MEGA
JOURNAL OF CASE REPORTS

OPEN ACCESS

Review Article

Compiled Date: May 22, 2026

Vitrification and High-Altitude Mountain Environments: Two Alternative Ways to Achieve Organ Preservation

María D Gallardo[1#], Mario Bernaski[2#], Mauricio A Girard[1], Claudia B Hereñu[3] and Rodolfo G Goya[4,5*]

[1]INIBIOLP- Histology B-Pathology B, Faculty of Medicine, National University of La Plata (UNLP), La Plata, Argentina

[2]Museum of High Mountain Archeology (MAAM), Salta city, Argentina

[3]Institute for Experimental Pharmacology (IFEC), School of Chemical Sciences, National University of Cordoba, Cordoba, Argentina

[4]UNLP

[5]Biomedical Research and Longevity Society (BRLS), Fort Lauderdale, FL, USA

[#]These authors contributed equally to this article.

[*]**Corresponding author:** Rodolfo G Goya, National University of La Plata (UNLP), Street 527# 824, 1906 La Plata, Argentina, Tel: (54-221) 5853988

Abstract

Organ vitrification and mummification in high-mountain burial sites are two different facets of organ and body preservation. Organ vitrification is a technology expected to enable the efficient development of biobanks. On the other hand, natural mummification at high-mountain sites constitutes an alternative instance of body preservation. In the vitrification protocols the organ is rapidly cooled to extremely low temperatures, below the glass transition, avoiding ice crystal formation and its associated damage to tissues. Vitrification relies on cryoprotectant agent suspensions that are perfused through the organ to prevent ice formation at practically feasible cooling rates. Since the Llullaillaco children constitute the most remarkable (although little known) example of high-mountain body preservation, we will focus our analysis of natural (CPA-free) body preservation on this case, which will be described rather extensively. We

believe that a detailed comparison of both processes could offer insights that may contribute to the development of efficient organ banks.

Keywords: Organ vitrification; Cryoprotectants; Llullalaco children; Natural mummification; Body preservation

Introductory Remarks

Organ vitrification and mummification in high-mountain burial sites are two different facets of organ and body preservation. Organ vitrification is a technology expected to enable the efficient development of biobanks. On the other hand, natural mummification at high-mountain sites constitutes an alternative instance of body preservation. Since the Llullaillaco children constitute the most remarkable (although little known) example of high mountain body preservation, we will focus our analysis of natural body preservation on this case, which will be described rather extensively. We believe that a detailed comparison of both processes could offer insights that might contribute to the development of efficient organ banks.

Vitrification for Biobanking

One approach to biobanking of organs is cryopreservation by vitrification, where the organ is rapidly cooled to extremely low temperatures, below the glass transition, avoiding ice crystal formation and its associated damage to tissues [9]. Vitrification relies on the use of Cryoprotectant Agent (CPA) suspensions that are perfused through the organ to prevent ice formation at practically feasible cooling rates [9]. Studies suggest that to implement organ biobanking by vitrification it is necessary to overcome challenges associated with ice crystal formation and temperature gradients during rewarming [9,15]. To avoid ice crystal formation due to devitrification during the rewarming step, sufficiently fast heating rates must be achieved [9]. As an example, the commonly used cryopreservation suspension VS55 has a Critical Cooling Rate (CCR) of −2.5°C/min and a Critical Warming Rate (CWR) of 50°C/min, illustrating the substantial difference in cooling and heating rates required for typical CPA suspensions for vitrification [17]. Also, as the volume of the cryopreserved tissue increases, thermomechanical stress resulting from temperature gradients hinders organ survivability [9,7]. Thus, technologies that achieve uniform, fast, controlled warming of cryopreserved organs are required to make cryopreservation by vitrification feasible for biobanking of whole organs. One potential solution to the heat transfer challenges associated with the rewarming step is nanowarming [8]. Nanowarming, a procedure introduced by Bischof and collaborators in 2014 [8], is a volumetric heating approach where Magnetic CPA (mCPA) suspensions containing biocompatible magnetic iron oxide nanoparticles are used to vitrify a perfused organ or immersed tissue specimen [15]. When nanowarming is used, at the time of organ rewarming and transplantation, an Alternating Magnetic Field (AMF) is applied, which results in heat generation by the MNP, rewarming the organ rapidly and uniformly. The AMF used penetrates the entire volume of tissue without attenuation, enabling uniform heating even in large organs [3].

