Chapter 10
Cryopreservation of Cell Lines

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10.1 Introduction

Metabolic activities have been observed to drastically reduce or cease to exist at extremely low temperatures in all living systems including humans; however such phenomenon if applied judiciously and effectively can result in ensuring long-term preservation of viable cells and tissues. Nevertheless, one of key and scientifically proven facts is that freezing alters the chemical profile inside the cells which summons distinct cellular mechanical constraints and damages which are fatal to almost all living organisms. One of the primary and the most crucial hurdles for animal cells at low temperatures is to overcome the phasic transition of water-to-ice which paves the way for several cellular injuries. Moreover there are several attributes which contribute to these injuries such as fast cooling rates leading to intracellular ice crystal formation, whereas slow cooling rates inflict osmotic damage due to concentration alteration in intracellular and extracellular solutions or hampered mechanical interaction between ice crystals and cells. Cryopreservation aids in maintaining biological samples in an animated suspended state at cryogenic temperatures for extended spells of time and simultaneously preserving cellular components. Several research studies have investigated and observed that cryoprotective agents (CPA) have distinct attribute which aids in altering cellular behaviour in cryogenic temperatures by influencing water transport, growth of the ice crystal and nucleation as cell survival in these freezing states depends upon several biophysical properties during the cooling and warming cycles of the cryopreservation. Apart from copious of such attributes, CPA also caters to several vital requirements necessary for ensuring optimum cell vitality under cryogenic environment, and some of the most common as

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well as successful CPAs employed in research facilities are dimethyl sulphoxide (DMSO), glycerol, protein and specific polymers. Modus of operandi for conventional cryopreservation has been found to be relatively simple which can be directly translated into several lab−/industry-level applications where large cell volumes can be subjected to cryopreservation in one vial however exhibit substandard survival rates. Therefore, despite having the potential of being an exceptional preservation method, it's slightly more complex than what it seems. Thus necessity to employ programmed freezing modules operating at specific and sustained cooling rates becomes imperative in ensuring cell vitality. Moreover, exploring novel cryopreservation techniques such as vitrification has also been developing in order to reduce cost and optimise several process parameters along the way. Though various evidence suggests that cryopreserved cells/tissues reap more benefits in research and future application as sustained availability of these cells ensures extensive quality control testing and reproducible results in cases of transplantation without the requirement to obtain fresh organ/tissue samples (Jang et al., 2017).

10.2 Fundamentals of Cryopreservation

The basic principle of cryopreservation deals with the utilisation of extremely low temperatures for the preservation and maintenance of cell's mechanical integrity for long storage applications. There are several cellular moieties which are classed depending upon their respective species, thereby imparting their influences on a distinct biological and survival response of the cells with respect to the cryogenic conditions and its subsequent thawing cycle. Moreover, for quality and reproducible results, it's necessary to identify some the characteristic attributes associated with the process of cryopreservation which are stated as follows:

- Prior to cooling, ensure proper mixing of CPAs with cells or tissues.
- Freezing and storage of cells or tissues at cryogenic temperatures.
- Thawing or warming cycle of cells or tissues.
- Efficient separation of CPAs from cell or tissues subsequently after thawing.

Amidst various extremities playing their part, the cooling rate of the process dominates all the biological effects as 80% of the cell's mass is occupied by water which gets converted to ice crystals. Such crystal formation generally employs either of the two processes heterogeneous or homogeneous ice nucleation as freezing has been found to be a nucleation induced event. Heterogeneous ice nucleation (generally at −35 °C) has been reported to be more prevalent in the process as compared to its counterpart, due to the higher thermodynamic stability offered by the random stacking of water molecule which further reinforces the stability than to cause thermodynamic decay. Homogeneous ice nucleation requires extremely low temperature (> -35 °C) to induce ice crystallisation catalysed by appropriate liquid or solid interface adjacent to the sample solution. Subsequent ice crystal formation has been observed to exhibit a pure crystalline water profile with no trace of

dissolved solute which encompasses the solute particles along with cells in a liquid phase at cryogenic temperatures. To ensure the viability of the cell samples under such temperatures reminds us the importance of CPAs in cryopreservation even more (Gupta et al., 2017).

