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Encapsulation for efficient cryopreservation

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Abstract

Cryopreservation is a fundamental technology in biomedical research, regenerative medicine, and tissue engineering, enabling the long-term storage of cells, tissues, and organs. However, its effectiveness is limited by challenges such as intracellular ice formation, cryoprotectant toxicity, and reduced post-thaw viability. This review explores the crucial role of encapsulation in enhancing cryopreservation efficiency, with a focus on recent advances in materials science, bioengineering, and cryobiology. Emerging technologies, such as nanotechnology and stimuli-responsive polymers, are transforming encapsulation strategies. Innovations such as microfluidic systems offer precise control over cooling rates and cryoprotectant distribution, thereby mitigating conventional limitations. The review also addresses current obstacles related to scaling up encapsulation processes and ensuring the long-term biocompatibility and stability of preserved specimens. By synthesizing recent findings, this work provides a comprehensive resource for researchers and clinicians seeking to enhance biopreservation techniques and their applications in contemporary medicine and biotechnology. Finally, the review identifies critical knowledge gaps that must be addressed to improve the efficacy of cryopreservation strategies and advance their clinical translation.

Keywords

cryopreservation; encapsulation; hydrogels; biomaterials; tissue engineering; regenerative medicine; nanotechnology; smart polymers

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1 Introduction

Cryopreservation is a critical technology in biomedicine, enabling the long-term storage of biological materials such as cells, tissues, and organs, at sub-zero temperatures, typically ranging from -150°C to -196°C[1]. By halting biological activity, cryopreservation preserves the viability and functionality of these samples, making them available for future therapeutic applications. Despite its widespread use, conventional cryopreservation methods encounter significant challenges, particularly the formation of intracellular and extracellular ice crystals during the freezing process, which can cause substantial damage to cells and tissues[2-3]. These ice crystals induce mechanical injury that compromises cellular integrity, while the resulting osmotic stress promotes cell dehydration and increases the risk of membrane rupture[2,4].

Traditional cryopreservation techniques, including slow freezing and vitrification, are widely used to mitigate the challenges associated with preserving biological materials. Slow freezing involves a controlled cooling process that gradually lowers the temperature of the sample, allowing water to exit cells, and thereby reducing intracellular ice crystal formation. Although this method is effective, it can result in osmotic imbalances, leading to cell shrinkage and potential membrane damage[5-6]. In contrast, vitrification employs rapid cooling to bypass ice formation altogether by inducing a glass-like, amorphous state within cells. While vitrification effectively prevents ice crystal formation, it requires high concentrations of Cryoprotective Agents (CPAs), which, although essential for cryoprotection, can be cytotoxic and impair cell viability and post-thaw functionality[7].

Recent advancements have identified encapsulation as a promising strategy to enhance cryopreservation outcomes, as depicted in Fig. 1. Encapsulation within protective hydrogel matrices provides a controlled microenvironment that mitigates the detrimental effects commonly associated with conventional techniques. In addition to serving as a physical barrier against ice crystals, hydrogels help regulate osmotic balance by

