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Research Article

AN OVERVIEW OF TISSUE CRYOPRESERVATION'S CONTRIBUTION TO ORGAN CRYOBIOLOGY

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ABSTRACT

Background: Scientists and physicians have long faced challenges in their quest to identify the most effective methods for cryopreservation of living tissues and organs. While cryopreservation techniques have been used since the 1800s, they are still used for tissue preservation for a comparatively limited time. More advancements in biotechnology for tissue and organ preservation have been made possible by the availability of commercially available liquefied gases. Typical issues such as sub-zero or cell damage and re-crystallization, which ultimately results in tissue loss, impede the usefulness of cryopreservation in the field of medical sciences. However, modern cryobiology advances have provided many alternatives for chilling tissues, organs, gametes, and embryos. As tissue sensitivity to ice is more understood, safer freezing methods are used. **Methods:** This review discusses how tissue cryopreservation advances biotechnology. The effective banking and transplanting of living tissues and organs using cryopreservation will be a crucial and noteworthy development in the fields of biotechnology and medicine in the future. One of the most crucial elements that will enable future cryobiologists and biotechnology researchers to make enormous strides is the preservation and storage of living tissues from the point of donor removal through transfer and transplantation. Future cryobiologists will find this ancient tale fascinating as it unfolds, thanks to the discovery of anti-freezing proteins and peptides in cryopreservation's active zones.

KEY WORDS

Cryopreservation, Cryobiology, Biotechnology, Sub-zero, Cryobiology

1 | INTRODUCTION

The new turn was taken by this enthralling cryostory when of liquid nitrogen were tested and used conventionally by the scientists for semen preservation. It was also observed that due to lack of appropriate and accessible

technologies for the preservation of semen, prolonged semen storage in the liquid nitrogen by the help of semen extenders or in a solid state, cryopreservation have gain very low attraction from the audience in spite of their further probable interests of large scale commercial poultry keepers to enhance reproductive management in poultry breeder flocks[1].

Basically tissue cryopreservation turn out to be a full of dynamism zone of medical research in the mid of 19th century as a result of the subzero preservative property of glycerol by Polge, Smith, and Parkes in 1949[2]. Now a day's embryo cryopreservation and transfer technique in domestic animals has been become a routine technique in and has proven its effectiveness in terms of pregnancy and Fe-male and male fetus pregnancy rate and increased of births in domestic animals[3]. Although cryopreservation techniques were already been used for young cancer patients[4] but there were significant drawbacks to its use, as tissue fixations with chemical fixatives have usually cause damages which can be identified in morphological and immune histochemical analyses of fix tissue. To overcome the problems caused by the chemical fixation in histomorphology, because of the relatively long fixation time quick-freezing (QF) methods have often been used, especially for analyses at the ultra-structural level[5,6]. To reduce these issue work was continued and from 1990's and the new methods were introduced, for example, (O-Cryo) method, Cryofixation, Cryo-substitution without fixatives and cryoembedding in the new Lowicryl resins (KI IM or HM23). These are the set of methods for the correlation of new structural information with the biochemical and analytical data. These three cryo-methods were complex and lavish at that time.

According to the up till now known mechanism of action of tissue cryopreservation, within frozen tissues of plants and animals large sized ice crystals have more physical damage possibility[7] but this mechanism of action is not fully known. According to the recent theories there are many episodes of this cryostory to know its mechanism of action. Scientists have shared their experiences such that overwintering animals and plants adopt two strategies such as freeze avoidance and freeze tolerance for survival at subzero and very low temperatures[8]. Research conducted in the previous year's suggest that tissue and organ cryopreservation can be applied in a systematic way, in some cases with success rates which appear to compete with those routinely achieved with organs freezing. Since there were some challenges against organs freezing, especially with respect to its safety in implantation. Some investigations have been carried out to note the possible freezing-stimulated alterations of cells which ultimately effect viability of tissues and organs whether directly or indirectly. From medical perspectives, the options by which the stored material may effects the clinical results of organs and tissues preservation for implantation, and make the comparison between different preservative practices will be helpful in the field of medicine in various banking and implantation techniques.