10.3 Cryoprotective Agents

Copious research studies have been conducted to explore as well to elucidate the chemical and physicochemical profile of various cryoprotective agents. Detailed assessment of these parameters deduced a high biological acceptance rate towards CPAs having liquids or aqueous profile as such attributes impart least strain, high penetration along with stunted toxicity under cryogenic environment. There have been several neoteric research approaches selected to devise an optimised form CPAs which enables to reduce the ice crystal formation at lower temperatures depending upon various factors such as warming rate, cooling rate and cell types. To accomplish the optimum survival rate of cells, cooling and warming rates, volume and CPA concentrations ought to be enhanced relying upon the distinctive cell types and setting of tissues (Jesus et al., 2021). It ought to be referenced that the macroscopic components of the tissue is a significant highlight be elucidated in cryopreservation convention due to warmth and mass exchange impediments in these mass frameworks. CPAs can be isolated and classed into two primary classes: first and foremost, cell layer saturating cryoprotectants (cell membrane permeating), for instance, dimethyl sulphoxide (DMSO), glycerol and propylene glycol (1, 2-propanediol). There are certain non-permeating cryoprotectants as well such as

Table 10.1 Various attributes of commonly employed cryoprotective agents

Cryoprotective agents	Toxicity profile	Potential applied cryoprotective application
Dimethyl sulphoxide (DMSO)	Cell membrane toxicity	Adipose tissue, bone marrow, amniotic fluid, umbilical cord, hepatocytes, platelets
Glycerol	Renal toxicity/failure	Amniotic fluid, red blood cell, spermatozoa
Ethylene glycol	Pulmonary oedema and gastrointestinal irritation	Amniotic fluid, dental pulp
Cell banker series	Low cell membrane toxicity	Adipose tissue-derived stem cells, bone marrow, synovium, amniotic fluid
Propylene glycol	Impairment in developmental potential of germ cells	Embryo, hepatocytes

2-methyl-2,4-pentanediol and polymers which have been intricately classed in accordance with their applied cryopreservation application (Table 10.1). In contrast to engineered synthetics and biomaterials, this can be further utilised to obstruct ice crystal development, alongside conventional little particles. The immediate restraint of ice gem arrangement and utilisation of cancer prevention agents and different mixes have been utilised to endeavour to diminish cell passing from cycles leading to apoptosis during the freezing and defrosting cycle (Taylor et al., 2019).

10.4 Polymers

Amidst several modus of operandi, resuspending encapsulated CPA within the capsule in the wake of cryopreservation tends to serve as a complex technique in manipulating the cell's location in the solution. Recent developments in modern science has offered several exemplifying materials which could be potentially be engineered to synthesis non-infiltrating polymers which tends to provide protection to cells moieties within the scaffold at cryogenic temperatures and thereby eluding several constraints associated with diffusion at higher-dimensional cryopreservation applications. However, there are also various conventional polymer class vinyl-determined polymers which further encompasses polyethylene glycol (C2nH4n+2On+1), polyvinyl liquor [(C2H4O)n] and hydroxyethyl starch with subatomic load of 200–9500 Da, 30–70 kDA and 130–200 kDa, respectively. These listed polymers have reported to exhibit extensive capabilities in reducing the crystal size, thereby offering better alternatives than the existing polymers employed as CPAs (Fuller & Paynter, 2007).

10.5 Glycerol

Observations of glycerol having cryoprotectant credits were first expressed by Polge et al. in 1949. Even after decades of research and development, there have been quite few compounds which can serve as a potential alternative of glycerol. The significance as well as usefulness of glycerol is second to none and has remained as highly effective CPA until DMSO was assessed and exhibited in 1959 (Lovelock & Bishop, 1959). Several studies have revealed that glycerol comes across as a non-electrolyte, and thereby its expression is associated with a decreased electrolyte concentration of the remaining aqueous regions of the freezing solution. Such phenomenon is more frequently and consistently observed near as well as around all the sample cells at all temperature levels. Its primary and widely accepted application has been identified in chronic storage of animals as well as bacterial cell samples in medical facilities. The defensive attributes of glycerol have been observed to be restricted to substances that themselves don't exhibit toxic profile and have a low

atomic weight, a high solvency in fluid electrolyte arrangements and a capacity to penetrate living cells. The quantity of solutes equipped for satisfying these conditions is little; thus far glycerol most intently moves towards the ideal cryoprotectant agent (Hubálek, 2003).