Natural Mummification at High-Mountain Sites

Discovery of the Llullallaco Children

The discovery of three human bodies buried about 500 years ago, at 6,700 meters of altitude, near the top of the Llullaillaco volcano, located on the Northern portion of the Andes Mountains in the Northwestern Argentine province of Salta, constitutes one of the most important findings in the field of high mountain archeology. The three mummified bodies were identified as Inca children, an estimated 15 year old girl known as the “Llullaillaco Maiden,” **(Figure 1)** a 7 year old boy known as the “Llullaillaco Boy,” **(Figure 2)** and a 6 year old girl, partially damaged by lighting while she was buried; she is known as the “Lighting Girl” **(Figure 3)**. Evidence gathered at the burial site suggests that the children had been sedated with an alcoholic beverage (chicha) and coca leaves **[13]** and placed inside a small chamber 2.5 meters beneath the ground, where they were left to die **[1].** The three bodies displayed a remarkably good level of preservation when they were found, in March 1999. Clearly, the environmental conditions that exist at the top of the volcano were a key causal factor for such unique integrity of the mummies.

Figure 1: (Top) - The Maiden. This young woman was approximately fifteen years of age. She was sitting with her legs crossed, her arms resting on her lower abdomen. Her long hair is styled in small braids as it was customary in certain villages of the Andes. Her face is painted with a red pigment and there are small fragments of coca leaves above her upper lip. The insets are intended to enlarge the details of the skin of the face **(inset A),** the skin of the hands and forearms **(inset B)** and the skin of one leg and the dorsal surface of a foot **(inset C).** The maiden offers a unique opportunity to appreciate in high detail the remarkable integrity of the skin which retains a good degree of its original appearance, color, and texture, allowing for detailed macroscopic study. The environmental conditions prevailing at the burial site for some 500 years, resulted in extraordinarily well-preserved skin, facial features and hair, which appear supple and largely intact **(From 22).**

