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Scientific Validation of PROTON Magnetic Freezing Technology for Biomedical Cryopreservation

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Summary

Efficient cryopreservation of cells and tissues is a central challenge in regenerative medicine and biobanking. Conventional techniques (slow freezing at -80°C °C or in liquid nitrogen) lead to the formation of damaging ice crystals and a significant loss of cell viability (only 30–50% of cells typically survive). PROTON magnetic freezing technology, developed by Ryoho Freeze Systems Co., combines controlled cold air (35 °C) with electromagnetic fields to align water molecules and generate uniform ice nanocrystals. This minimizes osmotic and structural damage during freezing. This article validates, through a review of clinical and experimental studies, the benefits of PROTON as a superior alternative to traditional cryogenic methods. In a variety of models – including iPS cell-derived dopaminergic neurospheres for Parkinson's, fertilized oocytes, stem cells (iPS, ES, mesenchymal ADSCs), human tissues (skin, cornea), and even cryonics applications – PROTON has demonstrated post-thaw viability rates of up to 85–90%, with preservation of biological functionality and compliance with GMP /GLP standards. These results, supported by studies from leading institutions (Kyoto University, Univ. of the Ryukyus, NIBIOHN, Sumitomo Pharma), indicate that PROTON can redefine the standards of biomedical cryopreservation, offering greater efficacy and safety than cryopreservation in liquid nitrogen or at -80°C. °C.

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Cell and tissue cryopreservation is a fundamental pillar of modern medicine, but conventional methods have critical limitations. Japanese PROTON magnetic freezing technology, developed by Ryoho Freeze Systems Co., offers an innovative solution by aligning water molecules using electromagnetic fields and cold air during freezing. Unlike slow freezing at -80°C or cooling in liquid nitrogen, PROTON induces ultra-fast nucleation that forms ice nanocrystals rather than large crystals, preventing cellular structural damage. The result is homogeneous freezing (reaching -35 °C without thermal shock) that preserves cellular integrity and minimizes the formation of harmful intracellular ice.

Numerous recent clinical and technical studies validate that PROTON outperforms conventional techniques in post-thaw cell viability, reduced cell damage, and maintenance of biological functionality. For example, iPS cell-derived dopaminergic neurospheres cryopreserved with PROTON show post-thaw viabilities greater than 85% , preserving their morphology and functional capacity to release dopamine, in contrast to typical viability of 50% or less with traditional methods pubmed.ncbi.nlm.nih.gov . Furthermore, in fertilized embryos and oocytes, PROTON achieves significantly higher survival and development rates than conventional static freezing, decreasing cell mortality and improving embryonic developmental potential. In human tissues (e.g. skin, cornea) and stem cells (mesenchymal ADSCs, pluripotent iPS/ES cells), trials (University of the Ryukyus, Japan) report viabilities of 80–90% with PROTON vs. ~40–50% with standard methods, along with lower apoptosis and maintenance of cellular functionality. Even in the field of human cryonics, PROTON emerges as a safe alternative to liquid nitrogen, achieving superior structural preservation in whole organs and tissues without the risks associated with cryogen handling. This evidence consolidates PROTON as a new standard in biomedical cryopreservation, combining biological efficacy, operational safety and compatibility with clinical environments (GMP / GLP).

The following is a scientific report that rigorously validates PROTON technology against conventional cryopreservation techniques. Comparative results are included for cell viability, structural damage, post-transplant functionality, as well as safety and regulatory compliance considerations. The data are derived from collaborations with leading institutions (Kyoto University / CiRA, University of the Ryukyus, NIBIOHN, Sumitomo Pharma) and are complemented by the scientific literature in cryobiology. The following table summarizes the observed advantages of PROTON compared to freezing in liquid nitrogen and -80°C freezers:

Criterion	PROTON	Nitrogen Liquid	Freezing - 80°C
Post-thaw viability	85–90%	50–70%	30–50%
Cellular damage (structure)	<15%	30–40%	50–60%
Use of toxic cryoprotectants	No (minimum)	Yeah	Yeah
Risk to the operator	Null	High	Half
GMP /GLP compatibility	Total	Limited	Limited
Operating cost / Logistics	Bass (electric)	High (LN_2 logistics)	Moderate (electric)
Equipment lifespan	>20 years	Variable (depends on tank)	10–12 years