10.6 Dimethyl Sulphoxide (DMSO)

Several studies have reported and evaluated the application of DMSO in cryopreservation of animal cells as a result of its minimal effort and relatively low cytotoxic profile which was originally reported by a Russian scientist Alexander Zaytsev in 1866. DMSO functions have been closely observed to be analogues to that of glycerol as both of the CPA facilitates their expression by lowering the electrolyte concentration in the remaining thawed solution arrangement in and around a cell at any random temperature. Moreover, a reduced viability rate along with acceptance of cell separation brought about by DNA methylation and histone change has been accounted for. Adipose-derived mesenchymal stem cells (ASCs) in recent research have exhibited attributes which contributes in the preservation of the properties of subcutaneous fat tissue especially effectively; thus cryopreservation is right now preceded as a standard strategy for safeguarding ASCs to securely gain enormous quantities of cells. Notwithstanding, numerous investigations have revealed that cell movement subsequent to freezing and defrosting might be influenced by the arrangements utilised for cryopreservation. Besides copious complex attributes offered by DMSO, the primary characteristic of DMSO-mediated cryopreservation is that it diffuses across the plasma membrane into the cell and shields the cells from any kind of impairments/damages caused in a cryogenic environment (Ishizuka & Bramham, 2020).

10.7 Proteins

Among others one the most promising recently discovered proteins which exhibited attributes similar to that of CPA is a sticky hydrophilic protein sericin having a molecular weight of 30 kDa. Sericin was derived from the cocoon of silkworm. Wide range of extensive applications has seen to be associated with this distinct protein; however, its potential as a CPA specifically for human adipose tissue-derived stem and hepatocytes is quite valued. Apart from sericin, a distinct protein harnessing attributes similar to antifreeze compound has been extracted from salt water teleosts/fishes which have been garnering a lot of attention due to its potential of being quality CPAs; however, there hasn't been enough detailed exploratory studies to state anything conclusive (Naing & Kim, 2019).

10.8 Primary Mechanism of Action of Cryoprotectants

Several research studies have been conducted aimed to explore as well as elucidate the basic functioning of CPAs. Glycerol and DMSO are two of the most popular and widely used CPAs categorised as penetrating cryoprotectants among the many others. They have been found to function by and large with small non-ionic particles with elevated levels of water solubility which facilitate easy diffusion across the plasma membrane and position themselves into the intracellular compartment. The preferred mode of their action was explored when the total concentration of the solute particles was observed to have remained unfrozen and possessed an independent profile of a partially frozen solution. Nevertheless various deleterious impacts of salts are straightforwardly identified with concentration, thereby diluting the concentration and subsequently supplanting a segment of the harming solutes in the partially frozen solution with a CPA, which will bring about less cryoinjuries at cryogenic temperatures. Reduction in the extent of ice crystal formation in the solution due to the presence of CPA would further enable the cell structure to be equipped when exposed to lower temperature let's say −20 °C than solution without CPAs. The cryoinjuries caused by the salts have been reported to be connected not exclusively to the concentrations yet additionally to the varying cryogenic temperatures. Accordingly, within the sight of a CPA, the cell will be presented to a similar toxic salt concentration, yet exposure will happen at a lower freezing temperature where its harming impact is diminished (Mandawala et al., 2016). Nonetheless, during the freezing cycle, salts are not by any means the only solutes to be concentrated – the CPA goes through a comparable cryogenic concentration and has appeared to add to the haemolysis of frozen defrosted red cells. Hence, insurance includes some significant downfalls in those convergences of CPA that in general have an advantageous defensive impact can likewise contribute to the "solution effect" injury at moderate cooling rates. Moreover, the proof for such effect has been documented in several prior studies, and different systems of activity have been proposed including adjustment of the plasma film by the CPA. Non-penetrating CPAs are for the most part can be referred to as long chained polymers along with heavy molecular weight that don't cross the plasma layer and stay in the extracellular compartment during freezing. The preferred mechanism of action for operation is most probably up for discussion; however, it has been credited to both a colligative impact and the pre-freeze drying out of the cell; diminishing intracellular water molecules apart from improving the probability of the sustaining cell's equilibrium by accepting loss of water molecules rather than having ice crystal formation is even worse with respect to cell survival. Elevated levels of compound viscosity due to addition of CPAs further helps to inhibit the ice nucleation during the cooling cycle and recrystallisation during the warming cycle (Li et al., 2010) (Fig. 10.1).

