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Fundamental principles of cryopreservation and stem cell banking

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ABSTRACT

Cryopreservation and stem cell banking have become increasingly popular topics in recent years. The purpose of cryopreservation is to store frozen cells or tissues with minimal damage to their structures and functions. With the ability to freeze and store mammalian embryos, the complete genomes of different species can be preserved for extended periods. People wish to take precautions for potential future health issues for themselves or their family members. When selecting a bank for collecting stem cells, several factors need to be considered. These factors include quality control and assurance protocols, the donor selection process, storage methods, compliance with international standards, and customer services. It is important to examine these factors before registering with a stem cell bank. Cryopreservation is a process of storing human tissues and cells at very low temperatures. This method is used to create a backup plan for using these cells in case they are somehow damaged. Cryopreservation technology is employed to preserve tissues, cells, and organs for an extended period in case of freezing or other types of damage. Stem cell banks, on the other hand, can be a crucial resource for individuals looking to reduce the impact of a disease or undergo stem cell therapy. Stem cells can assist in repairing damaged tissues and, in some cases, even aid in the treatment of diseases like cancer. Additionally, the storage conditions of stem cells are of critical importance. Stem cells must be protected from factors such as temperature, humidity, and other variables, and should be kept away from factors that may cause changes during the storage period. This review discusses cryopreservation and stem cell banking.

Keywords: Cryopreservation, slow-freezing, stem cell banking, stem cells, vitrification.

Biological and chemical reactions in living cells significantly decrease at low temperatures, which has generated great excitement and hope in the field of genetics for the potential long-term preservation of cells and tissues.[1] Cryopreservation is the use of extremely low temperatures (typically around -196 degrees Celsius) to preserve structurally intact living cells and tissues. The successful freezing and storage of reproductive cells, and tissues, which can be thawed when needed, is an event of great importance to humanity. Cryopreservation finds applications in various fields such as medicine, biotechnology, agriculture, the food industry, and environmental sciences.[2,3] The initial concepts related to sperm and embryo freezing emerged in 1776. The first successful human sperm-freezing procedure was achieved using glycerol in 1949 by Polge et al.[4] Following the freezing and storage of a human sperm sample with glycerol in liquid nitrogen in 1964, the first live birth occurred.[5] The first live cell freezing bank was established in 1972. The first healthy baby born from sperm that had been frozen and stored for more than a year and then thawed was born in 1973, and in 1998, a live baby was born from sperm that had been stored for over 20 years. In addition to this, cryopreservation of stem cells is also one of the popular applications today. Stem cell transplantation is used in the treatment of many malignant and non-malignant diseases.[6] This technique involves freezing and storing bone marrow cells for future use. Various freezing and thawing techniques, as well as various cryoprotectants, have been employed. Cryopreservation is also a treatment method that has the potential to increase the success rate in infertile couples.[7] This method should be tailored to the specific cell characteristics. Some of these methods include desiccation, pre-enlargement, desiccation-pre-enlargement, vitrification, and more. The slow freezing approach, the original method, was initially developed for the cryoprotection of plant tissues. The primary difference between rapid

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freezing processes and slow freezing processes is the high concentration of cryoprotectant substances used in rapid freezing. Some studies have discussed whether vitrification could be a good alternative to traditional slow freezing. The reason for seeking alternatives is the detrimental effects of slow freezing on both structural and functional sperm properties. Vitrification provides much better quality sperm recovery rates compared to slow freezing. Currently, there are numerous endangered plant and animal species, as well as species with endemic features. Cryogenic gene banks have been established as a solution to potential life issues that may arise in the future with regard to these species. Worldwide, especially in developed countries in the last 30 years, genes of various animal species have been preserved in cryogenic gene banks in the form of sperm or embryos.[8]

In the last 25-30 years, thanks to advanced laboratory facilities, the use of human stem cells in tissue engineering and the treatment of diseases has rapidly increased. These developments offer hope for some diseases that were once considered very difficult or impossible to treat. Significant progress has been made in these developments, which hold promise for the future well-being of many patients. Cryopreservation is an exciting technique that has the potential to be beneficial to numerous patients in the future.

