Extracorporeal Liquid Nitrogen-Treated Autografts in Limb-Salvage Surgery : A Review of Biological and Mechanical Outcomes

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Abstract

Objective: This narrative literature review aims to evaluate liquid nitrogen treated autografts (-196°C) as a biological limb salvage alternative to metallic endoprosthesis and allografts in bone tumour management.

Methods: This review consists of clinical literature from 1994-2026 and compiled data on local recurrence, biomechanical integrity, bone union rates, and MSTS functional scores.

Results: Liquid nitrogen cryotherapy demonstrated high rates of local oncological control for the studied cohorts (8.7% cumulative rate of local recurrence within four years) and preservation of the extracellular matrix, but incomplete penetration proved to be a challenge for some specific subtypes (e.g. chondrosarcoma). Pooled data from the studies also demonstrated 75% to 92% bone union by creeping substitution and MSTS functional scores of 75% to 85%

Conclusion: Liquid nitrogen treated autografts provide a durable and cost effective biologically integrated reconstruction option especially in resource limited settings.

Introduction

Bones are rigid organs that form the skeleton in most vertebrates. They support and protect the body, produce red and white blood cells, store minerals, and enable mobility (Grabowski, 2009). This skeletal system is maintained by a process called bone remodelling where mature bone tissue is removed from the skeleton (resorption) and new bone tissue is formed (ossification). Bone tissues maintain their structural homeostasis by resorbing and depositing matrices (The non-living extracellular framework of bone tissue that provides structural support and are usually made of organic matter mainly collagen and inorganic matter like calcium and phosphate groups) in response to physical stress. This equilibrium is maintained by osteoblasts (specialized cells responsible for the formation and mineralization of bone tissue) and osteoclasts (large, multinucleated cells responsible for breaking down and resorbing bone tissue) this important for maintaining for skeletal density and integrity (Hadjidakis and Androulakis, 2006). However this physiological process is compromised when primary malignant bone neoplasms (Sarcomas), e.g. Osteosarcoma, Chondrosarcoma and Ewing Sarcoma. These malignant tumours develop when abnormal cells mutate, multiply uncontrollably, and invade nearby healthy tissues.

In the past the treatment for these malignancies was a limb amputation. This was necessary to achieve wide oncological margins, ensuring total removal of the tumour to prevent recurrence and systemic metastasis, while this addresses the oncological risk it results in a lifelong disability.

However with recent major advancements in medicine, the development of neoadjuvant chemotherapy and limb salvage surgery (LSS) are the main treatments for sarcomas. LSS aims to remove the segment of the bone containing the tumour while preserving the limb's functional anatomy (Pak et al., 2025). But the primary problem of this is that it results in a major skeletal defect. The replacement material (usually metallic endoprostheses) for this must have stable mechanical properties while also having osteoconductive properties so that the limb can still have its functional anatomy. Now newer advancements show that there is another option which is cryosurgical bone freezing (or frozen inactivated autograft replantation) . This method attempts to repurpose the patient's own tumour bearing bone as a graft.

Now although there have been numerous clinical successes in Limb Salvage surgery (LSS), the main problem as stated before, are skeletal defects. Currently, orthopaedic surgeons rely on metallic endoprosthesis, vascularized allografts and autoclaved autografts. While metallic endoprosthesis immediately solves the problem of skeletal defects they have limitations such as infections, ascetic loosening and hardware fatigue, which may require many additional surgeries (this can be a major concern for many young people who may require a durable and long term solution).

