in vivo *38*: 1-39 (2024)
doi:10.21873/invivo.13407

Review

Cryogenic Media in Biomedical Applications: Current Advances, Challenges, and Future Perspectives

KEFAH MOKBEL[1], ALEVTINA KODRESKO[2], HEBA GHAZAL[3], RAMIA MOKBEL[4], JON TREMBLEY[5] and HUSSAM JOUHARA[2,6]

[1]*The London Breast Institute, Princess Grace Hospital, London, U.K.;*
[2]*Heat Pipe and Thermal Management Research Group, College of Engineering, Design and Physical Sciences, Brunel University, London, U.K.;*
[3]*Kingston University, School of Pharmacy and Chemistry, Kingston Upon Thames, U.K.;*
[4]*The Princess Grace Hospital, part of HCA Healthcare UK, London, U.K.;*
[5]*Air Products PLC, Hersham Place Technology Park, Surrey, U.K.;*
[6]*Vytautas Magnus University, Kaunas, Lithuania*

Abstract. *This paper explores the crucial role of cryogenic mediums in driving breakthroughs within the biomedical sector. The objective was to investigate, critically discuss, and present the current knowledge and state-of-the-art practices, along with the challenges and perspectives of the most common applications. Through an extensive literature review, this work aims to supplement existing research, offering a comprehensive and up-to-date understanding of the subject. Biomedical research involving cryogenic mediums is advancing on multiple fronts, including the development of advanced medical technologies, clinical treatments for life-threatening conditions, high-quality biospecimen preservation, and antimicrobial interventions in industrial food processing. These advances open new horizons and present cutting-edge opportunities for research and the medical community. While the current body of evidence showcases the impressive impact of cryogenic mediums, such as nitrogen, helium, argon, and oxygen, on revolutionary developments, reaching definitive conclusions on their efficiency and safety remains challenging due to process complexity and research scarcity with a moderate certainty of evidence. Knowledge gaps further underline the need for additional studies to facilitate cryogenic research in developing innovative technological processes in biomedicine. These advancements have the potential to reshape the modern world and significantly enhance the quality of life for people worldwide.*

Correspondence to: Hussam Jouhara, Heat Pipe and Thermal Management Research Group, College of Engineering, Design and Physical Sciences, Brunel University, London, UB8 3PH, UK. E-mail: hussam.jouhara@brunel.ac.uk

Key Words: Campylobacter decontamination, cryogenic mediums, cryosurgery, cryotherapy, medical technology, sperm cryopreservation, review.

Cryogenics, dating back to 1877, saw its early beginnings when Louis Cailletet first liquefied oxygen in Paris using compression and immersion cooling in sulphur dioxide, resulting in a brief fog of oxygen droplets. Since then, significant research and remarkable theoretical and experimental advancements in materials' behaviour at ultra-low temperatures have fostered numerous cryogenic heat transfer applications, driving innovative technological processes that can transform the modern world. Cryogenic mediums, known as cryogens, play a critical role in healthcare, superconductivity, advanced manufacturing, machining methods, aerospace, and advanced scientific research (1). Among these mediums, argon (Ar), helium (He), nitrogen (N2), and oxygen (O2) have found applications in biomedicine due to their unique properties, which vary with pressure, temperature, and phase. Notably, N2 and He are widely used cryogenic mediums, owing to N2's inert behaviour, thermal properties, high availability, and cost-effectiveness, while He's capability to exist in a liquid state at near-absolute zero temperatures presents challenges in processing and storage optimization. O2, as the third most abundant element in the universe, possesses commercial

viability as a cryogen due to its natural abundance and accessibility (2, 3). Additionally, Ar, an abundant atmospheric gas obtained through liquid air fractional distillation, is widely used for its inert properties. Current research continuously investigates cryogenic mediums, focusing on heat transfer and material behaviour, often incorporating the development of new numerical models to improve technology performance. Innovative and efficient energy storage methods are also under study to cater to processing and storage needs.

However, it's important to note that despite discussions surrounding xenon (Xe) and carbon dioxide (CO_2) gases, both possessing low temperature boiling points of −108.1˚C and −78.5˚C, respectively, cryogenic experts and standards do not classify them as cryogenic mediums since the cryogenic range at ambient pressure is suggested to start below −150˚C.

Considering the impressive discoveries and technological advances in cryogenic mediums for biomedicine, this paper aims to investigate and critically analyse current knowledge and state-of-the-art practices while addressing challenges and offering perspectives on the most common applications. The article will provide a comprehensive and up-to-date review of current applications, identifying knowledge gaps and suggesting areas for future research.

Medical Technology

Magnetic resonance imaging. One of the most remarkable advances in medical technology is magnetic resonance imaging (MRI), a powerful tool that plays an increasingly significant role in clinical diagnosis and treatment monitoring. Unlike traditional imaging methods that utilize ionizing radiation, MRI produces detailed three-dimensional (3D) anatomical images without any harmful radiation exposure. This makes it a safe and highly valuable diagnostic tool for various medical conditions.

MRI excels in providing exceptional soft tissue contrast, making it particularly useful for examining conditions involving the spine, brain, or abdomen, including vascular abnormalities, infections, and multiple sclerosis. The ability to visualize these areas in such great detail enhances medical professionals' ability to make accurate diagnoses and design effective treatment plans.

A critical component in the efficient and reliable functioning of MRI machines is Liquid Helium (He), which serves as a cryogenic coolant. Operating at an incredibly low temperature of −269.1˚C, Liquid Helium enables the necessary levels of superconductivity required in the scanner's magnetic coils. This, in turn, allows electrical current to flow through the coils with minimal resistance, resulting in the generation of high-intensity magnetic fields essential for the imaging process, schematically represented in Figure 1 (4).

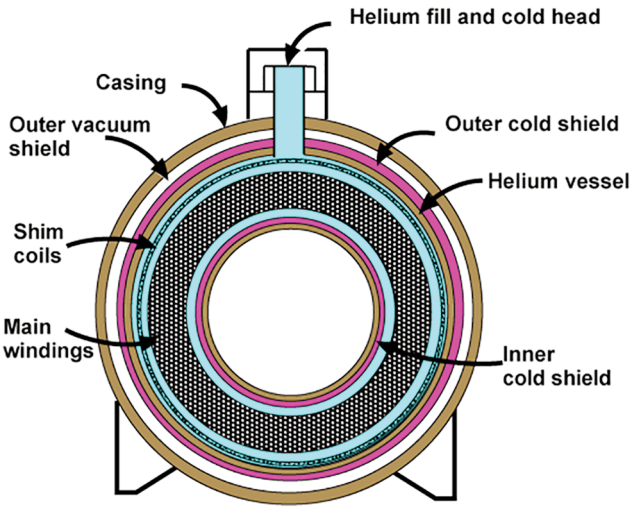

Figure 1. *Schematic representation of a typical superconducting magnet with liquid He chambers coloured blue (4).*

Throughout the years, helium's demand has outstripped supply due to various factors. Helium is a non-renewable natural resource with limited availability on Earth, and its importance has grown significantly in multiple industries, such as fibre-optic telecommunications and space propulsion. However, the number of producers remains relatively small. In 2012, this situation led to a global helium shortage, causing prices to soar by over 250% (5).

During the helium scarcity, the scientific community faced challenges due to limited purchasing power, especially concerning the operation of MRI scanners, which require a substantial amount of periodically topped-up liquid gas, approximately 1,700 litres per scanner. To address this issue, new scanner designs were developed, capable of functioning with only around 7 litres of gas sealed within the device. These innovative designs eliminate the need for constant refilling and prevent helium from escaping, offering more flexibility in placement as well.

One notable example of such a design is the Philips BlueSeal MRI magnet, which employs zero boil-off refrigeration and was introduced in 2018. This ground-breaking technology allows for helium-free operations, enhancing convenience and safety. Despite the impressive advancements in cryogen-free designs that have improved efficiency and reduced costs, further research is needed to address certain challenges. Some studies have identified complications arising from magnetic field variations caused by temporal magnetic field instability (6, 7).

Cystic fibrosis. Interestingly, cryogenic mediums play a vital role in MRI modality, serving as contrast agents to enhance visibility. They work on the principle of shortening the T1

relaxation time of protons within body tissues, which boosts the decay rate of induced polarization. This process facilitates high-resolution image construction with 3D spatial and temporal precision through systematic sampling across the examined tissue's spatial region.

Over the past few decades, this non-invasive assessment has proven crucial in studying lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF). CF is an inherited monogenic disorder affecting at least 162,000 people in 94 countries. It is caused by mutations in both gene copies that encode the cystic fibrosis transmembrane conductance regulator (CFTR) protein, responsible for the functioning of the epithelial ion channel. CFTR is essential for the transportation of chloride and bicarbonate, as well as mucus hydration and clearance (8, 9).

The hallmarks of CF include chronic upper respiratory tract diseases, such as nasal polyps and rhinosinusitis, along with lower respiratory tract diseases like neutrophilic and muco-obstructive inflammation. Additionally, CF can lead to pancreatic exocrine insufficiency, cystic fibrosis liver disease, and cystic fibrosis-related diabetes (10, 11). However, until recently, MRI imaging of low lung density regions posed challenges due to the inherently low-fat proton abundance and corresponding low signal (12).

Furthermore, the presence of multiple air-tissue interfaces in traditional pulmonary MRI has been causing susceptibility artefacts and magnetic field distortions. These issues compromise the magnetic resonance signal, resulting in degraded image quality of cardiac and respiratory motion during image acquisition. Consequently, this hampers the clinical utility of pulmonary MRI (13).

However, recent advancements in medical research have led to the development of a novel pulmonary MRI technique that utilizes the inhalation of hyperpolarized noble gases as contrast agents (referred to as HP MRI). This innovative approach has demonstrated the capability to detect changes in lung microstructure, perfusion, and ventilation associated with CF disease. Numerous studies have consistently shown that this new imaging technique holds promise as a valuable tool for early detection and longitudinal monitoring of CF progression. For instance, a study conducted by Mallallah *et al*. (14) highlighted the high sensitivity of HP MRI in comparison to the forced expiratory volume in 1 second (FEV1) modality for detecting CF structural and functional ventilation defects, as well as monitoring the response to treatment. HP MRI overcomes the limitations associated with low proton density in standard MRI.

Traditionally, FEV1 has been the primary measure used to characterize lung function. However, it has its limitations, such as its inability to depict total airway resistance and insensitivity to obstructions in small airways, which contribute to approximately 10% of overall healthy adults' resistance. Moreover, using high percentage predicted FEV1 (ppFEV1) values alone cannot determine the existence of damage or the potential for improvement in lung function through treatment, as it relies on population-based metrics with spirometry nature. Consequently, a highly sensitive technique like HP MRI holds great promise as a novel modality for pulmonary imaging in medical research, providing a more comprehensive assessment of lung function and potential treatment outcomes.

Hyperpolarized helium-3 (3He) is one of the most commonly used gases in HP MRI, obtained in gas mixtures with dilute concentrations through spin-exchange optical pumping (SEOP) and further cryogenic gas separation (15). Research has shown that 3He enables the detection of ventilation defects in CF (Cystic Fibrosis) patients with normal spirometry, and these abnormalities identified by HP MRI 3He are correlated with structural abnormalities observed through computed tomography (CT) (16-18). Moreover, the ventilation defects found in these patients have been observed to respond to conventional treatments such as mechanical airway clearance and bronchodilators (19, 20).

A significant study conducted by Altes *et al*. (21) supports this finding, demonstrating the capability of 3He MRI to assess and evaluate the effect of short-term and long-term ivacaftor treatment. This highlights the technique's potential as an efficient measure in clinical trials for determining the effect of treatments in CF by assessing regional lung ventilation, including lobar analysis, and personalizing disease monitoring.

Despite its relatively high cost and low availability, 3He has been found to be efficient and safe, even in vulnerable paediatric, elderly, and respiratory-compromised patients. However, there is another contrast gas agent - xenon-129 (129Xe) - which has been found to more efficiently dissolve in the blood and provide better information on gas exchange. Nonetheless, polarizing 129Xe is more challenging, and there are not fully developed clinical and research protocols for its application (22, 23). As a result, the transition to using naturally available 129Xe for CF HP MRI is still questionable due to the scarcity of studies.

Numerous previous comparative research studies have demonstrated that both HP 3He and 129Xe MRI are sensitive to CF lung function impairment, including mucus plugging, bronchial wall thickening, tissue destruction, airspace consolidation, and bronchiectasis (18-20, 24-27). Interestingly, Shammi *et al*. (23) concluded that the choice between 129Xe and 3He is largely inconsequential, but xenon could be more sensitive to partial obstructions. Their findings, along with MR images and associated defect analysis, are depicted in Figure 2.

However, there are contradictory findings as well. A study by Thomen *et al*. (28) identified that 49% of the 129Xe defect volume was not attributed to an apparent structural abnormality, highlighting the controversial 129Xe MRI

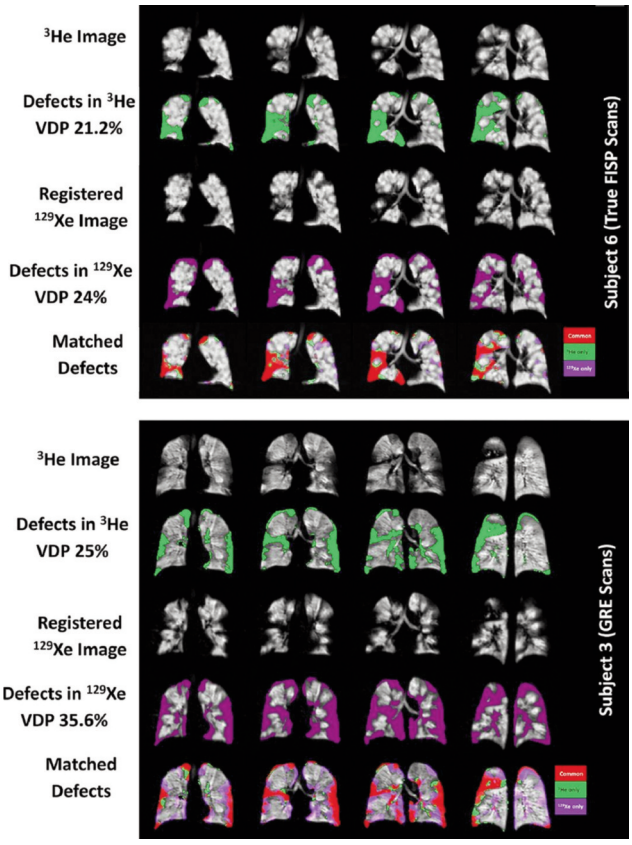

Figure 2. *MR images demonstrating defect analysis for both ^{129}Xe and ^{3}He gases from two representative subjects. Top set of images (Subject 6): excellent concordance between the ventilation defects with both ^{129}Xe and ^{3}He; bottom set of images (Subject 3): shows more and larger ventilation defects with ^{129}Xe compared to ^{3}He. MR, Magnetic resonance (23).*

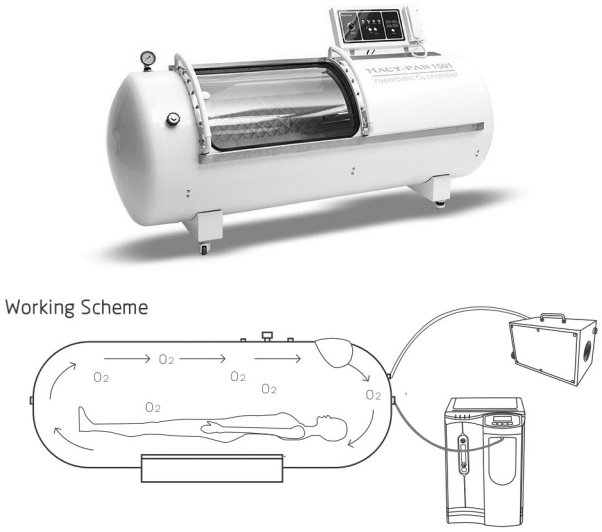

Figure 3. *Top: A typical HBOT mono chamber; Bottom: Diagram of HBOT working scheme including a chamber, air compressor, and oxygen concentrator. HBOT, Hyperbaric oxygen therapy (30).*

sensitivity to ventilatory impairment. More research is needed to clarify and establish the advantages and limitations of using 129Xe in CF HP MRI.

Overall, there is strong and robust evidence supporting the validity, reliability, and reproducibility of HP MRI studies in patients with cystic fibrosis. This innovative technique utilizes cryogenic 3He, offering a unique advantage in early disease detection and longitudinal monitoring. Its potential to improve the survival rates of CF patients, who still face significant unmet treatment needs, including minimally invasive and pharmaceutical interventions, is promising.

However, further validation studies are necessary to address certain aspects. These include investigating modality sensitivity, accessibility, and obtaining quantitative values that can justify the complexity and cost associated with HP MRI. By conducting such studies, we can enhance the acceptance and implementation of this advanced imaging technique in clinical practice.

Recent advances in low-helium zero boil-off systems have brought attention to potential areas of promising future developments. These areas warrant more comprehensive and cost-effective investigations to harness their full potential. Nevertheless, it's important to acknowledge that liquid helium remains a critical resource, acting as the lifeblood of scientific enterprise. It is indispensable in fuelling essential, life-saving standard MRI technologies, which have already contributed to numerous biomedical patents, fundamental scientific discoveries, and Nobel prizes.

Hyperbaric oxygen therapy. The significance of oxygen in medicine dates back to 1600, but its widespread use began in 1943 with hyperbaric oxygen therapy (HBOT) for decompression sickness in military divers and aviators. This involved administering 100% oxygen at higher pressures in a chamber to increase oxygen delivery to tissues. Hyperbaric chambers often use liquid oxygen for HBOT, along with converters and bottled gas backup (29). You can see a typical mono chamber in Figure 3 (30).

The treatment technique's mode of action is rooted in gas laws, along with the biochemical and physiological effects of hyperoxygenation. It can be categorized into primary and secondary effects (31). Firstly, the heightened oxygen concentration results in increased oxygen tension in tissues and enhanced diffusion. Secondly, hyperoxygenation triggers vasoconstriction, angiogenesis, fibroblast proliferation, and increased leukocyte oxidative killing in tissues. These processes are crucial for addressing conditions like central retinal artery occlusion, problem wounds, carbon monoxide poisoning,

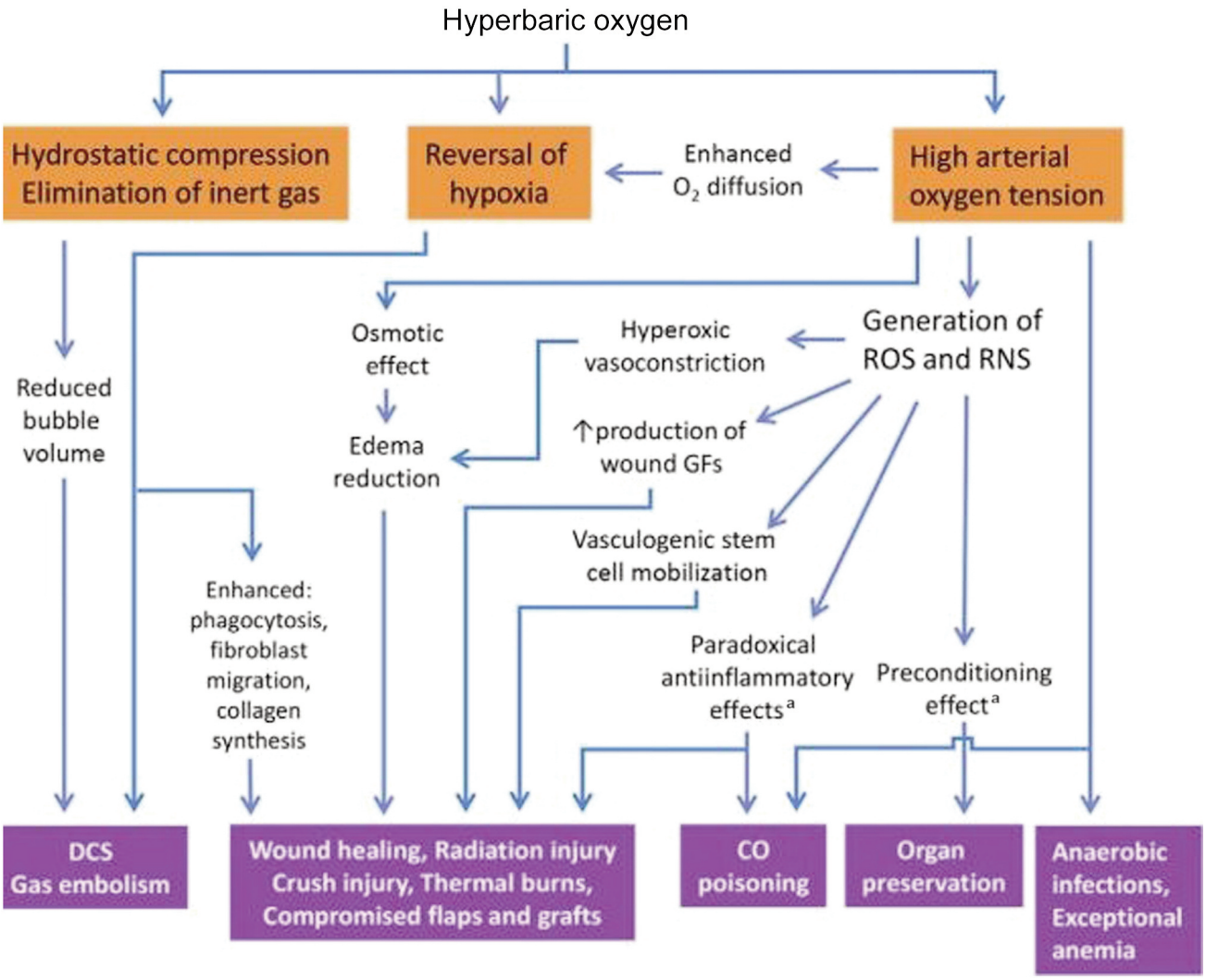

Figure 4. *Three principal therapeutic mechanisms of HBOT and associated clinical conditions, including multiple reactive species induction as hemoxygenase-1 and including heat shock proteins. DCS, Decompression sickness; GF, growth factors; HBOT, hyperbaric oxygen therapy; RNS, reactive nitrogen species (29).*

clostridial myonecrosis (gas gangrene), decompression sickness, and intracranial abscesses, such as necrotizing soft tissue infections. These mechanisms are visually illustrated in Figure 4, showcasing the principal therapeutic mechanisms and associated processes initiated by HBOT.

Problem wounds management. Based on statistical evidence, problem wounds represent a silent epidemic impacting quality of life and healthcare costs due to their failure to heal within a standard medical timeframe. They encompass skin perfusion restoration, pressure relief, metabolic control, infection treatment, and local wound care. A notable example is diabetic foot ulcers (DFUs), a severe complication of diabetes linked with peripheral arterial occlusive disease (PAOD) and lower extremity amputations (32-36). Hyperbaric oxygen therapy (HBOT) offers promise by inducing physiological changes like wound hypoxia correction. It enhances tissue oxygenation, vasoconstriction, fibroblast proliferation, suppresses inflammatory cytokines, elevates growth factors, boosts immune function, and stimulates angiogenesis (37). Notably, HBOT upregulates vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), preserving intracellular ATP levels (38). HBOT also stimulates nitric oxide synthase 3 (NOS-3) in bone marrow, crucial for circulating stem progenitor cell mobilization (39). Although HBOT usage shows no adverse events statistically, potential side effects like middle ear barotrauma (2%), myopia, lung collapse, and oxygen toxicity seizures should be considered (40-42).

Current studies consistently demonstrate HBOT's effectiveness in enhancing DFU healing and reducing amputations (43-46). Huang *et al*.'s study (47) highlights HBOT's role in fibroblast proliferation, angiogenesis regulator production (SDF-1 and VEGF), and activation of receptors CXCR4 and VEGFR. Fadol *et al*. (41) exhibit improved ulcer healing and reduced amputation rates in non-healing DFU cases, demonstrating safety and efficacy. Oley *et al*.'s study (48) shows accelerated healing through increased serum IL-6 and VEGF levels, as reflected in PEDIS score reduction. Systematic reviews like Brouwer *et al*. (49) suggest no difference in minor amputation rates or healing time, but major amputation rates decrease in PAOD patients with DFUs, contingent on good general condition and stamina. A study by Jenwitheesuk *et al*. (50) underscores poor prognosis in patients with problematic wounds due to factors like diabetes, arterial occlusion, and bone exposure. HBOT significantly improves short-term DFU healing, while its long-term benefits require confirmation. Bai *et al*.'s meta-analysis (51) supports HBOT's positive impact on venous leg ulcers, decreasing healing time, ulcer area, swelling regression time, colour improvement time, and VAS score. However, these effects were observed in surgical patients; non-surgical treatment outcomes need more investigation. HBOT appears effective as an adjunct to standard care for severe conditions, inducing beneficial physiological changes. Its universal use is limited due to lacking evidence and potential side effects. Future research targeting patient subgroups based on factors like occlusion location, ulcer site, and transcutaneous oxygen measurement could enhance effectiveness and quality of life.

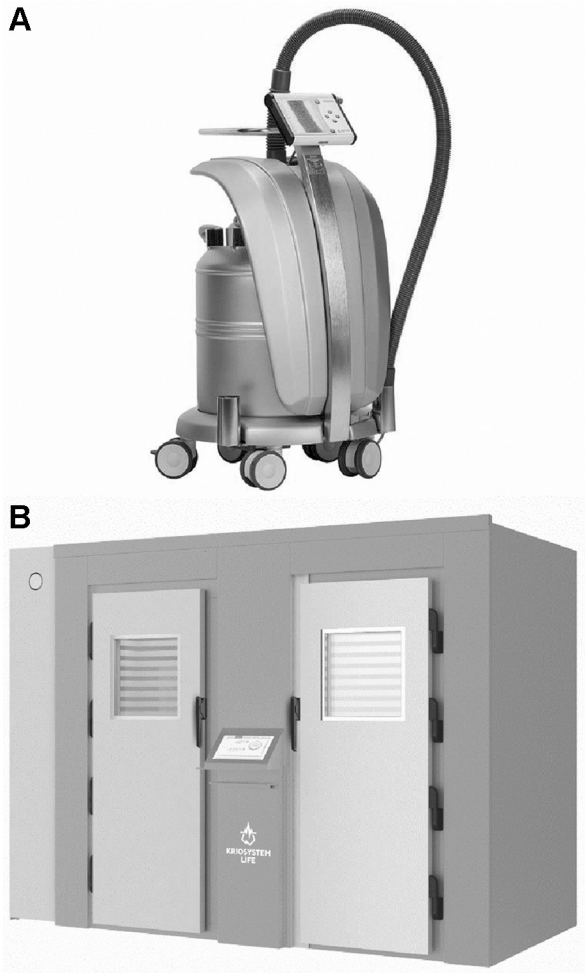

Figure 5. *(a) Localized cryotherapy device KRIOSAN (Wrocław, Poland); (b) Stationary cryo-chamber by KRYOSYSTEM (Wrocław, Poland) (54, 55).*

Cryotherapy

Mode of action. In health care, the origins of the use of low temperatures go back as far as 2500 BC, when the Egyptians used cold temperatures to treat injuries and inflammation (52). Many centuries of challenging research later have led to the development of impressive revolutionary discoveries and state-of-the-art modalities such as cryotherapy – a non-invasive technique which uses ultra-low temperatures of cryogenic mediums and works on the principle of heat withdrawal, assisted by core and tissue temperature reductions and blood flow alterations (53). Nowadays, liquid N_2 is the most effective and common cryogenic option for cryotherapy in the treatment of conditions associated with pain and inflammation. It is administered in the form of N_2 vapor or by air refrigeration applied locally at about −160˚C or as a whole-body cryotherapy (WBC), as shown in Figure 5, briefly exposing the body to a temperature of −110˚C to −160˚C in a chamber environment (54, 55).