STEM CELL BANKING

Stem cell banking is a medical facility that stores hematopoietic stem cells or other types of stem cells. It should have a high-level technological infrastructure for the acquisition, processing, testing, and storage of stem cells. Additionally, these facilities should conduct all their activities in compliance with international standards. These standards encompass various aspects, including globally accepted ethical principles and human resource management. Stem cells are used for a wide range of medical applications and can be obtained from different tissues in the body, such as bone marrow, blood, fat, dental pulp, placenta, liver, and brain. So far, hematopoietic stem cells derived from bone marrow have been the most extensively studied.[9]

Adipose-derived stem cells in clinical banking

Adipose tissue is a rich and accessible source of adult stem cells. The cryopreservation and banking of adipose-derived stem cells under the current good manufacturing practice conditions hold great importance for tissue engineering. Adipose-derived stem cells are routinely frozen and stored in a single-cell suspension. Research is needed to determine whether cryopreserved adipose tissue still preserves a sufficient quantity of viable and potent adult stem cells for clinical needs.[10]

Clinical banking of dental pulp-derived stem cells

Stem cells can be isolated from various tissues and organs today, including dental tissues. These stem cells include dental pulp cells, stem cells from exfoliated deciduous teeth, apical papilla stem cells, periodontal ligament stem cells, and dental follicle stem cells. Scientists reported no loss of differentiation capability in stem cells derived from frozen-stored pulp from third molar teeth. After being frozen for two years in liquid nitrogen, dental pulp stem cells were able to differentiate and proliferate normally. It has been determined that dental pulp-derived stem cells can be stored for at least six months at –85°C or –196°C without loss of functionality.[11]

Clinical banking of human amniotic fluid-derived mesenchymal stem cells

Mesenchymal stem cells are cells that can be isolated from various tissues in the body. While they were initially obtained from bone marrow, nowadays, they can be isolated from adipose tissue, cord blood, placenta, umbilical cord, amniotic fluid, peripheral blood, dermal connective tissue, and skeletal muscle.[12] The placenta is a complex structure. Many researchers have successfully isolated mesenchymal-like stem cells from various components of the placenta.[13] It has been shown that the amniotic fluid, which is surrounded by the amniotic membrane to protect the fetus, is also a rich source of mesenchymal stem cells.[14] Cryopreservation of the entire placenta can pose a significant technological challenge in terms of freezing

and thawing; however, minced placental tissue fragments can provide an alternative option.[15]

CRYOPRESERVATION

In relation to the freezing of biological materials, this method is commonly used in laboratory work. Freezing biological materials may be necessary for long-term storage or to have them readily available for continuous use. For example, live cell cultures, deoxyribonucleic acid, protein samples, or viruses can be stored at low temperatures and used when needed. The establishment of a stem cell bank should include cleanrooms that are well-organized and appropriately placed, with air systems designed adequately to prevent the spread of microorganisms. Stem cell banks should also be equipped with liquid nitrogen storage equipment, along with high-temperature alarms, to prevent the loss of cryopreserved cells.[16] Many stem cell banks, as they were established by blood banks, use blood bags for transport to clinical settings.

Legal and ethical aspects of cryopreservation

Cryopreservation is considered a legal procedure in many countries, while it remains controversial in some due to legal ambiguities. Therefore, legal aspects can vary from country to country. The establishment and design of stem cell banks have practical, financial, political, and ethical implications. The legal regulation of cryopreservation has been established under the "Regulation on Assisted Reproductive Practices and Assisted Reproductive Treatment Centers," which came into effect on March 6, 2010, prepared by the Turkish Ministry of Health. According to this regulation, except for medical necessity cases, it is prohibited to store male reproductive cells and gonadal tissues. In cases where surgical methods are used to obtain sperm, cryopreserved reproductive cells and gonadal tissues are recorded and destroyed by a commission established in the event of the individual's death, prior to surgeries that may lead to the loss of reproductive functions, such as removal of testicles, and before treatments that may damage gonadal cells, such as chemotherapy and radiotherapy. Although cryopreservation is allowed under certain conditions, many countries do not have a comprehensive legal framework for it. This situation can create a highly debatable and open-ended situation concerning ethical, health, and social issues related to the cryopreservation of human ovaries and embryos.[14-16]