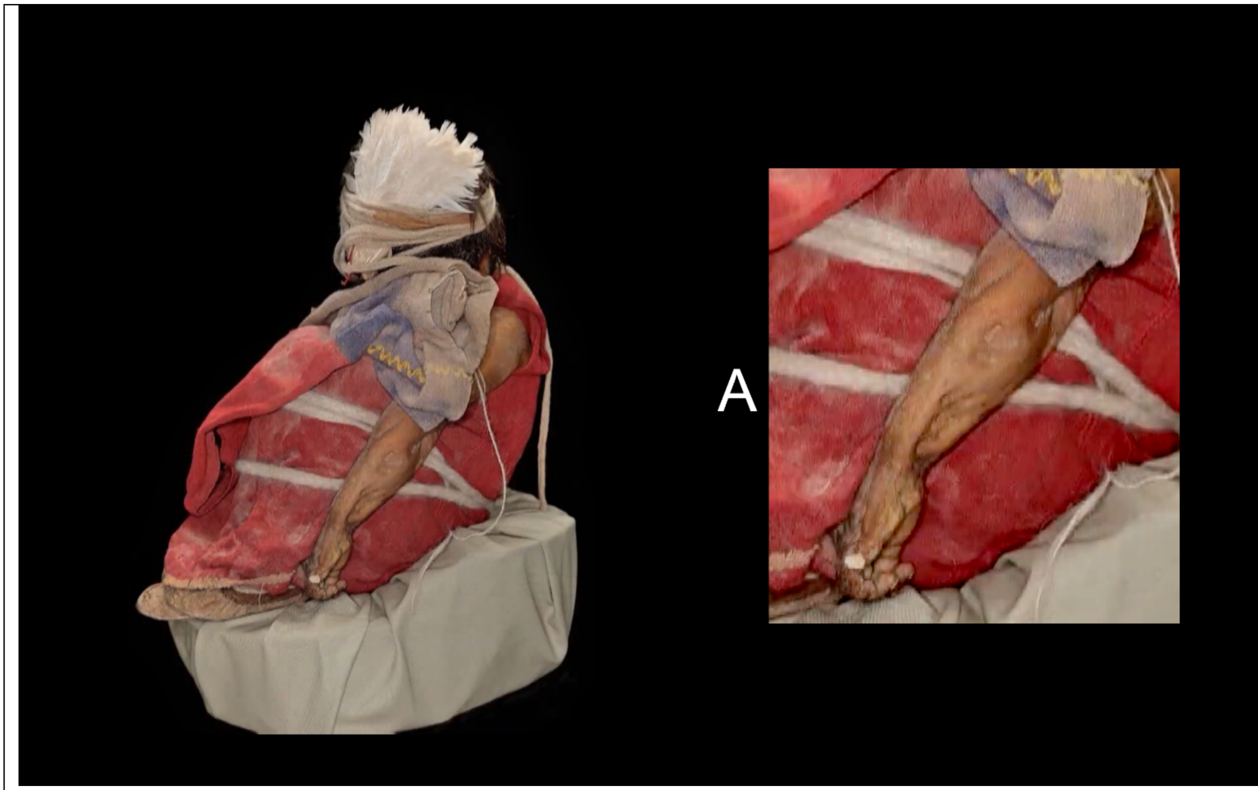

Figure 2: (Middle) - The Boy**.** He was seven years of age. He was seated on a gray tunic with his legs bent and his head -which faced the rising sun- resting on his knees. As every man belonging to the Inca elite, he has short hair. His hands are in a fist, his face is hidden and his eyes are semi closed. He has a slightly deformed skull that hints at his noble origin. In this figure, the inset allows for a closer view of the left forearm and hand. As in the case of the maiden, we can appreciate the remarkable integrity of the skin which retains a significant degree of its original appearance, color, and texture, additionally allowing for thorough macroscopic study of the anatomy of the arm and hand **(From 22).**

Figure 3: (Bottom) - The Lighting Girl. The girl was barely over six years old. She was seated with her legs bent, her partially open hands resting on her thighs and her head raised facing west-southwest. After her burial, at some point during these last centuries, the high temperature of a lightning bolt burnt a section of her face, neck, shoulders and arms as well as her clothing and part of her trove. Her eyes are closed and her mouth is partially open, revealing her teeth. Her skull was intentionally deformed to bear a conic shape as a symbol of her high social standing and hierarchy. The inset shows a magnified view of her flexed right hand. As for the other two mummies, the skin retains a significant degree of its original appearance, color, and texture **(From 22).**

Environment to which the mummies were exposed at the Llullaillaco Summit

The bodies were found separately entombed within a cubic shrine buried at 1.5 m depth near the summit. It is estimated that the environmental conditions that favored the natural mummification process and consequently their preservation, were: constantly low temperatures averaging -18ºC, low atmospheric pressure, low oxygen levels, presence of natural disinfectants and absence of sunlight. These values correspond to the air present in the bedrock surrounding the bodies, measured at a depth of 80 cm. The bodies were distributed on a square ceremonial platform, where they were found covered by a layer of rock, sand, volcanic ash, and ice, at depths of 1.67, 2.20, and 1.75 m for the Boy (El Niño), the Maiden (La Doncella), and the Lighting Girl (La Niña del Rayo), respectively [18]. According to Vigl report [22] the bodies suffered a weight loss of 8 kg, 5 kg and 2 kg for the boy, maiden and lighting girl, respectively. The corresponding reduction in tissue water content was estimated to be 54%, 35% and 25%, respectively. In the above study the authors considered racial differences, nutrition, individual height, and water loss in the last hours of life. Estimated initial body weight for the mummies was 18.23 kg, 33.73 kg and 16.52 kg for the boy, maiden and lighting girl, respectively. It is important to mention that a team of Argentine researchers developed a mathematical model that describes the processes of heat and mass transfer in bodies for the different stages and conditions to which the mummies have been exposed [14]. The numerical resolution of the models has contributed to understanding the physical dynamics of human mummification under the influence of the environmental factors prevailing at the volcano summit.

Immunological studies

The high integrity of mummy tissues allowed the use of shotgun proteomics to detect the protein expression profile of buccal swabs and cloth samples from two of the mummies, namely, the Boy and the Maiden. The profile of the Maiden was consistent with immune system response to severe pulmonary bacterial infection, likely caused by Mycobacterium sp [4]. No infectious processes were detected in the Boy.