According to experimental evidence, cryotherapy can be attributed to three general effects: reduction of pain, inflammation, and oedema. In terms of pain, it has been found that ultra-low temperatures induce the activation of thermal receptors associated with the blockage of the nociception – pain signals within the spinal cord (56). In addition, low temperature exposure can affect the neural cells' exchange between calcium and sodium, leading in action to a delay in potential generation (57). Local application of cooling also activates proprioceptors due to tissue pressure changes, resulting in the inhibition of the transmission of the nociceptive signal to the brain and therefore in the increase of spinal cord activity. This leads to reduced muscle spasm, muscle tone, and a consequent increase in blood flow. Prostaglandin E2 (PGE2) concentration, known as a mediator responsible for sensitisation to central and peripheral pain, has been also found to be reduced when ultra-low temperatures are applied (58, 59). In the case of the anti-inflammatory effect, a

hypermetabolic state within the treated tissue was recorded as well as compromised circulation of the blood (60). PGE2 which is also a marker of inflammation has been found to decrease in concentration during cryotherapy, indicating a positive anti-inflammatory effect. Cryogenic temperatures therefore can affect the regenerative process by its reduction from a hypermetabolic state with its capability of inducing secondary hypoxic injury, to a lower metabolic state that demonstrates lower oxygen demands and energy requirements, overall increasing the survival rate of the cells. The above-mentioned cellular metabolism additionally results in a reduction of swelling or oedema formation. The application of ultra-low temperatures on the tissue leads to an increased alpha-adrenoceptors sensitivity found in the blood vessel walls, resulting in vasoconstriction and a blood flow decrease within the tissue itself, interrupting sympathetic nerve conduction (61). The process has been found to be cyclic, also known as Hunting reaction, where the last activity before the cycle repeats itself, involves tissue rewarming by returned blood flow and nerve conduction re-establishment with increased alpha-adrenoceptors sensitivity.

Despite the widespread use of cryotherapy with established cost-effectiveness and safety, conflicting results have shown that its application can be associated with adverse side effects, such as frostbite, Raynaud's phenomenon, superficial nerve palsies, cold urticaria or a delayed regeneration process. When assessing therapeutic modalities, it is vital to compare any benefits with possible harm and risks as cryotherapy demonstrates the capability of modifying essential biochemical and physiological parameters in the human organism (62). Nevertheless, randomised studies and systematic reviews have been found to fail to provide solid evidence of cryotherapy safety. Therefore, this should remain a subject for future high-quality randomized controlled studies with evaluated timing, dose, temperature and frequency, using a standardised set of reliable and valid measurements of outcomes, enabling the performance of meta-analyses.

Professional sport athletes. WBC is widely used in rehabilitation programmes in sports medicine for both acute and chronic soft tissue injuries due to the biological and physiological effects which decrease the local inflammatory reaction and therefore reduce the severity and duration of musculoskeletal trauma recovery, maximising the performance of athletes (63). WBC has also been shown as a beneficial modality applied after intense training to minimize the risk of injury during competition seasons. Moreover, beneficial effects have been recorded, such as an increase in the endurance-associated margin for metabolic heat production as well as in the amount of time to touch the critical limiting temperature (64). Specifically, the mode of action of ultra-low temperatures on athletes could be divided into two major phases. Firstly, a stimulated sympathetic nervous system inhibits the heat loss, leading to narrowing of dermis and subcutaneous tissue blood vessels and increasing the body's insulating properties. Next, mechanisms that are responsible for intensifying heat production *via* the metabolic rate elevation are activated, consequently leading to tissue hyperaemia and concentrations of oxygen in the muscles with a number of recognized valuable biochemical, hormonal, and clinical effects persisting after the procedure. The important fact of WBC dose-dependency was recorded by Lubkowska *et al*. (65).

Importantly, research by Banfi *et al*. (66) showed that WBC is not deleterious for cardiac function when studying its effects on troponin I (TnI), high sensitivity C-reactive protein (hsCRP), and N-terminal pro B-type natriuretic peptide (NTproBNP) levels in rugby players. Furthermore, no negative effect was reported when investigating haematological values by measuring the concentration of haemoglobin and count of erythrocytes, leukocytes, platelets, and reticulocytes in peripheral blood. Beneficially improved antioxidant capacity exposure to professional athletes' intense training was discovered as well with associated increased antioxidant agent level activity, such as glutathione peroxidase and superoxide dismutase (66, 67). A general beneficial antioxidant effect was identified, addressing oxidative stress as the main performance-affecting factor, enhancing antioxidant capacities and counteracting the reactive oxygen species (ROS) production after muscle activity associated with membrane, cellular structure, and deoxyribonucleic acid (DNA) damage (68, 69). An additional study on rugby players by Banfi *et al*. (70) that focused on changes in immunological parameters, cytokines adhesion molecules, and muscle enzymes [creatine kinase (CK), lactate dehydrogenase (LAD)]. This demonstrated no change in immunological parameters and therefore no impairment of the immune function, with a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines, as well as significantly decreased CK and LAD levels after the cryotherapy treatment, closely associated with triggering a cascade of events leading to an improved recovery from exercise-induced muscle trauma and damage after intense physical training. In terms of the application of cryotherapy prior to training, researchers have identified improved recovery by beneficially affecting power output associated with pain alleviation, reduced muscle fibre recruitment and sense of fatigue by eliciting a post-activation potentiation (PAP) response (71, 72). PAP is a physiological phenomenon involving acute changes in muscular power output occurring by phosphorylation of myosin regulatory light chains making myosin and actin more sensitive to Ca^{2+}. Nevertheless, contrasting findings demonstrated that ultra-low temperatures applied locally can cause increased resistance to movement requiring the athlete to exert extra power on

the agonist muscle in order to achieve the desired performance, overall resulting in impaired muscle flexibility associated with an increased injury incidence. Similarly, a study by Alaca & Kablan (73) demonstrated that the rectus femoris muscle became harder and less elastic in terms of mechanical properties for 10 minutes after local spray cryotherapy. Accordingly, careful warming should be recommended to ensure muscle normalization and therefore to prevent potential injuries.

Another valuable study on the subject presented by Galliera *et al*. (63) focused its investigation on the effect of bone remodelling after WBC in athletes by measuring new bone remodelling osteoimmunological biomarkers, known as OPG – a bone formation marker, RANK – a marker of resorption, and RANKL – a strong marker of bone resorption, which defines the "osteoimmunology" concept as the main regulatory mechanism linking immune system and the bone (74-78). The study obtained consistent findings with previous research, demonstrating that the increase of the OPG/RANKL ratio is strongly associated with an osteogenic and osteoprotective effect and therefore improved bone formation and recovery. This indicates the beneficial effect of cryotherapy on musculoskeletal recovery in athletes after endurance physical activity. Nevertheless, due to the preliminary findings, further research is desired to evaluate further the effect of cryotherapy on bone metabolism.

Much of the current research agrees that hormones with an established circadian rhythm is one of the key mechanisms manipulated during the exposure to ultra-low temperatures. Specifically, salivary testosterone levels increased up to 28% have been found to elicit acute exercise-like effects in concentrations of hormone affecting the desire and motivation to compete, due to WBC increased testosterone concentrations especially beneficial to male athletes involved in sports played late in the evening (79). The relationship between training, cryotherapy and stress-related cortisol levels have been investigated recently and preliminary studies demonstrated a marked decrease in cortisol concentration and therefore positive performance consequences. In addition, the ratio of testosterone to cortisol is of special interest for professional athlete performance where the inverse relationship reflects the hormonal balance status (80). Returning to the aspect of stress, an increase in salivary alpha-amylase (sAA), a biomarker for stress-related changes and a measure of catecholamine concentration, such as epinephrine, norepinephrine, and dopamine, has been found to be beneficial for athletes' preparation and readiness in addition to a reduction in muscular fatigue and enhancement in muscle priming effects (81- 83).

Despite the wealth of literature on whole body cryotherapy, published data on professional sport athletes' rehabilitation are scarce and the effectiveness and viability are yet to be fully described. Further investigations, including a case-control protocol, are essential to provide more robust evidence about the findings on the topic. Consequently, they can determine the practical value and clinical significance of the use of ultra-low temperatures in sports medicine that have a potential benefit of minimising the risk of musculoskeletal injury and reducing the severity and duration of trauma; quick recovery is a primary concern for both sports physicians and athletes especially during the competition seasons.

Pain management. Apart from its application in professional sport, cryotherapy is used increasingly in pain management. According to the statistics, 66.6% of patients suffer from acute postoperative pain which exerts an enormous economic and personal burden (84). Several studies have already shown the advantages of cryotherapy as a post-operative treatment due to its effect on oedema, pain, and inflammation, in addition to relative safety, low cost, and ease in administration for healthcare professionals and patients. One clinical example is total knee arthroplasty (TKA) which is often accompanied with high postoperative pain ratings. A study conducted by Wyatt *et al*. (85) demonstrated that cryotherapy decreases opioid consumption in the first postoperative week when they are used the most extensively. Nevertheless, ultra-low temperature effects on pain, range of motion, and swelling are still being debated and heterogeneous protocols make comparisons challenging. Accordingly, more research is needed to draw conclusions on measures of outcomes, cryotherapy optimization, cost analysis, and postoperative protocols for patients recovering after the TKA.

Supportive findings were reported by Lizis *et al*. (86) investigating the effect of cryotherapy with mobilization (CM) or reinforced as well with home stretching exercises (CMS) on chronic neck pain. This affects from 5% to 14% of individuals, which causes major financial loss due to repeated absence at work, limitations on professional activity, sick leave, and potentially premature retirement (87-89). Evidence from clinical trials has shown that combinations of different treatment types including manual therapy, physiotherapy, and local cryotherapy induce pain relief significantly more effectively and function restoration. The study results were consistent with a great deal of previous research and demonstrated the efficiency of a combination of therapies in chronic neck pain, showing the potential of improving the patients' quality of life. In addition, a study by Salas-Fraire *et al*. (90) demonstrated WBC efficiency in the pain and disability treatment of patients with chronic low back pain due to a significant improvement in clinical score, specifically an increase in anti-inflammatory cytokine IL-10 and a decrease in IL-2 values.

To date, cryotherapy has also been shown as an effective treatment used extensively in dermatology, specifically for

plantar warts, pigmentation and melanocytic lesions, cysts, vascular lesions and naevi, acne vulgaris, rhinophyma, alopecia areata, and xanthelasma. Plantar warts are benign inwardly growing myrmecia or superficial mosaic wart clusters caused by infection with the human papillomavirus (HPV) (91). The warts can be painful, interfering with walking which leads to hip or knee pain. A broad range of therapeutic options can be offered from cyto-destructive methods, chemotherapeutic compounds, and antiviral therapy to topical acid and immunomodulators. Nevertheless, the condition still possesses a therapeutic challenge for clinicians due to the high recurrence rates as clinically untreated surrounding tissue often still harbours HPV (92). The most commonly applied treatments include topical acids, electrosurgery, and cryotherapy. Interestingly, evidence from the study presented by Singh & Neema (93), who compared the efficacy of cryotherapy and electrosurgery, found that the incidence of pain, infection of the wound, delayed wound healing afterwards, scarring, and recurrence was higher with electrosurgery when compared with cryotherapy. This demonstrates the successful, safe and painless use of ultra-low temperature treatment is suitable for treating plantar warts. In addition, a growing body of evidence, mainly from clinical experience, has shown cryotherapy to be an excellent second line treatment in the protocols when the initial agent application is unsuccessful (94). A study by Awad *et al*. (95) has shown that adding N_2 cryotherapy prior to intralesional (IL) antigen immunotherapy is a promising therapeutic approach in multiple and recalcitrant warts. It works on the principle of the effector immune cells attraction to HPV-loaded keratinocytes, highlighting the safety and efficacy of the combination therapy with the potential of reducing the number of treatment sessions.

To sum up, the findings of the current body of evidence in the pain management sector demonstrate cryotherapy efficiency in the treatment of patients after total knee arthroplasty, with chronic neck and low back pain, as well as in pain and recurrence of plantar warts. Nevertheless, due to the scarcity of studies and controversial findings, the interpretation of ultra-low therapy is still facing difficulties, highlighting the need for further research.

Neurocognition. Since the beginning of the 21st century, several studies have focused on the effect of ultra-low temperatures on neurocognition. According to the statistics, 970 million people are living with a mental health disorder, such as depression, schizophrenia, anxiety, bipolar disorder, and drug or substance use disorders, that have a direct impact on the quality of life of patients and their families and their income (96). Individuals with severe mental health disorders are more likely to be victims while being more dangerous to themselves than to others. What is of great significance is the fact that 70-75% of individuals receive no treatment, potentially due to the lack of effective, affordable and easily accessible treatment options. Therefore, the clinical community is still looking for effective add-on treatments targeting multiple symptom clusters, also known as transdiagnostic interventions. To address the demand, one of the studies conducted by Doets *et al*. (97) demonstrated preliminary evidence that application of WBC is an efficient add-on treatment for mental health problems, specifically depression, targeting symptoms like sleeping problems and inactivity. Nevertheless, further research in the form of randomized controlled trials with a larger number of participants is required due to the lack of previously established evidence, including systematic reviews and meta-analyses.

Today, the pace of population ageing is much quicker than ever in the past, closely associated with an increased prevalence of neurocognitive disorders like dementia. This is an incurable syndrome with no treatments modifying disease progression characterized by the decline of brain functioning, leading to memory loss, a decline in mental sharpness and language abilities, changes in perception as misperceptions, hallucinations, delusions, time-shifting, difficulties in movement and daily activities (98). Mild cognitive impairment (MCI) is a condition referring to the transition stage between normal cognitive functioning and dementia (99). Due to the irreversible and progressive nature of dementia, there is an urgent demand for intervention that could address cognitive impairment at its early stage. It is hypothesised that WBC might be beneficial in MCI and Alzheimer's disease (AD), the common form of dementia, *via* a protective effect on the central nervous system by induced hypothermia (100). The vast majority of studies have also identified an immunomodulation effect when exposed to ultra-low temperatures, leading to a decrease in pro-inflammatory cytokines as IL-1 and IL-2, as well as in oxidative stress, and an increase in the anti-inflammatory cytokines' concentration as IL-6 and IL-10. This in turn results in an increase in glutathione peroxidase and glutathione reductase activities, and concentrations of antioxidants, such as uric acid and extra-erythrocyte haemoglobin (70, 101). Furthermore, a c-reactive protein (CRP) and soluble intercellular adhesion molecule-1 (sICAM-1), markers of inflammatory response, have been found to be downregulated. This is especially important due to the correlation of neuroinflammation with cognitive impairment, such as an accumulation of amyloid beta peptide deposits inducing chronic neuroinflammation and the consequent apoptosis of neuronal cells in AD (102, 103). In addition, it has been found that a continuous pro-inflammatory reaction is associated with a reduction in microglia activity, and the changed secretion of chemokines, reactive oxygen species, cytokines, and nitric oxide (NO), subsequently inflicting damage to the blood-brain barrier.

Therefore, there is a growing body of evidence that alterations in the inflammatory events may precede the clinical development of cognitive impairment (104-108). One of the studies supporting this suggestion was conducted by Rymaszewska *et al*. (109), which demonstrated that WBC has the potential of increasing the performance of cognitive functions in MCI patients due to the systemic effect of ultra-low temperatures initiating neuro-muscular, analgesic, anti-inflammatory, antioedematous, circulatory, and hormonal reactions. Nevertheless, further research is needed as the study lacked sufficient information about regulatory role cryotherapy in MCI patients' immunological response and oxidative status. Another study by Misiak & Kiejna (100), based on accumulating a number of expert opinions, hypothesized that WBC is an effective modality with the potential of preventing AD acting on underlying path mechanisms of the disease *via* a multidirectional therapeutic approach, such as therapeutic hypothermia slowing down neurodegeneration, pro-hormones and antioxidant release, and affected lipid metabolism. Nevertheless, this field is still in its infancy and research including animal models is needed to determine underlying mechanisms of the cognitive impairment and evaluation of biological pathways in terms of ultra-low temperatures, in addition to putative targets for cryotherapy, and long-term results, due to the potential AD challenges as advanced neuropathological lesions being irreversible.

Returning to the aspect of the effect of WBC on lipid profile, a supportive study by Rymaszewska *et al*. (110) found that ultra-low temperatures can lower the levels of total cholesterol, triglycerides, and low-density lipoprotein, and this is of particular importance due to the evidence that dyslipidaemia is a risk factor for dementia. According to the evidence, cryotherapy induces the release of catecholamines leading to liver lipolysis and glycogenolysis. In addition, it can stimulate hepatic enzymes that convert cholesterol to bile acids, as well an increase in insulin sensitivity and brown adipose tissue, altogether accounting for the beneficial effects of cryotherapy on alterations of lipid profile. Therefore, WBC is proposed as a promising effective intervention. Nevertheless, more studies are required due to the small amount of supportive research and the considerable methodological heterogeneity of the studies.

Whilst the cryotherapy application method is open to change, the mechanisms of ultra-low temperature action and properties remain the same. The growing body of evidence highlights the positive perception that surrounds the application of cryotherapy in professional sport, pain management including after surgical treatment and to neurocognition, while being well tolerated with rarely reported side effects. In this sense, more research is desired in terms of eliciting the necessary physiological alterations while excluding the initiation of adverse pathopsychological effects, developing high-quality protocols, and therefore establishing a safe and efficient modality.

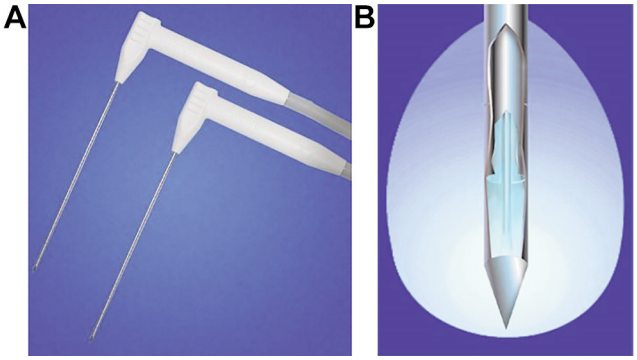

Figure 6. *(a) Percutaneous cryoablation probes; (b) Schematic representation of the active tip of a cryoablation probe and ice ball formed (112).*

Cryosurgery

Cryobiology. The modern application of cryosurgery in clinical practice dates back to the 1960's when Cooper & Lee (111) developed and applied a cryogenic probe, an ultra-small diameter vacuum-insulated tube-like tool served as a cryogen conduit, for the treatment of Parkinson's disease. It is important to point out that the term cryosurgery, or cryoablation, is usually misleadingly confused with cryotherapy, in contrast referring to an invasive closed system modality associated with total tissue destruction. The state-of-the-art third-generation cryosurgery systems work on the Joule-Thomson principle of temperature change accompanying gas expansion, exhibiting no heat exchange with the environment. Modern intra-operative ultrasound (US) in real-time monitoring aids cryoprobe placement and the extent of the freezing, while allowing easier atraumatic probe placement, greater control of the margins, and an even temperature distribution and as a result more efficient heat transfer. The rapid expansion of liquid N_2 or Ar gas, which is delivered through an uninsulated tip, results in extreme and rapid temperature drops while freezing adjacent tissues at −140˚C within a few seconds. The process is accompanied by an ice ball formation resulting in cell death out to 3 mm inside the ball margin as depicted in Figure 6, and the following thawing is achieved by instillation of the helium gas (112).

According to the growing body of experimental and clinical evidence, cryosurgery has been shown to be less invasive and associated with lower morbidity when compared with other surgical resection interventions in the management of life-threatening health conditions such as cancer. Several decades of challenging research led to fundamental scientific discoveries that allowed the

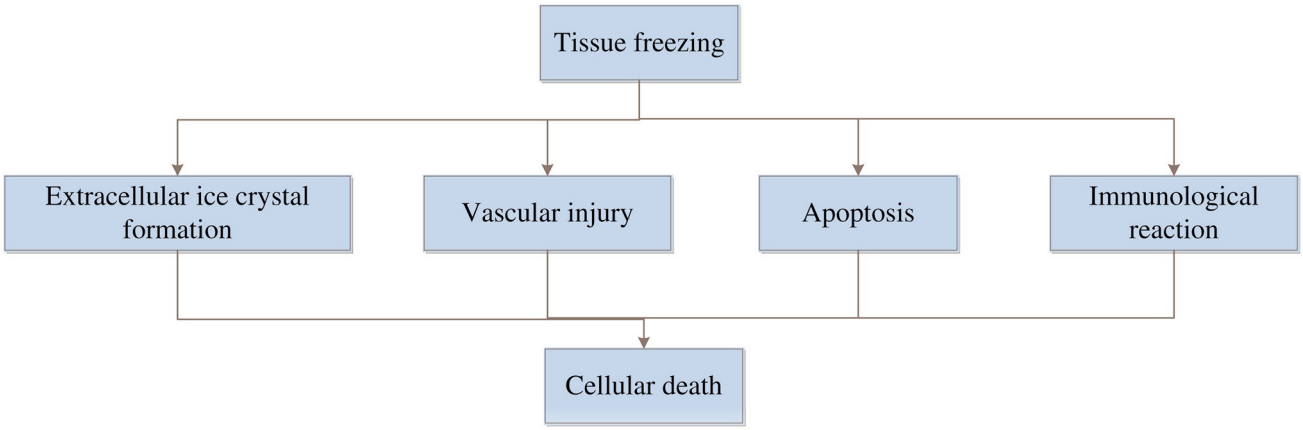

Figure 7. *Underlying pathophysiological mechanisms of cryo-destructive effects on biological tissues (113).*

underlying pathophysiological mechanisms of cryo-destructive effects on biological tissues, schematically represented in Figure 7, to be postulated (113).

Based on the findings on the direct cellular injury presented, extracellular compartment ice crystal formation has been found to give rise to a concentration gradient leading to enzymatic damage and denaturation of the proteins. At the same time, in the case of rapid freezing, crystal aggregation has been found to disrupt the mitochondria and organelles, ultimately resulting in cell membrane rupture at cell thawing (113). Furthermore, during thawing, hyperaemic injury reperfusion leads to toxic free radical release, inducing peroxidation of the cell wall lipid membrane.

In terms of a delayed mechanism vascular injury, it has been found to take place hours or days afterwards, when associated intracellular ice crystals cause damage to the endothelium, leading to vascular stasis, platelet aggregation, and intravascular thrombus formation, eventually resulting in ischemia and tissue necrosis reabsorbing over time (114). Regarding the cells that were not directly destroyed by cryoablation, these undergo a special cellular process of programmed cell death, known as apoptosis, usually at the periphery of the cryoprobe ice ball. Finally, cryogenic temperatures have been found to be associated with an immune-mediated cytotoxicity involving the activities of macrophages, neutrophils, and T cell infiltrates, leading to subsequent tumour cell death.

Nevertheless, it is of special importance to highlight that, according to the research study findings on the subject of the above-mentioned pathophysiological mechanisms, cryoablation has been found to demonstrate variable and less controllable effectiveness as each tissue cell is affected by and reacts to different thermal histories, such as cooling temperature and rate, time of exposure, hold time, and end temperature, resulting in variable degrees of cell injury. Therefore, despite extensive *in vitro* and *in vivo* experiments significantly contributing to the current understanding of cryobiology mechanisms and effects, more research to fill the knowledge gaps is required due to the complexity of the process and the multiple existing theories, which limit further developments in research.

Breast cancer. Nowadays, breast cancer is the most common malignancy among women due to the progression to deadly metastatic disease in 25-50% of cases, associated with an unfavourable prognosis and a low 5-year survival rate (115). Furthermore, while prodigious innovations in imaging allow the detection of breast cancers in its earliest stages, improving the prognosis for a 5-year survival rate up to 98%-99%, a close association has been found between current treatment protocols and overtreatment or unnecessary mastectomy interventions, which are a partial or complete surgical breast removal. Despite mastectomy historically being the gold standard of breast cancer treatment, its application is associated with anaesthesia, postoperative pain, longer recovery periods, as well as adverse cosmetic impacts, overall highlighting the need for de-escalation of treatment towards less invasive forms. According to the growing body of experimental and clinical evidence, cryosurgery possesses a confirmed feasibility, effectiveness, and tolerance in breast tumour destruction, given the method's simplicity, lack of pain, quick recovery, low associated morbidity, and cost-effectiveness (116, 117). Specifically, in regard to breast cancer, cryosurgery has been found to not only destroy cancer cells but additionally to induce the release of intact tumour-specific antigens into the circulation, essential for immune system recognition and consequent response (118). As cryosurgery is conducted in an ambulatory setting under local anaesthesia, sedation and operating room needs are decreased, as well as associated surgical complications, recovery times,

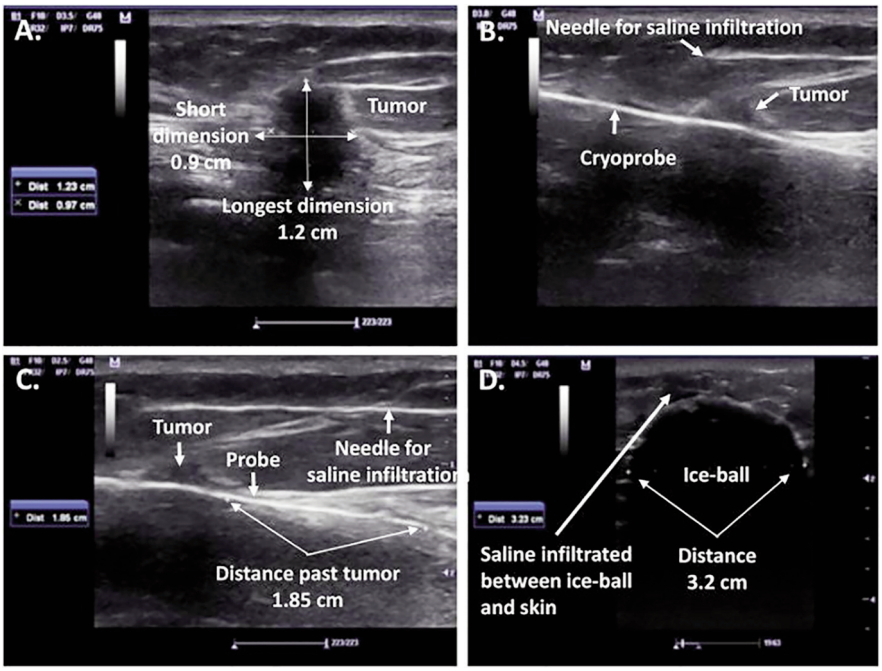

Figure 8. *Ultrasound (US) guided cryosurgery for right breast invasive ductal carcinoma. (a) Size of tumour visualized by US. (b) Visualization of cryoprobe penetration and saline hydro-dissection. (c) Visualization of distance of probe and hydro-dissection needle projection past tumour. (d) The ice-ball, tumour, and safety freeze margin visualization (123).*

and health-care costs, making the technique cost-effective. Importantly, cryosurgery does not involve caving within breast parenchyma which is a cause of breast asymmetry and poor cosmetic results, and therefore results in better cosmesis with no tissue loss, less breast deformity, and a smaller wound. Specifically, meta-analysis on small breast cancers showed cryosurgery satisfactory to be excellent cosmesis in more than 95% of the treated patients, in addition to lower complication rates of 5% compared to other ablation modalities including microwave ablation (MWA), high-intensity focused ultrasound (HIFU), radiofrequency ablation (RFA), and laser ablation (119). Minor adverse events are rare and include minor bleeding, bruising, or pain with anaesthetic injection, while being similar to those associated with needle biopsy application. Nevertheless, owing to the large heterogeneity of studies included, further large sample, randomized, controlled studies with a uniform protocol are necessary to consider other vital aspects, such as biological subtype or density category of the tumour in terms of modality response. Similarly, a study by Fine *et al.* (120) on the N_2-based cryosurgical system demonstrated no severe device-related adverse complications, while two thirds of moderate complications in 2.4% of the cases and mild complications in 18.4% of them have been demonstrated to be unrelated to the device or study procedure but rather were patient specific.