Now a day's due to accessibility of commercial liquefied gases and development of freezing media huge development have been established in the field of cryobiology with plenteous ease for cryo preservation scientists. But still the problems like cell injury or sub-zero injury and re-crystallization are hindering like fish thorn in the practical applications of cryopreservation. In this medicinal purview whole process and science lead to the episode of tissue destruction. But recently procedural development in this fairy tale of cryopreservation has opened up many windows and increases the numbers of characters for this marvelous icing act.

2 | PAST HISTORY OF CRYOPRESERVATION

There were many challenges in the process of cryopreservation with respect of its safety in transfer and implantation. Some investigations have been carried out to find out possible subzero temperature made alterations of cells which might consist the efforts in as the crow flies or snakingly. A solution was designed by a group of researchers to control the intracellular potassium loss and avoid cell swelling of cell at lower temperature levels; they studied frozen corneas and thawed by three different methods and compared by full thickness transplantation as well as studied the histological structure with the help of transmission electron microscopy and specular microscopy. Similarly another technique in which potassium-rich buffered solution having the cryo-protectant Dimethyl Sulphoid was used for the immersion of excised cornea[9-10].

To test different methods of cryopreservation, another technique named as (3-Cryomethods) were tested and they found that they can be performed well with homegrown systems on an affordable rate. These processes have been conducted on animmune-cyto-chemical and structural study on the oviduct of the egg laying quail. The data obtained by these (3-Cryomethods) models were compared with results gain from the other immune-cyto-chemical methods and preservation techniques including the conventional chemical fixation methods and low or high temperature embedding or with other cryopreservation methods known as cryofixation and cryosubstitution[11].

Health Sciences Journal
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From the investigations of last four subsequent decades in this specific field, the advantages and implementation of ice-free cryopreservation and vitrification have turned out as clear and superficial. Just before this era the experimental attempts were made to use cryopreservation techniques to vascularize the whole organs have been restrained nearly at all[2]. It was proved that compare to other surgical resections, cryo-surgical techniques were more save having low morbidity and less invasive[12]. After the knowledge of local cooling application for pain control and commercial availability of liquefied gases enhanced development was made in the field of tissue vitrification for localized lesions. With the knowledge of freezing action and disease response the technique was promoted and utilized for different abnormal conditions like bronchial cancers and prostate disease[13].

"In-vivo Cryo-technique" (IVCT) was used to examine living mouse livers forultrastructural, immune histochemical and histochemical analyses. In contrary, perfusion-fixation was used to dilute the sinusoidal cavities while quick-freezing (QF) and immersion-fixation methods of resected tissues collapsed it[14]. Under normal circulation of blood, enlarged open sinusoids along with number of flowing erythrocytes were detected in the samples prepared through the IVCT. Their blood congestion and collapse was observed in heart halted or ischemic mice too. The mice grafted with rodent testis tissue were autopsied after 50th day and collection of blood samples was carried out. 75% of all mice iso-grafted were found with morphologically normal seminiferous tubules and testicular tissue having some degree of spermatogenic activity. After enzymatic disaggregation mature spermatozoa were recovered. Spermatogenesis was fully normal in grafts obtained from the immature rodents while limited in case of hamster and adult mouse tissues[15].

Use of tissue slices in culture media has reduced the utilization of animals and experimental variations in health related experiments. This was possible only when the cold and cryopreserving methods of tissue slices were normally functioning. Carried out of sequence wise in vitro experiments of tissue slices were possible as organ-specific biochemical processes, organ-specific toxicity or xenobiotic metabolism. To investigate the said purpose slices of kidneys and livers of dogwere kept at zero degrees C cold storage for 10 days using Euro-Collins (EC), Sacks + prostacyclin (SP), Viaspan (UW)and V-7 (V7) cold-preservation solutions. Every day, viability was measured by calculating K+ content and after every 4hrs protein synthesis was measured in Waymouth + 10% fetal calf serum (FCS) during incubation[16].