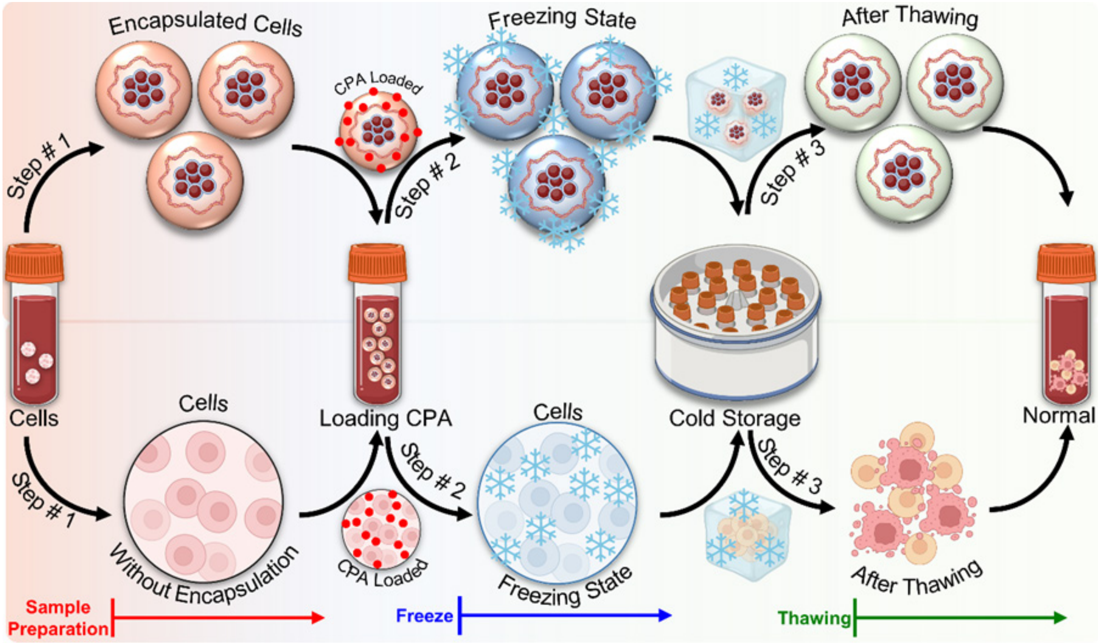

Fig. 1 Schematic diagram illustrating the cryopreservation process with and without encapsulation. CPA, cryoprotective agents

controlling the diffusion of water molecules and cryoprotectants during the freezing process. This approach reduces ice crystal formation, alleviates osmotic stress, and minimizes cellular exposure to potentially toxic concentrations of CPAs, thereby enhancing overall cell survival[8-9].

The properties of hydrogel materials play a pivotal role in the success of encapsulation-based cryopreservation. Key parameters such as pore size, cross-linking density, and mechanical strength significantly influence the effectiveness of cell protection. Optimized pore sizes allow for efficient diffusion of nutrients and waste products while shielding cells from ice-induced damage. Moreover, the use of biocompatible and non-toxic materials, such as alginate and Polyethylene Glycol (PEG), is essential for maintaining cell viability and functionality after thawing[10-11].

The interaction between encapsulated cells and CPAs is a critical determinant of cryopreservation success. CPAs like Dimethyl Sulfoxide (DMSO) and ethylene glycol are commonly used due to their efficacy in suppressing ice formation; however, their cytotoxic effects remain a major limitation. Encapsulation strategies can mitigate these effects by facilitating the controlled release of CPAs, thereby reducing the concentration needed for effective cryopreservation. Additionally, novel CPAs such as trehalose have shown promise in reducing cytotoxicity when used in conjunction with encapsulation systems, while maintaining adequate cryoprotective function[9,12]. Encapsulation techniques have demonstrated considerable success across a range of sensitive cell types, including stem cells, hepatocytes, and neural cells, which are particularly vulnerable to cryoinjury. Emerging innovations in hybrid hydrogels, microfluidic encapsulation, and stimuli-responsive polymers have addressed longstanding limitations in cryopreservation, opening new avenues for clinical applications such as organ banking, on-demand cell therapies, and personalized regenerative medicine. These next-generation hydrogels can dynamically adjust their properties in response to environmental changes such as temperature or pH offering continuous c protection during freezing and thawing cycles[13-14].

This review is organized into sections that examine encapsulation materials, mechanisms of action, recent technological advances, and ongoing challenges, providing a comprehensive resource for researchers and clinicians in cryopreservation. It provides a comprehensive analysis of encapsulation strategies in cryopreservation, emphasizing innovations such as nanotechnology integration and smart polymer development. Furthermore, it discusses critical issues related to the scalability of encapsulation techniques and the long-term biocompatibility of preserved specimens.

2 Encapsulation Materials and Techniques

The cryopreservation of cells and tissues has been significantly advanced by the development of sophisticated encapsulation materials and techniques designed to mitigate the stresses associated with freezing and thawing. This section explores recent innovations in encapsulation materials, their mechanisms of action, and state-of-the-art fabrication methods that enhance cryopreservation efficiency.

2.1 Natural Polymers

Natural polymers such as alginate and gelatin are widely used in encapsulation due to their excellent biocompatibility and functional versatility. Alginate, a polysaccharide derived from brown seaweed, forms hydrogels through ionic cross-linking with divalent cations. This structure provides a physical barrier that inhibits ice crystal formation while maintaining a hydrated microenvironment around cells. Recent studies have highlighted its effectiveness in preserving cell viability; for example, one report noted a 92% post-thaw viability rate for human mesenchymal stem cells encapsulated in alginate hydrogels, compared to 67% for non-encapsulated cells[15]. However, challenges remain in controlling the pore size and degradation rate of alginate hydrogels, which can affect the long-term stability of stored cells[16-17]. Gelatin, a denatured form of collagen, offers distinct advantages in tissue engineering due to its natural cell-adhesion properties[18].