The cryosurgery procedure under the guidance of US or MRI is performed by placing a single cryoprobe or multiprobes percutaneously into the lesion, while generating an ice ball covering the entire tumour. An appropriate surrounding tissue margin of 5 to 10 mm has been found to be sufficient and efficient for breast tumours (121, 122). An additional procedure, known as saline hydro-dissection, is required to protect overlying skin from the ultra-low temperatures of the ice ball. Figure 8 is a representative example of imaging during breast cancer cryosurgery, demonstrating cryoprobe and saline hydro-dissection needle placement in the tumour and the consequent ice ball formation (123).

During surgical preparation, patient eligibility criteria must be always followed, and ideal cryosurgery candidates are those with well visualized low-grade invasive ductal carcinomas (IDC) <1.5 cm without an extensive intraductal component (EIC), that are lower grade, less aggressive tumours located 1 cm from the skin and less likely to be multifocal, multicentric, or contralateral (124, 125). Patients with a pure IDC, ductal carcinoma in situ (DCIS) with EIC, or invasive lobular carcinoma (ILC) should be excluded. Since, according to the vast majority of supportive studies, the size of tumour has been found to be an essential predictor of residual cancer, US, MRI and mammography assessment together with multiple core biopsies of the tissues surrounding the tumour are required for optimal patient selection, as well

as to exclude extensive intraductal components associated with the lack of cryosurgery radicality and thus with diminishing modality efficiency (122, 124, 126-131).

Importantly, in the light of a quicker pace of population ageing than ever in the past, the significant progress in breast cancer genomics has allowed a more precise patient-specific management approach that led to a reconsideration of how to treat elderly patients. Cryosurgery being a minimally invasive ablation technique demonstrated efficacy equal to that of breast conservation therapy, while avoiding the risk associated with surgical intervention. A study on the subject by Habrawi *et al*. (123) in patients over 60 years old with infiltrating ductal carcinomas size ≤1.5 cm showed good tolerance without serious complications, good cosmesis, and no evidence of tumour recurrence, demonstrating that elderly patients with early breast cancers up to 1.5 cm with a favourable low-risk profile benefit from a single session of cryosurgery and do not require subsequent surgical intervention. Nevertheless, as for other similar studies, this small patient number series with a short follow-up needs further larger-sample long-term follow-up to record local control. Another study by Manenti *et al*. (132) in post-menopausal women between 64 and 82 years with larger ductal invasive unifocal breast cancers ≤2 cm demonstrated the successful target lesion destruction in 14 of the 15 cases, highlighting the cryosurgery efficiency of single small breast cancers resulting in complete necrosis, excellent cosmesis, and patient satisfaction. However, large multi-centre randomized control trials are desirable to determine its long-term advantages in order to confirm a similar efficacy to other breast conservation treatment options.

Evidently, dynamically evolving cryosurgery has demonstrated its antitumoral immunity effects and metastases development control in patients with stage IV metastatic disease and clinically indolent or nonpalpable tumours with stable metastatic disease, that, despite substantial discoveries and advances in treatment modalities, still lack an effective treatment regimen. While lumpectomy as a resection of the primary tumour could offer a survival benefit, removing potential tumour stem cell sources that have been found to support distant metastases through different neoplastic cell lines, resection could expose the patient to serious complications, delaying a systemic treatment and affecting the survival benefit (133). It has found that ultra-low temperature applications can affect tumour-infiltrating lymphocytes (TILs) in distant tumours, stimulating a tumour-specific response, while reflecting a systemic link in terms of local response, and therefore resulting in a regression of metastatic lesions. Interestingly, a greater number of TILs have been found to be associated with a better response to neoadjuvant chemotherapy treatment and therefore with better survival rates. The above-mentioned mechanism was demonstrated in experimental studies in animal models that recorded an increased number of immune effector cells in the distant tumours' microenvironment and fewer immunosuppressive regulatory T cells (Tregs) closely associated with tumour growth promotion. Specifically, the findings of the retrospective study conducted by Pusceddu *et al*. (134) demonstrated cryosurgery as an effective, well-tolerated, and feasible treatment option for primary advanced breast cancers in patients with bone metastatic ductal invasive breast lesions previously treated with systemic therapy, as complete regression was achieved in 88% of cases. Pusceddu *et al*. (135) supported this view in a further study, additionally demonstrating cryosurgery safety and efficiency in primary tumours in stage IV breast cancer patients, resulting in complete tumour necrosis in 85.7% at 2-month and in 100% at 6-month follow-up. Additional retrospective analysis by Niu *et al*. (115) comparing the therapeutic effects of cryosurgery to other therapies, such as immunotherapy and chemotherapy in metastatic patients after failure of radical surgery demonstrated the highest median overall survival of 83 months in the group treated with both cryosurgery and immunotherapy. Therefore, there is a beneficial effect of cryosurgery when applied in conjunction with other treatment modalities. The major study limitation as for nearly all breast ablation studies is its retrospective nature and sample size. Therefore, prospective large, well-designed studies comparing cryosurgery with other treatment options used in metastatic breast disease is vital for a deeper understanding of the cryobiology and tumour response in this subgroup of patients and the associated survival benefits. Furthermore, as most of the cryosurgery research has been focused on patients with early small breast cancers, the use of the modality in metastatic breast cancer is scarce, in addition to the presence of controversial studies, therefore more studies are awaited.

Finally, despite being in its infancy, it is vital to mention the promising potential of the synergism of cryosurgery and immunotherapy in breast cancer management, where ultra-low temperature induced tumour-specific immune responses have been found to increase the efficacy of the checkpoint inhibitors. The beneficial effect of this synergism is associated with critical components of *de novo* adaptive immune response that includes tumour antigen release and presentation, immune suppression regression, and tumour antigen-specific T cell activation. One of the clinical pilot studies by McArthur *et al*. (136) on cryosurgery and preoperative single dose ipilimumab (anti-CTLA-4) in early-stage breast cancer showed safety and favourable systemic immunologic and intra-tumoral effects, including a higher inducible costimulator expression playing a vital role in increased antitumor activity. The continuous CD4 and CD8 cell proliferation has been also recorded. A study by Page *et al*. (137) on T cell clonality and intra-tumoral T cell density

presented supportive conclusions while demonstrating the cryosurgery-induced death of both tumour and TILs as well as the release of a broader variety of tumour specific antigens essential for the immune system recognition, with a proliferation of a small subset of T cell clones mediated by the synergy of cryosurgery and immunotherapy. Nevertheless, despite promising findings, the research is limited by the use of animal models and small sample sizes. To date, several ongoing clinical trials have been initiated and are underway to examine further the above-mentioned relationship, aiming to treat both small breast cancers and metastatic disease. One such trial is focusing on the adverse side events, such as the primary outcome of cryosurgery, Nivolumab (anti-PD-l) and Ipilimumab (anti-CTLA-4) treatment in patients with early-stage breast cancer (138).

To date, cryosurgery has been explored with the intention of achieving equal efficacy to that of mastectomy and conservation therapies, while eliminating the associated risks and complications in the management of early breast cancer, metastatic disease and patients not suitable for the standard form of treatment. Experimental evidence agrees on cryosurgery effectiveness as minimally invasive, cosmetically preferable, safe, and a cost-effective treatment modality, in addition to recorded ease in its use. Comparison with standard treatment regimens, development of the optimal protocols, and establishment of the precise patient selection criteria are essential and are yet to be confirmed before accepting cryosurgery as a method of standard clinical practice. Accordingly, more studies are required for modality efficiency and validity evaluation due to the overall research scarcity and heterogeneity in study methods and outcomes.

Intralesional cryosurgery in keloid scars. Keloid and hypertrophic scars are benign fibrous nodules composed of type I or type III collagen resulting from an abnormal healing response following skin injuries, such as burns, injections, surgery or dermatitis, including acne vulgaris and bites (139). The scars can significantly reduce the quality of life causing a cosmetic burden accompanied by pruritis and pain (140-143). To date, numerous treatment options are available based on scar type, cause, recurrence rate, recovery rate, and complications, and include surgery, corticosteroid, 5-fluorouracil, or interferon intralesional injection, silicone coating and compressing, intense pulsed light (IPL), and cryosurgery (144-148). For decades, cryotherapy in the form of liquid N_2 applied externally was used in clinical practice. Nevertheless, this modality was associated with hypopigmentation, blistering and infection due to prolonged hold time, and attempts to minimize the damage of surface epithelium led to less volume decrease and higher recurrence rates (149, 150). To address these serious side effects and drawbacks, intralesional (IL) cryosurgery was introduced, freezing the scar from the inside using a hollow needle with the cryogen administered directly to the core of the scar, targeting the exact location of the pathology and not affecting the surface epithelium (151). The standard treatment regimen comprising 20 therapeutic sessions attended every two weeks has been reported together with a recovery rate of 76% (152). Nevertheless, the considerably greater length of the treatment period compared to other treatment modalities has been found to be the main shortcoming.

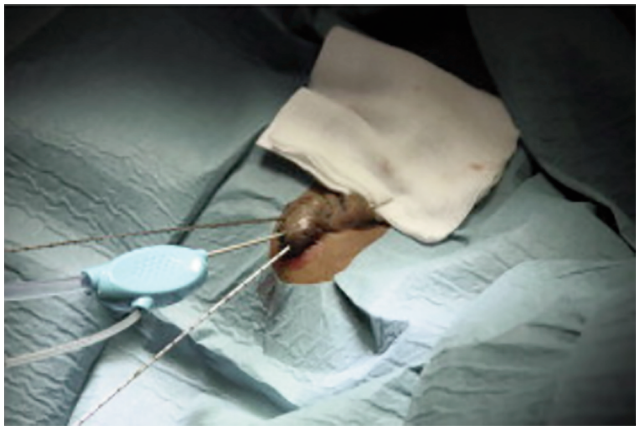

Figure 9. *Intralesional cryotherapy with the liquid nitrogen-based device and two thermocouples placement prior to the procedure on keloid scar (153).*

Liquid N_2 and Ar gas-based IL cryosurgery devices are currently available, both showing a similar volume decease. A study of the thermal behaviour by Van Leeuwen *et al*. (153) comparing the two different gas type IL cryosurgery devices demonstrated that the Ar gas-based device showed a faster freezing rate and a lower end temperature, resulting in lower recurrence rates. More hypopigmentation was recorded for the Ar-based device, as well as low outer surface temperatures of the keloid scars measured by the thermal camera for both devices, suggesting that some post-treatment hypopigmentation is inevitable as fast freezing rates are associated with cell necrosis, while slow freezing rates are associated with an irreversible apoptosis process. Figure 9 shows the IL cryotherapy liquid N_2 based device used prior to the procedure on keloid scar.

The study findings were consistent with previous research and clinical practice experience, where N_2 gas devices based on a simple Dewar cylinder were associated with a limited freezing capacity and elongated freezing times that led to dysfunctional treatments, in addition to an inability to closely control and monitor the freezing process. In contrast, Ar-based devices relying on the Joule-Thomson effect demonstrated controlled and accurate freezing, allowing the internal tissue temperature to be monitored. Similarly, a study by Van Leeuwen *et al*. (154) showed promising results

for an Ar-based device for the treatment of keloid scars in terms of a reduction in the volume and low recurrence rates. On the other hand, there is the growing body of evidence of contrasting findings highlighting N_2-based device efficiency in scar management. Supporting this view, Meymandi *et al*. (155) showed that a combination of intralesional corticosteroid injection and IPL or cryosurgery based on N_2 gas is an effective treatment for keloid and hypertrophic scars with high rates of participant satisfaction and no significant complications. Nevertheless, it should be highlighted that the number of complications in the N_2 gas device treatment group was higher when compared to the one treated by IPL.

To conclude, N_2 and Ar based IL cryosurgery alone or in combination with other treatments is a promising new technique for keloid and hypertrophic scars. More research is required due to the scarcity of published studies as well as their heterogeneity in study methods and outcomes in addition to the absence of a clear definition of recurrence, outcome measures, differentiation between scar types, and the type of cryosurgery device used.

Urologic malignancies. Renal cancer. Over the past years, cryosurgery been used with success in the treatment of the two most common urologic malignancies – prostate and renal cancer. Small renal masses (SRMs), as abnormal growths in kidneys in size ≤4 cm, also referred to as clinical stage T1a, are increasingly detected incidentally from the widespread use of cross-sectional imaging performed for unrelated indications. SRMs vary in histology and include benign renal tumours and potentially aggressive renal cell carcinomas. Most of these lesions are commonly seen in older or frail patients with concomitant comorbidities, such as hypertension, diabetes, or congestive cardiac failure. Therefore, management options of renal preservation are preferable, aiming to reduce the radical nephrectomy or morbidity associated with nephron-sparing surgery (113, 156). Cryosurgery may also confer benefit for patients with congenital syndromes, renal masses in solitary kidneys, as well as for patients having preferences for minimally invasive treatment. The major contra-indications recorded include widespread metastases, less favourable size or location of the tumour, and a life expectancy of less than a year. Renal cryosurgery is applied through either a percutaneous or a laparoscopic approach using probes of various shapes and sizes defining the ice ball characteristics. In recent years, despite two renal cryosurgery approaches, similar 5-year recurrence-free survival (RFS), cancer-specific survival (CSS), and OS rates, the percutaneous approach has gained prominence due to its application under sedation and the capability of decreasing pain as well as the length of hospitalization, hence showing its overall cost-effectiveness (157-160).

A great deal of previous research agrees on SRM cryosurgery effectiveness and safety with minimal morbidity and the absence of renal impairment, both as a primary treatment and for recurrent disease after partial nephrectomy (161). Moreover, recent data suggest that cryosurgery could be indicated as part of a multimodal approach for oligometastatic renal cell carcinoma (162). The rate of recorded complications is 7.8-20.0% and they include renal abscess formation or bleeding; pancreas, spleen, or bowel injury; or paraesthesia and pain at the entry site of the probe (163). Specifically, research conducted by Rodriguez Faba *et al*. (164) demonstrated that focal cryoablation with the use of Ar cryogen is safe and effective due to a low risk of complications and good functional outcomes. Renal function preservation is one of the main objectives in cryosurgery, especially for patients with pre-existing chronic kidney disease. Nevertheless, oncological outcomes require further research due to the lack of robust evidence, making cryoablation a favourable modality only for selected patients with contra-indications to extirpative approaches. An additional study on the subject presented by Carvalhal *et al*. (165) addressed two important concerns that remain in current research as a need for long-term follow-up and functional impact as regards hypertension and renal function. The study findings of a mean of 20.6 months laparoscopic renal cryoablation follow-up revealed no significant differences in terms of modality impact on the researched postoperative parameters.

When defining the role of a new modality in clinical practice, comparisons with existing standard approaches are crucial. According to the meta-analysis by Tang *et al*. (166), laparoscopic renal cryoablation demonstrated less blood loss, significantly shorter operative time, fewer complications, as well as a lower risk of conversion, while laparoscopic partial nephrectomy (PN) to which it was compared was associated with better oncological outcomes with a lower risk for distant metastasis and local recurrences. Similarly, a study by Klatte *et al*. (156) showed that laparoscopic renal cryoablation was correlated with lower estimated blood loss, shorter operative time and length of hospital stay, a lower risk of conversion, in addition to a lower risk of complications. However, a significantly increased risk of local tumour progression and a risk ratio for metastatic tumour progression was recorded for the modality compared to PN. Additionally, both laparoscopic cryosurgery and PN on multiple ipsilateral tumours showed a similar renal function, complication rates, and intermediate-term survival rates, as stated in the study by Lin *et al*. (157). The vast majority of studies were consistent with the finding that renal cryosurgery is associated with higher recurrence rates than for standard PN. In contrast, a study by Thompson *et al*. (159) comparing RFA with percutaneous cryosurgery and PN demonstrated similar local recurrence-free survival among

the three modalities while metastasis-free survival was significantly higher for the PN and cryosurgery groups. In addition, there were no statistically significant differences of metastasis-free survival and local recurrence-free survival when PN was compared with cryosurgery.

To sum up, the findings of the current body of evidence present renal cryosurgery as an effective, minimally invasive modality with minimal morbidity and the absence of renal impairment and a low rate of complications, especially for older and frail patients. Nevertheless, due to the lack of robust research evidence and long-term data, the high variability across case series, and publication bias, further research is needed to give definite conclusions regarding this novel modality in the management of renal malignancies.

Prostate cancer. To date, it is evident that slow growing cancers of the prostate, a male reproductive system gland surrounding the urethra, have been increasingly treated by cryosurgery as whole-gland therapy in primary cancer, as salvage therapy in disease recurrence, or as a focal therapy for localized cases. The procedure is performed in the patient extended Lloyd-Davies position with the use of a transrectal ultrasound scan (TRUS) probe and a 17-G brachytherapy template for accurate cryoprobe placement, where the formation of the ice ball starts at the anterior region extending laterally into the periprostatic tissues. There are no absolute contra-indications except for rectal fistulas and haemorrhagic diathesis, when transurethral resection of the prostate (TURP) is associated with an increased risk of urethral sloughing due to the difficulty for the coaptation related to the application of urethral warming device. In addition, previous history in obstructive lower urinary tract symptoms increases the risk of urinary obstruction after treatment (167). Furthermore, previous urethral and pelvic surgery could disturb the anatomy and is a contra-indication for cryosurgery, while the prostate mid lobe requires pre-cryosurgery treatment. It is a common practice to monitor pretreatment clinical parameters to predict the outcome as prostate-specific antigen (PSA), Gleason score, and clinical stage. Interestingly, Fushimi *et al.* (168) suggested the measurement of the levels of γ-globulin and IgE, in that high levels are contra-indicated for cryosurgery, associated with deaths due to cryo-shock, a syndrome of multiorgan failure, or cachexia, a complex syndrome of not entirely reversed ongoing muscle loss.

It has been reported that molecular adjuvants, as pre-cryosurgery treatment, could enhance the injury within the ice ball, and include thermophysical adjuvants targeting ice crystal formation injury, chemotherapeutic approaches targeting apoptosis, intravascular agents assisting in vessel damage, and immunomodulators assisting in immune-mediated tumour damage. In particular, a study by Jiang *et al.* (169) showed that tumour necrosis factor alpha (TNF-α) as a 4-hour pre-conditioning enhances cryosurgical lesions *via* vascular mechanisms, consequently leading to injury to tumour cells by initiation of inflammation and leukocyte recruitment. The findings are schematically represented in Figure 10.

In terms of quality of life, according to statistics the most commonly reported prostate cryosurgery complications are erectile dysfunction (ED) in 3.7-88.0% of patients; urinary retention in 4.1-18.0%; urinary incontinence in 1.6-18.0%; and lower urinary tract infection in 3.0-16.7% (170). Despite high rates of ED, the research suggested that other salvage modalities have a similar effect. Cryosurgery-related data vary but it was reported that only 8.8% of men returned to a normal sexual function without the assistance of injectables, phosphodiesterase type 5 inhibitors, or mechanical devices (171). Urinary retention-associated complications included lower urinary tract symptoms and dysuria, consequently progressing and requiring TURP in 13% of cases, as reported in a larger retrospective study by Long *et al.* (172). In addition, according to the data from the Cryo On-Line Data (COLD) registry, prolonged catheterization was recorded in 3.6% of cases, where 2.1% required TURP.

In the matter of primary whole-gland cryosurgery, the longest series conducted by Cohen *et al.* (173) studied recurrence rates of PSA and reported a 10-year biochemical disease-free survival (BDFS) as 80.56% for low-risk stratification, 74.16% for intermediate-risk stratification, and 45.54% for high-risk stratification, with an overall rate of negative biopsy of 76.96%. A further study by Levy *et al.* (174) on the COLD registry reported 14.5% of positive biopsy rates in patients with no suspicion of recurrence or biochemical failure, with 38.0% in defined biochemical failure. An additional study by Donnelly *et al.* (175) demonstrated 89.0% of overall 5-year survival and 98.6% of disease-specific survival. Despite enormous progress made in the research, it is difficult to predict a biochemical recurrence of prostate cancer as well as the patients who would potentially have a positive biopsy rate, as cryosurgery leaves viable periurethral prostate tissue which consequently produces PSA. Cryosurgery is also used in a form of salvage therapy following primary radiation therapy that resulted in biochemical relapse seen in 30% of patients as local recurrence or metastases (176). According to the evidence, third-generation cryosurgery achieved BDFS rates of 86% at 1 year and 74% at 2 years (177). Another study on the subject conducted by Williams *et al.* (178) identified BDFS values of 47%, 39%, and 39%, as well as overall survival of 95%, 91%, and 87% at 5, 8, and 10 years, respectively. Despite a lack of long-term data related to efficacy and survival, focussed prostate cryosurgery targeting only parts of the gland and attempting to minimize the risk of associated complications has been found to offer comparable functional and oncologic outcomes to radical prostatectomy

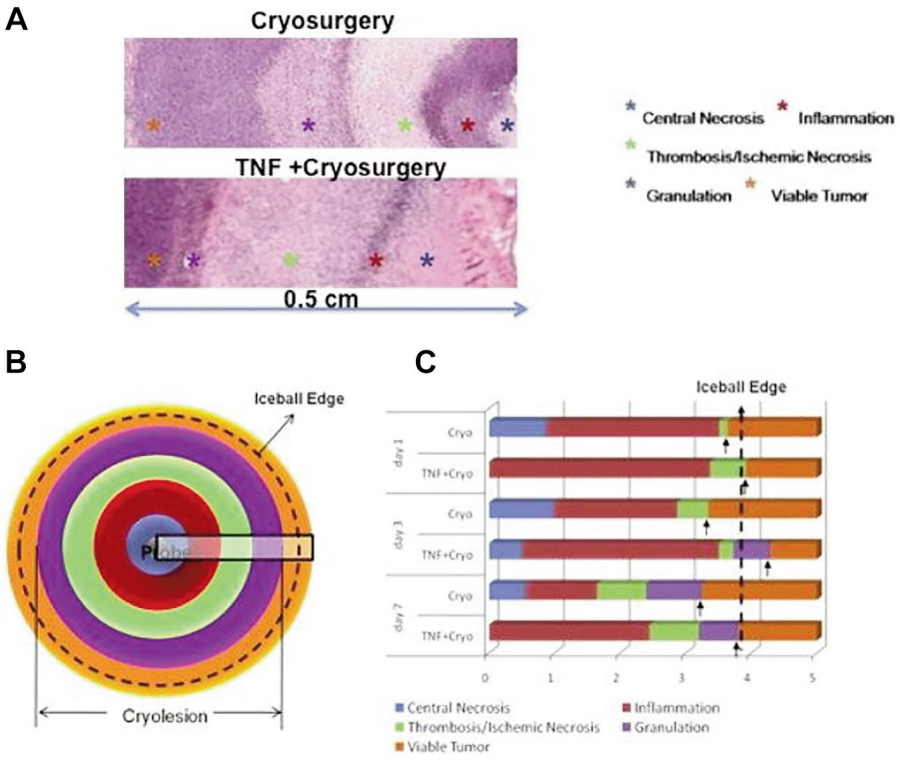

Figure 10. *(a) Histological changes following prostate cancer cryosurgery at day 7 with and without TNF-α pre-treatment. (b) Schematic representation of the ice ball and associated 5 histological zones. (c) Schematic representation of changes of histological zones over time. Arrows indicate histologic boundaries of granulation tissue zone (cryolesion edge). Dashed arrows represent the ice ball edge (169).*

for low to intermediate risk patients. Specifically, Bahn *et al*. (179) demonstrated a 70% reduction of PSA values with complete continence and patient erection sufficiency in 86%.

There is a growing body of research on the effectiveness of cryosurgery as a modality option for prostate cancer. Nevertheless, due to the scarcity of studies, the absence of an established definition of biochemical recurrence, and the studies using different definitions, a comparison of outcomes with other treatment modalities is difficult, therefore it is considered as experimental, while more research is required before accepting the method as standard clinical practice.

Gastroenterology. Barrett's oesophagus. Barrett's oesophagus (BE) is a clinical condition of metaplastic change in the mucosal cells of the oesophageal lower portion from normal squamous epithelium to simple columnar with goblet cells. The change is found to be premalignant, associated with a high incidence of further development of oesophageal adenocarcinoma with an estimated progression risk of 0.12% to 0.40%, and 5.60% to 6.60% per year for nondysplastic and dysplastic condition types, respectively (180). Figure 11 represents the progression from normal squamous epithelium to non-dysplastic Barrett's oesophagus, low-grade dysplasia (LGD), high-grade dysplasia (HGD) and oesophageal adenocarcinoma (EAC) with associated specific molecular hallmarks, such as mutation rate and signature to mutations in canonical cancer driver genes (181).

Due to the considerable oesophagostomy associated morbidity, endoscopic ablative therapies such as cryosurgery were developed to eradicate BE and prevent progression to oesophageal adenocarcinoma by restoring oesophageal epithelium. With cryosurgery, as a novel noncontact endoscopic modality for abnormal gastrointestinal (GI) mucosa treatment, ablation is performed with the use of cryogens, such as liquid nitrogen or carbon dioxide gas sprayed onto the target tissue. No serious adverse events related to the modality were identified, but reported adverse events included bleeding, stricture formation, and perforation (180).