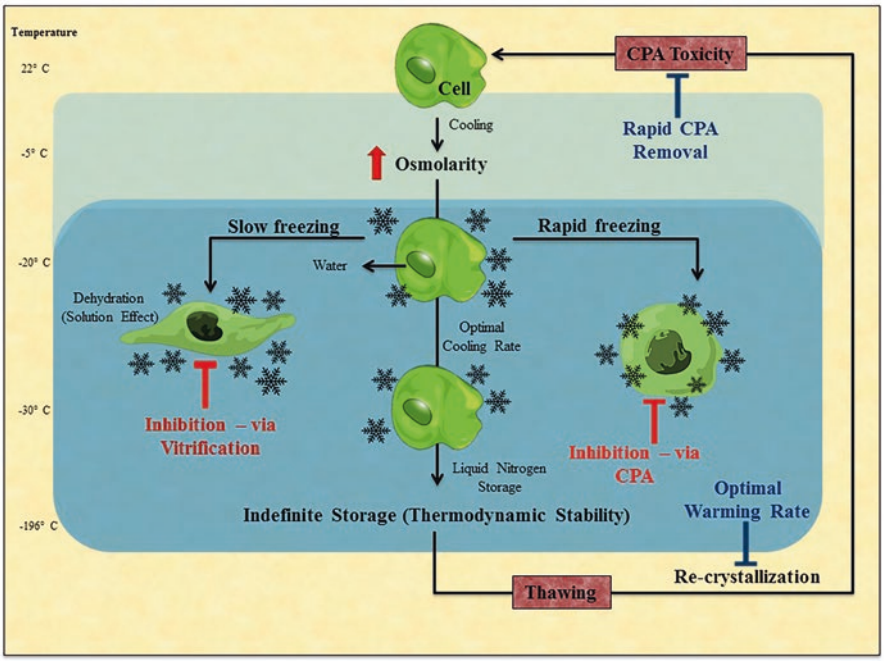

Fig. 10.1 Schematic representation of several facets employed in cryopreservation

10.9 The Cell Banker Series

One the recently devolved technology which is generally referred to as cell banker series (CBS) (established by Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) which was aimed to expedite the process of cryopreservation of cell samples at −80 °C and has been reported to accomplish better endurance and viability rates after subsequent freezing and defrosting cycles. This specific cell banker series of cryopreservation media holds distinct composition of glucose, an endorsed high-grade polymer, pH modulator and 10% DMSO. Moreover, their certain variations in distinct cell banker series on the basis of their constituents, for instance, CBS 1 and 1+ have been reported as serum-containing variants which can be utilised for the cryopreservation of a broad range of animal cells. To be sure, regular media employed for cryopreservation include the employment of foetal bovine serum which serves as a pool of copious cytokines, growth factors and vague substances (encompassing bovine exosomes) which made it illegal and unsafe in the foundation of a normalised cryopreservation convention for any kind of clinical application/experimentation associated with human subjects. While CBS2 serves as a potential alternative to CBS1 as it employs non-serum-type media for cryopreservation in a culture condition specifically engineered to compliment CBS2 which is a serum free environment. CBS3 which is more commonly also known as stem cell

banker is made out of 10% DMSO and other inorganic mixes (US20130198876) and fulfils the model of artificially characterised chemical components which are free from any xenobiotic origin and is subsequently appropriate for induced pluripotent stem cells and protection of somatic stem cells.

10.10 Cryoinjury

Cryopreservation indulges in the phase change of water within intracellular, and extracellular at cryogenic temperature leads to serious cell damage. There have been several damaging factors such as cooling and thawing velocities which are the primary cause in inflicting biophysical and physicochemical interaction-mediated impairment and further deteriorate the cell survival rates. Apart from these factors, osmotic rupture in the cell tends to allow ice crystal formation which has been observed to be directly regulated by the cooling rate. Additionally retention of semipermeable attributes exhibited by plasma membrane tends to aid in illustrating comprehensively the cell viability; however, retention of such properties still might not be able to influence the survival rate of organelles within cells. Several reports exploring the role of CPAs have clearly articulated various types of damages posed by the cryoprotectant themselves. The cyclic entry and exit of CPAs significantly cause the cell structure to expand and shrink sequentially causing the damage to the cells (McGann et al., 1988).

