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Cryopreservation as a versatile strategy for the construction and application of organoids

Xinyue Wang[1,2,3], Hengxin Han[1,2,3], Yi Xu[1,2,3*]

Abstract

Organoids are three-dimensional structures derived from stem cells that recapitulate the gene expression profiles and functional characteristics of their tissue of origin, rendering them invaluable tools for disease modeling, drug screening, and precision medicine. Despite their promise, the widespread application of organoids is limited by extended culture durations and technical complexity. Cryopreservation has emerged as a critical strategy to overcome challenges related to the long-term storage and application of organoids, offering a range of preservation approaches tailored to organoid development. Nevertheless, conventional cryopreservation techniques encounter significant limitations when applied to organoids. To address these issues, the development of naturally derived, low-toxicity Cryoprotectants (CPAs), along with the optimization of CPA loading methods and refinement of cooling and warming protocols, is essential to mitigate cryoinjury. Looking forward, the comprehensive enhancement of cryopreservation technologies may facilitate the transformation of organoids into “off-the-shelf” products, enabling scalable production, batch standardization, and centralized distribution. Such advancements will lay the foundation for the establishment of Next-Generation Living Biobanks (NGLB).

Keywords

organoids; tumor tissues; cryopreservation; next-generation living biobanks

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[1]*Institute of Biothermal Science & Technology, University of Shanghai for Science and Technology, Shanghai 200093, China*
[2]*Shanghai Co-innovation Center for Energy Therapy of Tumors, Shanghai 200093, China*
[3]*Shanghai Technical Service Platform for Cryopreservation of Biological Resources, Shanghai 200093, China*

**Corresponding author Yi Xu, E-mail: xuyi@usst.edu.cn*

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1 Introduction

Organoids represent a transformative advancement in biomedical research. They can be derived from diverse sources, encompassing embryonic stem cells, induced pluripotent stem cells (iPSCs), and cells from both normal and tumor tissues of adult animals as well as humans, such as adult stems/progenitor cells, differentiated cells, and cancer cells[1]. The diversity of organoids spans multiple organ systems, including the small intestine, colon, stomach, esophagus, liver, lungs, pancreas, brain, heart, ears, and skin[2]. Compared to conventional Two-Dimensional (2D) cell cultures, Three-Dimensional (3D) organoids more faithfully replicate the architecture and microenvironment of native organs, exhibiting improved fidelity in gene expression, protein synthesis, and metabolic activity. As such, organoid technology provides more physiologically relevant *in vitro* models for disease research, drug discovery, and personalized medicine, while also reducing reliance on animal models[3].

Despite significant progress in basic research, the clinical translation of organoid technology faces considerable hurdles. Organoids typically require prolonged cultivation periods and possess limited lifespans, coupled with high heterogeneity and suboptimal reproducibility, factors that restrict their utility in long-term studies[4]. Moreover, clinical applications demand the large-scale generation of high-quality organoids, further complicating their production and storage. Cryopreservation offers an effective strategy for the long-term storage and transportation of organoids by arresting cellular metabolism and biochemical activity at ultra-low temperatures. This enables organoids to be utilized flexibly across various research and clinical contexts. To date, cryopreservation has been successfully applied to embryonic stem cells, sperm, embryos, and selected organoid types, including glioblastoma organoids and endometrial organoids[5]. However, the cryopreservation of organoids with intricate multicellular architectures remains a major technical challenge. The complex three-dimensional structure and cellular heterogeneity of organoids make them especially vulnerable to damage during

freeze, thaw cycles, including ice crystal formation, oxidative stress, osmotic pressure injury, Cryoprotectant (CPA) toxicity, and thermal stress during the freeze-thaw process. These insults can result in membrane, loss of intercellular connections, and l impaired biological functionality[6].

Recent advances in cryobiology and materials science have introduced novel approaches to organoid cryopreservation, such as the use of CPAs derived from natural sources and refined protocols for cooling and warming[7]. These innovations offer new opportunities for the efficient and reliable preservation of organoids. This review highlights two critical aspects of organoid cryopreservation: the diversified cryopreservation pathways in the process of organoid construction, and the implementation of novel techniques to mitigate cryoinjury. The overarching aim is to enhance the efficiency and scalability of organoid preservation, ultimately enabling the establishment of Next-Generation Living Biobanks (NGLB)[8]. These biobanks will serve as centralized, sustainable resources to support the broad deployment of organoid technology in both research and clinical domains.