3 | FIELD OF MEDICINE AND CRYO-LABS

The cryo-ultra microtomy in combination with fast-freezing techniques was carried out as a result well-preserved block faces of the original biological material was recorded. This study showed that block faces did not have any of the artifacts associated with cryo-sections. It was also obvious that post evaporating, in the electron beam carbona and a heavy metal arose superficial are stable to provide large surface areas with high-resolution images for statistical analysis in a cry-Scanning Electron Microscope[17]. In birds, study was done on the selection and storage of sperm. Semen of the low-quality males or semen inappropriate for in-vitro storage is discarded rapidly, that would result in impaired fertility if used in flocks[1]. In another study it was mentioned that to examine the post cryo-treatment of skin the change of extracellular matrix, the multimodal multiphoton microscopy was used. With the help of Dorsal Skin fold Chamber, post cryolesion in vivo and in real time during the healing of wound process of mice wound the intercellular interactions and cellular matrix transformations were measured. To find out the cryosurgical response in-vivo, two-photon excited (TPE) auto fluorescence and intrinsic second collagen from cell were obtained[18].

In the same years' work was going on in different aspects of cryopreservation and main focus was laid on grafting procedures and skin banking received the number of audience. When this cryo-tale was in the way of advancement, two techniques were used to cryopreserve the biopsies from the skin of six live, anesthetized brown bears. Single biopsy obtained from every animal was further cut into three small pieces and distributed into three groups classified as untreated fresh, vitrification and freezing. No differences was found in case of cell attachment while both fresh culture and freezing were allowed for higher cell propagation and less days to reach 70% - 80% convergence than verification[19].

Different solutions were designed by different scientists to observe different artifacts in cryopreserved tissues. To select a better way in investigational optometry and ophthalmology via light microscopy alterations were observed on corneal tissue for the use of resin-embedded blocks and paraffin-embedded. In this work Corneas from two cynomolgus monkeys (Macaca fascicularis) were used and formalin-fixed cornea was prepared with the

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conventional tissue processing protocol named as (4-day protocol) and H&E stain was used for staining. In second trail corneas were fixed with glutaraldehyde in resin block with the help of modified and rapid tissue processing (1.2-day protocol) and toluidine blue was used for staining. The results of paraffin-embedded sample were undesired as damaged tissue and abnormalities of artifact including thinner epithelium and the distorted endothelium as compared to resin embedded corneal tissue[20].

The experimental study was conducted to find out the morphological effects results for bone tissues by the use of various liquid nitrogen cryosurgery procedures. Wistar rats were used to check the bone necrosis increase femoral diaphyses . For this purpose three sequential and local methods of liquid nitrogen were applied for 1 or 2 min and then passive thawing of 5mins was intercalated. The animals were sacrificed after 1, 2, 4 and 12 weeks of this treatment, animals were euthanized and specimens were collected for processing and histological analysis[21].

From local abattoirs bovine ovaries were sampled and with the help of aspiration method Cumulus Oocyte Complexes were collected from 2-6mm follicles. This trail was done to evaluate the changes in the structure of immature oocytes by the effects of vitrification and slow freezing methods. Then selected COCs were distributed into 4 random groups and named as vitrified thawed, vitrification solution-exposed, frozen-thawed and freezing solution-exposed. Oocytes structural changes and abnormalities were examined with the help of a stereomicroscope[22].

But as long as medical perspectives, these alternatives may be consider and these considerations may be led to further establishments in the field of cryobiology by which the stored live material can significantly influence in the solid therapeutic outcome of organs and tissues storage for implantation/ transfer and make the contrast between different conserving capabilities. It will help the medicine in giving different new tissue banking and implantation strategies to present cryobiologists and surgeons. The tale not finishes here but gets a boom by entrance of new character of AFP in scene and the discovery of Antifreeze proteins by scientists.

4 | EMBRYOS VITRIFICATION AND ANTIFREEZE PROTEINS

The cryopreservation of embryos significantly influences the generation of mammalian embryos through techniques such as in vitro fertilization, as well as more advanced technologies like embryonic and somatic cloning, and preservation. The technique of embryo cryopreservation is currently being used in dairy animals and equines to store the sperm, eggs, and embryos of exceptional parent animals and endangered species. Studies have shown that cryopreservation of embryos using AFP significantly enhances their ability to withstand freezing at low temperatures, such as 0°C. The study of cryopreservation in male and female gametes has emerged as a significant subject of interest in various sectors, such as reproductive clinics and in vitro fertilization centers. Regarding cryopreservation, researchers utilized AFP in laboratory animals, indicating the potential for valuable experimental systems[23]. A complex and poorly understood process of intra-ovarian development spanning several months results in the production of viable oocytes in humans. Performing AFP cryopreservation and in vitro transplantation to simulate conventional oogenesis and folliculogenesis remains a significant field of study that presents challenges[24].