The vascularized allografts use the donor's bone to solve this issue but again there are limitations such as a risk of immunological rejection, disease transmission and non fusion (where grafts fail to fuse with the patient's bone) (Barbeck et al., 2019, 111. p.). Looking at these issues with allografts it may seem that autografts (using the patients own bone) may seem ideal because there are no issues like immunological rejection or non fusion (Chen and Liu, 2016, 103. p.) but it provides other problems mainly the requirement to eliminate malignant cells from the autograft (extracorporeal sterilization) which is a rather difficult task

Some sterilization techniques include high pressure steam autoclaving or extracorporeal irradiation (ERCT), but these sterilization techniques can denature the bones extracellular matrix (ECM) and destroy signaling proteins e.g. Bone Morphogenetic Proteins (BMPs)(Ijiri et al., 1994). By doing this it results in a weak graft that does not have sufficient osteoconductive and osteoinductive properties; this compromises the process of creeping substitution. Thus this paper addresses this issue by looking over to cryotherapy where the use of liquid nitrogen is used as the sterilization medium (Migliorini et al., 2021, 123. p.). So the purpose of the paper is to investigate whether cryotherapy is a viable solution which can achieve oncological sterilization and preserve the structural integrity and biological viability of the scaffold.

The importance of this review is that there is a need for viable sterilization method in orthopaedic oncology, a method that can provide structural integrity and biological integration these two conditions rule out sterilization methods like high pressure steam autoclaving or extracorporeal irradiation (ERCT) (as they result in weak grafts that do not have sufficient osteoconductive and osteoinductive properties) and by assessing liquid nitrogen sterilization techniques this paper analyzes whether the incorporation of cryotherapy is a viable solution to provide a viable autograft.

This paper looks at prior clinical data to find out whether cryotherapy achieves localized tumour necrosis (eliminating malignant cells within the autograft) without degrading the extracellular matrix (ECM). This paper also evaluates whether this technique preserves osteoconductive signals e.g. Bone Morphogenetic Proteins (BMPs). This review also tests if the integrity of the collagen scaffold is maintained and if this is so the process of creeping substitution is significantly enhanced and could significantly improve postoperative union rates.

Additionally, this review also examines a gap in global health equality. metallic endoprosthesis and extracorporeal irradiation which may require specialized and expensive clinical infrastructure which can be unavailable in resource constrained areas . in comparison to liquid nitrogen cryotherapy which may be more cost effective thus if liquid nitrogen cryotherapy is a viable solution to sarcomas it can also promote global health equality.

Keeping all of this in mind the main objectives of this review is to evaluate the effectiveness of liquid nitrogen in eliminating malignant cells within the haversian systems, Understanding how the extreme temperature of the liquid nitrogen impacts the bones collagen matrix and overall structural stability and focusing of clinical rates of non union and pathological fractures, This paper also assess the autografts ability to facilitate the process of creeping substitution and fusion with the patients skeletal structure. And Finally to determine whether this technique is viable, sustainable and cost effective for limb preservation is developed. developing and underdeveloped areas of the world.

Methods/Approach to the Review

This narrative literature review draws upon previous clinical and experimental studies. To ensure transparency in the identification of the literature, an AI-assisted search tool (Jenni AI, https://app.jenni.ai) was used to aid in the initial search for relevant clinical studies and reviews, followed by a structured electronic search of Google Scholar and PubMed from 1994 to 2026 to supplement and verify the initial search. Boolean operators were used to search for strings combining free-text terms and MeSH headings (e.g. (“Liquid nitrogen cryotherapy” OR “Cryo-treated autograft”) AND (“Limb salvage surgery” OR “Bone sarcoma”) AND (“Biological reconstruction” OR “Creeping substitution”)).

Articles were screened against pre-specified inclusion/exclusion criteria. Inclusion criteria were peer-reviewed studies of humans (clinical studies, retrospective cohorts) and animals (animal model studies) that directly reported on oncological safety (local recurrence rates), cortical biomechanical stability, bone union rates by creeping substitution, or standardised MSTS functional scores. Exclusion criteria included non-peer reviewed sources, articles without full text, and studies without quantitative outcome data.

Limitations of this approach include the use of an initial AI-assisted tool, lack of a formal independent dual-reviewer screening process, and the integration of heterogeneous retrospective cohorts and animal models. These all have inherent risks of selection bias and clinical variability that should be borne in mind when interpreting aggregate outcomes.