Guan *et al.* developed a gelatin-based composite hydrogel that enhanced both cell viability and differentiation potential of encapsulated neural stem cells following cryopreservation. This approach resulted in a 25% increase in neuronal differentiation compared to conventional methods[19]. Additionally, the thermoreversible nature of gelatin allows for easier cell recovery post-thaw. Nevertheless, its relatively low mechanical strength may limit its application in settings that require structural robustness.

2.2 Synthetic Polymers

Synthetic polymers provide greater control over material properties, allowing for customized solutions tailored to specific cell types and cryopreservation protocols. Polyvinyl alcohol (PVA) hydrogels, for instance, exhibit strong resistance to mechanical stress during freezing[20]. A recent study by Liu *et al.*[21] employed molecular dynamics simulations to optimize PVA hydrogel composition, achieving a 30% reduction in ice crystal formation and improved cell viability. These benefits are attributed to PVA's capacity to form hydrogen bonds with water molecules, thereby mitigating water crystallization. Nonetheless, the high molecular weight of PVA can interfere with cellular metabolism, necessitating careful optimization of polymer concentration to balance protective efficacy and cytotoxicity[22].

PVA is another widely used synthetic polymer, valued for its ability to modify the surface characteristics of encapsulation materials. PEG-based copolymers have been shown to enhance cryoprotection for hepatocytes by reducing protein adsorption, a critical factor for preserving cell viability during freezing and thawing. This effect is largely due to PEG's ability to form a hydrated shell around cells, which helps prevent ice crystal damage. Despite these benefits, PEG may disrupt cell-cell interactions, which can pose limitations in certain tissue-specific applications[23].

2.3 Hybrid Materials

The integration of natural and synthetic polymers has led to the development of advanced hybrid materials that address multiple challenges in cryopreservation. For instance, alginate-PEG hybrid hydrogels can be engineered with adjustable mechanical properties, improving the post-thaw viability of pancreatic islets by enhancing control over cryoprotectant diffusion and ice crystal formation[24]. This hybrid approach combines the biocompatibility of natural polymers with the customizable features of synthetic materials. Nevertheless, optimizing the ratio and compatibility of these components remains a significant challenge[25-27].

2.4 Encapsulation Techniques

Recent advancements in encapsulation technologies have greatly improved the precision of material design and cellular microenvironments, thereby transforming the field of cell cryopreservation[14]. Among these, microfluidic encapsulation techniques offer unparalleled control over the size, composition, and architecture of hydrogel beads. Emerging microfluidic platforms have been developed to produce alginate beads with uniform size distribution, leading to enhanced post-thaw cell viability compared to traditional bulk encapsulation approaches. These systems allow fine-tuned control over encapsulated cell morphology and dimensions, ensuring consistent freezing and thawing rates across samples. This precision is crucial for maintaining cellular integrity by minimizing variations in ice crystal formation and cryoprotectant distribution[14,28-29].

The principal advantage of microfluidic encapsulation lies in its ability to generate highly uniform structures, which promotes reproducible outcomes in cryopreservation. These methods significantly reduce heterogeneity in encapsulation performance, a common limitation in bulk methods[29].

In parallel, Three-Dimensional (3D) bioprinting has emerged as a powerful tool for constructing complex, cell-laden architectures suitable for cryopreservation[30]. This technology enables the precise spatial arrangement of cells within the encapsulation matrix, mimicking native tissue organization[31]. Notably, recent studies have demonstrated the use of novel bioinks, such as gelatin methacrylate and alginate to print vascularized tissue constructs, with significant preservation of microvascular structures after cryopreservation[32]. The ability to reconstruct intricate tissue-like architectures prior to freezing expands the potential for preserving organ-like constructs and, eventually, whole organs[33]. These advanced fabrication techniques also enable the creation of multi-layered or compartmentalized encapsulation systems[34]. Such innovations allow differential protection of heterogeneous cell populations within a single construct or enable the controlled release of cryoprotectants during freezing and thawing. For example, combining microfluidics with layer-by-layer assembly has yielded core-shell microcapsules that significantly improve the viability of encapsulated pancreatic islets over traditional single-layer methods[35].

