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Mesenchymal stem cells and Cryopreservation, a new concept

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Abstract

Recently, there has been an increasing interest in the study of mesenchymal stem cells (MSCs), which are considered as subtype of adult stem cells with the ability of differentiation into ectoderm (into neural cells), endoderm (into hepatocytes, insulin B producing cells) or mesoderm (into adipocytes, osteocytes, chondrocytes), this was associated with increased clinical application of such cells in the management of autoimmune diseases such as (Type 1 diabetes and Rheumatoid arthritis), degenerative diseases as (Parkinson's disease, Amylotrophic lateral sclerosis, and Alzheimer) and inflammatory diseases, and consequently increased the need for and effective and safe way of preservation of these cells for a long time without affecting the viability of the MSCs after the long-term preservation.

This review discusses criteria of mesenchymal stem cells (MSCs), their markers, sources, methods of isolation and culturing, and their clinical applications, and then it discusses types of cryopreservation of MSCs and factors that may have an influence on viability of MSCs following the process of cryopreservation.

Keywords: MSCs, Isolation, Culturing, cryopreservation

1. Introduction:

There had been an interest in cell biology since the emergence of microscopes in the 1800s. In the early 1900s scientists realized that various types of blood cells such as red blood cells, white blood cells, and platelets all originate from a particular 'stem cell'. However, in 1963 the researchers achieved the first descriptions of self-renewing activities of transplanted bone marrow cells in mice [(1)].

The launch of stem cell research began in1998, when one of the scientists at Wisconsin university, James Thomson, managed to remove cells from spare embryos, found them at fertility clinics, and grew these cells in the laboratory. And in this way, he was able to establish the world's first human embryonic stem cell line, and this was considered the beginning of a new era of regenerative medicine [(2)].

2. Stem cells:

Stem cells were defined as those cells with the ability of self-renewal with multiple potencies and with the ability to differentiate into multiple lineages. Acc-

ording to the potency, stem cells were subdivided into totipotent, pluripotent, multipotent, and unipotent [3]. While according to the origin, stem cells were subdivided into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and somatic or adult stem cells [4].

Somatic stem cells were considered less replicative life span and less totipotent compared to ESCs which existed in mature tissues such as hematopoietic, neural, gastrointestinal, and mesenchymal tissues with high proliferative potential and higher capacity of differentiation into various cell types [5].

3. Mesenchymal stem cells (MSCs):

3.1. Definition of MSCs

Mesenchymal stem cells (MSCs) were considered a subtype of adult stem cells that were non-hematopoietic, multi-potent cells with the ability to differentiation into ectoderm (into neural cells), endoderm (into hepatocytes, insulin B producing cells) or mesoderm (into adipocytes, osteocytes, chondrocytes) [6].

The MSCs were first discovered when Virchow suggested the presence of an undifferentiated non-hematopoietic cell type in the human body in 1855, however, Friedenstein was the first one to demonstrate that some cells of the bone marrow were able of forming fibroblastic colonies [7].

The current concept of MSCs was originated by Arnold Caplan when he analyzed the culture conditions and differentiation experiments for the development of cartilage and muscle of embryonic limb buds of the chick [8].

3.2. Criteria of MSCs:

In 2006, the International Society of Cellular Therapy demonstrated the minimal criteria regarding the definition of MSCs which included:**1-** Adherence ability to plastic, **2-** Expression of specific surface markers expression (e.g. > 95% positive expression for CD73, CD105, CD90 ,and < 2% positive expression for CD45, CD34, CD14, CD79a, HLA-DR), **3-** the potential for multipotent differentiation [9].

3.3.Markers of MSCs:

CD73 (cluster of differenetiation73) which is also known as ecto-5'-nucleotidase was recognized by the monoclonal antibody (MAb) SH3 and SH4 are considered one of the surface markers that define MSCs [10].

CD105, also known as endoglin was originally recognized by MAb SH2 and CD90, known as Thy-1 [11].

Studies had also shown that stage-specific embryonic antigen (SSEA)-4, CD1-46 and stromal precursor antigen-1 (Stro-1) can be considered markers for MSCs [12-15]

3.4. Sources of MSCS:

There are multiple sources of MSCs which include bone marrow, adipose tissue, placenta, amniotic fluid, umbilical cord blood, peripheral blood, synovial fluid, dental pulp, muscles, skin, endometrium, and menstrual blood [16].

3.5. Isolation and Culturing of MSCs:

Having a good procedure for the isolateion and culturing of MSCs was considered one of the main keys to their success in different applications.

Isolation of MSCs was mostly done by using the ficoll density gradient centrifugation method (with FicollTM, LymphoprepTM, or PercollTM density mediums) to separate the mononuclear cells (MNCs) from other components such as red cells and plasma [17].

This is followed by culturing of MNCs using condition media such as Dulb-ecco's modified Eagle's media (DMEM) supplemented with 10% Phosphate buffer saline (PBS) [18].

After culturing it's very important to measure the viability of the cells using trypan blue dye to differentiate between healthy (unstained) and dead cells (stained blue) (19).

3.6. Clinical application of MSCS:

MSCs hold great potential for the treatment of autoimmune diseases such as (Type 1 diabetes and Rheumatoid arthritis), degenerative diseases such as (Parkinson's disease, Amylotrophic lateral sclerosis, and Alzheimer's) and inflamematory diseases because of their ability of self -renewal and differentiation into multiple lineages in addition to their paracrine and immunomodulatory properties [20].