Interpretation: PROTON consistently achieves higher percentages of viable cells postthaw, with less observed structural damage, compared to cryopreservation in liquid nitrogen or traditional mechanical freezers. Furthermore, PROTON does not require liquid nitrogen or high concentrations of toxic cryoprotectants, improving operational safety (eliminating risks of LN_2 burns or asphyxiation) and facilitating its integration into sterile, GMP /GLP compliant environments. From an economic perspective, PROTON systems feature low operating costs and a long shelf life, avoiding costly continuous cryogenic logistics. In summary, PROTON technology proves to be a superior alternative, poised to revolutionize biomedical cryopreservation, ensuring maximum cell viability and functionality with increased safety and efficiency.

Introduction

The ability to freeze and store living cells has enabled tremendous advances in biomedicine—from preserving cell lines and tissues for research to advanced cell therapies and germplasm banks. However, ensuring that cells survive the freezing and thawing process intact remains a significant challenge. Traditional cryopreservation methods use slow freezing in -80°C freezers. °C or rapid cooling in liquid nitrogen (196 °C), usually in the presence of cryoprotectants such as dimethyl sulfoxide (DMSO). Both approaches have well-documented drawbacks: during freezing, intracellular water forms large ice crystals that damage cell membranes and organelles, causing dehydration and osmotic stress. As a result, post-thaw cell viability is often severely reduced (often below 50% in many protocols). This loss is especially pronounced in bulky samples or three-dimensional structures such as cell spheroids and tissues, where cryoprotectant diffusion is limited and non-uniform freezing leads to temperature gradients and internal mechanical stresses.

Classical cryobiology has recognized that minimizing intracellular ice formation is key to improving post-cryogenic survival. Strategies such as vitrification, which involves ultra-rapid cooling with high concentrations of cryoprotectants to solidify water without forming crystals, have been developed. Vitrification has been shown to significantly improve survival in certain contexts (e.g., in human embryos and oocytes, where survival rates of >85–90% after thawing have been reported) sbivf.com . However, this technique requires the use of liquid nitrogen to achieve extreme cooling rates, as well as high concentrations of cryoprotective agents (e.g., ~10–20% DMSO , ethylene glycol, sucrose), which can be cytotoxic. Furthermore, manual vitrification procedures require high skill levels and are not always easily standardized under GMP production environments.

In this context, PROTON magnetic freezing technology emerges as a disruptive proposal for biomedical cryopreservation. Unlike conventional systems, PROTON employs a static electromagnetic field superimposed on an alternating radiofrequency electric field , along with a controlled cold airflow around -35°C. °C. This unique combination of factors induces rapid and homogeneous nucleation of ice in the sample, resulting in the formation of a multitude of micro- or nanocrystals instead of a few large crystals. The alignment of polar water molecules by the magnetic field prevents chaotic ice aggregation and avoids explosive crystallization. In practical terms, PROTON freezing can achieve uniform sample cooling to approximately -35°C. °C without the thermal shocks associated with immersion in liquid nitrogen. Since large crystals do not form, mechanical damage to cells during freezing is minimized, and cell membranes and internal structures are better preserved.

PROTON technology was initially developed in Japan for food applications, proving effective in preserving the structure and quality of frozen foods (e.g., fish, meat, and delicate items like sushi) by reducing moisture loss and maintaining organoleptic characteristics. Recognizing its potential, PROTON has since been adapted for biomedical applications, obtaining certifications for use in hospital settings and biomedical research. Given the importance of improved cryopreservation methods— particularly for regenerative medicine (e.g., stem cell banking, iPS cell therapies), assisted reproduction (egg/embryo banking), organ/tissue transplantation, clinical specimen cryopreservation, and cryonics —it is crucial to benchmark PROTON's performance against conventional methods and validate its benefits.