Methods of embryo cryopreservation

Application of vitrification to human embryo freezing

Initially, the slow freezing method, which requires a gradual cooling, was used in the freezing of embryos. In vitrification techniques, the rapid reduction of temperature is of critical importance. Slow freezing, which is a traditional method, requires very expensive and complex equipment for embryo freezing. Over the past 10-20 years, research in the field of cryopreservation has been geared towards making the system more straightforward. As a result of efforts to simplify the procedures, in 1985, a system called vitrification for embryo freezing was developed. Vitrification creates a glassy state where ice crystals do not form, allowing cells to be directly plunged into liquid nitrogen for freezing. To prevent cell damage during freezing and thawing, various substances called cryoprotectants are added to the freezing and thawing solutions. In a study comparing *in vitro* and *in vivo* development rates of domestic animal embryos frozen by vitrification, favorable results were obtained in favor of vitrification.[17,18]

Traditional slow freezing

The most common method for preserving sperm is slow freezing. It has also been the most widely used cryopreservation method for human embryos to date. In this method, embryos are exposed to sub-zero temperatures at a controlled rate using low-concentration cryoprotectants. Controlled slow freezing is achieved until the embryos reach temperatures between –30 and –70°C using an embryo freezing machine. Once the desired temperature is reached, storage is done in liquid nitrogen (-196°C).[19]

The slow freezing technique can cause two types of damage to biological structures:

- Formation of ice inside the cells as a result of rapid cooling.
- Very slow cooling leads to an increase in solute concentration due to the formation of ice crystals in the medium.[20]

Cryoprotectant materials

Cryoprotectant materials are chemicals that assist in protecting biological material, especially cells and tissues, from freezing damage during storage at low temperatures. These materials prevent ice crystals from causing damage to cells and tissues during freezing. They protect the cells from freezing damage during the process but can be toxic to the cells.[21] Cryoprotectants can be effective both inside and outside the cells.[22] In cryopreservation, ice formation increases with the rate of cooling. Ice formation within the cell can lead to cell death. Without the use of cryoprotectant materials, damage cannot be prevented and can also lead to cell death. External cryoprotectants make cell membranes more flexible and resistant to osmotic stress.[23] This also prevents cellular swelling during the thawing process.

Cryopreservation stages

The cryopreservation process can be divided into the following stages:

- Initial interaction with cryoprotectants to reduce cellular damage from water crystallization.
- Freezing below zero degrees.
- Storage.
- Thawing.

The removal of cryoprotectants from the environment can be categorized into two stages:

- Advancement.
- Return to the physiological microenvironment.[24]

Sperm cryopreservation

Sperm cells can be stored live for an extended period after freezing and can be used in assisted reproductive techniques when needed. The first pregnancy achieved using frozen-thawed sperm was reported in 1953. Parkes and Smith[25] published an article stating that she had successfully frozen bull sperm using glycerol and achieved a successful pregnancy with the thawed sperm. As a result, research in this field has gained momentum.[26]

Sperm cryopreservation is used for various purposes, including:

- Storing samples from patients who will undergo chemotherapy and radiotherapy.
- Storing samples taken from azoospermic patients to prevent repeated surgical procedures for desired pregnancies after successful or unsuccessful surgery, etc.

Specific cryopreservation can be performed for sperm at different stages of development. The lipid and protein membranes of sperm released from the testes undergo physical and chemical changes that can have a negative impact on sperm viability after freezing. To prevent this, some researchers suggest adding antioxidants (such as ascorbic acid, catalase, vitamin E, etc.) to the culture medium.[27]

Oocyte cryopreservation

Women choose to preserve their eggs to use them in the future, mainly as they have undergone medical or surgical interventions that could jeopardize their future fertility, such as ovarian surgery or chemotherapy. The slow-freezing technique provided successful pregnancies from frozen oocytes in the late 1980s.[28] However, the success rates were low, and not much progress was made. The hardening of the zona pellucida presented another challenge. When vitrification emerged as an alternative to slow freezing, the damage decreased, and success increased.[29] Oocyte cryopreservation begins with the selection of a group of cells found in a woman's ovaries. These selected cells are then subjected to a special freezing process under laboratory conditions. This process has a protective effect, preventing cell damage during freezing. The frozen eggs can be stored and later thawed for use when needed.[30]

In conclusion, at this point, important strides have been made in cryopreservation studies. It is being used to safeguard genetic resources in the long term and has become a new field of study for humanity. However, it is crucial to avoid dangerous and unethical practices. Stem cell banking is of great importance for the storage of stem cells, preparing them for treatment, and their proper utilization during the treatment process. The collection, freezing, and preservation of these cells from various tissues, cells, and organs have brought about a significant revolution for humanity, and this process continues to evolve. It is essential for our country to focus on research and efforts in this field.

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