Future directions include incorporating stimuli-responsive materials capable of dynamically adjusting their properties throughout the cryopreservation process. Thermosensitive polymers integrated into 3D-printed scaffolds, for instance, can enable controlled expansion during thawing, thereby reducing mechanical stress on cells. Moreover, the integration of fabrication techniques with emerging technologies, such as nano-encapsulated cryoprotectants, temperature-responsive smart polymers, artificial intelligence, and machine learning, promises to optimize encapsulation parameters (*e.g.*, material composition and printing conditions) and enhance the accuracy of post-thaw outcome predictions.

Despite these advances, the scalability of such technologies for clinical use remains a formidable challenge[36]. Enhancing the throughput of microfluidic platforms and 3D bioprinters is critical for meeting the demands of large-scale applications. Additionally, developing bio-inks that simultaneously meet the mechanical requirements for printing and the functional criteria for cryoprotection continues to be an area of active investigation, as these properties often conflict[33].

3 Mechanisms of Encapsulation

Encapsulation mechanisms are fundamental to effective cryopreservation, offering protection against ice crystal formation and regulating osmotic stress. Hydrogels, composed of natural or synthetic polymers, form a protective matrix around cells, significantly reducing the risk of intracellular and extracellular ice crystal damage. These hydrogels also moderate the diffusion of CPAs, helping to maintain an osmotic equilibrium between intra- and extracellular environments during freezing and thawing. By controlling the rate at which CPAs permeate the cell membrane, hydrogel systems minimize cellular shrinkage or swelling caused by abrupt osmotic pressure changes[8,37].

Studies have demonstrated that alginate hydrogels effectively support cell viability during cryopreservation by modulating CPA exposure and providing a stable microenvironment. These hydrogels help shield cells from direct contact with high CPA concentrations, which can otherwise compromise membrane integrity. For example, encapsulating cells in alginate hydrogels has been associated with enhanced cell survival, attributed to the mitigation of osmotic and cryo-induced stresses[38-39]. These mechanisms are critical for preserving both cell structure and function throughout the cryopreservation cycle.

4 Advancements in Encapsulation Technologies

The incorporation of nanotechnology and smart polymer systems has introduced a new era in cryopreservation, enabling dynamic and highly effective encapsulation strategies. Nanotechnology has contributed to the development of nano-encapsulated CPAs, which improve cellular uptake and targeted delivery. These nanoscale systems offer enhanced protection by ensuring more uniform CPA distribution and controlled release, thereby reducing cellular damage typically associated with conventional cryopreservation techniques.

Smart polymers, which respond to environmental stimuli such as temperature or pH, offer real-time adaptability during the cryopreservation process. These materials alter their properties under specific conditions, enabling them to optimize protective functions dynamically. For instance, thermosensitive polymers can expand during thawing, mitigating mechanical stress on cells and improving post-thaw outcomes.

As illustrated in Fig. 2, multiple encapsulation strategies have been developed to enhance cryopreservation efficacy: (1) A capillary microfluidic core-shell device encapsulates cells in droplets that are subsequently cross-linked into microcapsules with a calcium alginate shell. This method allows for precise control over microcapsule size and shell thickness by adjusting flow rates (Fig. 2A, E, I)[40]; (2) Another approach utilizes a nonplanar microfluidic flow-focusing device to encapsulate murine Embryonic Stem Cells (mESCs) or human Adipose-Derived Stem Cells (hADSCs). This technique integrates vitrification and cryomicroscopy to assess encapsulation efficiency and demonstrates a significant reduction in intracellular ice formation (Fig. 2B, G, J)[41]; (3) Electrostatic spraying has also been employed to generate core-shell microcapsules, with real-time imaging used to monitor the process. Post-vitrification