I - Oncological Efficacy of Liquid Nitrogen Cryotherapy

The main aim in the management of sarcomas is the achievement of a tumour free surgical margin. By adopting cryotherapy as a sterilization method for autografts due to its ability to induce completely, irreversible necrosis of neoplastic cells within the haversian system and the medullary spaces in the autograft. This section synthesizes prior and current clinical data regarding the oncological safety of this sterilization method, focusing on recurrence rates and evidence supporting that cryotherapy via liquid nitrogen is a viable sterilization method.

Currently, clinical evidence suggests that the immersion of the autograft in liquid nitrogen should (At -196 C) for a fixed period (Approximately 20 minutes) is sufficient to remove malignant cells (Zekry et al., 2017, 1481. p.). This is explained by intracellular and extracellular ice formations. As the bone is rapidly cooled, the formation of ice crystal disrupts the cellular membranes and organelles of the malignant cells and the dehydration (which happens simultaneously) this increases intracellular osmotic pressure leading to an irreversible metabolic collapse and protein denaturation in the malignant cells. Unlike other sterilization techniques like high pressure steam autoclaving or extracorporeal irradiation (ERCT) which can cause the degradation of the collagen matrix due to high temperatures, cryotherapy does not.

Also, a literature review of 280 patients in multiple cohorts from 2008 to 2023 suggests an overall local recurrence rate of malignant cells of about 8.7% in the first four years after reconstruction. (Belzarena and Cook, 2024. There are few direct head-to-head comparative trials with the traditional limb salvage modalities such as allografts or metallic endoprostheses in the current literature but these findings suggest that liquid nitrogen-treated autografts can achieve favourable local oncologic control.

Now an important aspect of this paper is to compare between liquid nitrogen, extracorporeal irradiation (ERCT), and thermal sterilization. Firstly, ERCT can be highly effective to remove malignant cells (Sharma et al., 2013) but it requires specialized medical grade particle

accelerators, a disadvantage because this type of equipment may not be available in disadvantaged areas (resource constrained environments). Now thermal sterilization requires the use of very high temperatures (autoclaving) that can be deemed acceptable to remove malignant cells from the autograft; however , it degrades bone collagen structures and significantly reduces the graft's load-bearing capacity. It can also destroy all osteocytes which can negatively impact the graft's natural osteoinductive and osteoconductive capabilities, this substantially slows down the process of creeping substitution and can increase the rate of non unions.

It is important to know that clinical success heavily relies on technical execution of the resection. Now in Cryotherapy there are 2 types of freezing techniques : the pedicle freezing procedure and the free freezing procedure (Zekry et al., 2017, 1482. p.), both of these methods present challenges. Firstly, the pedicle freezing procedure is excellent for localized treatment but the free freezing procedure (where the bone is fully separated) ensures a 360 degree exposure to cryogen which eliminates the chances of blind spots where malignant cells could exist even after cryotherapy. Most data suggests that recurrence cases mostly occur in cartilaginous components (Mohler et al., 2010) e.g. chondrosarcoma, where the dense matrix may interfere with the penetration of cryogenic temperatures (-196 C) this means that the autograft may have to be immersed in liquid nitrogen for longer.

Now assessments on the re-implanted autograft retrieved during revision surgeries provide key evidence of the technique's efficacy. And postoperative biopsies and haematoxylin and eosin staining have told us that there is a total absence of malignant cells. In the tissue was seen having vacant lacunae where malignant cells and osteocytes had resided, this made sure that the structural scaffold was maintained while the threat of a malignant tumour was removed.