Much of the current research agrees on cryosurgery safety and efficacy in the treatment of BE despite the lack of long-term follow-up and comparison with other ablative technologies, as well as the limited number of studies (182). One of the studies supporting this suggestion was conducted by Johnston *et al*. (183) on BE patients ranging from without dysplasia to with multifocal dysplasia. The treatment with a

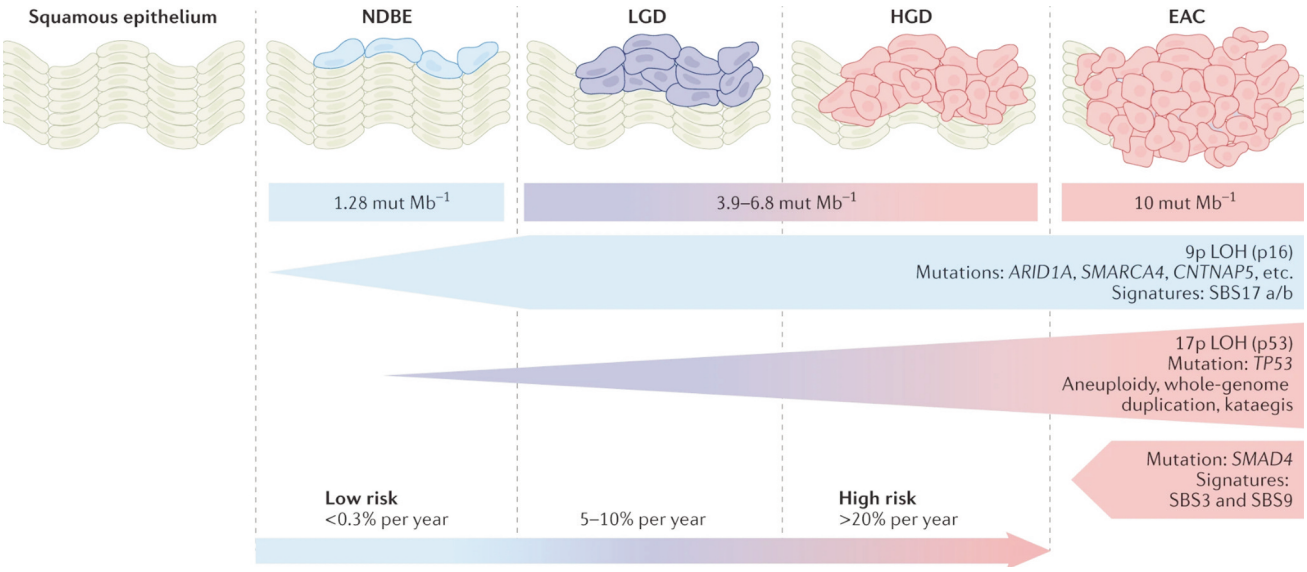

Figure 11. *Schematic representation of progression from squamous epithelium to non-dysplastic Barrett's oesophagus, LGD, HGD and EAC with associated specific molecular hallmarks as mutation rate and signature to mutations in canonical cancer driver genes. Blue colour represents a lower risk of progression, purple represents an increased risk and red represents high risk. LGD, Low-grade dysplasia; HGD, high-grade dysplasia; EAC, oesophageal adenocarcinoma (181).*

low-pressure liquid N_2 spray device demonstrated that no dysplasia, no sub squamous Barrett's, or procedure-related complications were identified at 6-month follow-up, highlighting modality efficiency and safety, in addition to being technically easy to perform. An additional study by Dumot *et al*. (184) with the application of a liquid N_2 device in patients with HGD and early oesophageal cancer in Barrett's, including those with previous ablation or endoscopic mucosal resection (EMR), showed a 56% response for those without dysplasia, and 60% for 5 patients with intramucosal cancer. Despite no procedure-related mortality being observed, one Marfan syndrome patient suffered a gastric perforation. Furthermore, using the same liquid N_2-based technology, two studies were conducted where the first showed encouraging results with minimal side effects in a group of clinically difficult patients with adenocarcinoma, and the second showed no procedure-related complications, a 93% response for HGD, 67% for all dysplasia, and 56% demonstrating total elimination of intestinal metaplasia (185, 186). Figure 12 and Figure 13 depict the endoscopic appearance of cryoablated oesophageal mucosa and associated procedural endoscopic images of oesophageal adenocarcinoma, respectively.

Moreover, a study using a low-pressure portable CO_2 cryosurgery system on HGD or early adenocarcinoma in BE recorded 50% complete reversal of intestinal metaplasia for all Barrett's patients, 86% for HGD, and 84% for LGD, while no serious complications were noted, highlighting the CO_2 system safety and efficiency as an alternative endoscopic

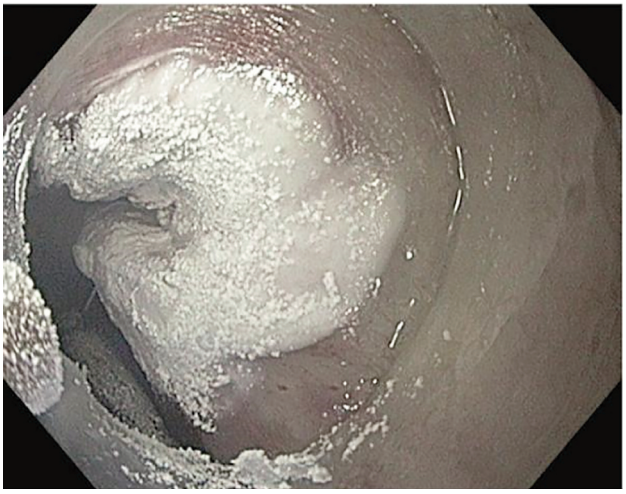

Figure 12. *Endoscopic appearance of cryoablated oesophageal mucosa using liquid N_2 (185).*

ablative modality for Barrett's dysplasia, carcinoma, and in refractory disease (187). A study by Sharma *et al*. (188) on liquid N_2-based cryosurgery quantified the risk of significant bleeding events in patients either with or without concurrent antithrombotic therapy applied in cardiovascular disease management and confirmed that major bleeding complications are extremely rare with no significant increases in transfusion and hospitalization rates, overall supporting current practice. Interestingly, evidence from the study of

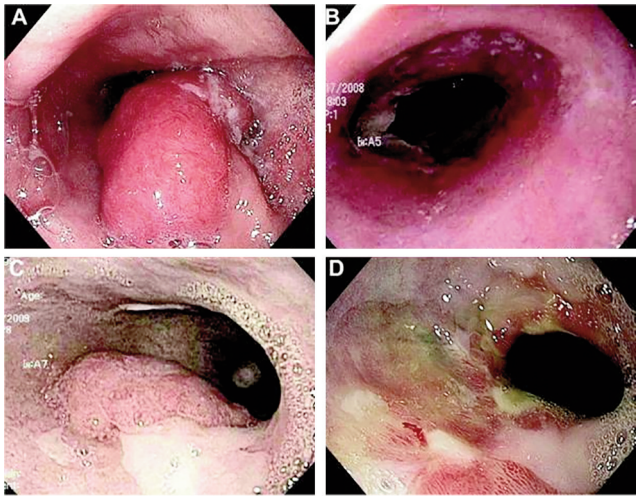

Figure 13. *Endoscopic images of oesophageal adenocarcinoma after chemotherapy and chemoradiation. (a) Initial appearance of adenocarcinoma before chemoradiation. (b) 1 month post chemoradiation with no adenocarcinoma detectable. (c) 5 months post chemoradiation with adenocarcinoma and residual intestinal metaplasia present. (d) After 3 cryoablation sessions, demonstrating no visible residual cancer (185).*

Jahromi *et al*. (189) revealed that Barrett's recurrence durability after complete remission of intestinal metaplasia is comparable to the other endoscopic methods available.

Cryoablation performed with the use of liquid N_2 or CO_2 demonstrated encouraging results in the treatment of abnormal GI mucosa such as in Barrett's oesophagus. The modality has been found to have a high success rate for eliminating intestinal metaplasia and dysplasia, low rates of recurrence and progression to oesophageal cancer in a long-term follow-up, as well as an acceptable safety profile. In addition, it is a robust and user-friendly technology, overall highlighting the great potential in BE management and the consequent improvement of patients' quality of life. Nevertheless, more studies are awaited due to the limited duration of follow-ups and number of patients. The optimal protocol is yet to be confirmed as to the number and duration of freeze cycles, session frequency, and treatment endpoints. More research is required to address the differences between CO_2 and N_2-based devices as well as the treatment regimen, including cryosurgery as a stand-alone monotherapy or as part of a multimodal management.

Liver cancer. Hepatocellular carcinoma. Hepatocellular carcinoma (HCC) as a primary cancer of the liver is a global health-care challenge with an estimated incidence of more than a million patients by the year 2025, while being the fifth most common cause of cancer and the second leading cause of cancer death in men worldwide (190, 191). Risk factors for the condition include viral hepatitis, alcoholic liver disease, as well as non-alcoholic liver steatohepatitis associated with diabetes mellitus or metabolic syndrome (192, 193). The presentation of HCC varies depending on the tumour stage and cirrhosis, liver scarring background. While non-cirrhotic related HCC could be asymptomatic in the early stage of the condition, cirrhotic patients have major implications for prognosis and management options and demonstrate symptoms of decompensated liver failure as abdominal pain, pruritus, ascites, worsening jaundice, hepatic encephalopathy, fever, abdominal distension, malaise, and cachexia. Paraneoplastic syndrome symptoms in HCC patients due to the cancer presence in the body induce the production of chemical signalling molecules or an immune response, which include erythrocytosis, hypercalcemia, diarrhoea, hypoglycaemia, and cutaneous findings. Intrahepatic metastases are more frequent, nevertheless, less common extrahepatic metastases could spread to intra-abdominal lymph nodes, lungs, bones, and adrenal glands. Despite liver resection representing the main curative treatment option outlined in national and international guidelines for patients with HCC, the modality would significantly benefit patients with single tumours and maintain function of the liver (194-199). Although, given that many patients have cirrhosis, poor hepatic reserve, multicentric tumours or extrahepatic disease type, as well as the fact that patients with resectable colorectal metastases have from 30% to 40% 5-year survival rates, non-surgical tumour ablation therapies, such as percutaneous ethanol injection, RFA, MWA, and cryoablation have been developed, demonstrating substantial advantages. These represent the first option for those patients (200). To date, the vast majority of research studies were consistent in that ablation techniques are associated with a minimally invasive nature, minimal toxicity profiles, effective tumour responses not affecting the normal parenchyma, larger tumour ablation volumes, higher intra-tumoral temperatures, faster ablation times, the use of multiple probes simultaneously, optimal heating of tumours that are close to the vessels as well as of cystic masses, and less procedural pain (201-204). Historically, cryoablation of the liver using liquid N_2 is an open technique, only in 10% of the cases performed laparoscopically. Nevertheless, with the advent of Ar gas-based device systems and an associated decrease in the cryoprobes' size, percutaneous application has become an option, inducing less diaphragmatic injury in addition to significantly reduced post procedural pain (205). Additionally, it has been found that cryoablation can be successfully applied in close proximity to larger vessels when compared to other ablative techniques. Nevertheless, caution must be exercised when treating regions close to the hepatic hilum due to unwanted induction or adverse effects on bile ducts, subsequently leading to stricture or rupture. Multiple probes can be inserted simultaneously, and their

number and location are determined by the size and shape of the ablated mass. The recent advances in imaging techniques allow accurate visualization of the tumour and ablation ice ball formation and therefore precise procedural real time control, and include the use of US, CT, and MRI. An animal model study conducted by Niu *et al*. (206) demonstrated the high coherence between the CT image of the ice ball and histological examination findings, both after the hepatic cryoablation and 7 days later.

Nevertheless, despite cryoablation being an important option for unresectable malignant hepatic tumours, the results of only a limited amount of research are available with no randomized studies with long-term follow-up to prove the safety and advantage over other ablation therapies. Furthermore, cryoablation usually requires multiple probes making the procedure time consuming and not cost-effective. To date, it has been recorded that the technical success rate for HCC cryoablation is between 55-100%, and local recurrence between 8-53% (207-211). Specifically, a study on the subject presented by Kerkar *et al*. (212) demonstrated 76%, 42%, 24%, and 16% hepatic recurrence–free survival rates at 1, 2, 3, and 5 years, and overall survival rates of 81%, 62%, 48%, and 28%, respectively. Another large series study demonstrated a 3-year survival rate of 40% and a 5-year rate of 27% (213). A study of medium to large lesions showed a 5-year local progression rate of 24% and a survival rate of 23% (214, 215). In contrast, Zhou *et al*. (216) reported a 5-year survival rate of 34.8% while concluding that the cryoablation results were compatible with liver resection. Similarly, a study conducted by Goering *et al*. (217) showed no significant difference in the survival of patients with metastases treated by cryoablation only, resection only, or by a combined treatment. In the matter of metastatic tumour cases, a study with mixed lesions, including HCC, colorectal metastases, and other metastatic tumours, demonstrated no significant difference in survival rates that were 57% and 48% for 3-year and 5-year for HCC patients, 43% and 22% for colorectal metastases, and 44% and 28% for non-colorectal metastases, respectively (212).

To conclude, cryoablation is a robust technology offering the precision attained in the shape and size of the ablation zone, as well as the ability to produce large zones of ablation compared to other ablation modalities. Nevertheless, according to the aforementioned contrasting overall survival, recurrence–free survival, local recurrence and progression rates of hepatic cryoablation, it is obvious that more research on the inherent complexity of parameters associated with HCC is needed to confirm the modality effectiveness.

Complications and the phenomenon of "cryoshock". The main reason liver cancer in terms of cryosurgery treatment was chosen in this work is due to the increasing amount of literature on associated complication rates following the modality application. According to data, an overall complication rate of 35% to up to 40% has been documented in 11% of patients with major complications (218-220). Haemorrhage, liver parenchyma fracture, fever, myoglobinuria, thrombocytopenia, biliary fistula, coagulopathy, pulmonary oedema, cold-induced lesions, and cryoshock are the most common and major associated complications (221). The so-called post-ablation syndrome, including general malaise and low-grade fevers, can develop in the postprocedural period and the severity correlates with the volume of tissue ablated. It has been found that every ablated patient had this complication to a degree, nevertheless, clinically significant haemorrhage is not frequent. One of the catastrophic complications associated that could occur is so-called liver "cracking" resulting in massive and rapid blood loss, caused by the air-ice interface in addition to tissue reperfusion as the ice ball undergo the thaw stage. Nevertheless, studies failed to identify experimentally an increased risk of bleeding when analysing healthy animal model livers as well as, to date, no data on cirrhotic liver studies are available (222). Experimental evidence has demonstrated that biliary complications in central lesions in close proximity to portahepatis or close to the central portal venous system are associated with accidental central biliary tree damage, consequently leading to severe biliary ductal structuring and obstruction. Abscesses requiring drainage catheters or intravenous antibiotics are rare but despite well-established risk factors no management protocols are available. The history of biliary interventions is the primary risk factor creating a conduit allowing bacteria migrating into the necrotic ablation zone and producing an infection.

Importantly, research on the subject found that the direct exposure to the bloodstream of tissues undergoing necrosis during cryoablation can lead to a severe systemic reaction termed as the phenomenon of "cryoshock", already mentioned when discussing prostate cancer associated deaths, resulting in thrombocytopenia, severe coagulopathy, disseminated intravascular coagulation, and multiorgan failure (213). Milder nonspecific phenomena, known as cryoreaction, can occur, including symptoms of tachypnoea, tachycardia, chills fever, or the temporary renal damage recorded in 1% of cryoablation sessions (112). *In vivo* comparison of cryoablation and radiofrequency ablation has demonstrated more severe systemic effects with cryoablation carrying a high mortality of up to 40%. Another study reported overall mortality of 1.5%, where 18% of deaths were due to cryoshock (218). The aetiology of cryoshock has been found to be mediated by the cytokine TNF-alpha, IL-1, and IL-6 release and associated with the volume of and duration of cryoablation, as well as with double freeze cycles or complete prior procedural thaw (223). In particular, research analysing underlying pathophysiologic processes in

cryoablation using liquid nitrogen, RF, and laser-induced thermotherapy (LITT) in rat liver conducted by Jansen *et al*. (224) showed similar a volume of complete liver parenchyma destruction in all tested modalities, whereas cryoablation in particular induced significant inflammatory and coagulation responses that bidirectionally affected the evolution of the cryoshock. Specifically, levels of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly higher following cryoablation, indicating increased cell membrane destruction compared to RF and LITT, consequently leading to elevated leakage of enzymes into the circulation. One of the explanations of the cell membrane being more vulnerable to cryosurgery is the time required for restoration of baseline temperature values, where for RF they were 7 minutes following a 2-cycle period, while more than 15 minutes was needed after cryoablation before normal tissue temperatures return. In the matter of increased levels of cytokines as reflected by high IL-6 and IL-10 levels during cryoablation, it was suggested that the sustained inflammatory response was caused by protein release, protein that was simultaneously frozen and consequently re-activated during the thawing, whereas during RF and LITT the proteins were immediately denatured *via* the application of extremely high local temperatures, inactivating both cytokines and liver enzymes. Supplementary study findings by Chapman *et al*. (225) suggested that cryoablation results in hepatocyte plasma membrane disruption with hepatocyte organelles extending into the space of Disse. Furthermore, thrombocytopenia, a condition of low blood platelet count responsible for blood clotting, was significantly more profound following cryoablation compared to RF and LITT. The findings were explained by inhibited endogenous fibrinolytic activity due to increased PAI-1, suggesting that a suppressed fibrinolysis and systemic procoagulant state could be induced after cryoablation, resulting in disseminated intravascular coagulation. The procoagulant changes themselves contribute to imbalance of thrombin generation that results to systemic intravascular fibrin deposition and disseminated intravascular coagulation, overall leading to severe consequences such as organ failure.

Returning to the aspect of ALT and AST levels, a retrospective study on large HCC by Alnaggar *et al*. (226) demonstrated that these values are valuable indicators of liver function impairment following cryoablation, which is important for patients with the hepatitis B virus, and liver-protective treatment is a promising option for alleviating impairment of liver function.

More research on the topic conducted by Niu *et al*. (206) compared imaging and pathological changes for argon–helium cryoablation and MW in a porcine liver model. The findings indicated that complete tissue necrosis could be achieved after both modalities, but the depth and extent of necrosis would differ. MW resulted in a larger necrosis, suggesting that MW is more suitable for larger volume tumours with simple anatomical structures, whereas cryoablation AH is more suitable for tumours located near important organs or with complex anatomical structures. Specifically, the findings demonstrated that the main difference between the two modalities was the inflammation area, where cryoablation induced the greater one. The results were consistent with the previous research which found that more severe systemic inflammation is associated with the phenomenon of cryoshock and it is a key factor influencing cancer development.

Despite enormous progress made in the research and development of cryogenic technologies used with hepatic malignancies and studies demonstrating acceptable results in hepatocellular carcinoma, which are unresectable tumours, more research is crucial to develop a safe modality with an efficient ablation protocol and hence to demonstrate long-term survival. This is in light of serious postsurgical complications such as cryoshock and the inherent complexity of mechanisms and parameters associated with the modality mode of action.

Industrial Food Processing

Campylobacter contamination of chicken carcasses. Another valuable biomedical application of cryogenic mediums is the industrial food processing of chicken carcasses to address *Campylobacter* spp. contamination. Campylobacters, Gram-negative rods with characteristic ‘s’ or spiral shape, is a leading cause of foodborne gastrointestinal bacterial infections worldwide. Most common self-limiting symptoms of Campylobacter infection that typically resolve in 7 days include diarrhoea, fever, abdominal pain, nausea, headache, and vomiting (227). Nevertheless, the latest studies registered other serious complications such as triggering the Guillain-Barré syndrome, possibly leading to permanent nerve damage or muscle weakness and paralysis, as well as irritable bowel syndrome in 5-20% of cases and arthritis in 1-5% of cases (228-231). Figure 14 schematically represents other associated clinical manifestations as well as environmental reservoirs and routes of transmission (232).

As a Campylobacter infection can spread to the bloodstream, special attention should be paid to immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS), blood disorders, or those undergoing chemotherapy, due to a high susceptibility to life-threatening complications. According to epidemiological data, 143 outbreaks of Campylobacter infection were reported in England and Wales between 1992 and 2009, schematically represented in Figure 15 in a form of timeline with outbreaks worldwide (232). Importantly, 21 deaths per year were registered in UK, highlighting the

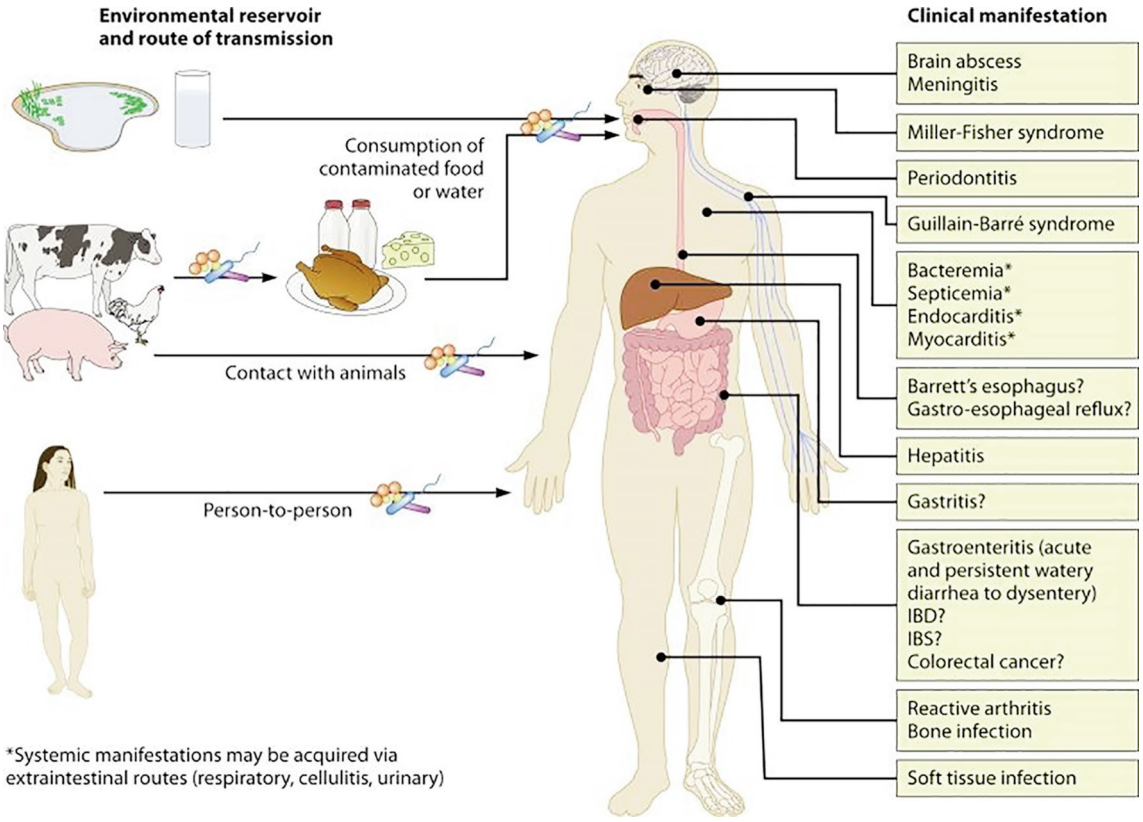

Figure 14. *Schematic representation of Campylobacter species associated environmental reservoirs, routes of transmission, and clinical manifestations. IBD, Inflammatory bowel diseases; IBS, irritable bowel syndrome. Question marks represent clinical conditions for which a role for Campylobacter has been recorded but is uncertain (232).*

potential severity of this infection as well as demonstrating the importance of preventative measures which need to be addressed (233).

The poultry reservoir has been identified as the main source of Campylobacter infection in humans where the infection is transmitted through consumption of undercooked meat or other products cross-contaminated by raw poultry. *Campylobacter* in chicken has been found to colonize the epithelial cell mucus in the small intestines, nevertheless, the bacteria can also be found in other parts of the digestive system, such as the ceca, spleen or liver (234). The transmission of infection between chickens in the broiler house spreads very fast due to the bird coprophagic behaviour in addition to other routes such as a contaminated drinking water system and factors including flock size, the number of poultry houses, insufficient biosecurity, wild birds, rodents, litter, farm personnel, equipment, and transport vehicles (235-239). According to data, it takes from 4 to 7 days after infection of the first chicken to colonize a 20,000-bird flock with *Campylobacter* shed at high numbers until slaughter (240). Nowadays, strictly followed biosecurity protocols are the most important measures to reduce primary farm bacterial level production, in addition to farmer education and training, improvement of the vehicles and container materials and design. A probabilistic study by Dogan *et al*. (241) that applied a farm-to-fork quantitative microbial risk assessment (QMRA) demonstrated that contamination can be significantly reduced during processing stages with the use of antimicrobial processing aids, where chemicals have been reported to be the most efficient ones, including peroxyacetic acid or cetylpyridinium chloride spraying, and acidified sodium chlorite or peroxyacetic acid immersion. While no chemical aids are currently authorized in the EU according to EC Regulation No 853/2004, these aids were associated with a need for occupational safety precautions and constant monitoring due to toxicity to humans. In addition to the cost of implementation and maintenance at high concentrations, the efficacy and the importance of implementing aids not compromising the safety of the final product is highlighted.