Discussion

General considerations

Vitrification is an experimental procedure intended to reversibly cryopreserve organs, particularly kidneys, heart, and lungs. In contrast, natural mummification at high altitudes is an example of body preservation without therapeutic purposes. However, we believe that a description of the natural factors present at high-altitude environments could reveal ways to capitalize on the cryopreservative effects of some of these factors, adding them to the organ vitrification arsenal. From a translational perspective, the long-term vision of building clinically suitable organ biobanks rests on the demonstration that whole organs can be cooled, stored, and rewarmed with preservation of their highly integrated structure–function relationships. Early work by Fahy and co-workers established that appropriately formulated CPAs, such as VMP and M22, can support life-sustaining function rabbit kidneys after cooling to around −45 °C, provided that cryoprotectant toxicity, devitrification, and chilling injury are rigorously controlled [10]. Building on this foundation, Bischof and colleagues have shown that inductive nanowarming using superparamagnetic iron oxide nanoparticles dispersed within the cryoprotectant can overcome the rewarming bottleneck by achieving volumetric heating rates that exceed the critical warming rate of clinically relevant CPA cocktails, thereby minimizing ice recrystallization and thermomechanical stress in large tissues and whole organs [12,16,19]. Recent proof-of-concept studies report long-term cryogenic storage of vitrified rat kidneys followed by nanowarming, transplantation, and recovery of near-normal renal function, indicating that true organ banking is technically achievable at least in small- animal models [12,19]. Current reviews emphasize that the remaining stages needed to achieve clinical translation are related not only to further mitigating CPA toxicity and ensuring homogeneous perfusion and washout of both cryoprotectants and nanoparticles, but also in scaling these protocols to human-sized organs and embedding them within realistic regulatory and logistical frameworks for transplantation [2,15]. In parallel with these technological developments, naturally occurring models like the Llullaillaco children constitute a rare “experiment” in long-term preservation of human tissues under a convergent combination of low temperature, low atmospheric pressure, hypoxia, aridity, and reduced microbial activity **[1,4,13,14,18,22].** It is also worth noting the case of freeze-tolerant amphibians such as the wood frog Rana *sylvatica* which routinely survive repeated freeze–thaw cycles of experimental cooling to approximately −6 °C in temperate populations and at least −16 °C in subarctic Alaskan populations by rapidly mobilizing hepatic glycogen into high systemic concentrations of glucose and other cryoprotective osmolytes that limit intracellular ice formation and stabilize proteins and membranes **[5,6,20].** Taken together, these technological and natural exemplars suggest that clinically robust organ vitrification will ultimately require multi-factorial strategies that integrate optimized CPA chemistry, controlled abiotic boundary conditions inspired by high-altitude environments, and bioinspired manipulations of cellular metabolism to emulate, at least in part, the evolved cryoprotective mechanisms of freeze-tolerant species.

Lessons of high-mountain preservation to vitrification technology

The low pressure prevalent at the summit of high mountains is a factor that could be added to the organ vitrification protocols. In this regard, the use of gaseous nitrogen instead of liquid nitrogen would allow manipulation of the pressure to which the vitrified organs are exposed, which could lead to optimized results, particularly concerning the reversibility of the process. Considering that low oxygen levels are thought to favor the preservation of mummies at high altitudes, it might be worthwhile exploring the potential benefits of completely replacing oxygen in vitrification protocols, with nitrogen or some other inert gases. Building on what is known about the conditions that favor the preservation of mummies at high-altitude, other researchers could envision alternative ways to apply the protective factors present at high altitudes, to organ vitrification and the development of biobanks. Undoubtedly, organ vitrification technology is in an early stage of development and could benefit from the adoption of components found in related processes, such as the preservation process of the Llullaillaco children.

Acknowledgements

The authors thank Ms. Yolanda E. Sosa for technical and editorial assistance and Architect Aylen Gonzalez Crespo for figure design. Part of the work reviewed here was supported by grant BRLS# 2024-02 from the Biomedical Research and Longevity Society to RGG and grant PIP2021# 11220200103136 to CBH.

Credit authorship contribution statement

RGG, MB, MDG: Writing – review & editing, Writing – original draft, MAG, CBH-Conceptualization – review & editing, RGG, CBH-Funding acquisition.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Submission declaration

The work described here has not been published previously (except in the form of an abstract), it is not under consideration for publication elsewhere, its publication is approved by all authors. This work will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

