CHAPTER 1

Biophysical Principles and Properties of Cryoablation

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Background

More than 4000 years have passed since the first documented medical use of cooling therapy, when the ancient Egyptian Edwin Smith Papyrus described applying cold compresses made up of figs, honey, and grease to battlefield injuries.[1] Not until 1947 did Hass and Taylor first describe the creation of myocardial lesions using cold energy generated by carbon dioxide as a refrigerant.[2] In contrast to the destructive nature of heat energy, which produces diffuse areas of hemorrhage and necrosis with thrombus formation and aneurysmal dilation, cryoablation involves a unique biophysical process that gives it the distinctive safety and efficacy profile.[3] Cryoablation induces cellular damage mainly via disruption of membranous organelles, such that destruction to the gross myocardial architectures is reduced. Furthermore, cryomapping is feasible as lesions created at a less cool temperature (>−30 °C) are reversible. These potential advantages nurtured the extensive clinical applications of cryoablation in the treatment of cardiac arrhythmias, such as atrioventricular nodal reentrant tachycardia, septal accessory pathways, atrial fibrillation, and ventricular tachycardia, where a high degree of precision is desirable.

Thermodynamics of the cryoablation system

Heat flows from higher temperature to lower temperature zones. Cryoablation destroys tissue by removing heat from it via a probe that is cooled down to freezing temperatures, which has been made feasible by the invention of refrigerants that permit ultra-effective cooling.

Joule–Thompson effect

In the 1850s, James Prescott Joule and William Thomson described the temperature change of a gas when it is forced through a valve and allowed to expand in an insulated environment. Above the inversion temperature, gas molecules move faster. When they collide with each other, kinetic energy is temporarily converted into potential energy. The average distance between molecules increases as gas expands. This results in significantly fewer collisions between molecules, thus lowers the stored

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potential energy. Because the total energy is conserved, there is a parallel increase in the kinetic energy of the gas. Temperature increases.

In contrast, gas molecules move slower at temperatures below the inversion point. The effect of collision-associated energy conversion becomes less important. The average distance between molecules increases when the gas is allowed to expand. The intermolecular attractive forces (van der Waals forces) increase, and so does the stored potential energy. As the total energy is conserved, there is a parallel decrease in the kinetic energy of the gas. Temperature decreases.[4]

Invention of refrigerant

In the 1870s, Carl Paul Gottfried von Linde applied the Joule–Thompson effect to develop the first commercial refrigeration machine. In his original design, liquefied air was first cooled down by a series of heat exchangers, followed by rapid expansion through a nozzle into an isolated chamber, such that the gas rapidly cooled down to freezing temperature. The cold air generated was then coupled with a countercurrent heat exchanger, where ambient air was chilled before expansion began. This further lowered the temperature of the compressed air entering the apparatus, and it increased the efficiency of the machine (Figure 1.1).[5]

According to the principles of the Joule–Thompson effect, only gases with a high inversion temperature can be used as refrigerants. This is because gases with a low inversion temperature under atmospheric pressure, such as hydrogen and helium, warm up rather than cool down during expansion.[6]

Modern cryoablation system

A cryoprobe is a high-pressure, closed-loop gas expansion system. The cryogen travels along the vacuum's central lumen under pressure to the distal electrode, where it is forced through a throttle and rapidly expands to atmospheric pressure. This causes a dramatic drop in the temperature of the metallic tip, so that the heat of tissue in contact with it is rapidly carried away by conduction and convection. The depressurized gas then returns to the console, where it is restored to the liquid state (Figure 1.2).[3,6]

The probe temperature varies with the cryogen used. The most widely used cryogens in surgery are liquid nitrogen, which can attain a temperature as low as −196 °C; and argon gas, which can achieve a temperature as low as −186 °C.[7] Nevertheless, the complex and bulky delivery systems for these agents limit their utility in percutaneous cardiac procedures. To date, only a nitrous oxide–based cryocatheter is commercially available for use by cardiologists, and its lowest achievable temperature is −89.5 °C.[3,7,8]

The minimal temperature and maximal cooling rate occur at the tissue in contact with the metal tip. With increasing distance from the tip, the nadir temperature rises, cooling rates decrease, and

