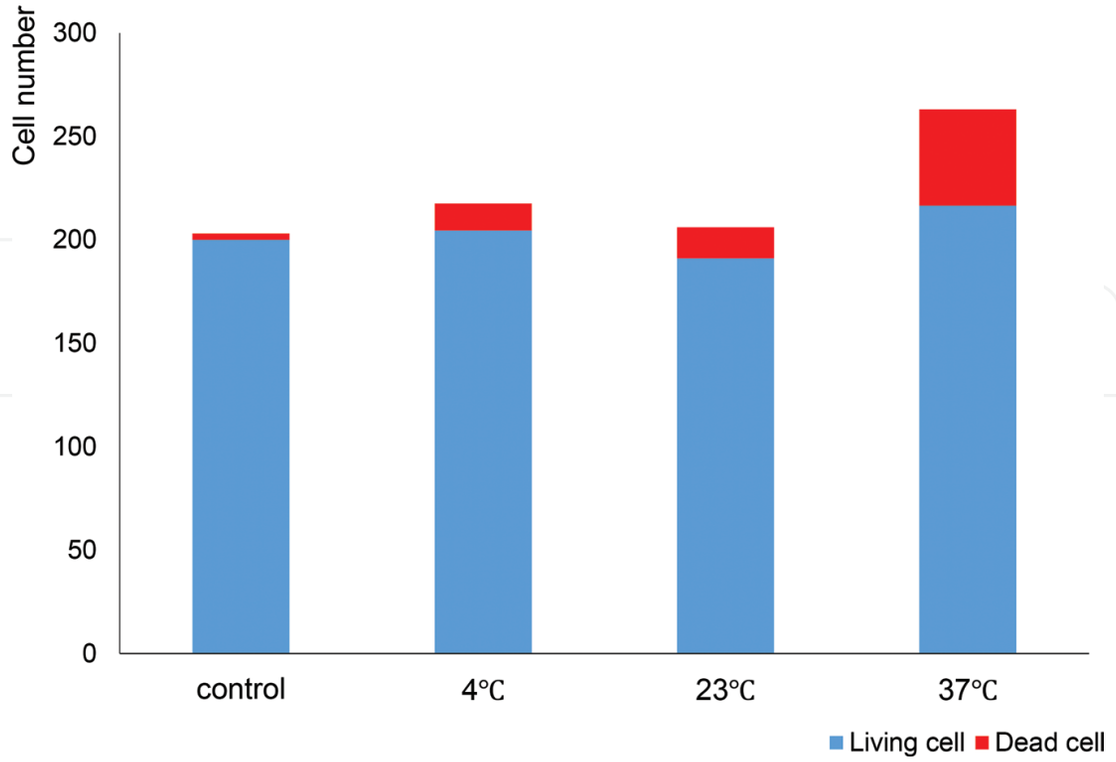

Figure 3. The cell number of MSCs peeled from a culture dish and incubated at 4, 20, or 37°C in a culture medium for 24 h. Living cell and dead cell numbers were determined by means of the Live/Dead Staining Kit II (Takara Bio Inc., Shiga, Japan).

The frequency range was 0–1900 Hz [22]. Peak vibration acceleration for the longitudinal axis was 0.10*g*, transverse axis 0.19*g*, and vertical axis 0.52*g* on a train. The frequency range was 0–750 Hz [23]. Strong shocks should be prevented from destroying the extracellular matrix of products. Microvibration should be considered for prevention of changes in the characteristics of MSCs. The lipid membrane, primary cilium, extracellular matrix, and intracellular cytoskeleton respond to mechanical stimuli. They transduce mechanical stimuli via ion channels, integrins, and focal adhesion kinase and thus alter gene expression and protein synthesis. As a consequence, survival, apoptosis, proliferation, and differentiation properties are affected [24]. For example, MSCs were stimulated by vibration from 1 to 1000 Hz, and myogenic transcription factors were found to be upregulated by low frequencies (1 and 50 Hz), but osteogenic transcription factors were upregulated by high frequencies (500 and 1000 Hz) [25]. These data suggest that some shock absorber is needed to prevent the shocks and vibration.