References

1. J Castro. "Final Moments of Incan Child Mummies' Lives Revealed" (https://www.livescience.com/38504-incan-child-mummies- livesrevealed.html). Live Science. 2013.
2. J Chen, X Zhang, IV Khaydukova, et al. Cryopreservation of tissues and organs: present, applications, and challenges. Front Vet Sci. 2023;10:1201794.
3. A Chiu-Lam, C Rinaldi. Nanoscale thermal phenomena in the vicinity of magnetic nanoparticles in alternating magnetic fields. Adv Funct Mater. 2016;26,3933–3941.
4. A Corthals, A Koller, DW Martin, R Rieger, Chen EI, et al. Detecting the Immune System Response of a 500 Year-Old Inca Mummy. PLoS ONE. 2012;7(7):e41244.
5. JP Costanzo, MCF do Amaral, AJ Rosendale, RE Lee. Seasonality of freeze tolerance in a subarctic population of the wood frog, Rana sylvatica. Int J Zool 2014;750153.
6. JP Costanzo, MCF do Amaral, AJ Rosendale, RE Lee. Cryoprotectants and extreme freeze tolerance in a subarctic population of the wood frog. Rana Sylvatica. 2014.
7. DP Eisenberg, JC Bischof, Y Rabin. Thermomechanical stress in cryopreservation via vitrification with nanoparticle heating as a stress-moderating effect. J Biomech Eng. 2015;138:11010.
8. ML Etheridge, Y Xu, L Rott, J Choi, B Glasmacher, JC Bischof. RF heating of magnetic nanoparticles improves the thawing of cryopreserved biomaterials. Technology. 2014;02:229–242.
9. GM Fahy, B Wowk, in Cryopreservation and Freeze-Drying Protocols, WF Wolkers, H Oldenhof, Eds. Springer New York, 2015;21–82.
10. GM Fahy, B Wowk, J Wu J, et al. Cryopreservation of organs by vitrification: perspectives and recent advances. Cryobiology. 2004;48:157–178.
11. Guidebook to the Museum of High-Mountain Archaeology (original title: Catálogo del Museo de Arqueología de Alta Montaña (MAAM), Salta Capital), 2nd Ed, 2007.
12. Z Han, A Chiu-Lam, Gangwar L, et al. Vitrification and nanowarming enable long-term organ banking for transplantation. Nat Commun. 2023;14:3309.
13. B Handwerk. "Inca Child Sacrifice Victims Were Drugged" (https://web.archive.org/web/20210218050038/https://www.nationalgeographic.com/culture/article/130729-inca-mummy-maiden-sacrifice-coca-alcohol-drugmountain-andes-children). National Geographic Society. 2021.
14. AR Lespinard, M Bernaski, G Recagno, RH Mascheroni. Mathematical modeling and simulation of physical phenomenology in children of Comechingonia Virtual: Nº 1: 119-153 Llullaillaco (in Spanish). 2013.
15. JK Lewis, JC Bischof, I Braslavsky, KGM Brockbank, GM Fahy, BJ Fuller, et al. The grand challenges of organ banking: Proceedings from the first global summit on

complex tissue cryopreservation. Cryobiology. 2016;72:169–182.
16. N Manuchehrabadi, Z Gao, Zhang J, et al. Improved tissue cryopreservation using inductive heating during nanowarming. Sci Transl Med. 2017;eaah4586.
17. PM Mehl. Nucleation and crystal growth in a vitrification solution tested for organ cryopreservation by vitrification, Cryobiology. 1993;30:509–518.
18. J Reinhard, Cerruti C. Investigaciones Arqueológicas en el volcán Llullaillaco. Ediciones de la Universidad Católica de Salta. Argentina; Comechingonia Virtual: año 2013, nº 1: 119-153 153. 2000. (in Spanish).
19. A Sharma, A Chiu-Lam, A Churski, et al. Vitrification and nanowarming of kidneys. Adv Sci. 2021;8:2101691.
20. KB Storey. Freeze tolerance in the frog, Rana sylvatica. Experientia. 1984;40:1261–1262.
21. MJ Taylor, YC Song, KGM. Brockbank. Vitrification in [Tissue Preservation: New Developments, in Life in the Frozen State, B. J. Fuller, N. Lane, E. E. Benson, Eds. CRC Press, 2004.
22. EE Vigl. "Los Niños del Llullaillaco" Scientific evidence on the condition of the three Inca mummies of Llullaillaco and proposals for their preservation. Summary of the Bolzano Report. J Glacial Archaeol. 2014;1(1):79–97.

Citation of this Article

Gallardo MD, Bernaski M, Girard MA, Hereñu CB and Goya RG. Vitrification and High-Altitude Mountain Environments: Two Alternative Ways to Achieve Organ Preservation. Mega J Case Rep. 2026;9(5):2001-2010.