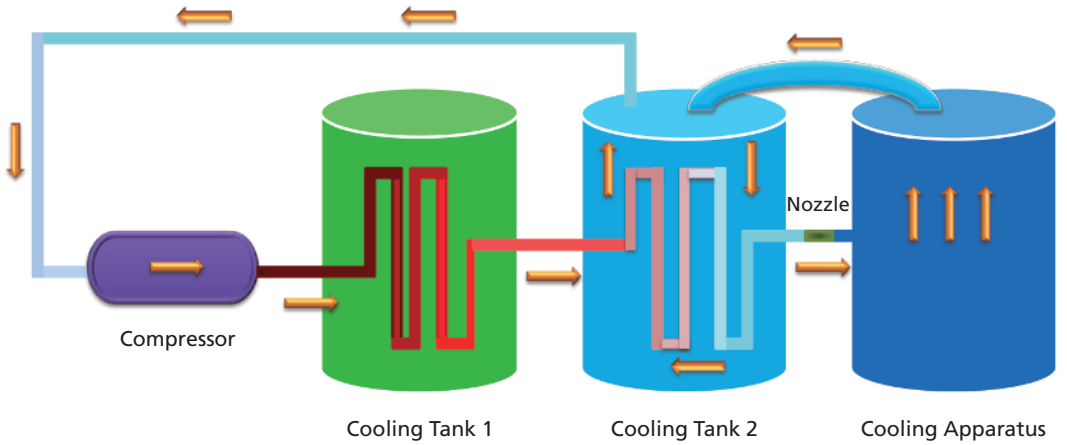


Figure 1.1. Schematic representation of the von Linde refrigerator. The direction of air flow is shown by the arrows.

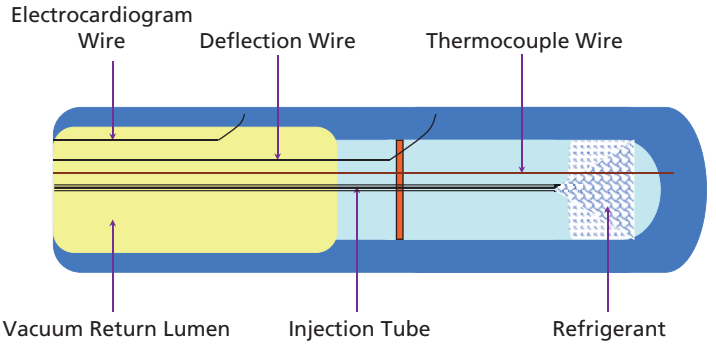


Figure 1.2. Schematic diagram to show the design of the cryoablation probe.

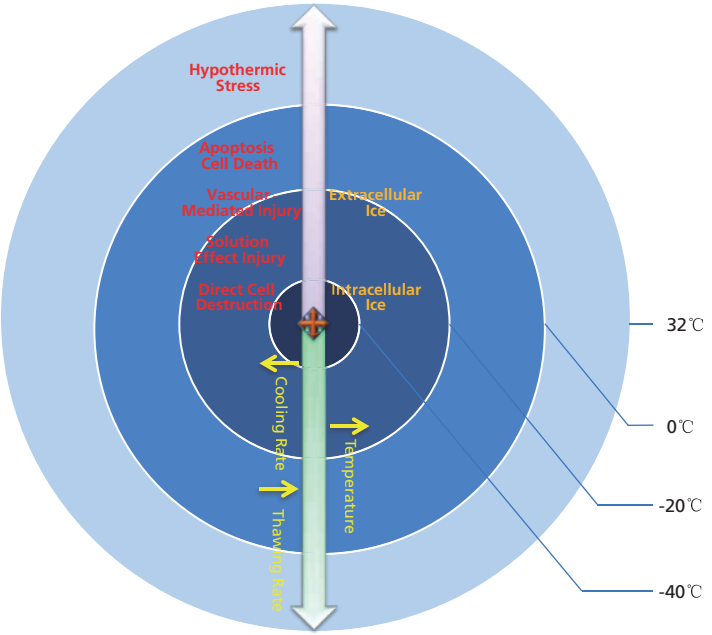


Figure 1.3. Isotherm map of the tip of the catheter electrode of a cryoablation probe (marked by the cross). As shown here, different mechanisms of cell injuries occur at different temperatures.