Despites these favourable outcomes we must look over to the challenges. For instance we must take into account the surgical resection one by an orthopaedic surgeon and if there is a use of poor surgical technique we cannot necessarily consider this a success. Furthermore, it’s also important to acknowledge that cryotherapy can no longer be of help when the malignant tumour has spread to soft tissues. In addition to this there is a variability in recurrence rates which may highlight a lack of standardized protocol (Malawer et al., 1999; Sumari et al., 2022) And finally Factors such subtypes of sarcomas (e.g. osteosarcoma or Ewing sarcoma), the tumour location and the depth of penetration required for freezing are variables that should be taken into account for future clinical trials.

II - Biomechanical Integrity of Cryo-treated Autografts

The successes in Limb Salvage surgery using a recycled autograft is primarily linked to the mechanical capabilities of the autograft. The main problem here is to check whether the extreme temperatures (-196 C) result in the micro-structural degradation, which can cause the bone

scaffold to be susceptible to mechanical failure or fracture. Thus this section primarily synthesizes current and prior experimental and clinical data regarding the impact of cryogenic sterilization on cortical bone density.

In comparative biomechanical analyses, cryotherapy (Performed under standardized protocols) does not degrade the structural integrity of the bones to significant degree where it may be in danger of mechanical failure or fracture when compared to a native, untreated bone. In experiments (Controlled) e.g. Four Point Bending Compression Tests Conducted by Nik Abdul Adel et al. (2022) (Adel et al., 2022), found no substantial differences in maximum compressive load, stress or strain between liquid nitrogen treated specimens and control specimens. These findings suggest that cryogenic treatment preserves the biomechanical properties of the cortical scaffold unlike other sterilization techniques such as High dose radiation or Prolonged Thermal Exposure (However it is important to note that this study was experimented on sheep bones).

Additionally, clinical data supports the observation that these autografts provide adequate stability for physiological load-bearing. In the study done by Xu et al. on frozen autograft-prosthesis composite (FAPC) reconstructions, researchers found that it provided satisfactory biomechanical stability, with no cases of greater trochanter avulsion or prosthesis loosening in long term follow up (Xu et al., 2019). Furthermore, an important factor of the stability of these constructs is the precision of the fixation methods e.g. double plate osteosynthesis that fixes the issue of mechanical risk in the early stages of graft incorporation (Idulhaq et al., 2026).

Since it has been concluded that these autografts can provide sufficient mechanical strength there is still a risk of pathological fracture during the biological healing process, specifically the time required for host-derived creeping substitution to replace the devitalized matrix. In early cryosurgical techniques, for instance the direct pour method which was associated with high fracture rates (17 - 25 %) due to difficulties in controlling depth of freezing and the extent of bone damage (Lee et al., 2011).

But due to modern advancement in application methods such as the pressurized-spray technique and the free freezing protocols having reduced those risks. In recent clinical reviews the risks of pathological fractures are significantly lower and with many reporting negligible or zero fracture rates prophylactic internal fixation was used (Lee et al., 2011). And the main reasons for failure for recycled autografts is usually infection or non-union rather than direct mechanical fatigue of the graft (Klangjorhor et al., 2026).

In conclusion, evidence points out that using liquid nitrogen as a sterilization technique is biomechanically neutral regarding the structural strength of the cortical bone matrix. and The risks that do exist are largely due to biological transition rather than weakness caused by the freezing process.

III - Biological Integration and Creeping Substitution

The primary advantage of using an autograft rather than allografts or metallic prosthesis lies in the properties of osteoinduction and osteoconduction and unlike allografts and synthetic implants, an autograft remains a biological template. This section of the review evaluates the process of creeping substitution in which the osteoclasts of the host resorb the autograft and replace it with new vital bone; it also examines the clinical rates of union seen in literature.

Creeping substitution is the biological process by which dead, necrotic, or transplanted bone tissue is slowly resorbed by the body and simultaneously replaced with new, living bone. In autograft treated with liquid nitrogen the internal structure of the cortical bone remains intact (this includes the haversian systems and interstitial lamellae). And 6 to 12 months after the implantation of the autograft, host derived blood vessels (angiogenesis) and osteoprogenitor cells begin to infiltrate these channels. This infiltration happens efficiently because the cryogenic process which can kill malignant cells does not harm it does not denature the bones extracellular matrix (ECM) proteins(Yamamoto and Tsuchiya, 2017).