One such technique is rapid cooling usually applied in a pre-chilling step using N_2 in the form of a spray. The results demonstrated 63% efficiency in reducing the risk estimates,

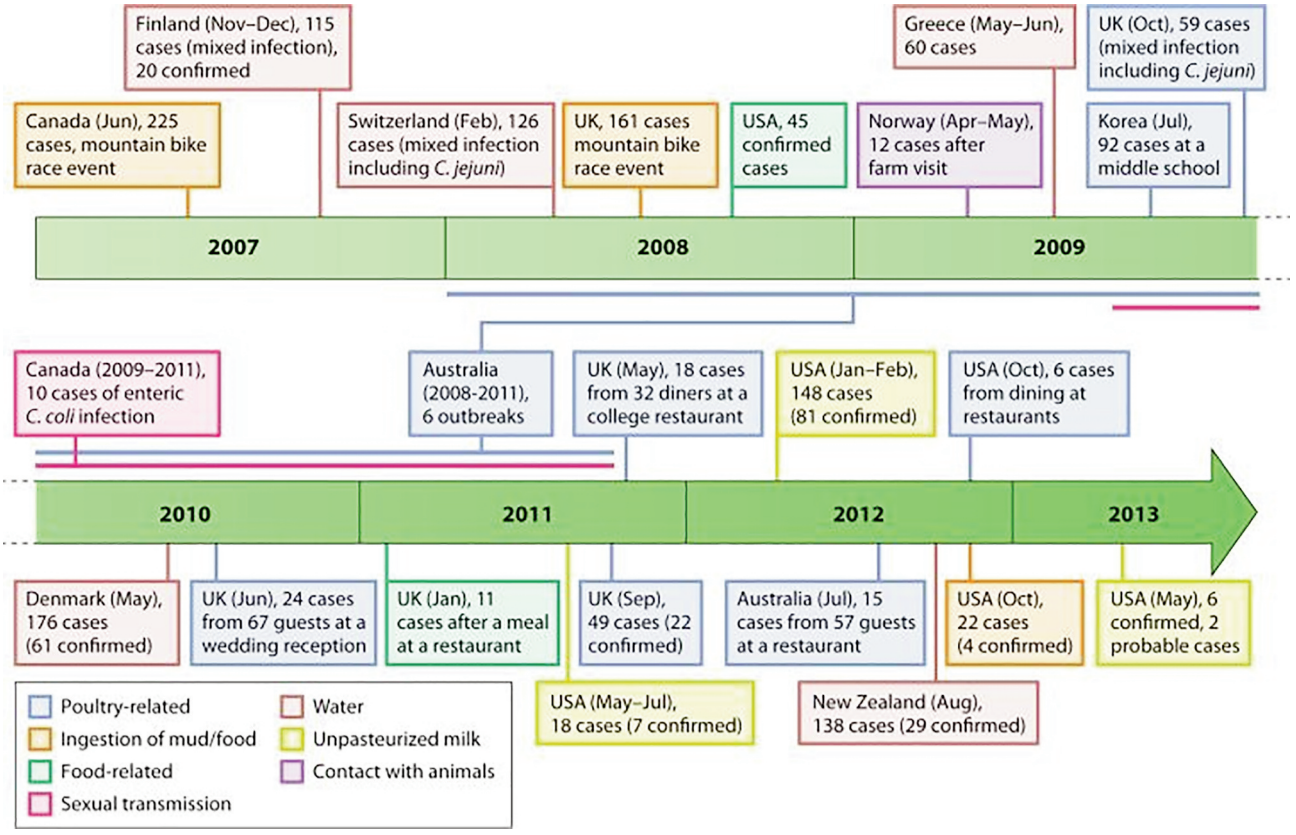

Figure 15. *Schematic representation of worldwide Campylobacter spp. infection outbreaks since 2007 (232).*

especially when applied in addition to another processing aid. Research conducted by Gunther *et al*. (242) showed that the rapid freezing technique to –62.2˚C with N_2 vapor applied to ground turkey *Campylobacter jejuni* (RM1221) and *Campylobacter coli* (RM1403) strains resulted in an efficient 2.5- to 3.7-log bacterial number reduction compared to previously reported reductions using gradual freezing at –20˚C. The effects of cryogenic freezing with N_2 or CO_2 were similarly reported in a study by Rasschaert *et al*. (234) that demonstrated the benefits of carcass outer surface crust freezing, resulting in unfrozen meat remains, while eliminating the need for additional freezers. A significant disadvantage was reported by Haughton *et al*. (243) as increased drip loss during thawing due to ice crystal formation puncturing the cell walls, making this technique economically undesirable. Nevertheless, this variable has been found to be dependent on temperatures and freezing times used, while a growing body of evidence reported that crust freezing has little negative effect on meat quality. Similarly, the study conducted by Zhao *et al*. (244) showed that liquid N_2 freezing could substantially reduce *Campylobacter jejuni* populations, but more research is needed on temperature, storage time, and freezing conditions to improve the rate and degree of reduction in the poultry industry. An additional study by James *et al*. (245) concluded that the crust freezer illustrated in Figure 16, used in combination with thermal steam decontamination treatment, is an effective method for reducing *Campylobacter* bacteria without degrading the carcass appearance, highlighting the potential efficacy of the concept and the importance of implementing combinations of several antimicrobial processing aids.

One advanced food processing product available in the market is the Air Products N_2 based Freshline SafeChill System, which is patented as a fully automated intervention used to effectively and safely reduce the *Campylobacter* spp. presence on contaminated broiler carcasses. This allows fully preserved quality and organoleptic properties of products with super-chilled skin with fresh raw poultry meat inside. This is demonstrated schematically in Figure 17 (246).

Despite the availability of a wide range of options, more research is required due to the number of drawbacks associated with existing and new intervention technologies, making decisions on the adoption of an intervention challenging in terms of practicality and economy. Nonetheless, much of the current research agrees that

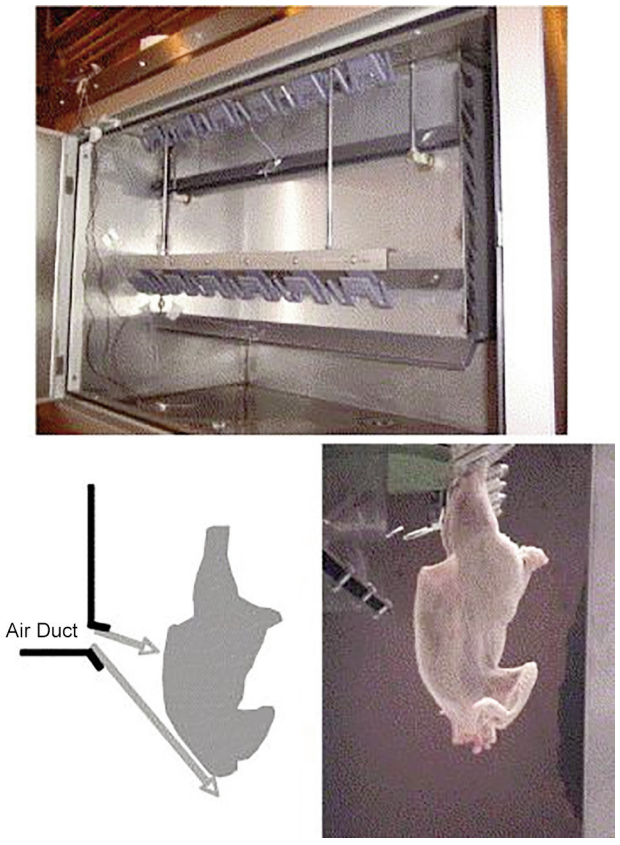

Figure 16. *Experimental rapid chilling system for poultry meat (245).*

Campylobacter contamination could be prevented and reduced at farm and processing levels by antimicrobial interventions, such as taking the benefit of ultra-low cryogenic medium temperatures as with liquid N_2. In addition, consumer education is critical for infection prevention, since undercooking has been shown to be the most valuable input parameter affecting the risk estimates. With the implementation of these measures, the risk of *Campylobacter* contamination and infection can be effectively managed in the poultry industry, aiming to meet the Appropriate Level of Protection (ALOP) of public health and Food Safety Objectives (FSO) and thus reducing the overall disease burden.

Cryopreservation

The state-of-the-art practice. A biobank is a biorepository infrastructure responsible for identification, collection, annotation, storage, and retrieval of high-quality biospecimens providing accurate clinical data for research, including advanced personalized technologies, such as transcriptomic and genomic sequencing, metabolomics, and proteomics (247). The biospecimens, or biological materials, are body fluids, blood, solid tissues, nail clippings, hair, specimens of infectious diseases, or laboratory-derived products including plasma, serum, cell lines, proteins, DNA, ribonucleic acid (RNA), metabolites, and proteins. A high quality of biospecimens is crucial for the molecular examination of complex disease processes prerequisite for personalized precision medicine, as well as for the development of diagnostic modalities and treatment options, where erroneous research data could potentially result in misleading both the investigator and the entire research community. One of the major challenges in ensuring high-quality are fluctuations in the variables in biospecimens following removal from the body, which results in abrupt temperature changes leading to numerous changes in protein phosphorylation or gene expression at various degrees and rates affecting metabolites, lipids, proteins, mRNA, and miRNA. To address this problem, cryopreservation – a cell preservation technology was developed to store biospecimens at ultra-low temperatures from −80 to −196˚C for prolonged periods of time of up to several years or decades (248). Nowadays, the technique involves the use of cryogen N_2 and a cryoprotective agent (CPA), which allows ultra-low temperature processing of the biospecimens and high-level functionality recovery *via* a wide range of biophysical and metabolic effects (249). Dimethyl sulfoxide (DMSO) and glycerol are the two most widely used penetrating type CPAs. Nevertheless, research still faces challenges with their application due to their penetrating nature, and thus their ability to influence cellular components such as enzymes, consequently possessing intrinsic toxicity and inducing clinical side effects in patients, including gastrointestinal or renal dysfunctions, allergies, and respiratory disorders (250-256). It has been demonstrated that a mixture of CPAs could maximise cell viability through glass formation avoiding the crystallization of ice during both cooling and thawing cryopreservation of mammalian and bacterial cells, overall showing the reduced toxicity associated with penetrating type CPAs (257-262). Additionally, it has been found that toxicity could be reduced by introducing CPAs in lower temperature protocols, as well as by modification of equilibration times and temperatures (263).

To date, in general two types of freezers are used for cryopreservation in clinical practice. A liquid phase N_2 storage under steady ultra-low (−196˚C) temperature shows simplicity and mechanical reliability while requiring the use of large N_2 volumes, which is a potential hazard, in addition to questionable cost-effectiveness. Moreover, viral cross contamination was documented shifting the application towards the most commonly used vapor phase N_2 storage freezers that possess no risk of cross-contamination while being simple and reliable. Nevertheless, they require a regular supply of liquid N_2 and have high running costs due to regular maintenance to avoid extreme temperature

Figure 17. *Top: Air Products PLC nitrogen based Freshline SafeChill System (Cheshire, England, UK) designed to effectively and safely reduce the Campylobacter spp. presence on contaminated broiler carcasses. Bottom: Detailed schematic representation of the equipment (246).*

fluctuations between −190˚C and −135˚C in the upper regions of the storage vessel. Nonetheless, thanks to recent technological advances, various modern designs of liquid N_2 storage vessels for vapor phase storage freezers have been developed and are routinely used both in research and clinical settings.

Despite an impressive set of discoveries, a great deal of previous research has identified continuous cell stresses associated with cryopreservation freeze-thaw cycles, specifically ROS, ice-induced osmotic and mechanical injuries, where some studies indicated the last two as the major causes of cellular death. Initial crystalline ice formation outside the cells leads to a solute freeze-concentration development with higher concentrations of salts, sugars, and proteins in a co-existing unfrozen fraction compared to the isotonic solution. Consequently, this results in cell damage *via* changes in protein conformations and bilayer structure, or the creation of osmotic stress on thawing (258, 264-266). The relative water removal by ice *via* hypotonic stress leads to dehydration and further membrane phase changes with cell damage (267-271). It has been found that cell damage in slow cooling protocols is associated with intracellular water loss, osmotic injury, leading to cell apoptosis or death, and the damage is dependent on the presence of nucleation sites, the concentration of solutes, and the rate of freeze/thaw. It is important to highlight, in terms of other cryopreservation cell injuries, that specifically ROS injuries have been found to be associated with slow freezing protocols, showing induced cellular redox imbalance, imposing the cell to the oxidative stress damaging essential macromolecules, and consequently leading to apoptosis and necrosis. On the other hand, in rapid cooling protocols the intracellular water could puncture cellular organelles and membranes, also known as mechanical injury (249, 272). The above-mentioned effects are represented in Figure 18. The analysis of these contrasting mechanisms led to the development of the 'Two Factor Hypothesis' proposing an ideal intermediate cooling

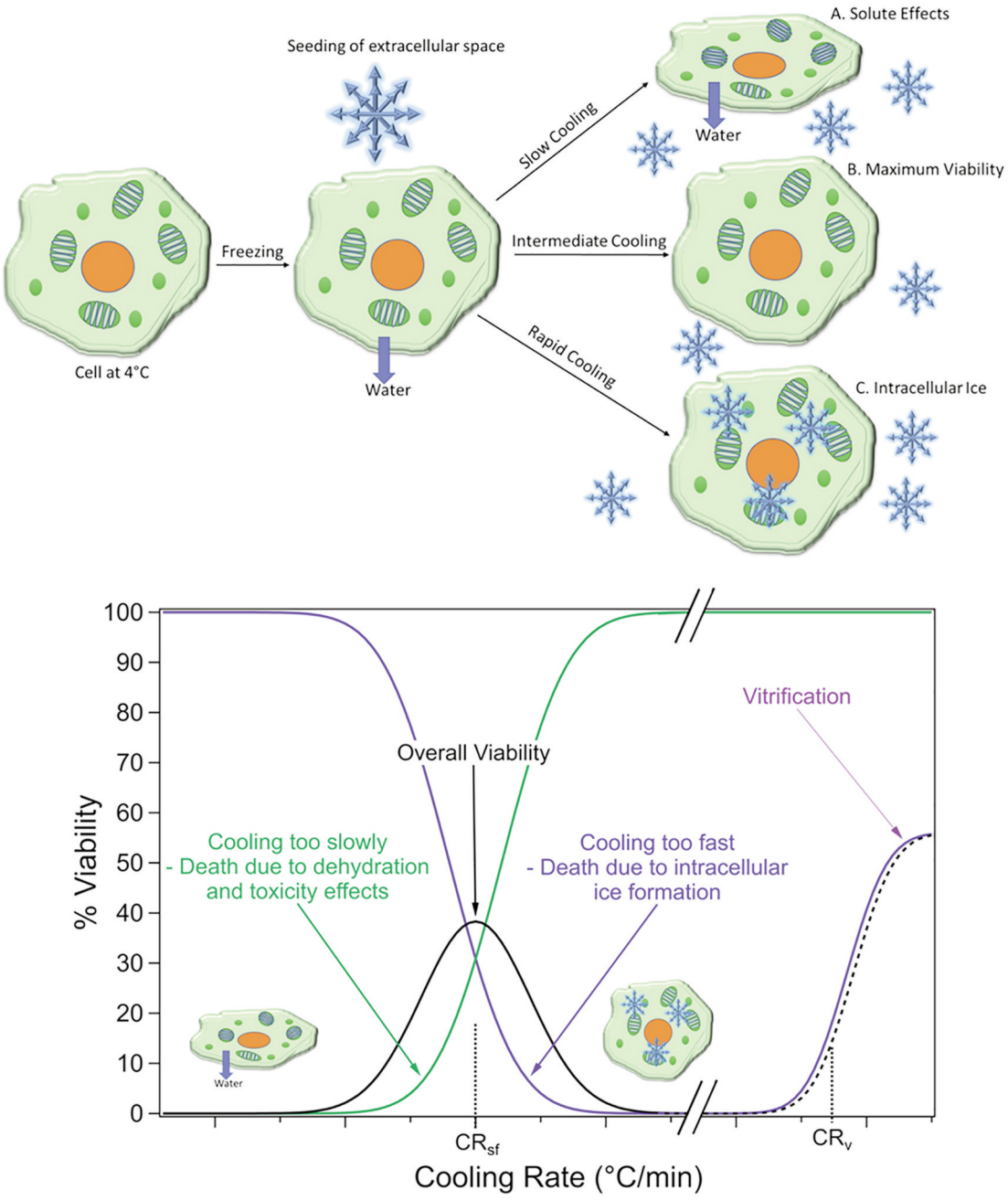

Figure 18. *Top: Schematic representation of cell damage at different cooling rates. Bottom: Graphical representation of Mazur's Two-Factor Hypothesis highlighting an optimal cooling rate (CRsf) which maximises cell survival (249).*

rate potentially leading to maximum cell viability, represented by the inverted 'U' curve shown in black in the bottom figure of Figure 18 (272, 273). Nevertheless, it must be highlighted that the ideal cooling rate as well as cryobiological responses are cell type specific, therefore cell-specific biological and biophysiological characteristics have to be evaluated prior to preservation to maximize post-cryo viability (274). Interestingly, the graph depicts the phenomenon of ultra-fast freezing, known as cryopreservation vitrification, a method which uses high concentrations of CPA (excess of 40% w/v) to cool the biospecimen below glass transition temperature while not causing ice formation or freeze concentration, subsequently resulting in an amorphous matrix which is an ultra-cold

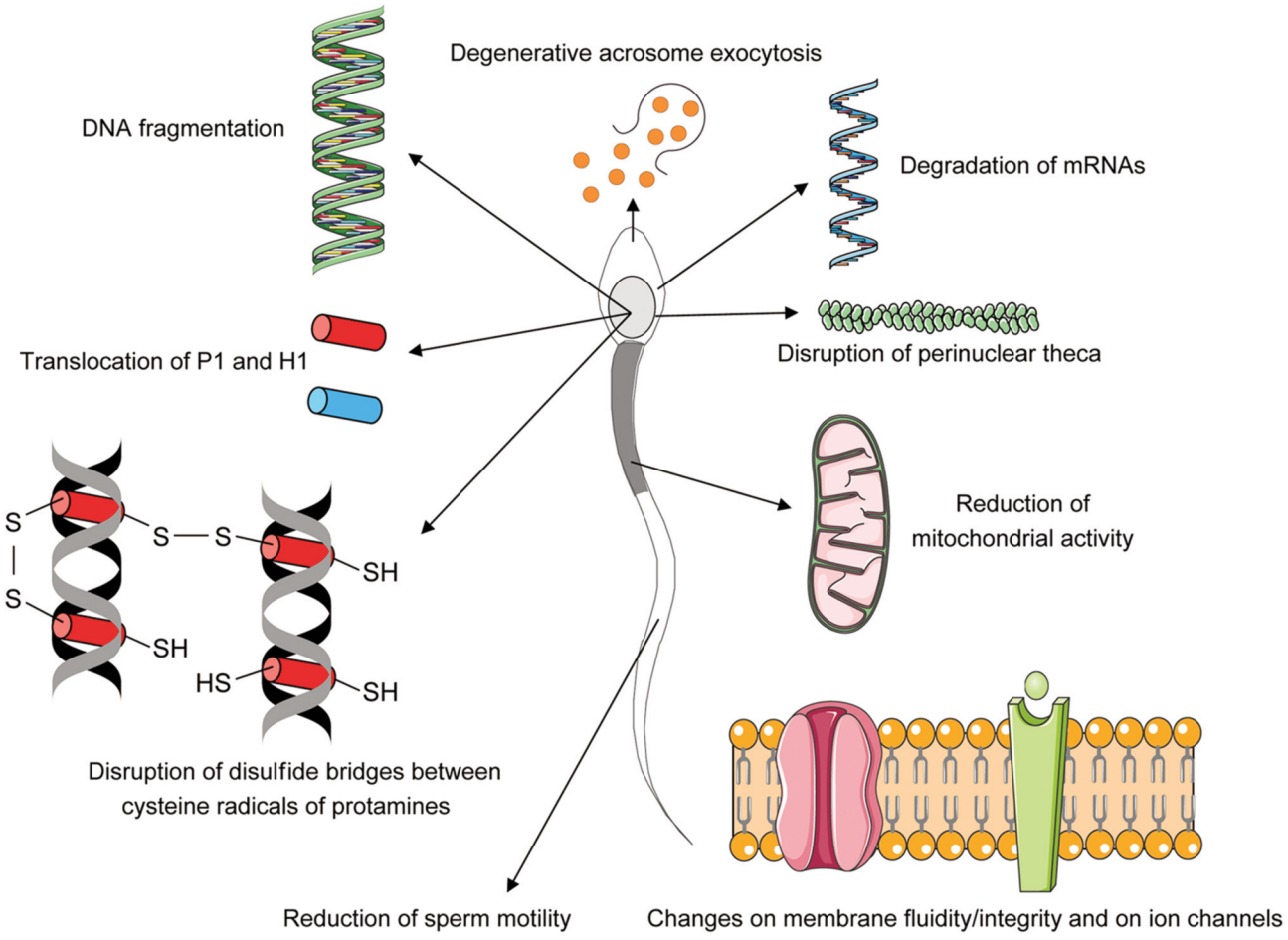

Figure 19. *Schematic representation of a sperm and associated damage caused by freeze-thawing procedures as in the fluidity and permeability of plasma membrane, and acrosome integrity resulting in degradation of mRNAs and proteins; DNA fragmentation, disruption of disulfide bridges between cysteine radicals of P1, and translocation of nucleoproteins leading to detrimental effects on sperm nucleus (283).*

viscous liquid (275). Nonetheless, the use of high concentrations of CPA has been found to induce toxicity to mammalian cells (276-278). As shown on the graph, the traditional 'U' curve is modified and extended into the region of ultra-fast cooling where cell viability increases with increasing cooling rate.

After many decades of research, it's evident that the advancement of effective cryopreservation protocols, along with the crucial use of non-toxic CPAs, is still at an early stage. Several studies have indicated the need to address various factors like CPA selection, its ability to penetrate cells and inhibit ice formation, cooling rate, and unique biophysical cell properties to enhance cryoprotection efficiency and biocompatibility. These efforts hold the potential to greatly enhance biobanking for personalized precision medicine, leading to improved disease diagnosis, monitoring, and treatment outcomes.

Clinical applications. The recent advancements in cryopreservation have expanded possibilities in research and medicine. For instance, assisted reproductive technology (ART) benefits from techniques like intracytoplasmic sperm injection (ICSI), *in vitro* fertilisation (IVF), and gamete/embryo cryopreservation, effectively treating infertility cases (279). Cryopreserved sperm maintain viability and motility after thawing (280). Long-term cryopreservation doesn't affect pregnancy or live birth rates, but sperm quality might decline after 5 years (281). Post-thaw damage can impact various sperm aspects, like acrosome integrity and fertilisation capability, due to DNA fragmentation and gene lesions, as summarized in Figure 19 (282, 283).

To tackle this aspect, extensive prior research has revealed that assessing pre-cryo sperm viability and motility can assist in predicting outcomes and cryosurvival post-thawing.

Additionally, the duration of the pre-freezing holding period of the semen, identified as a critical variable (284, 285), must be taken into account. Other essential considerations encompass evaluating initial sperm motility one hour after delivery, where cells are primarily exhibiting forward motility, noting a substantial reduction in forward motility four hours after delivery, and observing the decay in sperm motility (286).

Another exciting technological advance in reproductive health that provides cryopreservation is the transplantation of cryostored spermatogonial stem cells (SSCs) for pre-pubertal boys or adult males with non-obstructive azoospermia (NOA) for fertility preservation. This is conducted before fertility threatening therapies such as cytotoxic therapy that leads to SSCs depletion, as investigated in the study conducted by Kanbar *et al*. (287). Nonetheless, despite encouraging results obtained in animal studies and preclinical experiments, the development of a reproducible, improved, and efficient cryopreservation method is awaited, in addition to optimal culture media, injection techniques, and a standardized protocol for SSCs. This is required to address safety and technical issues such as the risk of cancer cell contamination or epigenetic and genetic stability of cultured SSCs prior to re-transplantation. In addition, a study by Amidi *et al*. (288) identified spermatozoa susceptibility to ROS injury, highlighting the need for antioxidants during cryopreservation to help the cellular antioxidant defence system to maintain the balance. An additional study on the cryostorage of white cachama (*Piaractus orinoquensis*) sperm presented by Medina-Robles *et al*. (289) demonstrated significantly affected motility and viability of spermatozoa after 24-hour cryostorage, with no significant difference across the year of storage as well as for other variables, such as pH, motility duration, and total antioxidant capacity. On the other hand, ATP content and DNA integrity were stable for 24 hours and 1 month, but drastically decreased when stored for 6 and 12 months, in addition to the observed substantial ultrastructural damage mostly at the head level depicted in Figure 20. This highlights that liquid N_2 storage does not terminate the metabolic processes, therefore more research is required to investigate, evaluate and optimize the cryostorage of gametes.

Alikani (290) highlighted similar conclusions regarding cryostorage of human gametes and embryos, emphasised contamination risks from liquid N2 exposure and reduced viability. This indicates a need for comprehensive experimentation and correction in cryostorage procedures for successful and safe reproductive treatment. Despite progress, challenges in protocols, agents, and biospecimen changes persist, demanding further research for improved disease management through cryopreservation.

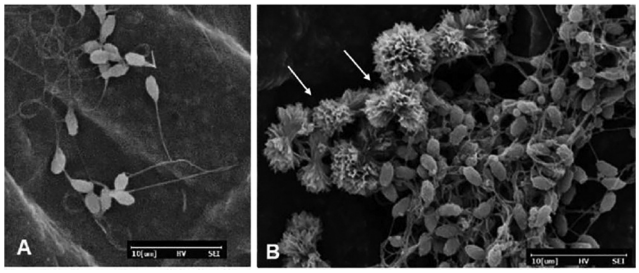

Figure 20. *Scanning electron microscope imaging demonstration: (a) Fresh spermatozoa; (b) Spermatozoa cryostored for 12 months with white arrows depicting cryo-damage at head level (289).*

Conclusion

The purpose of this article was to explore and discuss the current biomedical applications of cryogenic mediums, addressing challenges and perspectives. It provides an extended literature review to supplement existing research and offer a comprehensive view of these applications. The research covers various aspects, including advanced medical technologies, clinical treatments, biospecimen preservation, and food processing. While these applications show promise, there's a need for further validation and investigation in certain areas. For instance, MRI using cryogenic helium-3 for disease detection requires more validation, and hyperbaric oxygen therapy using liquid oxygen as a cryogenic medium needs more evidence to enhance its effectiveness. Cryotherapy with liquid nitrogen has conflicting results, demanding further studies. Cryosurgery, antimicrobial intervention, and cryopreservation also require more research to establish protocols and confirm efficiency. In conclusion, while the current article contributes to understanding cryogenic mediums' potential, more clinical and experimental studies are essential to address gaps and advance biomedical research using cryogens. The review's limitations include scarcity of information, restricted accessibility, and database reliability. Nonetheless, this work serves as a valuable preliminary resource for future research.

Funding

The reported work was supported by Air Products PLC under grant agreement: 216-206-P-F. Grant Holder: Professor Hussam Jouhara.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

HJ and AK were responsible for conceptualization; AK was responsible for writing—original draft preparation; AK, HJ, and KM were responsible for writing—review and editing; AK, HJ, KM,

HG, RM, and JT were responsible for visualization; HJ was responsible for funding acquisition. All authors have read and agreed to the published version of the manuscript.