thawing rates increase. The resultant isotherm map determines the mechanism of injury of those cells lying within each temperature zone, and hence the outcome of the procedure (Figure 1.3).[8]

Mechanisms of injury

Freezing results in both immediate and delayed damage to the targeted tissue. Immediate effects include hypothermic stress and direct cell injury, while delayed consequences are the results of vascular-mediated injury and apoptotic cell death.[5]

Hypothermic stress

When the temperature is lowered to below 32 °C, the membranes of the cells and organelles become less fluid, causing ion pumps to lose their transport capabilities. Electrophysiologically, this is reflected by a decrease in the amplitude of action potential, an increase in its duration, and an extension of the repolarization period. As the temperature continues to decline, metabolism slows, ion imbalances occur, intracellular pH lowers, and adenosine triphosphate levels decrease.[9] Intracellular calcium accumulation secondary to ion pump inactivity and

failure of the sarcoplasmic reticulum reuptake mechanism may lead to further free radical generation and cellular disruption.[5] Nevertheless, these effects are entirely transient, provided that the duration of cooling does not exceed a few minutes. The rapidity of recovery is inversely related to the duration of hypothermic exposure.[3]

Direct cell injury

Contrasting the transient effect of hypothermia, ice formation is the basis of permanent cell injury. When the tissue approaches freezing temperature, ice formation begins and results in cryoadhesion. It acts as a "heat sink" by which heat is rapidly extracted from the tissue.[5] With further lowering of temperature, ice crystals form in both extracellular and intracellular compartments.[3,10,11] Water crystallization begins inside the cells (heterogeneous nucleation) at −15 °C, but intracellular ice generally forms (homogeneous nucleation) at temperatures below −40 °C.[11] Besides, intracellular ice formation is more likely to occur under rapid cooling and at the sites where cells are tightly packed, as water cannot diffuse fast enough through the cellular membrane to equilibrate the intracellular and extracellular compartments.[6,8,10] Intracellular ice compresses and deforms the nuclei and cytoplasmic components, induces pore formation in the plasma membranes, and results in permanent dysfunction of the cellular transport systems and leakage of cellular components.[3,6,8,11] All these events lead to irreversible cell damage and ultimately cell death.

Extracellular ice usually forms under moderate freezing temperatures and slower cooling rates.[3,11] The ice crystals sequestrate free water, which increases solute concentration and hence tonicity of the extracellular compartment. Water is withdrawn from the cells along the osmotic gradient, causing cellular dehydration and elevated intracellular solute concentration. As the process continues, these alterations in the internal environment damage intracellular constituents and destabilize the cell membranes. This is termed *solution–effect injury*.[3,6,11]

Cells densely packed in a tissue are subjected to shearing forces generated between ice crystals, which can result in mechanical destruction.[8,11] However, a previous study has shown that membrane integrity was preserved for up to 2 min after thawing, questioning the actual importance of this theoretical effect.[12]

During thawing, extracellular ice melts and results in hypotonicity of the extracellular compartment. Water is shifted back to the intracellular space, causing cell swelling and bursting. It also perpetuates the growth of intracellular ice crystals, exacerbating cell destruction and cell death. This process of recrystallization occurs at temperatures between −40 and −15 °C, predominantly from −25 to −20 °C.[8,9,11]

Delayed cell death

Cooling results in vasoconstriction, which jeopardizes blood flow to the tissue supplied.[11] At −20 to −10 °C, vascular stasis occurs, water crystallizes, and endothelial cell injury ensues.[11,13] When the blood flow restores at the thawing phase, platelets aggregate and form thrombi at the sites of endothelial injury, leading to small vessel occlusion.[11] The resultant ischemia triggers an influx of vasoactive substances that lead to regional hyperemia and tissue edema, and migration of inflammatory cells that clear up cell debris.[4,6,11] The chance of cell survival is minimized, and uniform coagulation necrosis develops.[4,6,8]

Cells that survive the initial freeze and thaw phases may also die from apoptosis in the next few hours to days.[8] This is because cellular injuries, especially damage to the mitochondria, activate caspases, which cleave proteins and cause membrane blebbing, chromatin condensation, genomic fragmentation, and programmed cell death.[8,13] This is particularly important at the peripheral zone of ablation, where temperatures and cooling rates achieved are less likely to be immediately lethal to the cells.