Now the best measure of this integration can be the clinical union rate of the percentage of cases where the autograft fuses with the host bone. A review done by Garg et al. suggests union rates to be range from 75 - 92% (Garg et al., 2020) which can seem favourable when compared to rates of non union when using allografts (likely due to immunological incompatibility and a lack of osteoinductive capacity).

Moreover the time taken for this clinical union to happen is also an important factor. Most data indicates the clinical union is achieved within 9 to 15 months but radiological evidence suggests that complete graft remodelling can take much longer (up to 24 months) (Rabin et al., 2026). And during this period the graft remains vulnerable because the resorption phase of creeping substitution can significantly decrease the grafts total mineral density.

Recently there has been the use of adjunctive therapy to speed up the process of creeping substitution. This is because cryogenic treatment can sometimes inhibit the release of endogenous growth factors. Due to this, applying bone marrow aspirate concentrate (BMAC) or Bone Morphogenetic Proteins (BMPs) to the surface of the autograft before implantation can bridge the gap between simple osteoconduction and active osteoinduction.

Additionally, individual clinical case reports have shown successful bone healing timelines using liquid nitrogen-treated autografts, but comparative studies are needed to quantify exact reductions in union times compared to standard techniques (Idulhaq et al., 2026) These results suggest that along with the sterilization process (treating the autograft with liquid nitrogen) the autograft must also be treated by cellular therapies which can increase the mechanical reliability of the autograft.

IV - Clinical Utility and Functional Sustainability

Another important measure of the liquid nitrogen treated autograft techniques is its ability to restore long term limb function and quality of life. And unlike prosthetic reconstruction which may be prone to mechanical failure and are finite, an autograft aims to provide a permanent solution. So this section looks over data on functional outcomes and sustainability of the technique as a limb-salvage modality.

Musculoskeletal Tumour Society (MSTS) scoring system is used frequently to quantify clinical success it evaluates based on numerous criteria e.g. pain, function, emotional acceptance and support requirements. Recent data suggest patients treated with cryo-treated autografts usually achieve MSTS scores of 75% to 85% (Zekry et al., 2017). These scores demonstrate favorable patient recovery and limb preservation, indicating strong functional rehabilitation following reconstruction. This is mainly due to the autograft to maintain soft tissue attachment, primarily the tendons and ligaments that facilitate joint movement. And because the autograft is the patient's own bone the anatomical insertion site remains intact allowing for greater physiological range of motion and muscle power.

In Orthopedic oncology, sustainability is defined by the longevity of the construct and avoidance or revision surgeries (the need for more surgeries after failed attempts). Standard endoprosthetic reconstructions often result in long-term mechanical and biological complications, including aseptic loosening and periprosthetic infection, potentially requiring revision surgeries in the future (Sanders et al., 2026). However the nature of the cryo-treated autograft is so that it offers a solution to this as when the graft achieves complete union with the host bone it becomes a permanent part of the patient's body and can adapt to the changing physiological load that the patient experiences through their life. Another point to note is that since the patient's own bone is used rather than an expensive specialized prosthetic or a complex particle accelerator (required for autografts treated with extracorporeal irradiation) this make it reliable in underdeveloped and developing countries which may not have access to such infrastructure (Idulhaq et al., 2026).

Patient satisfaction is another aspect that must be looked into. Clinical results indicate that biological limb preservation with liquid nitrogen-treated autografts has excellent structural recovery and good long-term functional recovery compared to conventional prosthetic replacement (Zekry et al., 2017). Furthermore the capacity of the bone to respond to natural stress implies that the limb remains dynamic which is an advantage over prosthetic limbs which remain static.