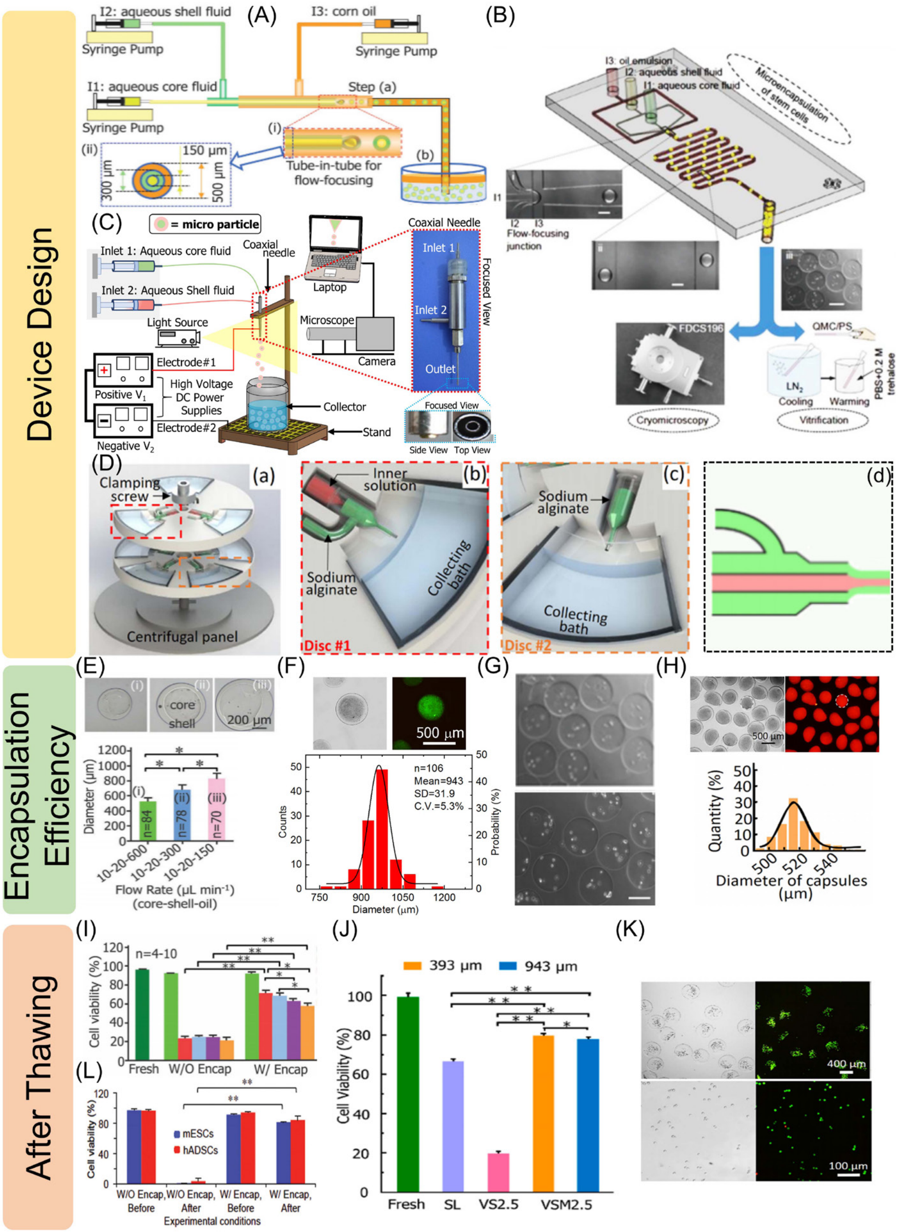

Fig. 2 Overview of encapsulation techniques for cryopreservation

(A) Schematic of a capillary microfluidics-based core-shell device, in which cells are encapsulated in droplets using a tube-in-tube system and cross-linked in $CaCl_2$ solution to form stable microcapsules. (B) Illustration of stem cell encapsulation and vitrification using quartz microcapillaries and plastic straws, including cryomicroscopy to monitor ice formation inhibition. Insets show alginate hydrogel microdroplets and stem cell-laden capsules. (C) Diagram of an electrostatic spraying system used to generate core-shell microcapsules and monitor vitrification in a water bath. (D) Schematic of a centrifugal platform used to fabricate core-shell structures and hydrogel beads/fibers. (E) Characterization of microcapsule formation as a function of oil flow rate, demonstrating control over microcapsule diameter and shell thickness. (F) Fluorescence microscopy images and size distribution of electrostatically sprayed microcapsules. (G) Microscopic evaluation of encapsulation efficiency for murine embryonic stem cells and human adipose-derived stem cells. (H) Morphological characterization and size distribution of hydrogel fibers and capsules generated *via* centrifugal techniques. (I) Comparison of perivascular Adipose-Derived Stem Cell (pADSC) viability among fresh, CPA-treated, and cryopreserved samples. (J) Viability assessment of encapsulated versus non-encapsulated stem cells before and after vitrification. (K) Cell viability comparison between conventional slow freezing and core-shell encapsulation methods for HUVECs. (L) Short-term viability analysis of cells encapsulated in simple hydrogel beads/fibers versus core-shell microcapsules. All panels reproduced with permission: Figure (A, E, I)[40], (B, G, J)[41], (C, F, K)[37], (D, H, L)[42]. * indicates $P < 0.05$, ** indicates $P < 0.01$.