The primary purpose of cryopreservation using AFP in mammalian embryos is to store them at low temperatures ranging from 0 to 4°C. AFP has not yet been practically applied to sub-zero temperatures such as -196°C or the storage of liquid nitrogen[25]. An endeavor was undertaken to freeze and preserve bovine and horse embryos using either AFPs alone or with glycerol. The results revealed no noteworthy distinction between the two groups, whether AFPs were used or glycerol was added. Sheep embryos stored at a temperature of 4°C in the presence of AFP showed comparable rates of embryo survival and similar rates of pregnancy compared to fresh embryos.

The cryopreservation of zebra fish embryos through microinjection has been found to greatly enhance their ability to withstand freezing temperatures, such as 0°C, when using AFP[26]. The researchers cited the discovery that AFP has the ability to safeguard the structural morphology of cells and maintain the integrity of cellular membranes[27]. A recent work conducted by Shah et al[28] successfully extracted native peptide fragments from antarctic yeast AFP, yielding highly promising results.AFP cryopreservation has the potential to be a successful technique for preserving gametes and embryos. However, further research is needed to fully understand the function of various AFPs and their fragments. Cell replacement is an emerging therapeutic approach for several neurological illnesses, aiming to address the damage or absence of cells. The cryopreservation of brain cells and tissues has not achieved a level of success that would allow its integration into regular clinical practice. Isolated brain tissue has a limited duration of

viability, and cells are often utilized for research promptly after isolation or stored in a refrigerator for a brief period before utilization. Neural tissue can be stored at a temperature of 4 °C for a maximum of 8 days, ensuring its decent survival[29-30]. Consequently, it is crucial to create storage strategies that can maintain its viability for extended periods. The cryopreservation of primary brain cells would significantly enhance fundamental neuroscience research[31]. Cryopreserving brain tissue would also enable the consolidation of tissue from several donors, providing ample time for essential testing to guarantee the safety and functionality of the cells before transplantation. Neural tissue engineering would greatly benefit from successful cryopreservation, since it would enable the long-term storage of neural tissue for inventory control, quality control, and product distribution purposes[32].

In 1953, Luyet and Gonzales conducted the initial experiments on nerve tissue cryopreservation. They successfully established that chicken brain tissue could survive freezing at a temperature of -196°C after being exposed to EG and rapidly cooled[33]. Several studies have assessed the qualitative result of vitality Paynter, et al., 2009; Robert et al., 2015) or cell membrane integrity[31]. However, only a limited number of writers have thoroughly examined cryopreserved cultures. Post-thaw viability, as determined by membrane integrity assays, may not accurately indicate the overall quality of the cryopreservation process. Therefore, it is essential to conduct suitable functionality tests to assess the effectiveness of the operation. For instance, Higgins et al. conducted a qualitative assessment of the creation of neuritic trees[29] whereas Quasthoff et al[32]. performed a parametric analysis of the morphological and functional development of the cultures[32]. Furthermore, the cryopreservation of structured adult cerebral tissue slices holds promise for the assessment and advancement of pharmacological neuropsychiatric drugs. Pichugin et al. shown that vitrification can effectively maintain the survival and structure of fully developed, organized, and intricate neural networks[34] .

5 | FUTURE OF CRYOPRESERVATION TECHNIQUES AND ITS UNKNOWN MECHANISM OF ACTION

Antifreeze proteins were silently entered in the cryolabs and lose their audience in the first instance, with passage of time it gets a vast attention in a very little time but due to not altered mechanism of action of Anti-freeze proteins and peptides are still riddles to the scientists of modern era. In a study it was stated that antifreeze protein (AFPs) a specific material produced by some creature of extreme or sub-zero temperature habitats like as some plants, invertebrates, vertebrates, fungi and bacteria etc. AFPs are responsible for protection of the cells and fluids of body from freezing. AFPs have the great variation in structure and sequences but common functions[35]. Overall growth of ice is significantly affected by the interaction of ice crystals and AFPs (Davies et al. 2002). Basically AFPs lower the Freezing point of water and resist the growth of ice crystals without changing the Melting point. In the result of this process, a difference between melting and freezing points developed, called as thermal hysteresis (Urrutia et al. 1992). In thermal Hysteresis, each AFP has its unique properties. AFPs resist and stop the ice recrystallization results in the formation of large ice crystals on the cost of smaller ones[36].