References

1 Jouhara H, Chauhan A, Guichet V, Delpech B, Abdelkareem MA, Olabi A, Trembley J: Low-temperature heat transfer mediums for cryogenic applications. J Taiwan Inst Chem Eng 148: 104709, 2023. DOI: 10.1016/J.JTICE.2023.104709

2 Ershov BG: Radiation-chemical decomposition of seawater: The appearance and accumulation of oxygen in the Earth's atmosphere. Radiat Phys Chem 168: 108530, 2020. DOI: 10.1016/J.RADPHYSCHEM.2019.108530

3 Park DH, Park JJ, Olawuyi IF, Lee WY: Quality of White mushroom (Agaricus bisporus) under argon- and nitrogen-based controlled atmosphere storage. Sci Hortic 265: 109229, 2020. DOI: 10.1016/J.SCIENTA.2020.109229

4 Superconducting magnets. Questions and Answers in MRI. Available at: https://mri-q.com/superconductive-design.html [Last accessed on June 9, 2023]

5 Mahesh M, Barker PB: The MRI helium crisis: Past and future. J Am Coll Radiol 13(12 Pt A): 1536-1537, 2016. DOI: 10.1016/j.jacr.2016.07.038

6 Kryukov E, Owczarkowski M, Phillipps D, Perez Linde AJ, Burgess S, Good J: A new method to measure the temporal magnetic field instabilities in cryogen-free magnets for magnetic resonance. Solid State Nucl Magn Reson 113: 101732, 2021. DOI: 10.1016/j.ssnmr.2021.101732

7 Kryukov E, Perez Linde AJ, Raghunathan S, Burgess S, Jonsen P, Good J: On the magnetic field stability of cryogen-free magnets for magnetic resonance applications. Solid State Nucl Magn Reson 105: 101639, 2020. DOI: 10.1016/j.ssnmr.2019.101639

8 Guo J, Garratt A, Hill A: Worldwide rates of diagnosis and effective treatment for cystic fibrosis. J Cyst Fibros 21(3): 456-462, 2022. DOI: 10.1016/j.jcf.2022.01.009

9 Randell SH, Boucher RC, University of North Carolina Virtual Lung Group: Effective mucus clearance is essential for respiratory health. Am J Respir Cell Mol Biol 35(1): 20-28, 2006. DOI: 10.1165/rcmb.2006-0082SF

10 Boucher RC: New concepts of the pathogenesis of cystic fibrosis lung disease. Eur Respir J 23(1): 146-158, 2004. DOI: 10.1183/09031936.03.00057003

11 Woodworth BA, Ahn C, Flume PA, Schlosser RJ: The Delta F508 mutation in cystic fibrosis and impact on sinus development. Am J Rhinol 21(1): 122-127, 2007. DOI: 10.2500/ajr.2007.21.2905

12 Fain S, Schiebler ML, McCormack DG, Parraga G: Imaging of lung function using hyperpolarized helium-3 magnetic resonance imaging: Review of current and emerging translational methods and applications. J Magn Reson Imaging 32(6): 1398-1408, 2010. DOI: 10.1002/jmri.22375

13 McBennett K, MacAskill CJ, Keshock E, Mahani MG, Mata J, Towbin AJ, Sankararaman S, Drumm ML, Yu X, Ren CL, Nasr SZ, Kutney K, Flask CA: Magnetic resonance imaging of cystic fibrosis: Multi-organ imaging in the age of CFTR modulator therapies. J Cyst Fibros 21(2): e148-e157, 2022. DOI: 10.1016/j.jcf.2021.11.006

14 Mallallah F, Packham A, Lee E, Hind D: Is hyperpolarised gas magnetic resonance imaging a valid and reliable tool to detect lung health in cystic fibrosis patients? a cosmin systematic review. J Cyst Fibros 20(6): 906-919, 2021. DOI: 10.1016/j.jcf.2020.12.020

15 Six JS, Hughes-Riley T, Stupic KF, Pavlovskaya GE, Meersmann T: Pathway to cryogen free production of hyperpolarized Krypton-83 and Xenon-129. PLoS One 7(11): e49927, 2012. DOI: 10.1371/journal.pone.0049927

16 Bannier E, Cieslar K, Mosbah K, Aubert F, Duboeuf F, Salhi Z, Gaillard S, Berthezène Y, Crémillieux Y, Reix P: Hyperpolarized ^{3}He MR for sensitive imaging of ventilation function and treatment efficiency in young cystic fibrosis patients with normal lung function. Radiology 255(1): 225-232, 2010. DOI: 10.1148/radiol.09090039

17 McMahon CJ, Dodd JD, Hill C, Woodhouse N, Wild JM, Fichele S, Gallagher CG, Skehan SJ, van Beek EJR, Masterson JB: Hyperpolarized 3helium magnetic resonance ventilation imaging of the lung in cystic fibrosis: comparison with high resolution CT and spirometry. Eur Radiol 16(11): 2483-2490, 2006. DOI: 10.1007/s00330-006-0311-5

18 Mentore K, Froh DK, De Lange EE, Brookeman JR, Paget-Brown AO, Altes TA: Hyperpolarized HHe 3 MRI of the lung in cystic fibrosis. Acad Radiol 12(11): 1423-1429, 2005. DOI: 10.1016/j.acra.2005.07.008

19 Sun Y, O'Sullivan BP, Roche JP, Walvick R, Reno A, Baker D, Mansour JK, Albert MS: Using hyperpolarized 3He MRI to evaluate treatment efficacy in cystic fibrosis patients. J Magn Reson Imag 34(5): 1206-1211, 2011. DOI: 10.1002/jmri.22724

20 Woodhouse N, Wild JM, Van Beek EJ, Hoggard N, Barker N, Taylor CJ: Assessment of hyperpolarized ^{3}He lung MRI for regional evaluation of interventional therapy: A pilot study in pediatric cystic fibrosis. J Magn Reson Imag 30(5): 981-988, 2009. DOI: 10.1002/jmri.21949

21 Altes TA, Johnson M, Fidler M, Botfield M, Tustison NJ, Leiva-Salinas C, de Lange EE, Froh D, Mugler JP: Use of hyperpolarized helium-3 MRI to assess response to ivacaftor treatment in patients with cystic fibrosis. J Cyst Fibros 16(2): 267-274, 2017. DOI: 10.1016/j.jcf.2016.12.004

22 Santyr G, Kanhere N, Morgado F, Rayment JH, Ratjen F, Couch MJ: Hyperpolarized gas magnetic resonance imaging of pediatric cystic fibrosis lung disease. Acad Radiol 26(3): 344-354, 2019. DOI: 10.1016/j.acra.2018.04.024

23 Shammi UA, D'Alessandro MF, Altes T, Hersman FW, Ruset IC, Mugler J, Meyer C, Mata J, Qing K, Thomen R: Comparison of hyperpolarized 3He and 129Xe MR imaging in cystic fibrosis patients. Acad Radiol 29: S82-S90, 2022. DOI: 10.1016/j.acra.2021.01.007

24 Kanhere N, Couch MJ, Kowalik K, Zanette B, Rayment JH, Manson D, Subbarao P, Ratjen F, Santyr G: Correlation of lung clearance index with hyperpolarized ^{129}Xe magnetic resonance imaging in pediatric subjects with cystic fibrosis. Am J Respir Crit Care Med 196(8): 1073-1075, 2017. DOI: 10.1164/rccm.201611-2228LE

25 Smith L, Marshall H, Aldag I, Horn F, Collier G, Hughes D, West N, Horsley A, Taylor CJ, Wild J: Longitudinal assessment of children with mild cystic fibrosis using hyperpolarized gas lung magnetic resonance imaging and lung clearance index. Am J Respir Crit Care Med 197(3): 397-400, 2018. DOI: 10.1164/rccm.201705-0894LE

26 Thomen RP, Walkup LL, Roach DJ, Cleveland ZI, Clancy JP, Woods JC: Hyperpolarized (129)Xe for investigation of mild

cystic fibrosis lung disease in pediatric patients. J Cyst Fibros 16(2): 275-282, 2017. DOI: 10.1016/j.jcf.2016.07.008

27 Walkup LL, Thomen RP, Akinyi TG, Watters E, Ruppert K, Clancy JP, Woods JC, Cleveland ZI: Feasibility, tolerability and safety of pediatric hyperpolarized (129)Xe magnetic resonance imaging in healthy volunteers and children with cystic fibrosis. Pediatr Radiol 46(12): 1651-1662, 2016. DOI: 10.1007/s00247-016-3672-1

28 Thomen RP, Walkup LL, Roach DJ, Higano N, Schapiro A, Brody A, Clancy JP, Cleveland ZI, Woods JC: Regional structure-function in cystic fibrosis lung disease using hyperpolarized (129)Xe and ultrashort echo magnetic resonance imaging. Am J Respir Crit Care Med 202(2): 290-292, 2020. DOI: 10.1164/rccm.202001-0031LE

29 Sethuraman KN, Smolin R, Henry S: Is there a place for hyperbaric oxygen therapy? Adv Surg 56(1): 169-204, 2022. DOI: 10.1016/j.yasu.2022.02.011

30 Hyperbaric oxygen chamber for health rehabilitation. LabsNova. Available at: https://labrotovap.com/portfolio-item/hyperbaric-oxygen-chamber-for-health-rehabilitation/?portfolioCats=691 [Last accessed on June 9, 2023]

31 Devaraj D, Srisakthi D: Hyperbaric oxygen therapy - can it be the new era in dentistry? J Clin Diagn Res 8(2): 263-265, 2014. DOI: 10.7860/JCDR/2014/7262.4077

32 Boulton AJ: The pathway to foot ulceration in diabetes. Med Clin North Am 97(5): 775-790, 2013. DOI: 10.1016/j.mcna.2013.03.007

33 Claessen H, Narres M, Haastert B, Arend W, Hoffmann F, Morbach S, Rümenapf G, Kvitkina T, Friedel H, Günster C, Schubert I, Ullrich W, Westerhoff B, Wilk A, Icks A: Lower-extremity amputations in people with and without diabetes in Germany, 2008-2012 - an analysis of more than 30 million inhabitants. Clin Epidemiol 10: 475-488, 2018. DOI: 10.2147/CLEP.S146484

34 Hicks CW, Selvin E: Epidemiology of peripheral neuropathy and lower extremity disease in diabetes. Curr Diab Rep 19(10): 86, 2019. DOI: 10.1007/s11892-019-1212-8

35 Kavitha KV, Tiwari S, Purandare VB, Khedkar S, Bhosale SS, Unnikrishnan AG: Choice of wound care in diabetic foot ulcer: A practical approach. World J Diabetes 5(4): 546-556, 2014. DOI: 10.4239/wjd.v5.i4.546

36 Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y: Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Ann Med 49(2): 106-116, 2017. DOI: 10.1080/07853890.2016.1231932

37 Choudhury R: Hypoxia and hyperbaric oxygen therapy: a review. Int J Gen Med 11: 431-442, 2018. DOI: 10.2147/IJGM.S172460

38 Thom SR: Hyperbaric oxygen: its mechanisms and efficacy. Plast Reconstr Surg 127 Suppl 1(Suppl 1): 131S-141S, 2011. DOI: 10.1097/PRS.0b013e3181fbe2bf

39 Tiaka EK, Papanas N, Manolakis AC, Maltezos E: The role of hyperbaric oxygen in the treatment of diabetic foot ulcers. Angiology 63(4): 302-314, 2012. DOI: 10.1177/0003319711416804

40 Camporesi EM: Side effects of hyperbaric oxygen therapy. Undersea Hyperb Med 41(3): 253-257, 2014.

41 Fadol EM, Suliman HM, Osman B, Abdalla SA, Osman WJ, Mohamed EM, Abdoon IH: Therapeutic outcomes evaluation of adjuvant hyperbaric oxygen therapy for non-healing diabetic foot ulcers among sudanese patients. Diabetes Metab Syndr 15(4): 102173, 2021. DOI: 10.1016/j.dsx.2021.06.010

42 Heyboer M 3rd, Sharma D, Santiago W, McCulloch N: Hyperbaric oxygen therapy: Side effects defined and quantified. Adv Wound Care (New Rochelle) 6(6): 210-224, 2017. DOI: 10.1089/wound.2016.0718

43 Everett E, Mathioudakis N: Update on management of diabetic foot ulcers. Ann N Y Acad Sci 1411(1): 153-165, 2018. DOI: 10.1111/nyas.13569

44 Health Quality Ontario: Hyperbaric oxygen therapy for the treatment of diabetic foot ulcers: A health technology assessment. Ont Health Technol Assess Ser 17(5): 1-142, 2017.

45 van Neck JW, Tuk B, Fijneman EMG, Redeker JJ, Talahatu EM, Tong M: Hyperbaric oxygen therapy for wound healing in diabetic rats: Varying efficacy after a clinically-based protocol. PLoS One 12(5): e0177766, 2017. DOI: 10.1371/journal.pone.0177766

46 Vas P, Rayman G, Dhatariya K, Driver V, Hartemann A, Londahl M, Piaggesi A, Apelqvist J, Attinger C, Game F: Effectiveness of interventions to enhance healing of chronic foot ulcers in diabetes: a systematic review. Diabetes Metab Res Rev 36(S1): e3284, 2020. DOI: 10.1002/dmrr.3284

47 Huang X, Liang P, Jiang B, Zhang P, Yu W, Duan M, Guo L, Cui X, Huang M, Huang X: Hyperbaric oxygen potentiates diabetic wound healing by promoting fibroblast cell proliferation and endothelial cell angiogenesis. Life Sci 259: 118246, 2020. DOI: 10.1016/j.lfs.2020.118246

48 Oley MH, Oley MC, Tjandra DE, Sedu SW, Sumarauw ER, Aling DMR, Kalangi JA, Islam AA, Hatta M, Faruk M: Hyperbaric oxygen therapy in the healing process of foot ulcers in diabetic type 2 patients marked by interleukin 6, vascular endothelial growth factor, and PEDIS score: A randomized controlled trial study. Int J Surg Open 27: 154-161, 2020. DOI: 10.1016/j.ijso.2020.11.012

49 Brouwer RJ, Lalieu RC, Hoencamp R, van Hulst RA, Ubbink DT: A systematic review and meta-analysis of hyperbaric oxygen therapy for diabetic foot ulcers with arterial insufficiency. J Vasc Surg 71(2): 682-692.e1, 2020. DOI: 10.1016/j.jvs.2019.07.082

50 Jenwitheesuk K, Mahakkanukrauh A, Punjaruk W, Jenwitheesuk K, Winaikosol K, Punyavong P, Wongkonkitsin N, Prasertcharoensuk S, Limrattanapimpa P: Predictors for success of hyperbaric oxygen therapy for problematic wounds. Wound Med 30: 100187, 2020. DOI: 10.1016/j.wndm.2020.100187

51 Bai Z, Wang H, Sun H, Cui L: Effect of hyperbaric oxygen therapy on the patients with venous leg ulcer: A systematic review and meta-analysis. Asian J Surg 46(10): 4131-4137, 2023. DOI: 10.1016/j.asjsur.2023.01.068

52 Bouganim N, Freiman A: History of cryotherapy. Dermatol Online J 11(2): 9, 2005. DOI: 10.5070/d34f62h9vt

53 Allan R, Malone J, Alexander J, Vorajee S, Ihsan M, Gregson W, Kwiecien S, Mawhinney C: Cold for centuries: a brief history of cryotherapies to improve health, injury and post-exercise recovery. Eur J Appl Physiol 122(5): 1153-1162, 2022. DOI: 10.1007/s00421-022-04915-5

54 Kriosan. KRYOSYSTEM. Available at: https://kryosystem.pl/en/kriosan-eng/ [Last accessed on June 9, 2023]

55 Stationary chamber. KRYOSYSTEM. Available at: https://kryosystem.pl/en/stationary-chamber/ [Last accessed on June 9, 2023]

56 Nadler SF: The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. Pain Physician 3;7(7;3): 395-399, 2004. DOI: 10.36076/ppj.2004/7/395

57 Reid G, Babes A, Pluteanu F: A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. J Physiol 545(2): 595-614, 2002. DOI: 10.1113/jphysiol.2002.024331

58 McNearney T, Baethge BA, Cao S, Alam R, Lisse JR, Westlund KN: Excitatory amino acids, TNF-alpha, and chemokine levels in synovial fluids of patients with active arthropathies. Clin Exp Immunol 137(3): 621-627, 2004. DOI: 10.1111/j.1365-2249.2004.02563.x

59 Stålman A, Berglund L, Dungnerc E, Arner P, Felländer-Tsai L: Temperature-sensitive release of prostaglandin E2 and diminished energy requirements in synovial tissue with postoperative cryotherapy. J Bone Joint Surg 93(21): 1961-1968, 2011. DOI: 10.2106/JBJS.J.01790

60 Klintberg IH, Larsson ME: Shall we use cryotherapy in the treatment in surgical procedures, in acute pain or injury, or in long term pain or dysfunction? - A systematic review. J Bodyw Mov Ther 27: 368-387, 2021. DOI: 10.1016/J.JBMT.2021.03.002

61 Shepherd JT, Rusch NJ, Vanhoutte PM: Effect of cold on the blood vessel wall. Gen Pharmacol 14(1): 61-64, 1983. DOI: 10.1016/0306-3623(83)90064-2

62 Bleakley CM, Bieuzen F, Davison GW, Costello JT: Whole-body cryotherapy: empirical evidence and theoretical perspectives. Open Access J Sports Med 5: 25-36, 2014. DOI: 10.2147/OAJSM.S41655

63 Galliera E, Dogliotti G, Melegati G, Corsi Romanelli MM, Cabitza P, Banfi G: Bone remodelling biomarkers after whole body cryotherapy (WBC) in elite rugby players. Injury 44(8): 1117-1121, 2013. DOI: 10.1016/j.injury.2012.08.057

64 Hessemer V, Langusch D, Bruck LK, Bödeker RH, Breidenbach T: Effect of slightly lowered body temperatures on endurance performance in humans. J Appl Physiol Respir Environ Exerc Physiol 57(6): 1731-1737, 1984. DOI: 10.1152/jappl.1984.57.6.1731

65 Lubkowska A, Dołęgowska B, Szyguła Z: Whole-body cryostimulation—potential beneficial treatment for improving antioxidant capacity in healthy men—significance of the number of sessions. PLoS One 7(10): e46352, 2012. DOI: 10.1371/journal.pone.0046352

66 Banfi G, Melegati G, Barassi A, d'Eril GM: Effects of the whole-body cryotherapy on NTproBNP, hsCRP and troponin I in athletes. J Sci Med Sport 12(6): 609-610, 2009. DOI: 10.1016/j.jsams.2008.06.004

67 Mila-Kierzenkowska C, Woźniak A, Woźniak B, Drewa G, Rakowski A, Jurecka A, Rajewski R: Whole-body cryostimulation in kayaker women: A study of the effect of cryogenic temperatures on oxidative stress after the exercise. J Sports Med Phys Fitness 49(2): 201-207, 2009.

68 Lombardi G, Ziemann E, Banfi G: Whole-body cryotherapy in athletes: from therapy to stimulation. An updated review of the literature. Front Physiol 8: 258, 2017. DOI: 10.3389/fphys.2017.00258

69 Slattery K, Bentley D, Coutts AJ: The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: Implications of antioxidant supplementation on physiological adaptation during intensified physical training. Sports Med 45(4): 453-471, 2015. DOI: 10.1007/S40279-014-0282-7

70 Banfi G, Melegati G, Barassi A, Dogliotti G, Melzi d'Eril G, Dugué B, Corsi MM: Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. J Therm Biol 34(2): 55-59, 2009. DOI: 10.1016/j.jtherbio.2008.10.003

71 Hodgson M, Docherty D, Robbins D: Post-activation potentiation. Sports Med 35(7): 585-595, 2005. DOI: 10.2165/00007256-200535070-00004

72 Partridge EM, Cooke J, McKune A, Pyne DB: Whole-body cryotherapy: Potential to enhance athlete preparation for competition? Front Physiol 10: 1007, 2019. DOI: 10.3389/fphys.2019.01007

73 Alaca N, Kablan N: Acute effects of cold spray application on mechanical properties of the rectus femoris muscle in athletes. J Bodyw Mov Ther 30: 100-104, 2022. DOI: 10.1016/j.jbmt.2022.02.010

74 Baud'huin M, Lamoureux F, Duplomb L, Rédini F, Heymann D: RANKL, RANK, osteoprotegerin: key partners of osteoimmunology and vascular diseases. Cell Mol Life Sci 64(18): 2334-2350, 2007. DOI: 10.1007/s00018-007-7104-0

75 Nakashima T, Takayanagi H: Osteoimmunology: Crosstalk between the immune and bone systems. J Clin Immunol 29(5): 555-567, 2009. DOI: 10.1007/s10875-009-9316-6

76 Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ: Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20(3): 345-357, 1999. DOI: 10.1210/edrv.20.3.0367

77 Takayanagi H: Osteoimmunology and the effects of the immune system on bone. Nat Rev Rheumatol 5(12): 667-676, 2009. DOI: 10.1038/nrrheum.2009.217

78 Teitelbaum SL: Bone resorption by osteoclasts. Science (1979) 289(5484): 1504-1508, 2000. DOI: 10.1126/SCIENCE.289.5484.1504

79 Russell M, Birch J, Love T, Cook CJ, Bracken RM, Taylor T, Swift E, Cockburn E, Finn C, Cunningham D, Wilson L, Kilduff LP: The effects of a single whole-body cryotherapy exposure on physiological, performance, and perceptual responses of professional academy soccer players after repeated sprint exercise. J Strength Cond Res 31(2): 415-421, 2017. DOI: 10.1519/JSC.0000000000001505

80 Strahorn J, Serpell BG, McKune A, Pumpa KL: Effect of physical and psychosocial interventions on hormone and performance outcomes in professional rugby union players: a systematic review. J Strength Cond Res 31(11): 3158-3169, 2017. DOI: 10.1519/JSC.0000000000002067

81 Christensen NJ, Galbo H: Sympathetic nervous activity during exercise. Annu Rev Physiol 45(1): 139-153, 1983. DOI: 10.1146/ANNUREV.PH.45.030183.001035

82 Chatterton RT, Vogelsong KM, Lu Y, Ellman AB, Hudgens GA: Salivary α-amylase as a measure of endogenous adrenergic activity. Clin Physiol 16(4): 433-448, 1996. DOI: 10.1111/j.1475-097X.1996.tb00731.x

83 Lim IS: Correlation between salivary alpha-amylase, anxiety, and game records in the archery competition. J Exerc Nutrition Biochem 20(4): 44-47, 2016. DOI: 10.20463/jenb.2016.0050

84 Kasahun HG, Agizew TB, Temesgen MM, Ashagrie HE: Assessment of acute postoperative pain management and

associated factors after elective surgery among adult surgical patients: a prospective cross-sectional study. IJS Short Rep 7(1): e37-e37, 2022. DOI: 10.1097/sr9.0000000000000037

85 Wyatt PB, Nelson CT, Cyrus JW, Goldman AH, Patel NK: The role of cryotherapy after total knee arthroplasty: a systematic review. J Arthroplasty 38(5): 950-956, 2023. DOI: 10.1016/j.arth.2022.12.004

86 Lizis P, Kobza W, Manko G, Jaszczur-Nowicki J, Perlinski J, Para B: Cryotherapy with mobilization *versus* cryotherapy with mobilization reinforced with home stretching exercises in treatment of chronic neck pain: a randomized trial. J Manipulative Physiol Ther 43(3): 197-205, 2020. DOI: 10.1016/j.jmpt.2018.11.030

87 Hogg-Johnson S, van der Velde G, Carroll LJ, Holm LW, Cassidy JD, Guzman J, Côté P, Haldeman S, Ammendolia C, Carragee E, Hurwitz E, Nordin M, Peloso P: The burden and determinants of neck pain in the general population. Spine 33(Supplement): S39-S51, 2008. DOI: 10.1097/BRS.0b013e31816454c8

88 Webb R, Brammah T, Lunt M, Urwin M, Allison T, Symmons D: Prevalence and predictors of intense, chronic, and disabling neck and back pain in the UK general population. Spine 28(11): 1195-1202, 2003. DOI: 10.1097/01.BRS.0000067430.49169.01

89 Ylinen J, Kautiainen H, Wirén K, Häkkinen A: Stretching exercises *vs*. manual therapy in treatment of chronic neck pain: a randomized, controlled cross-over trial. J Rehabil Med 39(2): 126-132, 2007. DOI: 10.2340/16501977-0015

90 Salas-Fraire O, Rivera-Pérez JA, Guevara-Neri NP, Urrutia-García K, Martínez-Gutiérrez OA, Salas-Longoria K, Morales-Avalos R: Efficacy of whole-body cryotherapy in the treatment of chronic low back pain: Quasi-experimental study. J Orthop Sci 28(1): 112-116, 2023. DOI: 10.1016/j.jos.2021.10.006

91 Lipke MM: An armamentarium of wart treatments. Clin Med Res 4(4): 273-293, 2006. DOI: 10.3121/cmr.4.4.273

92 Bonnez W, Oakes D, Choi A, D'Arcy SJ, Pappas PG, Corey L, Stoler MH, Demeter LM, Reichman RC: Therapeutic efficacy and complications of excisional biopsy of condyloma acuminatum. Sex Transm Dis 23(4): 273-276, 1996. DOI: 10.1097/00007435-199607000-00005

93 Singh S, Neema S: Comparison of electrosurgery by electrodessication *versus* cryotherapy by liquid nitrogen spray technique in the treatment of plantar warts. Med J Armed Forces India 76(2): 156-160, 2020. DOI: 10.1016/j.mjafi.2018.11.005

94 Benton EC: Therapy of cutaneous warts. Clin Dermatol 15(3): 449-455, 1997. DOI: 10.1016/S0738-081X(96)00153-8

95 Awad SM, Gomaa AS, Hassan HA, Tawfik YM: Efficacy of cryotherapy combined with intralesional purified protein derivative (PPD) *versus* intralesional PPD monotherapy in the treatment of multiple common warts. J Cutan Med Surg 27(2): 117-125, 2023. DOI: 10.1177/12034754231152224

96 Mental Health. Our World in Data. Available at: https://ourworldindata.org/mental-health [Last accessed on June 9, 2023]

97 Doets JJ, Topper M, Nugter AM: A systematic review and meta-analysis of the effect of whole body cryotherapy on mental health problems. Complement Ther Med 63: 102783, 2021. DOI: 10.1016/J.CTIM.2021.102783

98 Holmes C, Amin J: Dementia. Medicine (United Kingdom) 48(11): 742-745, 2020. DOI: 10.1016/j.mpmed.2020.08.014

99 Sanford AM: Mild cognitive impairment. Clin Geriatr Med 33(3): 325-337, 2017. DOI: 10.1016/j.cger.2017.02.005

100 Misiak B, Kiejna A: Translating whole-body cryotherapy into geriatric psychiatry – A proposed strategy for the prevention of Alzheimer's disease. Med Hypotheses 79(1): 56-58, 2012. DOI: 10.1016/J.MEHY.2012.03.033

101 Stanek A, Cholewka A, Wielkoszyński T, Romuk E, Sieroń A: Decreased oxidative stress in male patients with active phase ankylosing spondylitis who underwent whole-body cryotherapy in closed cryochamber. Oxid Med Cell Longev 2018: 7365490, 2018. DOI: 10.1155/2018/7365490

102 Bouzigon R, Grappe F, Ravier G, Dugue B: Whole- and partial-body cryostimulation/cryotherapy: Current technologies and practical applications. J Therm Biol 61: 67-81, 2016. DOI: 10.1016/j.jtherbio.2016.08.009