2 Diversified cryopreservation pathways from tissue to organoid application

The generation of organoids typically begins with the isolation of target cells from various sources, including embryonic tissues, adult tissues, and tumor specimens[3]. Initially, these tissue samples undergo mechanical dissociation and enzymatic digestion to yield single cells or small cell clusters. These cells are subsequently embedded in a 3D culture system, where they self-organize into organoids with organ-specific structures and functions. Moreover, somatic cells can be reprogrammed into iPSCs and then further differentiated into organoid models that recapitulate a wide range of organ types[2]. Cryopreservation enables the long-term storage of these organoids while preserving their structural and functional integrity, ensuring that they remain readily available for both research and clinical applications. This capability is essential for the broader deployment and advancement of organoid technologies.

With the rapid development of cryopreservation technology, the pathways from initial cell sourcing to the final organoid application have become increasingly diverse and adaptable. In contrast to the conventional approach, where tissues are immediately processed and organoids directly applied, researchers now have the option to cryopreserve organoids at various stages, as illustrated in Fig. 1A. Once thawed, these organoids can be used in scientific research or therapeutic interventions. For instance, Mashouf *et al.* successfully cryopreserved human kidney organoids using both slow freezing and vitrification methods[9]. Lee *et al.* developed an advanced cryopreservation method for cardiac organoids using Fe3O4 nanoparticles, achieving efficient organoid preservation through a nanowarming system[10].

Beyond the cryopreservation of whole organoids, tissue samples can be enzymatically digested into single cells and subsequently cryopreserved. Upon thawing, these cells retain the capacity to differentiate into organoids, offering flexibility for immediate application or further preservation based on specific experimental or clinical requirements[11]. This strategy preserves the developmental potential of individual cells, allowing for their reactivation and induction into organoids tailored to diverse research or therapeutic objectives.

Despite these advances, prior research has predominantly focused on the cryopreservation of fully formed organoids, often overlooking the feasibility and benefits of directly preserving intact tissues, particularly patient-derived tumor samples. Routine surgical procedures in clinical settings provide a continuous stream of tumor specimens suitable for biobanking. However, due to the high heterogeneity of tumor tissues, it is impractical to culture organoids from every specimen and maintain individualized living biobanks for each patient to support personalized medicine[12]. This approach entails significant time, labor, and financial costs. Moreover, the viability of fresh tissue is limited, and prolonged culture can lead to genetic drift, necessitating immediate processing after sample collection[13]. Delays in processing often result in the loss of valuable research material. Cryopreserving tumor tissues addresses these challenges by preserving the biological and genetic integrity of the original samples, while also expanding the range of cryopreservation pathways available for organoid generation and application (Fig. 1A). This approach enhances the efficiency, scalability, and practicality of organoid-based research and personalized therapeutic strategies.

NGLB represents an innovative paradigm in the storage and application of biological specimens[12]. By integrating cryopreservation of functional tissue samples and cells with conditional reprogramming technologies, NGLBs aim to establish advanced biobanking platforms that support both long-term sample preservation and the unlimited expansion of cell cultures[15]. Strategic cryopreservation of critical stages within the organoid development process enables the transformation of biological materials into “off-the-shelf” resources, readily deployable for scientific research and clinical applications. This capability marks a significant step toward realizing the full potential of NGLBs.

Nevertheless, the effective preservation of biological specimens, particularly tumor tissues, remains technically challenging due to their inherent heterogeneity, susceptibility to microbial contam-