4.5. Gas stability

In frozen-MSC transportation, gas control is not needed, but in non-frozen-MSC transportation, some gas control technology is necessary. When we consider the gas control, the knowledge about cellular senescence is crucial. Cellular senescence was discovered in the 1960s by Hayflick [26]. He showed that human diploid fibroblasts have a limited ability to proliferate; it is called senescence in ex vivo culture [26]. Some stressors, such as dysfunctional telomeres,

genotoxic stressors, DNA damage, UVR, and oxidative stress, induce cellular senescence [27]. This phenomenon is induced by the upregulation of $p16^{INK4A}$ in MSCs [28]. The expression of $p16^{INK4A}$ is associated with oxidative stress. When MSCs are cultured under hypoxic conditions (1% pO_2), their proliferation is increased by 8–20 population doublings as compared with normoxic conditions (20% pO_2) [29]. The *p16*^{INK4A} gene is downregulated in hypoxic culture [29]. An antioxidant agent that induces 5% pO_2 conditions [30] and 3% hydrogen gas treatment [31] prolongs the replicative lifespan. Not only cellular proliferation but also the characteristics of MSCs are influenced by gas conditions. The osteogenic differentiation potential is not influenced, but adipogenic and chondrogenic differentiation abilities are enhanced under hypoxic culture conditions [29]. Cell surface antigens are downregulated, and the ability to suppress T-cell proliferation is diminished by oxidative stress [32]. These results suggest that some gas control system is required for non-frozen-MSC transportation.

4.6. UVR shielding

UVR is a natural component of sunlight and is invisible to the human eye. There are three types of UVR by wavelength: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). UV-A and UV-B can reach the Earth's surface, but the ozone layer filters out UV-C [33]. Ageing of skin associated with UVR exposure is referred to as photoageing. Sun-exposed areas of the skin, such as the face and neck, undergo premature ageing [33]. UV-A induces production of reactive oxygen species (ROS) and activates cell cycle checkpoint proteins such as p53 and p21 [34]. UV-B exposure causes structural rearrangement of nucleotides that leads to defects in DNA strands. The UVR-induced ROS production and DNA damage to epidermal stem cells lead to photoageing [33]. Although there are many reports about the harmful effects of UVR on epidermal stem cells, there is little information about UVR effects on MSCs. The report on the gene expression profile, when MSCs were exposed to low-dose UV-B, showed that only a minimal change in gene expression was induced [35]. Further research is needed and some UVR shielding technology may be required, especially in case of air transportation.

4.7. Monitoring

Temperature data loggers are required for proof that transportation was completed without a problem. In the absence of temperature data loggers, consideration should be given to the use of chemical or other indicators to provide information on temperature during transportation [2]. If the shock resistance, gas control, and UV shielding are crucial for the transportation of MSCs, then acceleration sensors, gas monitoring, and radiation monitoring should be considered.

5. Transportation systems

Establishment of a transportation system is required in compliance with the government regulations in each country and international guidelines. At present, there is no specific guidance on transportation for regenerative medicine. Nonetheless, some guidelines issued by the ISCI [2] and WHO [3] and specific legislation in Europe [36] can be used for reference.

Education of delivery personnel to prevent man-made incidents, preparation of the documentation for clearance, a traceability system, and security control should be provided. Further research and consensus guidelines are needed for the transportation of materials related to MSCs and cell products for clinical use.

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Abbreviations

MSC	Mesenchymal stem cell
GMP	Good Manufacturing Practices
CPC	Cell-processing centre
GVHD	Graft-versus-host disease
DMSO	Dimethyl sulfoxide
$p16^{INK4A}$	Cyclin-dependent kinase 4 inhibitor p16
DNA	Deoxyribonucleic acid
UVR	Ultraviolet radiation
ROS	Reactive oxygen species

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