103 Kim JY, Kim DH, Kim JH, Lee D, Jeon HB, Kwon SJ, Kim SM, Yoo YJ, Lee EH, Choi SJ, Seo SW, Lee JI, Na DL, Yang YS, Oh W, Chang JW: Soluble intracellular adhesion molecule-1 secreted by human umbilical cord blood-derived mesenchymal stem cell reduces amyloid-β plaques. Cell Death Differ 19(4): 680-691, 2012. DOI: 10.1038/cdd.2011.140

104 Brosseron F, Krauthausen M, Kummer M, Heneka MT: Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. Mol Neurobiol 50(2): 534-544, 2014. DOI: 10.1007/s12035-014-8657-1

105 Faria MC, Gonçalves GS, Rocha NP, Moraes EN, Bicalho MA, Gualberto Cintra MT, Jardim de Paula J, José Ravic de Miranda LF, Clayton de Souza Ferreira A, Teixeira AL, Gomes KB, Carvalho MDG, Sousa LP: Increased plasma levels of BDNF and inflammatory markers in Alzheimer's disease. J Psychiatr Res 53: 166-172, 2014. DOI: 10.1016/j.jpsychires.2014.01.019

106 Janoutová J, Šerý O, Hosák L, Janout V: Is mild cognitive impairment a precursor of Alzheimer's disease? Short Review. Cent Eur J Public Health 23(4): 365-367, 2015. DOI: 10.21101/cejph.a4414

107 Magaki S, Mueller C, Dickson C, Kirsch W: Increased production of inflammatory cytokines in mild cognitive impairment. Exp Gerontol 42(3): 233-240, 2007. DOI: 10.1016/j.exger.2006.09.015

108 Saleem M, Herrmann N, Swardfager W, Eisen R, Lanctot KL: Inflammatory markers in mild cognitive impairment: A meta-analysis. J Alzheimers Dis 47(3): 669-679, 2015. DOI: 10.3233/JAD-150042

109 Rymaszewska J, Lion KM, Stańczykiewicz B, Rymaszewska JE, Trypka E, Pawlik-Sobecka L, Kokot I, Płaczkowska S, Zabłocka A, Szcześniak D: The improvement of cognitive deficits after whole-body cryotherapy – A randomised controlled trial. Exp Gerontol 146: 111237, 2021. DOI: 10.1016/J.EXGER.2021.111237

110 Rymaszewska JE, Stańczykiewicz B, Lion K, Misiak B: The impact of whole-body cryotherapy on lipid profile: A systematic review and meta-analysis. Complement Ther Med 55: 102568, 2020. DOI: 10.1016/J.CTIM.2020.102568

111 Cooper IS, Lee ASJ: Cryostatic congelation: A system for producing a limited, controlled region of cooling or freezing of biologic tissues. JNMD 133(3): 259-263, 1961. DOI: 10.1097/00005053-196109000-00013

112 Ullrick SR, Hebert JJ, Davis KW: Cryoablation in the musculoskeletal system. Curr Probl Diagn Radiol 37(1): 39-48, 2008. DOI: 10.1067/j.cpradiol.2007.05.001

113 Mohammed A, Miller S, Douglas-Moore J, Miller M: Cryotherapy and its applications in the management of urologic malignancies: A review of its use in prostate and renal cancers. Urol Oncol 32(1): 39.e19-39.e27, 2014. DOI: 10.1016/j.urolonc.2013.04.004

114 Yiu WK, Basco MT, Aruny JE, Cheng SW, Sumpio BE: Cryosurgery: A review. Int J Angiol 16(1): 1-6, 2007. DOI: 10.1055/s-0031-1278235

115 Niu L, Mu F, Zhang C, Li Y, Liu W, Jiang F, Li L, Liu C, Zeng J, Yao F, Chen J, Li J, Zuo J, Xu K: Cryotherapy protocols for metastatic breast cancer after failure of radical surgery. Cryobiology 67(1): 17-22, 2013. DOI: 10.1016/j.cryobiol.2013.04.004

116 Kaufman CS, Rewcastle JC: Cryosurgery for breast cancer. Technol Cancer Res Treat 3(2): 165-175, 2004. DOI: 10.1177/153303460400300209

117 Tarkowski R, Rzaca M: Cryosurgery in the treatment of women with breast cancer-a review. Gland Surg 3(2): 88-93, 2014. DOI: 10.3978/j.issn.2227-684X.2014.03.04

118 Olagunju A, Forsman T, Ward RC: An update on the use of cryoablation and immunotherapy for breast cancer. Front Immunol 13: 1026475, 2022. DOI: 10.3389/fimmu.2022.1026475

119 van de Voort EM, Struik GM, Birnie E, Moelker A, Verhoef C, Klem TM: Thermal ablation as an alternative for surgical resection of small (≤2 cm) breast cancers: a meta-analysis. Clin Breast Cancer 21(6): e715-e730, 2021. DOI: 10.1016/j.clbc.2021.03.004

120 Fine RE, Gilmore RC, Dietz JR, Boolbol SK, Berry MP, Han LK, Kenler AS, Sabel M, Tomkovich KR, Vanderwalde NA, Chen M, Columbus KS, Curcio LD, Feldman SM, Gold L, Hernandez L, Manahan ER, Seedman SA, Vaidya RP, Sevrukov AB, Aoun HD, Hicks RD, Simmons RM: Cryoablation without excision for low-risk early-stage breast cancer: 3-year interim analysis of ipsilateral breast tumor recurrence in the ICE3 trial. Ann Surg Oncol 28(10): 5525-5534, 2021. DOI: 10.1245/s10434-021-10501-4

121 Rabin Y, Julian TB, Olson P, Taylor MJ, Wolmark N: Long-term follow-up post-cryosurgery in a sheep breast model. Cryobiology 39(1): 29-46, 1999. DOI: 10.1006/CRYO.1999.2183

122 Roubidoux MA, Sabel MS, Bailey JE, Kleer CG, Klein KA, Helvie MA: Small (<2.0-cm) breast cancers: Mammographic and US findings at US-guided cryoablation—initial experience. Radiology 233(3): 857-867, 2004. DOI: 10.1148/RADIOL.2333031734

123 Habrawi Z, Melkus MW, Khan S, Henderson J, Brandi L, Chu V, Layeequr Rahman R: Cryoablation: A promising non-operative therapy for low-risk breast cancer. Am J Surg 221(1): 127-133, 2021. DOI: 10.1016/j.amjsurg.2020.07.028

124 Sabel MS, Kaufman CS, Whitworth P, Chang H, Stocks LH, Simmons R, Schultz M: Cryoablation of early-stage breast cancer: Work-in-progress report of a multi-institutional trial. Ann Surg Oncol 11(5): 542-549, 2004. DOI: 10.1245/ASO.2004.08.003

125 Simmons RM, Ballman KV, Cox C, Carp N, Sabol J, Hwang RF, Attai D, Sabel M, Nathanson D, Kenler A, Gold L, Kaufman C, Han L, Bleznak A, Stanley Smith J, Holmes D, Fornage B, Le-Petross C, Hoda S, McCall L, Hunt KK, ACOSOG investigators: A Phase II trial exploring the success of cryoablation therapy in the treatment of invasive breast carcinoma: results from ACOSOG (Alliance) Z1072. Ann Surg Oncol 23(8): 2438-2445, 2016. DOI: 10.1245/s10434-016-5275-3

126 Adachi T, Machida Y, Fukuma E, Tateishi U: Fluorodeoxyglucose positron emission tomography/computed tomography findings after percutaneous cryoablation of early breast cancer. Cancer Imaging 20(1): 49, 2020. DOI: 10.1186/s40644-020-00325-y

127 Kwong A, Co M, Fukuma E: Prospective clinical trial on expanding indications for cryosurgery for early breast cancers. Clin Breast Cancer 23(4): 363-368, 2023. DOI: 10.1016/j.clbc.2023.01.007

128 Littrup PJ, Jallad B, Chandiwala-Mody P, D'Agostini M, Adam BA, Bouwman D: Cryotherapy for breast cancer: a feasibility study without excision. J Vasc Interv Radiol 20(10): 1329-1341, 2009. DOI: 10.1016/j.jvir.2009.06.029

129 Machida Y, Shimauchi A, Igarashi T, Fukuma E: MRI findings after cryoablation of primary breast cancer without surgical resection. Acad Radiol 26(6): 744-751, 2019. DOI: 10.1016/J.ACRA.2018.07.012

130 Pfleiderer SO, Freesmeyer MG, Marx C, Kühne-Heid R, Schneider A, Kaiser WA: Cryotherapy of breast cancer under ultrasound guidance: initial results and limitations. Eur Radiol 12(12): 3009-3014, 2002. DOI: 10.1007/s00330-002-1511-2

131 Pfleiderer SOR, Marx C, Camara O, Gajda M, Kaiser WA: Ultrasound-guided, percutaneous cryotherapy of small (≤15 mm) breast cancers. Invest Radiol 40(7): 472-477, 2005. DOI: 10.1097/01.rli.0000166935.56971.ff

132 Manenti G, Scarano AL, Pistolese CA, Perretta T, Bonanno E, Orlandi A, Simonetti G: Subclinical breast cancer: Minimally invasive approaches. Our experience with percutaneous radiofrequency ablation *vs*. cryotherapy. Breast Care (Basel) 8(5): 356-360, 2013. DOI: 10.1159/000355707

133 Patrick J, Khan SA: Surgical management of *de novo* stage IV breast cancer. JNCCN 13(4): 487-493, 2015. DOI: 10.6004/jnccn.2015.0062

134 Pusceddu C, Sotgia B, Amucano G, Fele RM, Pilleri S, Meloni GB, Melis L: Breast cryoablation in patients with bone metastatic breast cancer. J Vasc Intervent Radiol 25(8): 1225-1232, 2014. DOI: 10.1016/J.JVIR.2014.05.001

135 Pusceddu C, Melis L, Ballicu N, Meloni P, Sanna V, Porcu A, Fancellu A: Cryoablation of primary breast cancer in patients with metastatic disease: Considerations arising from a single-centre data analysis. Biomed Res Int 2017: 3839012, 2017. DOI: 10.1155/2017/3839012

136 McArthur HL, Diab A, Page DB, Yuan J, Solomon SB, Sacchini V, Comstock C, Durack JC, Maybody M, Sung J, Ginsberg A, Wong P, Barlas A, Dong Z, Zhao C, Blum B, Patil S, Neville D, Comen EA, Morris EA, Kotin A, Brogi E, Wen YH, Morrow M, Lacouture ME, Sharma P, Allison JP, Hudis CA, Wolchok JD, Norton L: A pilot study of preoperative single-dose ipilimumab and/or cryoablation in women with early-stage breast cancer with comprehensive immune profiling. Clin Cancer Res 22(23): 5729-5737, 2016. DOI: 10.1158/1078-0432.CCR-16-0190

137 Page DB, Yuan J, Redmond D, Wen YH, Durack JC, Emerson R, Solomon S, Dong Z, Wong P, Comstock C, Diab A, Sung J, Maybody M, Morris E, Brogi E, Morrow M, Sacchini V, Elemento O, Robins H, Patil S, Allison JP, Wolchok JD, Hudis C, Norton L, McArthur HL: Deep sequencing of T-cell receptor

DNA as a biomarker of clonally expanded TILs in breast cancer after immunotherapy. Cancer Immunol Res 4(10): 835-844, 2016. DOI: 10.1158/2326-6066.CIR-16-0013

138 Comen E: A study of pre-operative treatment with cryoablation and immune therapy in early stage breast cancer. Identifier NCT02833233. Available at: https://clinicaltrials.gov/ct2/show/NCT02833233 [Last accessed on June 9, 2023]

139 Branski LK, Rennekampff HO, Vogt PM: [Keloid and hypertrophic scar treatment modalities: An update.] Chirurg 83(9): 831-846, 2012. DOI: 10.1007/S00104-011-2243-0/FIGURES/9

140 Bischof M, Krempien R, Debus J, Treiber M: Postoperative electron beam radiotherapy for keloids: objective findings and patient satisfaction in self-assessment. Int J Dermatol 46(9): 971-975, 2007. DOI: 10.1111/j.1365-4632.2007.03326.x

141 Bock O, Schmid-Ott G, Malewski P, Mrowietz U: Quality of life of patients with keloid and hypertrophic scarring. Arch Dermatol Res 297(10): 433-438, 2006. DOI: 10.1007/s00403-006-0651-7

142 Mustoe TA, Cooter RD, Gold MH, Richard Hobbs FD, Ramelet A, Shakespeare PG, Stella M, Téot L, Wood FM, Ziegler UE: International clinical recommendations on scar management. Plast Reconstr Surg 110(2): 560-571, 2002. DOI: 10.1097/00006534-200208000-00031

143 Niessen FB, Spauwen PHM, Schalkwijk J, Kon M: On the nature of hypertrophic scars and keloids: a review. Plast Reconstr Surg 104(5): 1435-1458, 1999. DOI: 10.1097/00006534-199910000-00031

144 Bellew SG, Weiss MA, Weiss RA: Comparison of intense pulsed light to 595-nm long-pulsed pulsed dye laser for treatment of hypertrophic surgical scars: A pilot study. J Drugs Dermatol 4(4): 448-452, 2005.

145 Ogawa R, Mitsuhashi K, Hyakusoku H, Miyashita T: Postoperative electron-beam irradiation therapy for keloids and hypertrophic scars: retrospective study of 147 cases followed for more than 18 months. Plast Reconstr Surg 111(2): 547-553, 2003. DOI: 10.1097/01.PRS.0000040466.55214.35

146 Robles DT, Berg D: Abnormal wound healing: keloids. Clin Dermatol 25(1): 26-32, 2007. DOI: 10.1016/J.CLINDERMATOL.2006.09.009

147 Uchida G, Yoshimura K, Kitano Y, Okazaki M, Harii K: Tretinoin reverses upregulation of matrix metalloproteinase-13 in human keloid-derived fibroblasts. Exp Dermatol 12(s2): 35-42, 2003. DOI: 10.1034/j.1600-0625.12.s2.6.x

148 Wolfram D, Tzankov A, Pülzl P, Piza-Katzer H: Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management. Dermatol Surg 35(2): 171-181, 2009. DOI: 10.1111/j.1524-4725.2008.34406.x

149 Zouboulis CC: Principles of cutaneous cryosurgery: an update. Dermatology 198(2): 111-117, 1999. DOI: 10.1159/000018084

150 Zouboulis CC, Blume U, Büttner P, Orfanos CE: Outcomes of cryosurgery in keloids and hypertrophic scars. Arch Dermatol 129(9): 1146, 1993. DOI: 10.1001/archderm.1993.01680300074011

151 Weshahy AH: Intralesional cryosurgery a new technique using cryoneedles. J Dermatol Surg Oncol 19(2): 123-126, 1993. DOI: 10.1111/J.1524-4725.1993.TB03440.X

152 Bloemen MC, van der Veer WM, Ulrich MM, van Zuijlen PP, Niessen FB, Middelkoop E: Prevention and curative management of hypertrophic scar formation. Burns 35(4): 463-475, 2009. DOI: 10.1016/j.burns.2008.07.016

153 van Leeuwen MC, Bulstra AEJ, van der Veen A, Bloem W, van Leeuwen P, Niessen F: Comparison of two devices for the treatment of keloid scars with the use of intralesional cryotherapy: An experimental study. Cryobiology 71(1): 146-150, 2015. DOI: 10.1016/j.cryobiol.2015.04.004

154 van Leeuwen MC, Bulstra AJ, Van Leeuwen PA, Niessen FB: A new argon gas-based device for the treatment of keloid scars with the use of intralesional cryotherapy. JPRAS 67(12): 1703-1710, 2014. DOI: 10.1016/j.bjps.2014.08.046

155 Meymandi SS, Moosazadeh M, Rezazadeh A: Comparing two methods of cryotherapy and intense pulsed light with triamcinolone injection in the treatment of keloid and hypertrophic scars: a clinical trial. Osong Public Health Res Perspect 7(5): 313-319, 2016. DOI: 10.1016/j.phrp.2016.08.005

156 Klatte T, Shariat SF, Remzi M: Systematic review and meta-analysis of perioperative and oncologic outcomes of laparoscopic cryoablation *versus* laparoscopic partial nephrectomy for the treatment of small renal tumors. J Urol 191(5): 1209-1217, 2014. DOI: 10.1016/J.JURO.2013.11.006

157 Lin Y, Turna B, Frota R, Aron M, Haber GP, Kamoi K, Koenig P, Gill IS: Laparoscopic partial nephrectomy *versus* laparoscopic cryoablation for multiple ipsilateral renal tumors. Eur Urol 53(6): 1210-1218, 2008. DOI: 10.1016/J.EURURO.2008.02.052

158 Sisul DM, Liss MA, Palazzi KL, Briles K, Mehrazin R, Gold RE, Masterson JH, Mirheydar HS, Jabaji R, Stroup SP, L'esperance JO, Wake RW, Rivera-sanfeliz G, Derweesh IH: RENAL nephrometry score is associated with complications after renal cryoablation: a multicenter analysis. Urology 81(4): 775-780, 2013. DOI: 10.1016/J.UROLOGY.2012.11.037

159 Thompson RH, Atwell T, Schmit G, Lohse CM, Kurup AN, Weisbrod A, Psutka SP, Stewart SB, Callstrom MR, Cheville JC, Boorjian SA, Leibovich BC: Comparison of partial nephrectomy and percutaneous ablation for cT1 renal masses. Eur Urol 67(2): 252-259, 2015. DOI: 10.1016/J.EURURO.2014.07.021

160 Zargar H, Samarasekera D, Khalifeh A, Remer EM, O'Malley C, Akca O, Autorino R, Kaouk JH: Laparoscopic *vs*. percutaneous cryoablation for the small renal mass: 15-year experience at a single center. Urology 85(4): 850-855, 2015. DOI: 10.1016/J.UROLOGY.2015.01.004

161 Yang B, Autorino R, Remer EM, Laydner HK, Hillyer S, Altunrende F, White MA, Khanna R, Stein RJ, Haber G, O'malley CM, Kaouk JH: Probe ablation as salvage therapy for renal tumors in von Hippel-Lindau patients: The Cleveland Clinic experience with 3 years follow-up. Urol Oncol 31(5): 686-692, 2013. DOI: 10.1016/J.UROLONC.2011.05.008

162 Welch BT, Callstrom MR, Morris JM, Kurup AN, Schmit GD, Weisbrod AJ, Lohse CM, Kohli M, Costello BA, Olivier KR, Thompson RH, Boorjian SA, Atwell TD: Feasibility and oncologic control after percutaneous image guided ablation of metastatic renal cell carcinoma. J Urol 192(2): 357-363, 2014. DOI: 10.1016/J.JURO.2014.03.006

163 Johnson DB, Solomon SB, Su L, Matsumoto ED, Kavoussi LR, Nakada SY, Moon TD, Shingleton WB, Cadeddu JA: Defining the complications of cryoablation and radio frequency ablation of small renal tumors: a multi-institutional review. J Urol 172(3): 874-877, 2004. DOI: 10.1097/01.JU.0000135833.67906.EC

164 Rodriguez Faba O, Akdogan B, Marszalek M, Langenhuijsen J, Brookman-May S, Stewart GD, Capitanio U, Sanguedolce F:

Current status of focal cryoablation for small renal masses. Urology 90: 9-15, 2016. DOI: 10.1016/J.UROLOGY.2015.11.041
165 Carvalhal EF, Gill IS, Meraney AM, Desai MM, Schweizer DK, Sung GT: Laparoscopic renal cryoablation: impact on renal function and blood pressure. Urology 58(3): 357-361, 2001. DOI: 10.1016/S0090-4295(01)01220-1
166 Tang K, Yao W, Li H, Guo X, Guan W, Ma X, Zhang X, Zeng G, He W, Xu H, Ye Z: Laparoscopic renal cryoablation *versus* laparoscopic partial nephrectomy for the treatment of small renal masses: a systematic review and meta-analysis of comparative studies. J Laparoendosc Adv Surg Tech 24(6): 403-410, 2014. DOI: 10.1089/LAP.2013.0550
167 Rodríguez SA, Arias Fúnez F, Bueno Bravo C, Rodríguez-Patrón Rodríguez R, Sanz Mayayo E, Palacios VH, Burgos Revilla FJ: Cryotherapy for primary treatment of prostate cancer: intermediate term results of a prospective study from a single institution. Prostate Cancer 2014: 571576, 2014. DOI: 10.1155/2014/571576
168 Fushimi N, Jinno H, Washida H, Ueda K, Otaguro K: Humoral immunity following double-freezing of the prostate in patients with prostatic cancer. Cryobiology 19(3): 242-246, 1982. DOI: 10.1016/0011-2240(82)90150-X
169 Jiang J, Goel R, Schmechel S, Vercellotti G, Forster C, Bischof J: Pre-conditioning cryosurgery: cellular and molecular mechanisms and dynamics of TNF-α enhanced cryotherapy in an *in vivo* prostate cancer model system. Cryobiology 61(3): 280-288, 2010. DOI: 10.1016/j.cryobiol.2010.09.006
170 Chin YF, Lynn N: Systematic review of focal and salvage cryotherapy for prostate cancer. Cureus 14(6): e26400, 2022. DOI: 10.7759/cureus.26400
171 Jones JS, Rewcastle JC, Donnelly BJ, Lugnani FM, Pisters LL, Katz AE: Whole gland primary prostate cryoablation: Initial results from the Cryo On-Line Data Registry. J Urol 180(2): 554-558, 2008. DOI: 10.1016/J.JURO.2008.04.027
172 Long JP, Bahn D, Lee F, Shinohara K, Chinn DO, Macaluso JN: Five-year retrospective, multi-institutional pooled analysis of cancer-related outcomes after cryosurgical ablation of the prostate. Urology 57(3): 518-523, 2001. DOI: 10.1016/S0090-4295(00)01060-8
173 Cohen JK, Miller RJ, Ahmed S, Lotz MJ, Baust J: Ten-year biochemical disease control for patients with prostate cancer treated with cryosurgery as primary therapy. Urology 71(3): 515-518, 2008. DOI: 10.1016/J.UROLOGY.2007.09.059
174 Levy DA, Pisters LL, Jones JS: Primary cryoablation nadir prostate specific antigen and biochemical failure. J Urol 182(3): 931-937, 2009. DOI: 10.1016/J.JURO.2009.05.041
175 Donnelly BJ, Saliken JC, Ernst DS, Ali-Ridha N, Brasher PMA, Robinson JW, Rewcastle JC: Prospective trial of cryosurgical ablation of the prostate: five-year results. Urology 60(4): 645-649, 2002. DOI: 10.1016/S0090-4295(02)01839-3
176 Agarwal PK, Sadetsky N, Konety BR, Resnick MI, Carroll PR, Cancer of the Prostate Strategic Urological Research Endeavor (CaPSURE) Investigators: Treatment failure after primary and salvage therapy for prostate cancer. Cancer 112(2): 307-314, 2008. DOI: 10.1002/CNCR.23161
177 Ghafar MA, Johnson CW, De La Taille A, Benson MC, Bagiella E, Fatal M, Olsson CA, Katz AE: Salvage cryotherapy using an argon based system for locally recurrent prostate cancer after radiation therapy: The Columbia experience. J Urol 166(4): 1333-1338, 2001. DOI: 10.1016/S0022-5347(05)65763-1
178 Williams AK, Martínez CH, Lu C, Ng CK, Pautler SE, Chin JL: Disease-free survival following salvage cryotherapy for biopsy-proven radio-recurrent prostate cancer. Eur Urol 60(3): 405-410, 2011. DOI: 10.1016/J.EURURO.2010.12.012
179 Bahn D, De Castro Abreu AL, Gill IS, Hung AJ, Silverman P, Gross ME, Lieskovsky G, Ukimura O: Focal cryotherapy for clinically unilateral, low-intermediate risk prostate cancer in 73 men with a median follow-up of 3.7 years. Eur Urol 62(1): 55-63, 2012. DOI: 10.1016/J.EURURO.2012.03.006
180 Gosain S, Mercer K, Twaddell WS, Uradomo L, Greenwald BD: Liquid nitrogen spray cryotherapy in Barrett's esophagus with high-grade dysplasia: long-term results. Gastrointest Endosc 78(2): 260-265, 2013. DOI: 10.1016/j.gie.2013.03.002
181 Killcoyne S, Fitzgerald RC: Evolution and progression of Barrett's oesophagus to oesophageal cancer. Nat Rev Cancer 21(11): 731-741, 2021. DOI: 10.1038/s41568-021-00400-x
182 Muguruma N, Marcon NE: Technique and emerging role of cryotherapy. Tech Gastrointest Endosc 12(1): 44-48, 2010. DOI: 10.1016/j.tgie.2010.02.002
183 Johnston MH, Eastone JA, Horwhat J, Cartledge J, Mathews JS, Foggy JR: Cryoablation of Barrett's esophagus: a pilot study. Gastrointest Endosc 62(6): 842-848, 2005. DOI: 10.1016/J.GIE.2005.05.008
184 Dumot JA, Vargo JJ, Falk GW, Frey L, Lopez R, Rice TW: An open-label, prospective trial of cryospray ablation for Barrett's esophagus high-grade dysplasia and early esophageal cancer in high-risk patients. Gastrointest Endosc 70(4): 635-644, 2009. DOI: 10.1016/J.GIE.2009.02.006
185 Greenwald BD, Dumot JA, Abrams JA, Lightdale CJ, David DS, Nishioka NS, Yachimski P, Johnston MH, Shaheen NJ, Zfass AM, Smith JO, Gill KR, Burdick JS, Mallat D, Wolfsen HC: Endoscopic spray cryotherapy for esophageal cancer: safety and efficacy. Gastrointest Endosc 71(4): 686-693, 2010. DOI: 10.1016/j.gie.2010.01.042
186 Shaheen NJ, Greenwald BD, Peery AF, Dumot JA, Nishioka NS, Wolfsen HC, Burdick JS, Abrams JA, Wang KK, Mallat D, Johnston MH, Zfass AM, Smith JO, Barthel JS, Lightdale CJ: Safety and efficacy of endoscopic spray cryotherapy for Barrett's esophagus with high-grade dysplasia. Gastrointest Endosc 71(4): 680-685, 2010. DOI: 10.1016/j.gie.2010.01.018
187 Canto MI, Gorospe EC, Shin EJ, Dunbar KB, Montgomery EA, Okolo P: Carbon dioxide (CO2) cryotherapy is a safe and effective treatment of Barrett's esophagus (BE) with HGD/intramucosal carcinoma. Gastrointest Endosc 69(5): AB341, 2009. DOI: 10.1016/j.gie.2009.03.994
188 Sharma NR, Perisetti A, Leibowitz RM, Sehmbhi M, Park E, Malik ZA, Mushtaq KR, Zelt CM, Talabiska NJ, Klein J, Hogan CT, Smith MS: Liquid nitrogen spray cryotherapy in the esophagus is performed with minimal bleeding risk regardless of concurrent antithrombotic therapy. Gastrointest Endosc 95(6): AB373-AB374, 2022. DOI: 10.1016/j.gie.2022.04.950
189 Jahromi BM, Prevallet A, Orendain N, Tsai F, Coyle WJ: Long-term outcomes for cryotherapy of barrett's esophagus. Gastrointest Endosc 95(6): AB383-AB384, 2022. DOI: 10.1016/j.gie.2022.04.963
190 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns

in GLOBOCAN 2012. Int J Cancer 136(5): E359-E386, 2015. DOI: 10.1002/IJC.29210

191 Ioannou GN, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP: Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol 5(8): 938-945.e4, 2007. DOI: 10.1016/J.CGH.2007.02.039