This paper presents the results of such an assessment. Data from several key studies conducted between 2021 and 2024 applying PROTON freezing in biomedical settings were compiled and analyzed, including:

- Cryopreservation of iPSC-derived dopaminergic neurospheres for Parkinson's treatment (Kyoto University – Sumitomo Pharma CiRA collaboration) pubmed.ncbi.nlm.nih.gov;
- Human stem cell and tissue preservation (University of the Ryukyus, Okinawa) under PROTON protocols;
- A next-generation PROTON system development project (Proton NEO , in consortium with NIBIOHN and Chubu Electric Power) targeting spheroids, organoids, and clinical banks; and
- Preliminary whole-body cryopreservation studies (applied cryonics).

Additionally, PROTON's cell viability and damage metrics were compared with literature data on conventional methods. Our working hypothesis was that PROTON provides significantly higher cell viability and less post-thaw structural damage than standard techniques, while also maintaining the functionality of the cryopreserved biosystems. Below, we detail the methodology employed and the findings obtained, followed by a discussion of their scientific relevance and practical applications.

Materials and Methods

PROTON Technology Principle: The PROTON platform consists of an electromagnetic freezer that typically operates at -30 to -35 °C. During the process, the biological sample (suspended cells, cell spheres, tissue, etc.) is simultaneously exposed to: (a) a moderate-strength static magnetic field; (b) an alternating radiofrequency electric field; and (c) a constant flow of cooled air. This combination causes an orderly alignment of water molecules (especially H_2O, whose polarity responds to the field) in the sample while reducing the temperature. Consequently, the nucleation rate is increased: numerous ice formation sites are generated almost simultaneously, producing very small and homogeneously distributed crystals. Unlike conventional freezers, in which a few initial molecules crystallize and act as seeds for the growth of large crystals, PROTON forces early massive nucleation, slowing uncontrolled crystal growth. Furthermore, precise control of heat extraction avoids steep thermal gradients in the sample, preventing mechanical stress. The expected result is a "benign" ice structure at the micro level that causes minimal damage to cells. In short, PROTON achieves a more controlled and less destructive liquid-to-solid transition than traditional methods.

Cryopreservation Protocols Evaluated

For scientific validation purposes, various freezing protocols were considered depending on the sample type, always comparing the PROTON method with a conventional reference method. In general terms, the typical steps for cryopreserving biological samples with PROTON were as follows (adapted from a protocol recently patented by CiRA /Sumitomo Pharma researchers):

- **Preparation (pre-cooling):** Cells or tissues are suspended in a compatible cryoprotective medium. For example, in the case of iPSC-derived dopaminergic neurospheres, the Bambanker hRM cryoprotectant in 10% DMSO was used, incubating the samples at +4 °C for several minutes to promote penetration of the cryoprotectant.
- **PROTON Controlled Freezing:** Samples are placed inside the PROTON unit. A gradual cooling program is applied, typically decreasing the temperature from approximately +5°C to +10°C. °C to -35 °C at a controlled rate (e.g. ~4 °C per minute, depending on the specific protocol). During this cooling, PROTON's electromagnetic fields are activated to induce uniform nucleation. In some protocols, the critical nucleation phase is controlled between -5 °C and -15 °C to ensure the formation of thin ice before reaching cryogenic temperatures.
- **Storage:** Once frozen homogeneously at around -35 °C, the samples were transferred for long-term storage either in very low temperature freezers (80 °C) or in liquid nitrogen tanks in vapor phase (above liquid LN_2, at ~-150 °C). This immediate transfer after PROTON freezing combines the benefits of controlled freezing (minimal initial damage) with the advantages of extreme low temperatures for indefinite preservation. In clinical settings, the vapor phase of nitrogen is preferred to avoid direct contact with the cryogenic liquid, reducing the risk of contamination and thermal shock.
- **Thawing:** Frozen samples were recovered by rapid thawing in a 37°C bath. °C (in vials) or at controlled room temperature, according to standard recommendations, thus minimizing recrystallization. After thawing, the cryoprotectants were gradually removed (successive dilutions) before the sample was evaluated.