assessments show increased cell viability in encapsulated samples compared to non-encapsulated controls (Fig. 2C, F, K)[37]; (4) A centrifugal platform enables the fabrication of core-shell capsules and hydrogel fibers, supporting short-term cell viability and allowing for greater customization of encapsulation structures (Fig. 2D, H, L)[42].

As these advanced fabrication techniques continue to evolve, they are anticipated to significantly influence the future of cryopreservation. The development of biomimetic encapsulation systems capable of mimicking native tissue environments could transform fields such as regenerative medicine and biobanking. Notably, these innovations are poised to enable breakthroughs in organ preservation and tissue engineering, offering promising solutions for the long-term storage and recovery of complex biological systems.

5 Challenges and Future Directions

Despite significant progress in encapsulation technologies for cryopreservation, several challenges remain in scaling these approaches for clinical and industrial applications. One of the primary obstacles is maintaining biocompatibility and structural stability under large-scale manufacturing conditions. As production scales up, achieving uniform encapsulation and precise control over material properties becomes increasingly complex, which may adversely affect post-thaw cell viability and the overall efficacy of cryopreservation.

The transition from laboratory-scale techniques, such as microfluidics and 3D bioprinting, to industrial-scale production is particularly challenging. These technologies, while highly effective at small volumes, often struggle with throughput limitations and process variability at larger scales. Addressing this gap requires innovations in bioreactor design, automation, and high-throughput encapsulation systems to ensure reproducibility, quality control, and regulatory compliance.

Another critical concern is the risk of immune rejection, especially in the context of allogeneic cell or tissue transplantation. This underscores the need for stable and inert hydrogel matrices that can shield cells from immune surveillance or for the incorporation of immunomodulatory strategies within the encapsulation system to promote tolerance and reduce inflammatory responses.

Looking ahead, the field of cryopreservation is poised for transformative advancements through breakthroughs in materials science and the integration of artificial intelligence (AI) and machine learning technologies. The development of hybrid materials combining the biocompatibility of natural polymers with the tunability of synthetic polymers is leading to improved encapsulation matrices with enhanced mechanical properties and tailored degradation profiles. Furthermore, the incorporation of nanoparticles and bioactive agents offers new opportunities to enhance cryoprotection, promote tissue regeneration, and facilitate post-thaw functional recovery.

AI and machine learning hold tremendous promise for optimizing encapsulation parameters in real-time. These technologies can analyze large-scale datasets to identify optimal material compositions, cryoprotectant concentrations, and process conditions. They also enable the dynamic adjustment of fabrication parameters during encapsulation or freezing processes, paving the way for personalized cryopreservation protocols tailored to specific cell types, tissues, or patient needs.

Together, these innovations are expected to revolutionize the field of cryopreservation, particularly in applications such as organ preservation, tissue engineering, and cell-based therapies. By overcoming current limitations, these advancements could significantly enhance the efficacy of regenerative medicine and enable personalized therapeutic strategies for diseases such as diabetes, liver failure, and neurodegenerative disorders.

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Research ethics

Not applicable.

Informed consent

Not applicable.

Author contributions

Concept and design: Zhao G; literature search: Memon K; drafting of the article: Memon K, Zhang B, and Fareed M A; revision of the article: Zhao G.

Use of large language models, AI and machine learning tools

Not applicable.

Conflict of interest

The authors declare no conflicts of interest.

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Data availability

Not applicable.

References

[1] Moniz I, Soares M, Sousa A P, *et al.* The low survivability of transplanted gonadal grafts: the impact of cryopreservation and transplantation conditions on mitochondrial function. Biology (Basel), 2024; 13(7): 542.