Lesion characteristics

A detailed description of the histological effect of cryoablation has been published elsewhere.[12] In summary, it can be divided into three phases: the immediate postthaw phase, hemorrhagic and inflammatory phase, and replacement fibrosis phase.

Immediate postthaw phase

Within 30 min of thawing, the myocytes become swollen and the myofilaments appear stretched. The

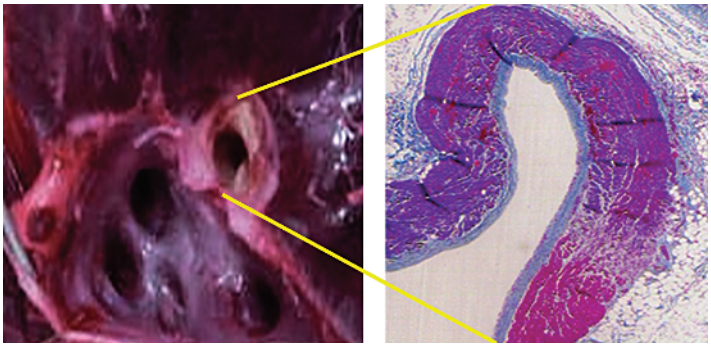


Figure 1.4. Examples of gross (left panel) and histological (right panel) sections show cryoablation lesions after percutaneous cryoablation at the pulmonary vein in a canine. Note the well-demarcated boundary and intact endothelial layer at the site of the cryoablation lesion.

increase in membrane permeability causes mitochondria to swell, which results in oxidation of the endogenous pyridine nucleotides, membrane lipid peroxidation, and enzymatic hydrolysis. This is followed by progressive loss in myofilament structure and irreversible mitochondrial damage.[12]

Hemorrhagic and inflammatory phase

Coagulation necrosis, characterized by hemorrhage, edema, and inflammation, becomes evident at the central part of the lesion within 48 hours after thawing.[12] At the peripheral zone, apoptosis progressively increases and becomes apparent in 8 to 12 hours. At 1 week, infiltrates of inflammatory cells, fibrin and collagen stranding, and capillary ingrowth sharply demarcate the periphery of the lesion.[12] Endothelial layers remain intact, and thrombus formation is uncommon compared with radiofrequency ablation.[14]

Replacement fibrosis phase

Necrotic tissue is largely cleared up by the end of the fourth week. The lesion now consists mainly of dense collagen fibers and fat infiltration, with new blood vessels reestablishing at the periphery. Healing continues for 3 months until a small, fibrotic scar with an intact endothelial layer and a well-demarcated boundary is formed (Figure 1.4).

Factors affecting cryoablation efficacy

The success of cryoablation depends on its ability to deliver a lethal condition to the targeted cells. Although it is more clinically relevant to define it by the completeness of tissue destruction or ablation outcomes, most of the literature has compared only the size of cryolesions produced under different conditions. A summary of our current knowledge in the optimal freezing parameters is discussed in this section.

Tissue temperature

A lower temperature probe creates a deeper lesion, with each 10 °C decrease in the nadir temperature increasing the depth of lesion by 0.4 mm.[3] Although many experiments have shown that extensive damage occurs between −30 and −20 °C, destruction may be incomplete for some types of tissue.[7,8,11] In particular, muscle cells including cardiomyocytes are very sensitive to freezing injury, while cancer cells appear to be much more resistant.[8,11] Generally speaking, a nadir temperature below −40 °C is preferred, as this is the temperature required to produce direct cell injury through lethal intracellular ice formation, and experiments have confirmed that almost all cell types died after rapid cooling to −40 °C.[6]

Cooling rate

Studies have shown that intracellular ice tends to form with rapid freezing. This is because a slow cooling rate increases the duration of exposure of the cells to a higher temperature environment, where extracellular ice is preferentially formed. This in turn causes cellular dehydration and elevated solute concentration, and lowers the intracellular freezing temperature. These alterations in the internal environment hamper the formation of intracellular ice crystals, making cellular destruction less effective.[6]