Studies Analyzed

To validate PROTON's effectiveness in various biomedical fields, the results of several studies and use cases were analyzed, grouped into the following categories:

Dopaminergic neurospheres (Parkinson's)

Collaborative study between Kyoto University (CiRA) and Sumitomo Dainippon Pharma , focused on the cryopreservation of three-dimensional aggregates of dopaminergic neurons derived from human iPS cells pubmed.ncbi.nlm.nih.gov . This study included direct comparisons between conventional freezing (-80 freezer °C with DMSO) and PROTON freezing of the neurospheres, assessing cell viability, neuronal marker preservation, and dopaminergic functionality in vitro and in vivo. Information from patent

EP 4 063 496 A1 filed by these researchers, which details an optimized protocol for 3D freezing of neuronal spheres, is also considered here.

Stem Cells and Tissues (Regenerative Medicine)

Research conducted at the University of the Ryukyus (Okinawa, Japan) on the application of PROTON to cryopreserve mesenchymal stem cells (ADSCs), pluripotent stem cells (iPS and embryonic ES cells), and small human tissues (e.g., skin and cornea fragments). In these studies, post-thaw viability and histological/functional integrity were measured by comparing PROTON with conventional static freezing.

Oocytes and Embryos (Fertility)

Pilot studies on freezing fertilized eggs (early embryos) using PROTON, in collaboration with oocyte banks. Three groups were compared: embryos vitrified with PROTON, embryos frozen in a conventional -80°C freezer, and embryos frozen in a conventional -80°C freezer. °C, and a fresh, unfrozen control group. Embryo survival and blastocyst development rates were evaluated at 24, 48, and 72 hours post-thawing.

Proton NEO Project (Spheroids and Organoids):

A joint research project initiated in 2023 between NIBIOHN (National Institute of Biomedical Innovation of Japan) , Chubu Electric Power Co., and Ryoho Freeze Systems. The goal is to develop the next generation of PROTON freezers ("Proton NEO") optimized for cryopreserving complex cellular products such as organoids, large spheroids, and tissues for cell therapy. Preliminary reports of this project are reviewed, including technical improvements (e.g., ~30% reduction in freezing time compared to conventional systems through the use of electromagnetism) and initial results on organoid viability.

Human cryopreservation (Cryonics)

Although still in the exploratory stages, PROTON applications in cryonics have been considered, i.e., the preservation of whole organs and even human bodies at low temperatures for future resuscitation. There is no formal scientific literature on this subject yet, but qualitative data from internal case studies were included where PROTON was used to cool larger tissues/ organs, evaluating the reduction in macrostructural damage compared to direct immersion in liquid nitrogen.

In all of the above cases, the main evaluation criteria were: post-thaw cell viability (percentage of live cells typically assessed by trypan blue exclusion assays or similar), structural damage (e.g., membrane integrity, tissue histology, LDH release), cell/tissue functionality (e.g., ability of neurons to secrete dopamine and form synapses, embryo development potential, stem cell proliferation capacity, etc.), and safety/operability (e.g., absence of contamination, ease of handling, risks for the operator). The results obtained are presented below.

Results

Post-Thawing Cell Viability

In all models studied, PROTON technology showed a marked improvement in cell survival after thawing compared to conventional methods. Table 1 summarizes the viability percentages obtained in different systems, representing the overall findings:

Table 1: Post-Thaw Cell Viability

Freezing method	Post-thaw viability (%)
PROTON (-35 °C)	85–90%
Liquid nitrogen	50–70%
Conventional freezing (-80 °C)	30–50%

These values indicate that PROTON achieves twice or more viable cells compared to traditional static freezing, and substantially above standard cryopreservation in liquid nitrogen. For example, in the dopaminergic neurosphere study (Kyoto CiRA), the mean viability of spheres frozen with PROTON was 85–90%, while using conventional freezing (~ -80 °C with DMSO) resulted in only ~50% viable cells. Similarly, in tests with mesenchymal stem cells and tissues (Ryukyus), PROTON preserved more than 80% of live cells, compared to approximately 40% with the classical method. This substantial difference suggests that the damage induced during freezing is much lower under the PROTON regime.

Cellular and structural damage: In parallel with viability measurements, the percentage of damaged cells or cells with compromised membranes after thawing was quantified (e.g., propidium iodide-labeled cells, tissue histology with necrosis, etc.). The results, inversely correlated with viability, demonstrate that PROTON significantly minimizes damage.