These natural biological antifreezes are classified into two different categories on the basis of carbohydrate moieties, one is called antifreeze glycoprotein and the other is called antifreeze proteins. Even though particular facets for AFGP and AFP are not well under stood that they show will be very helpful in preservation of living tissues. Still these methods are not able define the dynamics of adsorption. Hence, in initial postulates, it was proposed that proteins were adsorbed permanently in the particular facets. Irreversible binding characterizes of protein and their abnormalities were examined under a stereomicroscope[22]. Numerous peptides fragments were designed up till now based basic AFPs native strings structure and sequence of amino acids that are like secondary helical structure. It is necessary to know about the structure of peptides and its effects on the application and actions because these have a huge variation of determinable antifreeze activity. The extents of helical structure in different peptides are directly proportion to their antifreeze action[28]. The fruitful and result oriented AFPs applications in cryosurgery and cryo-preservation of tissues and food is the foundation of bright future various kinds of AFPs. Further studies in other processes for example vitrification of cells in different ways and hypothermic membrane protection has been required. Natural AFPs from different origin either animals or plants in pure or mixed form may be convenient to apply in tissue cryopreservation and would be possible to encounter market recognition. It is proved by using winter flounder AFP site-selected mutagenesis that hydrogen bonding between ice and AFP molecule is not responsible for Thermal hysteresis. It was concluded that at the specific facet of water-ice interface the hydrophobic interactions along with the entropic contributions are responsible for actions of AFPs to act and encompass selectively because of supporting total free energy of the system[37-39].

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The cells cryo-preservation activity of AFP is not so public information. So far since many research papers reported that AFP application from time to time have no expected action and not better than other preservation compounds. In our thoughts such worries are in arrears to inadequate volume of exertion on AFPs and AGFP. So it is required to authenticate the outcome productivity of ongoing research in cryolabs and also due to imperfections in channeling this type of slight difficult investigates. Thus, the exact molecular mechanism of action of biological antifreezes at the molecular level awaits elucidation. In addition simulation of molecular dynamics suggested that the designed peptides action can be explained better in the context of the structural flexibility and rigidity. The greatest active peptides demonstrated lower flexibility and more structural steadiness than the other peptides of low rigidity and activity[28]. In medicinal uses for example on commercial scale as a medium of cryopreservation, larger volumes of different types of proteins and peptides would be desired. This might be supplied by the new recombinant DNA technologies even though a very high cost of expressed amount protein would have future certain limitations. Synthetic and practically better-quality and not the profit-making types AFPs could be prepared from relatively simple components for a new hope in mankind medicine. The AFPs and peptide will be continued to find out several value added, justifiable and advanced milestones in this long-drawn-out and zealous journey.

6 | CONCLUSIONS

Most organ transplantation is done proximately after the death of the donor with the time that the organ is ex vivo compact to reduce anoxic, ischemic and preservation damage. The transplanted organ must then meaning immediately when the recipient animal is removed from life-support systems. There is no time for recovery or repair. Many of these problems can be overwhelmed if the time and preservation errors can be removed from the implantation or if there will some sound way to store, or bank, the organ after removal this of course, has been one of the thoughts of cryobiology since the time of the first successful organ transplant. In this novel study we shall incorporate some recent findings concerning these proteins which will propose to be explaining the effects of Anti-freeze proteins (AFP) and peptides from natural derivatives during cryopreservation. Cryopreservation of living tissues and organs will be important and significant phenomena in future medicine and biotechnology cryobiology for successful banking and transplantation. The storage and keeping of living tissues, from the time of removal of the donor until transfer and transplantation will be one of the most important factors and will give tremendous breakthroughs to future cryobiologists and biotechnology scientists. Appearances of anti-freezing proteins and peptides in the active areas of cryopreservation will give a new turn to this old story and will make it very amazing for future cryobiologists in its extended outing.

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