Clinically, DMSO has appeared to trigger several extreme allergic responses in patients which were reported to be treated with CPA-infused haematopoietic stem cell samples and also has additionally appeared to incite apoptosis machinery in specific locations of the central nervous system in mouse models. Numerous facilities have preferred to employ a relatively diluted form of DMSO before reinfusion of stem cells into beneficiaries, especially youngsters. Cells also can be influenced by varying molar concentrations of CPA which in general serves as a protecting shield for cells from freezing and defrosting cycle associated with cryopreservation. Differentiation process is strongly induced by DMSO on haematopoietic stem cells along with several other cells in vitro; furthermore, this process is associated with triggering apoptosis. The primary cause for initiation of both these processes is employment of DMSO at low concentrations for long spells of time frames at raised temperatures (+37 °C). Henceforth variations were reported with respect to the epigenetic profile of stem cells, and moreover the concentration ranging from 0.5 to 2 M has been seen to function at cryogenic temperatures during cryopreservation which offers apt exposure time and optimum survival chances along with temperatures which are limited to those adequate to permit equilibration of the cell. An elevated level of concentration utilised to accomplish vitrification tends to exhibit a serious toxic and osmotic profile which further requires various ways to deal with their presentation and expulsion (Yavin & Arav, 2007).

10.11 Varied Application of Cryopreservation Technique on Special Cell Cultures/Cells

Contemporary past has witnessed the evolution of copious neoteric applications of cryopreservation with respect to varying research field areas encompassing biochemical molecular biology, food technology, cryosurgery and ecology and plant anatomy along with several biomedical purposes (blood transfusion, artificial insemination, in vitro fertilisation (IVF) and bone marrow transplantation) with the primary application being cryopreservation of cells. Scientific communities in coherence have comprehensively observed the potential of cryopreservation in developing elaborate cell bank facilities for better and rapid analysis for human leukocyte antigen (HLA) typing during the organ transplantation process (Keck et al., 2011). This further enhances the distribution process efficiency of viable cell samples amidst various medical facilities. One of chief growing areas of application is the long-term storage of stem cells which further caters to several ancillary applications such as tissue engineering and cellular therapies. Besides that, there are various animal cell types associated with their specific application in relation to cryopreservation in modern medicine and are mentioned as follows.

10.12 Female Germ Cells (Oocytes and Embryos)

The principal instance associated with human embryo cryopreservation aimed to safeguard the fertility was reported in early 1996, which employed in vitro fertilisation on a female subject which was diagnosed with breast cancer, thus operating before undergoing chemotherapy. In the wake of 1996 success, cryopreservation of mature female germ cells has been viewed as a way of safeguarding the reproductive capacity of female subjects. Moreover, several retrospective exploratory studies have been conducted during the time frame of 1986 to 2007, investigating the efficacy and viability of the technique (Mandawala et al., 2016). The researchers didn't observe any conclusive evidence to support that the process of cryopreservation has affected or has any significant influence over pregnancy, childbirth, labour, birth rate or even miscarriages in comparison to that of natural or IVF. However, the results are highly promising; still scope of improvement and modification remains (Chang et al., 2017).

10.13 Male Germ Cells (Sperm, Testicular Tissue and Semen)

Last decade witnessed and faced several issues regarding the germ cell applications, not due to the lack of scientific resources but always depleting sample germ cells due deficient techniques which were unable to guard the sample cells from toxicity (chemical and physical) and infection/contamination during the course of experimentation, thus, the suboptimal success rate. However, nowadays cryopreservation has resolved the related concerns and ensured fertility as well quality of patients undergoing cancer treatment which involves radio and chemotherapies. Research has shown that proper cryopreservation of sperm and semen samples can preserve the vitality along with viability for unlimited periods of time. Moreover, novel trials encompassing testicular tissue-centric cryopreservation have been reported under early clinical phases. The trial focuses on achieving cell suspension of testicular tissues which further been subjected to cryopreservation, although in early stages yet trials hold huge potential for offering as well as safeguarding fertility of copious men facing therapeutic altercations which elevates the probability to compromise their fertility (Alotaibi et al., 2016).

10.14 Hepatocytes

Fundamentally there have been several significant discoveries in the field of research and medicine focused on hepatocytes centred application which have been documented in the last four decades, amidst various few of the primary applications encompassing varying physiological inspection of liver-related attributes such as metabolism, drug toxicology profile and organ/cell preservation along with other clinical approaches. Moreover, recent times have garnered a coherent support for developing cryopreservation facilities for hepatocytes in wake of soaring interest in liver-specific research along with dedicated employment of biotechnological aid to further expedite the related clinical applications (Ibars et al., 2016).