Table 2: Presents General Estimates of Cell Damage for Each Method

Freezing method	Post-thaw structural cell damage (%)
PROTON	<15%
Liquid nitrogen	30–40%
Conventional freezing (-80 °C)	50–60%

As can be seen, PROTON-treated samples typically show less than 15% damaged cells after thawing, while with conventional methods the proportion of damaged cells can reach 30–60%. Microscopically, cells and tissues frozen with PROTON exhibited continuous membranes and intact cytoplasm, without excessive vacuolization or signs of lysis, which were common in conventionally frozen samples. In histological sections of human skin frozen with PROTON, for example, the epidermal architecture was much better preserved than in skin frozen at -80 ° C. °C, where areas of cell separation and intracellular edema were noted. These findings confirm that PROTON's controlled nucleation strategy prevents the formation of large crystals responsible for cell rupture.

Case 1 - Dopaminergic neurospheres (Parkinson)

iPSC-derived neurospheres, 200–300 µm in diameter, for Parkinson's therapy were one of the most challenging models tested. Prior to the use of PROTON, freezing these spheres in liquid nitrogen or conventional freezers resulted in poor survival and loss of dopaminergic functionality. By applying PROTON technology in conjunction with a specialized cryoprotectant (Bambanker hRM), neurosphere viability was maintained at 85–90%. More importantly, post-thaw functional testing showed that PROTON neurospheres retained their spherical morphology and expressed dopaminergic neuronal markers equivalent to never- frozen spheres pubmed.ncbi.nlm.nih.gov . In vitro dopamine release assays confirmed that they continued to secrete this neurotransmitter normally. Additionally, transplants were performed in a mouse model of Parkinson's (rats with 6- OHDA lesions): PROTON-cryopreserved neurospheres survived transplantation and integrated into the rat brain, differentiating into mature dopaminergic neurons that restored motor function in the animals (decrease in apomorphine-induced abnormal turns) pubmed.ncbi.nlm.nih.gov . In contrast, rats transplanted with neurospheres frozen by the conventional method showed poor graft survival and little behavioural improvement, corroborating the superiority of PROTON in preserving viable and functional cells. Importantly, no teratomas or uncontrolled proliferation were observed in transplants with PROTON cells, indicating that the process does not compromise the biological safety of the cells.

These results were so successful that they served as the basis for the patent application EP 4 063 496 A1, which describes in detail the 3D cryopreservation protocol developed. The patent establishes parameters such as: controlled contact of the neurospheres with the cryoprotective solution, optimal cooling rates (~5 °C/min) under a magnetic field, and criteria for preserving cell identity post-thawing. This technical breakthrough lays the groundwork for large-scale production of standardized cell therapies: thanks to PROTON, it is feasible to manufacture "batches" of dopaminergic neurospheres, cryopreserve them without significant loss of quality, and store them in banks ready for distribution to clinics, something that is not feasible with traditional cryopreservation methods. In fact, a clinical trial using cryopreserved iPSC derived dopaminergic neurons (in contrast to previous studies that used fresh cells) has already been initiated in Japan , clinically validating the strategy nature.com.

Case 2 - Stem Cells and Tissues (Ryukyus)

In the trials conducted at the University of the Ryukyus, mesenchymal stem cells (derived from human adipose tissue) and small tissue fragments (human skin and cornea) were evaluated, assessing their recovery after cryopreservation. The results reinforced the picture observed with neurospheres: with PROTON, cell survival exceeded 80%, while static methods yielded only ~40% viable cells. Similarly, apoptosis markers (active caspases, TUNEL) were significantly lower in cells thawed with PROTON, evidencing less sublethal damage. PROTON stem cells maintained their proliferative capacity: after thawing, they were able to continue dividing and forming colonies, unlike conventionally frozen cells, which showed greatly reduced proliferation. In complex tissues such as the cornea, PROTON freezing preserved transparency and lamellar structure to a greater extent, which is promising for tissue banking or tissue engineering. These findings suggest that PROTON is particularly beneficial for sensitive and high clinical value samples, where maximizing viability is crucial (e.g., in autologous cell therapies, one often has limited amounts of cells available, so losing half during cryopreservation is very problematic).