192 Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J, Schirmacher P, Vilgrain V: EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 69(1): 182-236, 2018. DOI: 10.1016/J.JHEP.2018.03.019

193 Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK: Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 68(2): 723-750, 2018. DOI: 10.1002/HEP.29913

194 Bruix J, Chan SL, Galle PR, Rimassa L, Sangro B: Systemic treatment of hepatocellular carcinoma: An EASL position paper. J Hepatol 75(4): 960-974, 2021. DOI: 10.1016/J.JHEP.2021.07.004

195 Chen LT, Martinelli E, Cheng AL, Pentheroudakis G, Qin S, Bhattacharyya GS, Ikeda M, Lim HY, Ho GF, Choo SP, Ren Z, Malhotra H, Ueno M, Ryoo BY, Kiang TC, Tai D, Vogel A, Cervantes A, Lu SN, Yen CJ, Huang YH, Chen SC, Hsu C, Shen YC, Tabernero J, Yen Y, Hsu CH, Yoshino T, Douillard JY: Pan-Asian adapted ESMO Clinical Practice Guidelines for the management of patients with intermediate and advanced/relapsed hepatocellular carcinoma: a TOS-ESMO initiative endorsed by CSCO, ISMPO, JSMO, KSMO, MOS and SSO. Ann Oncol 31(3): 334-351, 2020. DOI: 10.1016/J.ANNONC.2019.12.001

196 Kudo M, Kawamura Y, Hasegawa K, Tateishi R, Kariyama K, Shiina S, Toyoda H, Imai Y, Hiraoka A, Ikeda M, Izumi N, Moriguchi M, Ogasawara S, Minami Y, Ueshima K, Murakami T, Miyayama S, Nakashima O, Yano H, Sakamoto M, Hatano E, Shimada M, Kokudo N, Mochida S, Takehara T: Management of hepatocellular carcinoma in Japan: JSH Consensus Statements and Recommendations 2021 update. Liver Cancer 10(3): 181-223, 2021. DOI: 10.1159/000514174

197 Lang H, Sotiropoulos GC, Dömland M, Frühauf NR, Paul A, Hüsing J, Malagó M, Broelsch CE: Liver resection for hepatocellular carcinoma in non-cirrhotic liver without underlying viral hepatitis. Br J Surg 92(2): 198-202, 2005. DOI: 10.1002/BJS.4763

198 Viganò L, Conci S, Cescon M, Fava C, Capelli P, D'Errico A, Torzilli G, Di Tommaso L, Giuliante F, Vecchio FM, Salizzoni M, David E, Pinna AD, Guglielmi A, Capussotti L: Liver resection for hepatocellular carcinoma in patients with metabolic syndrome: A multicenter matched analysis with HCV-related HCC. J Hepatol 63(1): 93-101, 2015. DOI: 10.1016/J.JHEP.2015.01.024

199 Vogel A, Martinelli E, ESMO Guidelines Committee: Updated treatment recommendations for hepatocellular carcinoma (HCC) from the ESMO Clinical Practice Guidelines. Ann Oncol 32(6): 801-805, 2021. DOI: 10.1016/j.annonc.2021.02.014

200 Fong Y: Surgical therapy of hepatic colorectal metastasis. CA Cancer J Clin 49(4): 231-255, 1999. DOI: 10.3322/CANJCLIN.49.4.231

201 Littrup PJ, Jallad B, Vorugu V, Littrup G, Currier B, George M, Herring D: Lethal isotherms of cryoablation in a phantom study: effects of heat load, probe size, and number. J Vasc Interv Radiol 20(10): 1343-1351, 2009. DOI: 10.1016/j.jvir.2009.05.038

202 Shock SA, Meredith K, Warner TF, Sampson LA, Wright AS, Winter TC 3rd, Mahvi DM, Fine JP, Lee FT Jr: Microwave ablation with loop antenna: *in vivo* porcine liver model. Radiology 231(1): 143-149, 2004. DOI: 10.1148/RADIOL.2311021342

203 Simon CJ, Dupuy DE, Mayo-Smith WW: Microwave ablation: Principles and applications. RadioGraphics 25(suppl_1): S69-S83, 2005. DOI: 10.1148/rg.25si055501

204 Wright AS, Lee FT, Mahvi DM: Hepatic microwave ablation with multiple antennae results in synergistically larger zones of coagulation necrosis. Springer 10(3): 275-283, 2003. DOI: 10.1245/ASO.2003.03.045

205 Orsi F, Varano G: Minimal invasive treatments for liver malignancies. Ultrason Sonochem 27: 659-667, 2015. DOI: 10.1016/j.ultsonch.2015.05.030

206 Niu L, Li J, Zeng J, Zhou L, Wang S, Zhou X, Sheng L, Chen J, Xu K: Comparison of percutaneous cryoablation with microwave ablation in a porcine liver model. Cryobiology 68(2): 194-199, 2014. DOI: 10.1016/j.cryobiol.2014.01.005

207 Adam R: A comparison of percutaneous cryosurgery and percutaneous radiofrequency for unresectable hepatic malignancies. Arch Surg 137(12): 1332, 2002. DOI: 10.1001/ARCHSURG.137.12.1332

208 Li Z, Zhang C, Lou C, Yan F, Mao Y, Hong X, Zhang Y: Comparison of percutaneous cryosurgery and surgical resection for the treatment of small hepatocellular carcinoma. Oncol Lett 6(1): 239-245, 2013. DOI: 10.3892/ol.2013.1314

209 Wu B, Xiao YY, Zhang X, Zhang AL, Li HJ, Gao DF: Magnetic resonance imaging-guided percutaneous cryoablation of hepatocellular carcinoma in special regions. Hepatobiliary Pancreat Dis Int 9(4): 384-392, 2010.

210 Xu KC, Niu LZ, He WB, Guo ZQ, Hu YZ, Zuo JS: Percutaneous cryoablation in combination with ethanol injection for unresectable hepatocellular carcinoma. World J Gastroenterol 9(12): 2686-2689, 2003. DOI: 10.3748/wjg.v9.i12.2686

211 Yang Y, Wang C, Lu Y, Bai W, An L, Qu J, Gao X, Chen Y, Zhou L, Wu Y, Feng Y, Zhang M, Chang X, Lv J: Outcomes of ultrasound-guided percutaneous argon-helium cryoablation of hepatocellular carcinoma. J Hepatobiliary Pancreat Sci 19(6): 674-684, 2012. DOI: 10.1007/s00534-011-0490-6

212 Kerkar S, Carlin AM, Sohn RL, Steffes C, Tyburski J, Littrup P, Weaver D: Long-term follow up and prognostic factors for cryotherapy of malignant liver tumors. Surgery 136(4): 770-779, 2004. DOI: 10.1016/J.SURG.2004.07.001

213 Knab LM, Salem R, Mahvi DM: Minimally invasive therapies for hepatic malignancy. Curr Probl Surg 50(4): 146-179, 2013. DOI: 10.1067/j.cpsurg.2013.01.001

214 Orlacchio A, Bazzocchi G, Pastorelli D, Bolacchi F, Angelico M, Almerighi C, Masala S, Simonetti G: Percutaneous cryoablation of small hepatocellular carcinoma with US guidance and CT monitoring: initial experience. Cardiovasc Intervent Radiol 31(3): 587-594, 2008. DOI: 10.1007/s00270-008-9293-9

215 Shimizu T, Sakuhara Y, Abo D, Hasegawa Y, Kodama Y, Endo H, Shirato H, Miyasaka K: Outcome of MR-guided percutaneous cryoablation for hepatocellular carcinoma. J Hepatobiliary Pancreat Surg 16(6): 816-823, 2009. DOI: 10.1007/S00534-009-0124-4

216 Zhou X, Tang Z, Yu Y, Weng J, Ma Z, Zhang B, Zheng Y: The role of cryosurgery in the treatment of hepatic cancer: a report of 113 cases. J Cancer Res Clin Oncol 120(1-2): 100-102, 1993. DOI: 10.1007/BF01200732
217 Goering JD, Mahvi DM, Niederhuber JE, Chicks D, Rikkers LF: Cryoablation and liver resection for noncolorectal liver metastases. Am J Surg 183(4): 384-389, 2002. DOI: 10.1016/S0002-9610(02)00806-1
218 Seifert JK, Morris DL: World survey on the complications of hepatic and prostate cryotherapy. World J Surg 23(2): 109-114, 1999. DOI: 10.1007/PL00013173
219 Sohn RL, Carlin AM, Steffes C, Tyburski JG, Wilson RF, Littrup PJ, Weaver DW: The extent of cryosurgery increases the complication rate after hepatic cryoablation. Am Surg 69: 317-323, 2003.
220 Tatli S, Acar M, Silverman S: Percutaneous cryoablation: techniques and clinical applications. Diagn Interv Radiol 16(1): 90-5, 2008. DOI: 10.4261/1305-3825.DIR.1922-08.0
221 Hinshaw JL, Lee FT: Cryoablation for liver cancer. Tech Vasc Interv Radiol 10(1): 47-57, 2007. DOI: 10.1053/j.tvir.2007.08.005
222 Shock SA, Laeseke PF, Sampson LA, Lewis WD, Winter TC 3rd, Fine JP, Lee FT Jr: Hepatic hemorrhage caused by percutaneous tumor ablation: Radiofrequency ablation *versus* cryoablation in a porcine model. Radiology 236(1): 125-131, 2005. DOI: 10.1148/RADIOL.2361040533
223 Ní Eochagáin A: Cryoshock following cryoablation for hepatocellular carcinoma. J Clin Anesth 77: 110641, 2022. DOI: 10.1016/j.jclinane.2021.110641
224 Jansen MC, van Hillegersberg R, Schoots IG, Levi M, Beek JF, Crezee H, van Gulik TM: Cryoablation induces greater inflammatory and coagulative responses than radiofrequency ablation or laser induced thermotherapy in a rat liver model. Surgery 147(5): 686-695, 2010. DOI: 10.1016/j.surg.2009.10.053
225 Chapman WC, Debelak JP, Blackwell TS, Gainer KA, Christman JW, Pinson CW, Brigham KL, Parker RE: Hepatic cryoablation-induced acute lung injury. Arch Surg 135(6): 667, 2000. DOI: 10.1001/ARCHSURG.135.6.667
226 Alnaggar M, Niu L, Li J, Yao F, Wang Y, Zeng J, Ye J, Chen J, Mu F, Xu K: Cryoprotective therapy for huge hepatocellular carcinoma: A study of 14 patients with a single lesion. Cryobiology 69(3): 457-461, 2014. DOI: 10.1016/j.cryobiol.2014.10.004
227 Robyn J, Rasschaert G, Pasmans F, Heyndrickx M: Thermotolerant Campylobacter during broiler rearing: Risk factors and intervention. Compr Rev Food Sci Food Saf 14(2): 81-105, 2015. DOI: 10.1111/1541-4337.12124
228 McCarthy N, Giesecke J: Incidence of Guillain-Barre syndrome following infection with Campylobacter jejuni. Am J Epidemiol 153(6): 610-614, 2001. DOI: 10.1093/aje/153.6.610
229 Nachamkin I, Allos BM, Ho T: Campylobacter species and Guillain-Barré syndrome. Clin Microbiol Rev 11(3): 555-567, 1998. DOI: 10.1128/CMR.11.3.555
230 Poropatich KO, Walker CL, Black RE: Quantifying the association between Campylobacter infection and Guillain-Barré syndrome: a systematic review. J Health Popul Nutr 28(6): 545-552, 2010. DOI: 10.3329/jhpn.v28i6.6602
231 Rees JH, Soudain SE, Gregson NA, Hughes RA: Campylobacter jejuni infection and Guillain-Barré syndrome. N Engl J Med 333(21): 1374-1379, 1995. DOI: 10.1056/NEJM199511233332102
232 Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM: Global epidemiology of Campylobacter infection. Clin Microbiol Rev 28(3): 687-720, 2015. DOI: 10.1128/CMR.00006-15
233 Holland D, Thomson L, Mahmoudzadeh N, Khaled A: Estimating deaths from foodborne disease in the UK for 11 key pathogens. BMJ Open Gastroenterol 7(1): e000377, 2020. DOI: 10.1136/bmjgast-2020-000377
234 Rasschaert G, De Zutter L, Herman L, Heyndrickx M: Campylobacter contamination of broilers: the role of transport and slaughterhouse. Int J Food Microbiol 322: 108564, 2020. DOI: 10.1016/J.IJFOODMICRO.2020.108564
235 Agunos A, Waddell L, Léger D, Taboada E: A systematic review characterizing on-farm sources of Campylobacter spp. for broiler chickens. PLoS One 9(8): e104905, 2014. DOI: 10.1371/journal.pone.0104905
236 Herman L, Heyndrickx M, Grijspeerdt K, Vandekerchove D, Rollier I, De Zutter L: Routes for Campylobacter contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiol Infect 131(3): 1169-1180, 2003. DOI: 10.1017/s0950268803001183
237 Hermans D, Van Deun K, Messens W, Martel A, Van Immerseel F, Haesebrouck F, Rasschaert G, Heyndrickx M, Pasmans F: Campylobacter control in poultry by current intervention measures ineffective: Urgent need for intensified fundamental research. Vet Microbiol 152(3-4): 219-228, 2011. DOI: 10.1016/J.VETMIC.2011.03.010
238 Newell DG, Fearnley C: Sources of Campylobacter colonization in broiler chickens. Appl Environ Microbiol 69(8): 4343-4351, 2003. DOI: 10.1128/AEM.69.8.4343-4351.2003
239 Sahin O, Kassem II, Shen Z, Lin J, Rajashekara G, Zhang Q: Campylobacter in poultry: Ecology and potential interventions. Avian Dis 59(2): 185-200, 2015. DOI: 10.1637/11072-032315-REVIEW
240 van Gerwe T, Miflin JK, Templeton JM, Bouma A, Wagenaar JA, Jacobs-Reitsma WF, Stegeman A, Klinkenberg D: Quantifying transmission of Campylobacter jejuni in commercial broiler flocks. Appl Environ Microbiol 75(3): 625-628, 2009. DOI: 10.1128/AEM.01912-08
241 Dogan OB, Clarke J, Mattos F, Wang B: A quantitative microbial risk assessment model of Campylobacter in broiler chickens: Evaluating processing interventions. Food Control 100: 97-110, 2019. DOI: 10.1016/J.FOODCONT.2019.01.003
242 Gunther NW, Rajkowski KT, Sommers C: Survival after cryogenic freezing of campylobacter species in ground turkey patties treated with polyphosphates. J Food Prot 78(2): 419-423, 2015. DOI: 10.4315/0362-028X.JFP-14-301
243 Haughton PN, Lyng J, Cronin D, Fanning S, Whyte P: Effect of crust freezing applied alone and in combination with ultraviolet light on the survival of Campylobacter on raw chicken. Food Microbiol 32(1): 147-151, 2012. DOI: 10.1016/J.FM.2012.05.004
244 Zhao T, Ezeike GO, Doyle MP, Hung Y, Howell RS: Reduction of Campylobacter jejuni on poultry by low-temperature treatment. J Food Prot 66(4): 652-655, 2003. DOI: 10.4315/0362-028X-66.4.652
245 James C, James SJ, Hannay N, Purnell G, Barbedo-Pinto C, Yaman H, Araujo M, Gonzalez ML, Calvo J, Howell M, Corry

JE: Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. Int J Food Microbiol 114(2): 195-203, 2007. DOI: 10.1016/J.IJFOODMICRO.2006.09.019

246 Campylobacter Intervention. Air Products. Available at: https://airproducts.co.uk/applications/campylobacter-intervention [Last accessed on June 9, 2023]

247 Chadwick D, Roehrl MH: High-quality biobanking for personalized precision medicine: BioSpecimen Sciences at the helm. Diagn Histopathol 19(12): 447-456, 2013. DOI: 10.1016/j.mpdhp.2013.11.009

248 Ma Y, Gao L, Tian Y, Chen P, Yang J, Zhang L: Advanced biomaterials in cell preservation: Hypothermic preservation and cryopreservation. Acta Biomater 131: 97-116, 2021. DOI: 10.1016/j.actbio.2021.07.001

249 Raju R, Bryant SJ, Wilkinson BL, Bryant G: The need for novel cryoprotectants and cryopreservation protocols: Insights into the importance of biophysical investigation and cell permeability. Biochim Biophys Acta Gen Subj 1865(1): 129749, 2021. DOI: 10.1016/j.bbagen.2020.129749

250 Arakawa T, Carpenter JF, Kita YA, Crowe JH: The basis for toxicity of certain cryoprotectants: A hypothesis. Cryobiology 27(4): 401-415, 1990. DOI: 10.1016/0011-2240(90)90017-X

251 Arakawa T, Kita Y, Timasheff SN: Protein precipitation and denaturation by dimethyl sulfoxide. Biophys Chem 131(1-3): 62-70, 2007. DOI: 10.1016/J.BPC.2007.09.004

252 Best BP: Cryoprotectant toxicity: Facts, issues, and questions. Rejuvenation Res 18(5): 422-436, 2015. DOI: 10.1089/rej.2014.1656

253 Demey HE, Daelemans RA, Verpooten GA, De Broe ME, Van Campenhout CM, Lakiere FV, Schepens PJ, Bossaert LL: Propylene glycol-induced side effects during intravenous nitroglycerin therapy. Intensive Care Med 14(3): 221-226, 1988. DOI: 10.1007/BF00717993

254 Shu Z, Heimfeld S, Gao D: Hematopoietic SCT with cryopreserved grafts: adverse reactions after transplantation and cryoprotectant removal before infusion. Bone Marrow Transplant 49(4): 469-476, 2014. DOI: 10.1038/bmt.2013.152

255 Wolkers WF, Oldenhof H, Tang F, Han J, Bigalk J, Sieme H: Factors affecting the membrane permeability barrier function of cells during preservation technologies. Langmuir 35(23): 7520-7528, 2019. DOI: 10.1021/ACS.LANGMUIR.8B02852

256 Yoon J, Yoo C, Ahn Y: N,N-dimethylformamide: evidence of carcinogenicity from national representative cohort study in South Korea. Scand J Work Environ 45(4): 396-401, 2019. DOI: 10.5271/sjweh.3802

257 Djerassi I, Roy A: A method for preservation of viable platelets: Combined effects of sugars and dimethylsulfoxide. Blood 22(6): 703-717, 1963. DOI: 10.1182/blood.v22.6.703.703

258 Elliott GD, Wang S, Fuller BJ: Cryoprotectants: A review of the actions and applications of cryoprotective solutes that modulate cell recovery from ultra-low temperatures. Cryobiology 76: 74-91, 2017. DOI: 10.1016/j.cryobiol.2017.04.004

259 Fahy G, Macfarlane D, Angell C, Meryman H: Vitrification as an approach to cryopreservation. Cryobiology 21(4): 407-426, 1984. DOI: 10.1016/0011-2240(84)90079-8

260 Mantri S, Kanungo S, Mohapatra PC: Cryoprotective effect of disaccharides on cord blood stem cells with minimal use of DMSO. Indian J Hematol Blood Transfus 31(2): 206-212, 2015. DOI: 10.1007/s12288-014-0352-x

261 Oldenhof H, Gojowsky M, Wang S, Henke S, Yu C, Rohn K, Wolkers WF, Sieme H: Osmotic stress and membrane phase changes during freezing of stallion sperm: mode of action of cryoprotective agents. Biol Reprod 88(3): 68, 2013. DOI: 10.1095/biolreprod.112.104661

262 Roy S, Arora S, Kumari P, Ta M: A simple and serum-free protocol for cryopreservation of human umbilical cord as source of Wharton's jelly mesenchymal stem cells. Cryobiology 68(3): 467-472, 2014. DOI: 10.1016/j.cryobiol.2014.03.010

263 Davidson AF, Glasscock C, McClanahan DR, Benson JD, Higgins AZ: Toxicity minimized cryoprotectant addition and removal procedures for adherent endothelial cells. PLoS One 10(11): e0142828, 2015. DOI: 10.1371/journal.pone.0142828

264 Gao D, Critser JK: Mechanisms of cryoinjury in living cells. ILAR J 41(4): 187-196, 2000. DOI: 10.1093/ilar.41.4.187

265 Wolfe J, Bryant G: Cellular cryobiology: thermodynamic and mechanical effects. Int J Refriger 24(5): 438-450, 2001. DOI: 10.1016/S0140-7007(00)00027-X

266 Zhao G, Fu J: Microfluidics for cryopreservation. Biotechnol Adv 35(2): 323-336, 2017. DOI: 10.1016/j.biotechadv.2017.01.006

267 Bryant G: DSC measurement of cell suspensions during successive freezing runs: implications for the mechanisms of intracellular ice formation. Cryobiology 32(2): 114-128, 1995. DOI: 10.1006/cryo.1995.1011

268 Crowe JH, Carpenter JF, Crowe LM, Anchordoguy TJ: Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology 27(3): 219-231, 1990. DOI: 10.1016/0011-2240(90)90023-W

269 Crowe JH, Hoekstra FA, Crowe LM: Anhydrobiosis. Annu Rev Physiol 54(1): 579-599, 1992. DOI: 10.1146/annurev.ph.54.030192.003051

270 Wolfe J, Bryant G: Freezing, drying, and/or vitrification of membrane– solute–water systems. Cryobiology 39(2): 103-129, 1999. DOI: 10.1006/cryo.1999.2195

271 Wolfe J, Bryant G, Koster KL: What is "unfreezable water", how unfreezable is it and how much is there? Cryo-Letters 23(3): 157-166, 2002.

272 Mazur P, Leibo S, Chu E: A two-factor hypothesis of freezing injury. Exp Cell Res 71(2): 345-355, 1972. DOI: 10.1016/0014-4827(72)90303-5

273 Mazur P: Freezing of living cells: mechanisms and implications. Am J Physiol 247(3): C125-C142, 1984. DOI: 10.1152/AJPCELL.1984.247.3.C125

274 Whaley D, Damyar K, Witek RP, Mendoza A, Alexander M, Lakey JR: Cryopreservation: an overview of principles and cell-specific considerations. Cell Transplant 30: 963689721999617, 2021. DOI: 10.1177/0963689721999617

275 Kuleshova L, Gouk S, Hutmacher D: Vitrification as a prospect for cryopreservation of tissue-engineered constructs. Biomaterials 28(9): 1585-1596, 2007. DOI: 10.1016/j.biomaterials.2006.11.047

276 Fahy GM, Wowk B, Pagotan R, Chang A, Phan J, Thomson B, Phan L: Physical and biological aspects of renal vitrification. Organogenesis 5(3): 167-175, 2009. DOI: 10.4161/org.5.3.9974

277 Kutluyer F, Kayim M, Öğretmen F, Büyükleblebici S, Tuncer PB: Cryopreservation of rainbow trout Oncorhynchus mykiss spermatozoa: Effects of extender supplemented with different antioxidants on sperm motility, velocity and fertility. Cryobiology 69(3): 462-466, 2014. DOI: 10.1016/j.cryobiol.2014.10.005

278 Vajta G, Rienzi L, Ubaldi FM: Open *vs*. closed systems for vitrification of human oocytes and embryos. Reprod Biomed Online 30(4): 325-333, 2015. DOI: 10.1016/j.rbmo.2014.12.012
279 Jain M, Singh M: Assisted reproductive technology (ART) techniques. Treasure Island, FL, USA, StatPearls Publishing, 2023.
280 Mahadevan M, Trounson A: Effect of cooling, freezing and thawing rates and storage conditions on preservation of human spermatozoa. Andrologia 16(1): 52-60, 2009. DOI: 10.1111/j.1439-0272.1984.tb00234.x
281 Huang C, Lei L, Wu H, Gan R, Yuan X, Fan L, Zhu W: Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes. Fertil Steril 112(4): 663-669.e1, 2019. DOI: 10.1016/J.FERTNSTERT.2019.06.008
282 Valcarce DG, Cartón-García F, Riesco MF, Herráez MP, Robles V: Analysis of DNA damage after human sperm cryopreservation in genes crucial for fertilization and early embryo development. Andrology 1(5): 723-730, 2013. DOI: 10.1111/j.2047-2927.2013.00116.x
283 Yeste M: Sperm cryopreservation update: Cryodamage, markers, and factors affecting the sperm freezability in pigs. Theriogenology 85(1): 47-64, 2016. DOI: 10.1016/j.theriogenology.2015.09.047
284 Degl'Innocenti S, Filimberti E, Magini A, Krausz C, Lombardi G, Fino MG, Rastrelli G, Maggi M, Baldi E: Semen cryopreservation for men banking for oligospermia, cancers, and other pathologies: Prediction of post-thaw outcome using basal semen quality. Fertil Steril 100: 1555-1563.e3, 2013. DOI: 10.1016/j.fertnstert.2013.08.005
285 WHO laboratory manual for the examination and processing of human semen. World Health Organization, 2021. Available at: https://www.who.int/publications/i/item/9789240030787 [Last accessed on June 9, 2023]
286 Yavetz H, Yogev L, Homonnai Z, Paz G: Prerequisites for successful human sperm cryobanking: sperm quality and prefreezing holding time. Fertil Steril 55(4): 812-816, 1991. DOI: 10.1016/S0015-0282(16)54253-8
287 Kanbar M, de Michele F, Wyns C: Cryostorage of testicular tissue and retransplantation of spermatogonial stem cells in the infertile male. Best Pract Res Clin Endocrinol Metab 33(1): 103-115, 2019. DOI: 10.1016/j.beem.2018.10.003
288 Amidi F, Pazhohan A, Shabani Nashtaei M, Khodarahmian M, Nekoonam S: The role of antioxidants in sperm freezing: a review. Cell Tissue Bank 17(4): 745-756, 2016. DOI: 10.1007/s10561-016-9566-5
289 Medina-Robles VM, Sandoval-Vargas LY, Suárez-Martínez RO, Gómez-Ramírez E, Guaje-Ramírez DN, Cruz-Casallas PE: Cryostorage of white cachama (Piaractus orinoquensis) sperm: Effects on cellular, biochemical and ultrastructural parameters. Aquac Rep 29: 101477, 2023. DOI: 10.1016/j.aqrep.2023.101477
290 Alikani M: Cryostorage of human gametes and embryos: a reckoning. Reprod Biomed Online 37(1): 1-3, 2018. DOI: 10.1016/j.rbmo.2018.05.004

Received August 10, 2023
Revised September 27, 2023
Accepted October 5, 2023