This method does have disadvantages because the time for complete recovery is long and during the time of recovery patients can not put stress on the limb this can lead to many patients

to develop a negative attitude and they may likely require strong support from experts e.g. physical therapists and sometimes even mental health counsellors.

Discussion

Liquid Nitrogen Cryotherapy eliminates malignant cells in the haversian systems and the medullary spaces. This happens because liquid nitrogen cryotherapy induces intracellular and extracellular ice formations and metabolic collapse while preserving the extracellular matrix (ECM) and proteins e.g.Bone morphogenetic proteins (BMPs). This is important as this eliminates the tumour within bone grafts while maintaining structural integrity.

In prior studies it was shown that cryotherapy avoids collagen degradation that is caused by high pressure steam autoclaving and extracorporeal irradiation (ERCT).

While older studies do show high fracture rates, this is because they mainly used direct pour methods, in contrast recent studies which show lower fracture due to the use of other methods like pressurized spray techniques or free freezing protocols.

Now the strengths of this review are that it evaluates the autograft systematically using four points : oncological safety, biomechanical strength, biological integration, and functional outcomes. Moreover it shows how cryotherapy may be an option in resource constrained areas as it avoids the need of particle accelerators needed for ERCT. It looks how cryotherapy preserve native soft tissue that provide higher MSTS functional scores and the psychological ownership compared to metallic endoprostheses

There are limitations in this review for instance it integrates retrospective cohorts, animal models (e.g. Sheep bone studies) and inconsistent technical parameters (such as different immersion times). Another important limitation to note is that during the resorption phase of creeping substations the grafts suffer from a decrease of total mineral density making them vulnerable. And cryotherapy can sometimes not eliminate sarcomas such as chondrosarcoma due to dense cartilaginous material and it is also important to note that this technique no longer works once the tumour spreads to soft tissue.

Although the positive clinical results in limb salvage reconstruction there are multiple critical questions as well as future research directions that need to be approached.

Firstly, there is a lack of global consensus concerning technical parameters e.g. concerns on the duration of immersion of the autograft in liquid nitrogen, the number of freeze-thaw cycles, consistent thawing protocols which eliminate the maximum amount of malignant cells while minimising structural damage to the graft.

Moreover, there must be further research done on the use of bone morphogenetic proteins or bone marrow aspirate concentrate (BMAC) to decrease the time taken for clinical union to happen.

Although Medium term survival rates are well researched on, prospective longitudinal studies with follow ups duration more than than 10 years are less common, this is important as it can evaluate late onset structural fatigue, cortical resorption and the long term durability.

Prosthetic composite optimization (reducing mechanical stress where 2 implants meet a frozen bone graft) is another key area that must be well researched on. And by understanding how these 2 material interactions can help to prevent aseptic loosening or structural specifically in active patients

Conclusion

This paper shows that liquid nitrogen cryotherapy provides a safe and biologically integrative solution which maintains the structural integrity of the bone graft (preserves the extracellular matrix) while eliminating malignant cells and it enables higher union rate when compared to other methods and it also offers a sustainable and cost effective alternative to other methods.

Furthermore, liquid nitrogen cryotherapy is a cost effective alternative to expensive metallic endoprostheses or techniques that require expensive infrastructure e.g. extracorporeal irradiation.

However, future research is needed to find standardised freezing protocols (e.g. immersion duration and temperature consistency) and there is a need for future longitudinal studies lasting more than 10 years to assess long term structural durability.

Recommendations include to make a standardised method which will eliminate technical variability, decrease recurrence rates and to use biological enhancements like BMPs and BMAC to speed up the process of creeping substitution.

Closing Statement : Liquid nitrogen cryotherapy is a viable solution to eliminating sarcoma in bone grafts as it eliminates malignant cells while maintaining the graft structural integrity, this provides a durable and sustainable graft.

Conflict of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

Author Contributions (Credit)

Kunwar Yoaab Jiwanmall: Conceptualization, literature research, data curation, writing of the original draft, and manuscript review and editing.

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