10.15 Stem Cells

Copious amounts of evidence support the potential application of cryopreservation specifically which has been reported to be associated with adult stem cells. Extraction of these cells has been primarily subjected to locations such as the umbilical cord, adipose tissues, bone marrow and amniotic fluids apart from several other sources of extraction. The primary functional profile of most stem cell types (such as stromal, embryonic, mesenchymal, etc.) makes them ideal candidates which cater to the requirements necessary to be employed as therapeutic altercations in

regenerative therapy. Although it's not just regenerative medicine; besides this there have been several others highly expensive as well as exclusive therapeutic medical applications which have been found to be closely associated with stem cells such as tissue grafting and engineering, gene therapy and organ transplantation. Thereby making a sustained and quality supply of stem cells even more important and therefore cryopreservation of such cells is one of the most valued applications in recent times (Li et al., 2010).

10.16 Other Frequently Cryopreserved Cells

Amidst several animal cells, there are some distinct classes of cells which extensively witness cryopreservation due to their lack of availability, and thus different neural cells as well as cell lines along with cardiomyocytes are being routinely utilised at medical and research facilities across the globe. Yet a best-quality level methodology for safeguarding their vitality has not been elucidated. Neoteric studies dealing with CPAs have revealed certain alternative therapeutic regimes avoiding any involvement of glucocorticoid immunosuppressive agents; however, a potential transplantation of pancreatic islet may serve as an ideal treatment of type I diabetes. Therefore, the improvement as well development in islet cryopreservation strategies has been progressing; however, the results reported weren't that promising, and one of the factors contributing to that was the suboptimal survival rate of the treated cells (Taylor et al., 2019).

10.17 Optimum Cooling Rate

The expressions moderate as well rapid are generally considered as relative and closely identified with the permeability of water along with the surface/volume proportion of the varied class of cell which is subjected to the process. Cooling operated at a moderate rate has been observed to be characterised as cooling at suboptimum pace beneath which cell could sustain a constant responsiveness with respect to steady change in temperature together with the resulting expansion in extracellular ice crystal formation, exclusively caused by efflux of water which further contributed to dwindling of cell. Operating under the influence of such conditions harm is inferable from arrangement impacts caused straightforwardly or in a roundabout way by the raised solute concentration. The impacts of moderate cooling injury aggregate with expanding openness time to harming solute focuses. Accordingly, expanding the cooling rate will bring about more limited spells of exposure that further elevated the endurance rates. Fast cooling might be characterised as any pace of cooling above which the cells neglect to keep up synthetic harmony by diffused water flow along with the balance re-establishment via nucleation further leading to ice crystal formation inside the cells. These newly formed ice

crystals would rely directly upon the subjected warming rate yet tend to prompt deadly occasions and diminished endurance. Endurance will keep on being diminished as cooling rates keep on expanding. The ideal rate of cooling is subsequently a trade-off amidst the two contending factors, primarily when the cooling is sufficiently fast to decrease harm caused by the solute impacts however tapered down to lessen rate in the cell populace.

10.18 Influence of Warming Rate

Warming rate can significantly influence the survival rate of the cells which were subjected to cooling at elevated rates post-defrost. Here, fast rewarming considerably elevated endurance contrasted with rewarming gradually. Several studies explain distinct facets of a relatively rapid warming rate which limits ice recrystallisation, consequently ensuring at the same time negating the conversion of tiny intracellular ice crystals to develop into sizable crystals which could result in cellular damage; sluggish rate of warming generally offers additional time at raised cryogenic temperatures which aids in the process of recrystallisation. Rates of warming have been observed to impact the cooled cells which is much more complex as a quick rewarming tends to exhibit analogous expression to that of cells which were cooled quickly, i.e. expanded endurance when operated at moderate rewarming rates. Studies have revealed the application of specific models where the influence imparted by the warming rate on the cryopreserved cells tends to exhibit more or less null effect which eventually proves to be extremely helpful in preserving cell's attributes (Jang et al., 2017). Cells which have been subjected to a steady rate of cooling have been observed to experience antagonistic effects of warming rates on the cell's survival rate as the damages or injuries incurred by the cell during its time at cryogenic temperatures get re-exposed during thawing. Thus, the process becomes extremely time-sensitive, and any kind of delay due to the sluggish rate of warming could further deteriorate and damage the cellular conformation of the cryopreserved cells (Hunt, 2017).