[2] Chang T, Zhao G. Ice inhibition for cryopreservation: materials, strategies, and challenges. Adv Sci (Weinh), 2021; 8(6): 2002425.

[3] Bojic S, Murray A, Bentley B L, *et al.* Winter is coming: the future of cryopreservation. BMC Biol, 2021; 19(1): 56.

[4] Pegg D E. The relevance of ice crystal formation for the cryopreservation of tissues and organs. Cryobiology, 2020; 93: 3-11.

[5] Netshirovha T R, Makumbane V, Sehlabela L D, *et al.* Cryopreservation of oocyte in livestock animals: principles, techniques and updated outcomes. 2024.

[6] von Bomhard A, Elsässer A, Ritschl L M, *et al.* Cryopreservation of endothelial cells in various cryoprotective agents and media - vitrification versus slow freezing methods. PLoS One, 2016; 11(2): e0149660.

[7] Rajan R, Matsumura K. Development and application of cryoprotectants. Survival strategies in extreme cold and desiccation: Adaptation mechanisms and their applications. Adv Exp Med Bio, 2018; 1081: 339-354.

[8] Zhang C, Zhou Y, Zhang L, *et al.* Hydrogel cryopreservation system: an effective method for cell storage. Int J Mol Sci, 2018; 19(11): 3330.

[9] Marcantonini G, Bartolini D, Zatini L, *et al.* Natural cryoprotective and cytoprotective agents in cryopreservation: a focus on melatonin. Molecules, 2022; 27(10): 3254.

[10] Naranjo-Alcazar R, Bendix S, Groth T, *et al.* Research progress in enzymatically cross-linked hydrogels as injectable systems for bioprinting and tissue engineering. Gels, 2023; 9(3): 230.

[11] Hu W , Wang Z , Xiao Y , *et al.* Advances in crosslinking strategies of biomedical hydrogels. Biomater Sci, 2019; 7(3): 843-855.

[12] Wang X, Wang E, Zhao G. Advanced cryopreservation engineering strategies: the critical step to utilize stem cell products. Cell Regen, 2023; 12(1): 28.

[13] Yan X, Chen Y R, Song Y F, *et al.* Advances in the application of supramolecular hydrogels for stem cell delivery and cartilage tissue engineering. Front Bioeng Biotechnol, 2020; 8: 847.

[14] Tianjin Da Xue. Transactions of Tianjin University. Tianjin: The University, 2005: Vol. 11.

[15] Aabling R R, Alstrup T, Kjær E M, *et al.* Reconstitution and post-thaw storage of cryopreserved human mesenchymal stromal cells: pitfalls and optimizations for clinically compatible formulants. Regen Ther, 2023; 23: 67-75.

[16] Watanabe T, Okitsu T, Ozawa F, *et al.* Millimeter-thick xenoislet-laden fibers as retrievable transplants mitigate foreign body reactions for long-term glycemic control in diabetic mice. Biomaterials, 2020; 255: 120162.

[17] Benson E E, Harding K, Ryan M, *et al.* Alginate encapsulation to enhance biopreservation scope and success: a multidisciplinary review of current ideas and applications in cryopreservation and non-freezing storage. Cryoletters, 2018; 39(1): 14-38.

[18] Han Y, Sun M, Lu X, *et al.* A 3D printable gelatin methacryloyl/chitosan hydrogel assembled with conductive PEDOT for neural tissue engineering. Composites Part B: Engineering, 2024; 273: 111241.

[19] Guan S, Wang Y, Xie F, *et al.* Carboxymethyl chitosan and gelatin hydrogel scaffolds incorporated with conductive PEDOT nanoparticles for improved neural stem cell proliferation and neuronal differentiation. Molecules, 2022; 27(23): 8326.

[20] Todros S, Spadoni S, Barbon S, *et al.* Compressive mechanical behavior of partially oxidized polyvinyl alcohol hydrogels for cartilage tissue repair. Bioengineering, 2022; 9(12): 789.

[21] Liu Y, Zhang D, Tang Y, *et al.* Development of a radical polymerization algorithm for molecular dynamics simulations of antifreezing hydrogels with double-network structures. NPJ Comput Mater, 2023; 9(1): 209.