3.7.In COVID-19:

Some of the published studies suggested the usage of MSCs as an anti-inflammatory strategy during the COVID-19 pandemic, as the virus can enter the target cells through the angiotensin-converting enzyme receptor 2 (ACE 2), which in turn is highly expressed in adipose tissue, bone marrow, or umbilical cord-derived MSC [21]. Another research suggested that using intravenous transplantation of umbilical cord-derived -MSCs is considered a safe and effective method in severe cases of COVID-19 [22].

4. Cryopreservation:

4.1.Definition:

Cryopreservation was considered the method that allow maintenance of cells at low temperatures [23].

The main objective of the process of cryopreservation is to preserve cells and limit their damage during storage. When the process of cryopreservation is done accurately, the resulting cells will be better, and this will be positively reflected in the transplantation with fewer post-transplantation complications [24].

4.2. Types of cryopreservation:

1- Slow freezing (also known as conventional cryopreservation): is considered to be the best way of cryopreservation during research because it is an easy process and associated with low contamination [25].

2- Vitrification: means solidification as the conversion of a liquid into a glass leads to increased viscosity. This method is suitable for small volumes like oocytes, bus not considered to be the best way while we are dealing with large volumes [26].

3- Programmable cryopreservation: This is a slow-rate freezing method that can be achieved by a programmable freezer controlled at a rate of 0.2C–0.4C/min [27].

4- Subzero non- freezing storage is used to preserve organs at sub-zero temperatures in a non-frozen condition [28].

5- Preservation in the dry state

4.3. Applications of cryopreservation:

Cryopreservation can be used for the following: [1] cryopreservation of cells or organs [2] biochemistry and molecular biology [3] cryosurgery [4] ecology and plant physiology [5] food sciences [6] medical applications including bone marrow transplantation, blood transfusion, in vitro fertilization (IVF) and artificial insemination [29, 30].

4.4. Cryopreservation and MSCs

Due to the increased demand for MSCs in clinical applications, cells have to be preserved for the long term in a good functioning status, and cryopreservation is considered the best way to do so [31].

However, Arutyunyan reported, in one of his publications, cryostorage may hurt the number of MSCs (what is the called "cryo stun effect"), and this can explain the failure of clinical trials which used cell transplants immediately after the process of thawing [32].

Shivakumar, also, reported some sort of reduction in the osteoblastic differentiation following cryopreservation while maintaining the chondrogenic and adipogenic differentiation [33].

Another study, done on peripheral blood mononuclear cells, reported a statistically significant decrease in CD73 expression on T cells following cryopreservation for more than 6 months when compared to fresh ones [34].

On the other hand, Shahensha et al.,[35] showed no statistically significant differrence in the expression of surface markers following short-term cryopreservation (for 3–7 years), long-term cryopreservation (more than or equal to 10 years), and fresh mesenchymal stem cells (never cryopreserved) derived from adipose tissue, which suggested that cryopreservation do not affect immunophenotyping.

Also, Yong et al.,[31] reported in their study that, cryopreservation for 3 months had no impact on MSCs derived from adipose tissue regarding the phenotyping, proliferation, or differentiation.

Jang et al.,[26] also excluded any significant association between cryopreserveation and post-thaw viability.

However, the effect of cryopreservation on cells can be related to other factors.

4.5. Factors affecting Cryopreservation:

Many factors that may affect the quality of cryopreservation include the type of used cryoprotective agents (glycerol, DMSO, cell banker series, polymers), the cooling rate, the thawing rate, and storage temperature. Any of these factors can influence the cryopreservation's outcome [24].

Cryoprotective agents (CPAs):

CPAs were used mainly to reduce the amount of ice formed that causes cell injury at any given temperature [36]. CPAs can be sorted into two main categories: **(1)** cell membrane-permeating cryoprotectants, such as glycerol and 1,2-propanediol, dimethyl sulfoxide (DMSO), **and (2)** non-membrane permeating cryoprotectants, such as 2-methyl-2, 4-pentanediol and polymers such as polyvinyl pyrrolidone, hydroxyethyl starch, and various sugars [37].

Dimethyl sulfoxide (DMSO):

DMSO is considered a strong cryoprotectant and the commonly used one which prevents intracellular ice formation, at any given temperature, by reducing electrolyte concentration of the residual unfrozen solution in and around a cell. However, at the same time, it had toxic effects on cells which may lead to post-transplantation complications [38].

To overcome these toxic effects caused by DEMSO, a significant reduction in the concentration of DMSO is associated with an improvement in the total number of recovered cells, compared to the standard DEMSO used method, which was reported by Murray et al. [39].

While Shivakumar's study suggested another way to reduce such toxicity, which is using a cocktail of cryoprotectants (0.05M glucose, 0.05M sucrose, and 1.5M ethylene glycol), which showed higher cell survival post-thawing

cell and better expression of surface markers compared to using DMSO as a cryoprotectant [33].

Isildar et al also published a study about different cryopreservation protocols for umbilical cord blood as a source of MSCs and stated that using two different cryopreservation solutions 10% DMSO and 10% 1,2 propanediol (PrOH) was associated with lower toxicity and can be used as a preferred cryoprotectant instead of DMSO alone regarding the process of cryopreservation of the umbilical cord [40].

5. Conclusion:

Mesenchymal stem cell therapy represented a new revolution in regenerative medicine and tissue engineering and its promising potential in the treatment of multiple diseases. Cryopreservation represented a process of storage of MSCs which became a mandatory need to keep the MSCs safe and available for some time.

However, there was a huge debate about the efficacy of cryopreservation and its effects on the cell's viability, properties, and the difference between using fresh and cryopreserved MSCs cells.

The more accurate the cryopreservation technique is, the better result could be obtained from the cells. Added to the quality of the isolated MSCs used. That's why the stem cell bank takes care of the high standared cryopreservation technique and insists on the good quality of the isolated because of its impact on the outcome results.

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