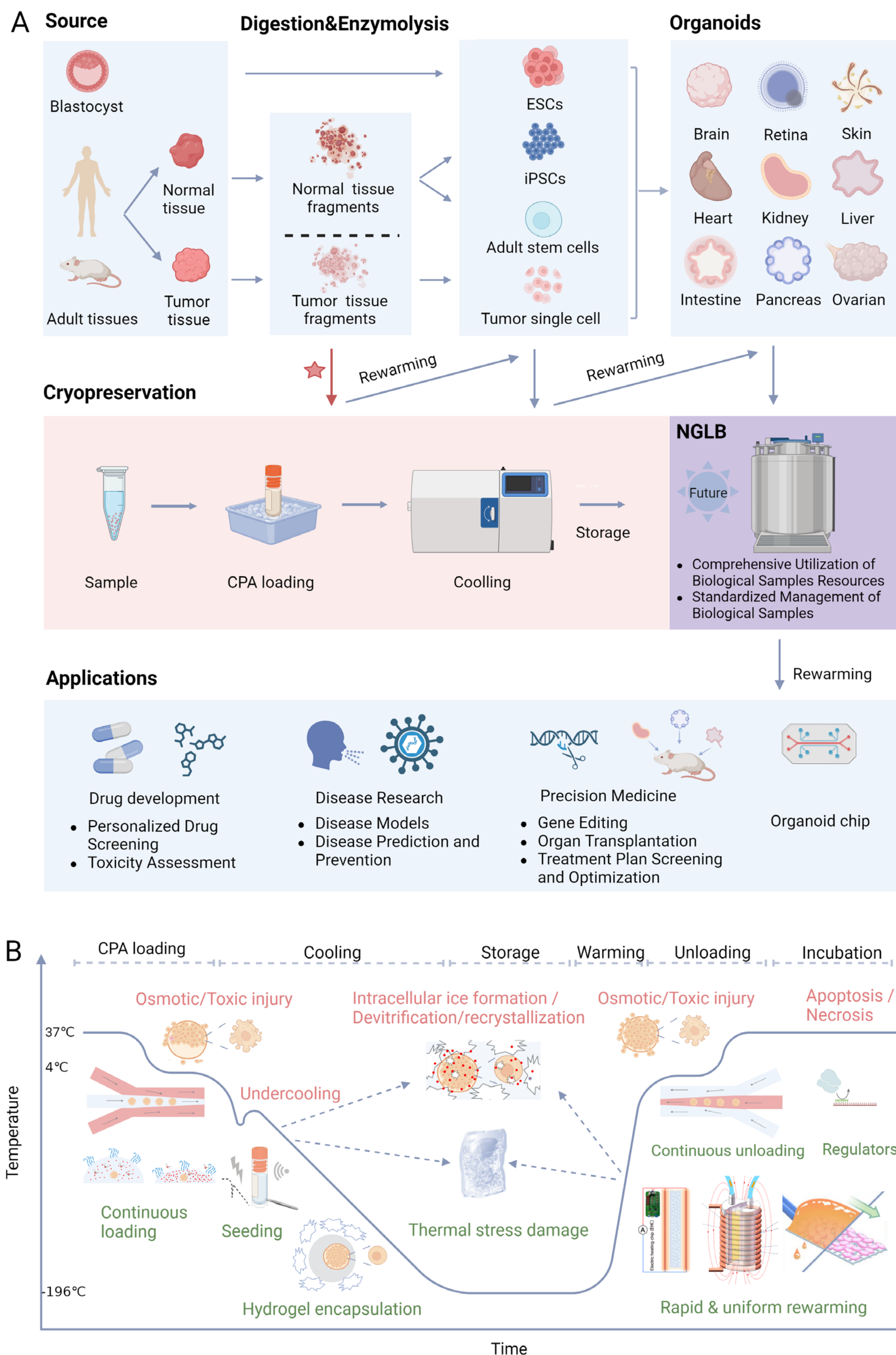

Fig. 1 Diverse cryopreservation pathways and damage mitigation strategies in organoid cryopreservation

(A) Schematic representation of diversified cryopreservation pathways, illustrating the stages from tissue acquisition to organoid application. This approach supports the flexible and scalable development of Next-Generation Living Biobanks (NGLB). CPA, cryoprotectant; ESCs, Embryonic stem cells; iPSCs, induced pluripotent stem cells. (B) Overview of the principal types of cryoinjury encountered during cryopreservation, along with corresponding strategies for mitigation, including optimization of cryoprotectants, cooling/warming protocols, and loading techniques[14]. (Copyright 2024, biotechnology journal. Created with BioRender.com).

ination, and issues related to experimental reproducibility. Continuous refinement of cryopreservation protocols is therefore essential to minimize cryoinjury and enhance the integrity of stored samples. Optimized cryopreservation methods can substantially reduce the risk of cellular and molecular damage during storage, thereby preserving genetic information and maintaining phenotypic fidelity. High-quality cryopreservation not only underpins reliable downstream applications such as disease modeling, drug screening, and regenerative therapies, but also serves as a critical foundation for the advancement of personalized and precision medicine initiatives[16].