[22] Bercea M. Recent advances in poly (vinyl alcohol)-based hydrogels. Polymers, 2024; 16(14): 2021.

[23] Tenório-Neto E T. Poly (ethylene glycol)-based hydrogels from preparation methods to applications. Journal of Colloid Science and Biotechnology, 2016; 5(1): 2-15.

[24] Li C, Qian Y, Zhao S, *et al.* Alginate/PEG based microcarriers with cleavable crosslinkage for expansion and non-invasive harvest of human umbilical cord blood mesenchymal stem cells. Mater Sci Eng C Mater Biol Appl, 2016; 64: 43-53.

[25] Underwood L A. Development and optimization of a preservable three-dimensional bio-construct. Michigan: University of Michigan-Dearborn, 2022.

[26] Mahou R, Wandrey C. Alginate-Poly (ethylene glycol) hybrid microspheres with adjustable physical properties. Macromolecules, 2010; 43(3): 1371-1378.

[27] Gok O. Physicochemical effects of PEG content in alginate-based double network hydrogels as hybrid scaffolds. TJST, 2024; 19(1): 249-256.

[28] Nakamura M, Matsumoto M, Ito T, *et al.* Microfluidic device for the high-throughput and selective encapsulation of single target cells. Lab Chip, 2024; 24(11): 2958-2967.

[29] Ling S D, Geng Y, Chen A, *et al.* Enhanced single-cell encapsulation in microfluidic devices: From droplet generation to single-cell analysis. Biomicrofluidics, 2020; 14(6): 061508.

[30] Kumar A, Brown R A, Roufaeil D B, *et al.* DeepFreeze 3D-biofabrication for bioengineering and storage of stem cells in thick and large-scale human tissue analogs. Adv Sci (Weinh), 2024; 11(11): e2306683.

[31] Zhan Y, Jiang W, Liu Z, *et al.* Utilizing bioprinting to engineer spatially organized tissues from the bottom-up. Stem Cell Res Ther, 2024; 15(1): 101.

[32] Li J, Moeinzadeh S, Kim C, *et al.* Development and systematic characterization of GelMA/alginate/PEGDMA/xanthan gum hydrogel bioink system for extrusion bioprinting. Biomaterials, 2023; 293: 121969.

[33] Warburton L, Rubinsky B. Cryopreservation of 3D bioprinted scaffolds with temperature-controlled-cryoprinting. Gels, 2023; 9(6): 502.

[34] Borges J, Zeng J, Liu X Q, *et al.* Recent developments in layer-by-layer assembly for drug delivery and tissue engineering applications. Adv Healthc Mater, 2024; 13(8): e2302713.

[35] Su R, Wang F, McAlpine M C. 3D printed microfluidics: advances in strategies, integration, and applications. Lab Chip, 2023; 23(5): 1279-1299.

[36] Dou M, Lu C, Rao W. Bioinspired materials and technology for advanced cryopreservation. Trends Biotechnol, 2022; 40(1): 93-106.

[37] Li Y, Memon K, Zheng Y, *et al*. Microencapsulation facilitates lowcryoprotectant vitrification of human umbilical vein endothelial cells. ACS Biomater Sci Eng, 2019; 5(10): 5273-5283.

[38] Meneghel J, Kilbride P, Morris G J. Cryopreservation as a key element in the successful delivery of cell-based therapies-a review. Front Med (Lausanne), 2020; 7: 592242.

[39] Kavand A, Noverraz F, Gerber-Lemaire S. Recent advances in alginate-based hydrogels for cell transplantation applications. Pharmaceutics, 2024; 16(4): 469.

[40] Zhao G, Liu X, Zhu K, *et al*. Hydrogel encapsulation facilitates rapidcooling cryopreservation of stem cell-laden core-shell microcapsules as cell-biomaterial constructs. Adv Healthc Mater, 2017; 6(23): 10.

[41] Huang H, Choi J K, Rao W, *et al*. Alginate hydrogel microencapsulation inhibits devitrification and enables large-volume low-CPA cell vitrification. Adv Funct Mater, 2015; 25(44): 6939-6850.

[42] Cheng Y, Zhang X Z, Cao Y, *et al*. Centrifugal microfluidics for ultrarapid fabrication of versatile hydrogel microcarriers. Applied Materials Today, 2018; 13: 116-125.