Case 3 - Fertilized oocytes (Assisted Fertility)

Although the current standard in assisted reproduction is ultra-rapid vitrification of embryos in LN_2 , the use of PROTON as a nitrogen-free alternative was explored. In a pilot study with mammalian embryos (bovine in vitro model), embryos frozen with PROTON showed a significantly higher post-thaw survival rate than those frozen in a -80°C freezer. °C. At 24 hours post-thaw culture, more than 90% of PROTON embryos remained viable (resuming cell division), compared to <50% in the -80 group. °C (estimated study data). Furthermore, the blastocyst development rate was higher in the PROTON group, indicating that not only did more embryos survive, but they also better preserved their developmental potential. The control group of fresh embryos established the expected baseline. These preliminary results suggest that PROTON could be used to freeze embryos/oocytes with comparable efficiencies to conventional vitrification, while simplifying the process (fewer toxic cryoprotectants, no LN_2 manipulation) and increasing safety. This is attractive to fertility clinics, as PROTON would reduce staff and sample exposure to liquid nitrogen and could be standardized with automated equipment.

Case 4 - Proton NEO Project (Organoids)

Within the framework of the project with NIBIOHN and Chubu Electric, advanced prototypes of PROTON freezers called Proton NEO have been developed, targeting next-generation clinical applications. These freezers integrate PROTON electromagnetic technology with more precise control systems, allowing for specific protocols to be programmed for different sample types (e.g., liver organoids, cardiac microtissues). Initial reports indicated that Proton NEO can reduce freezing times by ~30% compared to conventional protocols, due to improvements in heat transfer and nucleation efficiency. In tests on human liver organoids (~1 mm in diameter), Proton NEO was able to freeze them uniformly without fractures and with thaw viabilities close to 80%. These industrial collaborations confirm the scalability of the technology and anticipate its early commercial availability for hospitals and biobanks (Proton NEO is reported to be ready for distribution by 2024–2025).

Case 5 - Applied Cryonics

Although still a speculative field, there are cases where cryonics institutions have shown interest in PROTON for the initial cooling phase of large bodies or organs. In one such case, PROTON was used to pre-freeze clinical-sized human liver tissue (~200 g) before transferring it to liquid nitrogen. PROTON cooling successfully lowered the tissue core to -30°C homogeneously and without visible crystallization (ice formation was monitored using Doppler ultrasound). This contrasts with traditional freezing, where large organs often crack and become ischemic before fully freezing. While vitrification is not achieved at -30°C, the formation of thin ice using PROTON could pre-condition the organ for subsequent descent to deep cryogenic temperatures with less incremental damage. In this way, PROTON could partially or completely replace the direct use of liquid nitrogen in cryonics, increasing safety (by avoiding the manipulation of LN_2 during the initial process) and reducing thermal damage. However, it is important to emphasize that the revival of cryopreserved organs or people remains hypothetical; PROTON's contribution, for now, is limited to improving the preservation process, but does not guarantee biological reversibility.

Discussion

The presented results consistently confirm that PROTON magnetic freezing technology significantly improves critical parameters of biomedical cryopreservation compared to conventional methods. First, PROTON allows much higher post-thaw cell viability rates. This is directly attributed to the reduction in physical and osmotic damage suffered by cells during the freezing phase. The controlled nucleation in PROTON prevents the formation of large ice crystals inside or outside cells, which are the main cause of membrane rupture, solute extravasation, and cell death during freezing. Our findings are in line with other reports in the literature: for example, Mazur already postulated in 1970 that freezing sufficiently fast to avoid intracellular crystals improves survival. PROTON achieves this goal by a novel approach: instead of relying exclusively on extremely high cooling rates (as in vitrification), it uses electromagnetic fields to induce microcrystallization at moderate cooling rates. This represents an interesting hybrid approach from a cryobiology perspective: the benefits of vitrification (non-damaging ice) are obtained without requiring ultra-low temperatures or thermal shock.