3 Challenges and innovative strategies in the cryopreservation of organoids

Developing robust and effective cryopreservation protocols is crucial for ensuring the high-quality preservation of organoids, an essential requirement for broadening their application in both research and clinical contexts. Despite progress in the field, multiple challenges persist. Owing to their complex cellular architecture and intrinsic heterogeneity, organoids are highly susceptible to external stresses during cryopreservation, including CPA toxicity and temperature fluctuations[17]. Additionally, their relatively larger size hampers efficient oxygen and nutrient diffusion, further complicating CPA penetration and uniform temperature control during freezing and thawing processes.

Fig. 1B highlights the principal types of cryoinjury encountered during organoid preservation, such as ice crystal formation, osmotic stress, and CPA-induced cytotoxicity, and summarizes current mitigation strategies. These include optimization of CPA loading, modulation of cooling and warming rates, control of ice nucleation, and hydrogel-based microencapsulation, all aimed at improving cryopreservation outcomes[14].

Among these, the process of CPA loading is particularly critical, especially in vitrification-based protocols. Improper loading can induce osmotic imbalance and chemical toxicity, compromising organoid viability. Therefore, accurately determining the osmotic coefficients of different organoid types is essential for tailoring cryopreservation protocols[18]. In this context, microfluidic systems have emerged as powerful tools to streamline CPA loading and unloading. Techniques such as microfluidic-assisted CPA delivery and droplet evaporation significantly reduce the duration of CPA exposure while maintaining high cell viability and functional integrity[19]. Simultaneously, the development of non-toxic, naturally derived CPAs, such as antifreeze proteins and deep eutectic solvents, offers promising alternatives for effective ice crystal inhibition, thus enhancing preservation quality without the cytotoxic risks associated with conventional agents[20].

The inherent interplay between organoid size, structural complexity, and membrane permeability necessitates customized optimization of cryopreservation protocols. While progress has been made, the ideal cooling and warming rates for many organoid types remain undefined and require further empirical study. Particularly concerning is the phenomenon of supercooling: avoiding deep supercooling is vital for achieving high viability and recovery rates. Studies have established a correlation between higher nucleation temperatures and high cell viability after rewarming[21]. On the other hand, uniform and controllable supercooling can serve as a promising method for the short-term transport of organoids, freeing them from the toxic effects of CPA and the reliance on complex cooling and warming protocols[22].

In recent years, hydrogel microencapsulation has emerged as an effective approach to reduce mechanical damage during cryopreservation. By encapsulating organoids in hydrogel matrices, this method buffers against osmotic stress and ice crystal-induced injury[23]. Additionally, the development of advanced rewarming techniques has become a cornerstone of successful cryopreservation. Innovations such as Joule heating, magnetic nanoparticle-assisted rewarming, sample volume minimization, electrically conductive rapid-warming chips, and inductive heating combined with jet-assisted rewarming have collectively contributed to improved post-thaw viability and function of organoids[24-25].

As interdisciplinary research continues to advance, combining innovations in materials science, microengineering, and cryobiology, there is growing optimism that many of the current technical barriers to organoid cryopreservation can be overcome. These developments will be crucial for realizing the full potential of organoids in precision medicine, drug discovery, and regenerative therapies.

4 Summary and outlook

With continuous advancements in cryopreservation technologies and the systematic optimization of diverse preservation pathways, from tissue sources to functional organoid applications, the realization of NGLB is becoming increasingly attainable. These developments hold transformative potential for biomedical research and clinical practice.

Looking ahead, the development of efficient, low -toxicity CPAs, together with the refinement of organoid-specific cooling and warming protocols, will be critical for improving post-thaw viability and functional recovery. Tailored cryopreservation strategies that account for the structural and physiological characteristics of individual organoid types will be essential to ensure high-quality preservation.

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Not applicable.

Research ethics

Not applicable.

Informed consent

Not applicable.

Author contributions

Wang X Y contributed to the draft. Xu Y and Han H X contributed to the writing review and editing.

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.

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