10.19 Limitation of Cryopreservation

Albeit various uses of the cryopreservation methods exist, both in fundamental and clinical examination, a few limits actually exist. At cryogenic temperatures, the cell tends to cease all metabolic activities which has inescapable results which eventually pave the way for several associated complexities encompassing specific genetic alterations correlating to impairment to the cell's structural as well as functional conformation (Li et al., 2010). Generally, it has been witnessed that CPAs themselves can be harmful to cells, particularly when utilised in soaring levels of concentrations. Many exploratory studies have firmly suggested the likelihood of DMSO

expression may change chromosome steadiness, which can prompt a danger of tumor pro-genesis. Besides copious unwanted endogenous alterations in cell, the conceivable disease or contamination at the cellular level leads to several discrepancies such as tumours which ones ought to be forestalled (Luhur et al., 2019).

10.20 Vitrification

Although cryopreservation is one of the most preferred and favoured means of cell preservation, however, there are certain limitations such as ice crystal formation which significantly damage cell moiety and reduce cell viability; therefore an effective alternative to this method is vitrification. This is a process where the sample solution gets solidified without ice crystal formation. Broadly vitrification requires two primary criteria for successful attempt towards cryopreservation which includes highly concentrated solute systems and elevated cooling rates which evade ice crystal formation via ice nucleation inhibition due to raised viscosity levels. Sustained cooling rate leads to a steady increase in the solution's viscosity until the intermolecular motion is halted and solution turns into glass which displays all the prominent attributes of solids, yet retention of all molecular structures of liquid has been observed to be ensured (Reubinoff et al., 2001). Vitrification caters to several needs of the researchers by ensuring limited or no intracellular damage of ice crystals, henceforth subsequently evading varying types of solution effect injuries. Process is relatively cost-effective as no expensive and intricate equipment is needed if compared against traditional cryopreservation processes. Numerous mathematical models were utilised, and numerous rich sluggish cooling conventions were intended to make these stepwise systems successful for the cryopreservation of various kinds of cells and tissues. Be that as it may, ice ought to never be permitted to show up and develop inside the cells or tissue as this prompts harm and demise of the living framework. Realise that the last objective of both lethargic cooling and vitrification is the equivalent: to actuate a glasslike cementing inside cells to shield them from harm by ice gems at all phases of cryopreservation, which further guides in investigating and broadening the extent of its application at both lab and industrial scale. Nevertheless, there are copious serious complications associated with the process too such as its high toxic profile along with critical osmotic damage to the cellular structures which reinforces the apprehensions regarding vitrification and limits its application (Hunt, 2017).

10.21 Conclusion

Cryopreservation has been viewed as a relatively simple yet significant process which primarily aids to sketch a comprehensive exploratory analysis with respect to extraction and efficient employment of cryopreserved cells along with several

ancillary applications such as optimising the delivery of mechanism of cryopreserved cells and establishing facilities like cell banks to ensure quality and sustainability of clinical research approaches. Contemporary times have necessitated as well as validate the employment of optimised cryopreservation strategies along with the optimised logistics facilities. Vitrification has been viewed as a chief candidate which in the future can be the potential alternative for cryopreservation. The process has reported to negate all the major limitations associated with cryopreservation and also exhibit a phenomenal recuperative rate, yet there are still no concrete/standard protocols to optimise vitrification. Another major concern associated with vitrification is its inability to withstand scale processes which drastically limit its application to laboratory scale. Importance of optimising vital factors influencing the process of cryopreservation is necessary to attain higher levels of cell survival and cell vitality. Factors such as CPAs, toxicology and osmotic profile along with cooling and warming rates are the chief modulators operating the proper functioning required to achieve quality clinical applications. This chapter discusses several facets of cryopreservation encompassing copious strategies employed and role CPAs. Sustained efforts in exploring novel CPA were made which significantly contribute in determining numerous inalienable toxic profiles of many known specialists. Superior comprehension of the chemical as well as biochemical profile dictating the cryopreservation and warming rates would be essential for any kind of modern future advancements. Effectiveness of the process protocols highly influenced by the employed animal cell sample which is an essential part in examination associated with clinical utility for a wide range of human preliminaries. All in all, unmistakably all the future endeavours aimed with respect to cryopreservation must be zero in on the improvement in facilities along with application which would significantly influence the reproducibility, efficacy and popularity of cryopreservation not only in scientific but also amidst leman population.

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