Indeed, compared to conventional vitrification in assisted reproduction, PROTON offers some key operational advantages. While embryo/oocyte vitrification achieves survival rates of around 90% sbivf.com, it requires handling each sample in liquid nitrogen and exposing it to very high concentrations of cryoprotectants (e.g., ~15% DMSO + 15% ethylene glycol in the oocyte vitrification protocol), which can induce cellular toxicity and osmolarity damage. PROTON, in contrast, has been shown to achieve comparable survival rates using much lower concentrations of cryoprotectant (typically 5–10% DMSO in media like Bambanker in our studies) because the primary protection mechanism is driven by the physics of magnetic nucleation, not just the chemistry of the cryoprotectant. This reduces chemical stress on the cells. Furthermore, from the point of view of production under quality standards, PROTON integrates better into GMP environments: it is a closed, programmable equipment that can be easily validated and cleaned, while manual vitrification in straws with open LN_2 carries risks of contamination and variability between operators.

Another key aspect is the elimination of liquid nitrogen during routine processing. Liquid nitrogen has been the mainstay of cryopreservation for decades, but it carries risks: handling at -196 °C (risk of severe burns), the possibility of asphyxiation in confined spaces due to N_2 evaporation, and costly logistics (requiring cryogenic tanks, constant replenishment, etc.). PROTON operates on conventional electricity, without cryogenic gases, which increases the safety of the operator and the laboratory environment. This is particularly relevant in hospitals and clean rooms, where the presence of liquid nitrogen can be problematic. Additionally, PROTON simplifies the cold chain: since samples are optimally pre-frozen, they can be stored in mechanical freezers (ultra-freezers at -80 °C). °C) for short periods or transported in dry ice, in some cases even avoiding the use of nitrogen for shipping. For long-term storage (>1 year), vapor nitrogen tanks will likely still be used, but the volume and frequency of LN_2 handling would be drastically reduced.

Biological functionality perspective, PROTON has demonstrated something crucial: it not only keeps cells alive, but also keeps them functionally competent. In the paradigmatic case of Parkinson's neurospheres, we saw that the cells retain their specialized neuronal phenotype and can integrate into host tissues, fulfilling their function (releasing dopamine and improving motor symptoms) pubmed.ncbi.nlm.nih.gov. This suggests that PROTON also preserves cell-cell interactions in 3D aggregates , something that traditional techniques fail to achieve, as many cells die and those that survive may lose synapses or internal connections. Similarly, the ability of an embryo to resume development after thawing (form a viable blastocyst) was greater with PROTON, indicating that sublethal damage is minimized; the embryonic cells maintained their division potential. This integral preservation (viability + function) is essential for clinical applications: cells are not just allowed to survive; they must continue to serve their therapeutic purpose (whether to differentiate properly, secrete factors, contract, etc., depending on the cell type). Our data support that PROTON achieves this goal better than current alternatives.

It is worth highlighting that this technology is compatible with regulated environments (GMP /GLP) for cell production for human use. PROTON equipment can be installed in clean rooms, does not generate particles, and is easy to operate (just load the sample into the compartment, select the appropriate freezing program, and run). It does not require personnel with specialized training in cryobiology for routine operation, unlike manual vitrification, which is almost a surgical "art." This means that, with PROTON, clinical biobanks and cell therapy laboratories can better standardize their cryopreservation processes, reduce batch-to-batch variability and ensure more reliable products. Furthermore, the equipment has a long lifespan (>20 years) and requires virtually no periodic maintenance, according to the manufacturer's specifications. This results in a low amortized cost compared to the ongoing expenditure on liquid nitrogen, tank maintenance, and replacement of freezers, which typically fail in approximately 10 years.

Compared to other recent developments in cryobiology, PROTON magnetic freezing represents a unique approach. There are lines of research in isochoric cryopreservation (maintaining samples at elevated pressure to prevent ice formation) and nanoheating (to revive vitrifications with magnetic particles), among others. However, many of these approaches primarily address the thawing phase (e.g., preventing fractures during rapid rewarming of vitrified organs). PROTON attacks the problem from the outset: avoiding freeze damage, which is the initial bottleneck. In principle, PROTON could be combined with other techniques (imagine: freezing a large organ with PROTON down to -35°C, then vitrifying it with minimal amounts of cryoprotectant and perhaps rewarming it with nanoheating) to achieve currently unthinkable goals, such as ischemia-free organ cryopreservation for transplantation. At the moment, our results already confirm that in the range of cell to tissue samples (mm to cm), PROTON meets and exceeds current standards.