In reality, rapid freezing (i.e. more than −50 °C per min) occurs only at the cryotip. At about 1 cm from the tip, the cooling rate rapidly drops to −10 to −20 °C per min.[11] While affecting the mode of cellular injury, in vivo experiments, however, have not shown that cooling rate per second is a primary determinant of ablation outcomes.[7,11]

Duration of freezing

The duration of freezing is probably unimportant at the cryotip (where the tissue temperature rapidly reaches −50 °C), as all intracellular water is frozen immediately.[7,11] However, as the cooling effect reduces across the ablation zone, a large portion of tissue will only attain a lower nadir temperature over a longer period of time. Prolongation of freezing not only provides time for the peripheral tissue to reach its lowest achievable temperature such that lethal ice may form, but also increases cell death through solution–effect injury and water recrystallization.[7,11] Indeed, prior studies have shown that 5 min of freezing created significantly larger and deeper cryolesions when compared to 2.5 min of freezing,[15] although the optimal freezing duration for each tissue type has not yet been clearly defined.

Thawing rate

Studies have shown that time to electrode rewarming predicts lesion size.[16] It is thought that prolonged rewarming increases time for cell damage by solution–effect injury and water recrystallization, as both occur during tissue thawing.[7,8,11] In practice, this can be done by passive rewarming.

Freeze-thaw cycles

Early experiments have shown that by repeating the freeze-thaw cycle, both the size of the lesion and the extent of necrosis are increased. This is because thermal conduction is enhanced by the initial cellular breakdown, such that subsequent cycles may lead to more substantial tissue destruction.[7,8] This is especially critical at the peripheral zone of ablation, where the nadir temperature is higher and cell damage tends to be incomplete.

Although the development of newer cryoablation technology that enables much a lower freezing temperature and faster cooling rate may alter the benefit of repeating the freeze-thaw cycle,[3] it is probably still advisable in the treatment of malignancy, where complete tissue destruction is of utmost importance.[8]

Blood flow

Blood flow is a heat source that increases the difficulty of freezing by altering the cooling rate, thawing rate, and lowest attainable temperature.[17] Experimentation has shown that by lowering the blood flow velocity, lesion volume increases.[16,18,19] For this reason, cryoablation is particularly effective when used in low-flow regions such as areas with trabeculations.

Size of catheter tip

Studies have shown that both surface area and volume of cryolesions increase with the size of the catheter tip.[19,20] Possible explanations include an increase in the amount of tissue in direct contact with the cryotip, and a difference in tip-to-tissue contact angles. Nevertheless, lesion depth remains independent of the size of catheter used.

Electrode orientation

In contrast to radiofrequency ablation, in cryoablation significantly larger lesions are created using horizontal rather than vertical catheter tip-to-tissue orientation, probably due to the reduction in parts of the electrode exposed to the warming effect of the blood pool.[15,16,18] Again, only surface dimensions, but not depth of the lesions, are found to be affected.

Contact pressure

Although it is commonly believed that constant contact pressure is not necessary during cryoablation as the ice formed at the catheter tip acts as a reliable thermal conductor, studies have consistently proved that better tissue contact improves lesion sizes and is desirable.[16,18,19]

Conclusion

With its unique mechanism of tissue injury, cryoenergy has demonstrated various advantages over hyperthermic destruction: catheter stability can be improved by cryoadhesion formed from extracellular ice; ablation of vital structures can be prevented by cryomapping, as cell damage is largely reversible at the ablation onset; and thromboembolism can be avoided due to the lack of thrombus formation. All these factors allow cryoablation to gain favor for use among populations and procedures that desire high safety profiles. Nevertheless, optimal lesion creation still depends on catheter design and on freezing parameters, including duration, repeated freeze-thaw cycles, tissue contact, as well as the local warming effect from the surrounding blood flow. With better defined catheters and freezing param-

eters based on ablation outcomes, and the development of new cryogens and delivery systems, the safety and efficacy profiles of cryoablation will continue to improve. It is foreseeable that the application of cryoablation in the treatment of cardiac arrhythmias will continue to expand in the future.

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