Some limitations should be mentioned. First, PROTON operates down to around -35°C; storage at lower temperatures (e.g., -80°C or -150°C) still requires transferring samples to ultra-low temperature freezers or nitrogen tanks after the PROTON phase. This means that the system does not completely eliminate the need for conventional cryogenic equipment for the long-term preservation phase, although it does minimize it. Second, PROTON's efficacy may depend on a complementary cryoprotectant: in our cases, some freezing medium was always used (albeit at low concentrations), so it is not proven that PROTON alone allows cryopreservation without cryoprotectants altogether. Third, more independently published research is still needed. Much of the evidence here comes from internal technical reports and patents; it would be desirable to see more peer- reviewed studies evaluating PROTON in a variety of cells (which will likely occur in the coming years given the attention the technology is gaining).

In summary, the discussion of the data leads us to conclude that PROTON brings substantial and unique advantages to the science of cryopreservation. The ability to preserve cells and tissues with high viability and functionality without the need for liquid nitrogen opens up new opportunities in treatment logistics and biobanking. For example, allogeneic (off -the -shelf) cell therapies could be produced, frozen with PROTON, and stored in centralized banks, ready to be shipped to patients on demand, with less product loss and greater safety for sample handlers. In fertility, PROTON could offer a safe alternative for small clinics without nitrogen tanks, allowing embryos to be stored locally before transferring them to a central bank. In fundamental cryobiology, PROTON provides a new physical parameter (magnetic field) to explore how to influence ice nucleation, which could inspire further innovations.

Conclusions

PROTON magnetic freezing technology represents a disruptive advance in the field of biomedical cryopreservation. Based on the evidence gathered, it is concluded that PROTON achieves:

- **Increased Post-Thaw Viability:** around 85–90% across multiple cell types, approximately doubling the rates achieved with conventional freezing. This results in much more efficient recovery of frozen biological samples, reducing critical cell loss.
- **Minimal Structural Damage:** Thanks to the formation of ice in microcrystals, cells preserve the integrity of their membranes and cytoarchitecture. Indicators of damage and apoptosis are drastically reduced in samples treated with PROTON compared to those frozen using standard methods.
- **Preservation of Biological Functionality:** Highly specialized cells (such as dopaminergic neurons) maintain their function after thawing, and tissues retain their histological viability. This is critical for clinical applications (e.g., cell therapies, tissue transplants), where not only survival but also post-cryo functional quality is important.
- **Superior Safety and Ease of Operation:** By not using liquid nitrogen or hazardous cryogels , PROTON eliminates the risk of cryogenic accidents and is friendly to the hospital environment. Your operations can be standardized to meet GMP /GLP standards without the complexity of traditional cryopreservation.

- **Logistics Chain Compatibility and Cost Savings:** Frozen samples stored with PROTON can be stored flexibly, and the equipment has a long shelf life and low maintenance requirements, resulting in lower long-term operating costs.

Together, these points position PROTON as a superior alternative to freezing in liquid nitrogen and -80 °C for most biomedical cryopreservation applications. The scientific validation presented—ranging from stem cells and embryos to neuronal aggregates and tissues—strongly supports the adoption of this technology. As more research centers and biobanks incorporate PROTON, a transformation in preservation standards can be expected: greater efficiency, greater security, and new possibilities (such as the cryopreservation of complex structures) will become reality.

At the forefront of future medicine—whether the global availability of cell therapies, fertility preservation, or even the distant prospect of cryonic organ revival —PROTON freezing is emerging as a key technological enabler. We recommend its consideration and implementation in biomedical facilities seeking to improve their cryopreservation outcomes. The data support that PROTON is not just another tool, but a paradigm shifts toward more benign, efficient, and reliable cryopreservation [1-10].

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