Since 1959, cryotherapy for the selective destruction of pathological processes across a range of medical and surgical fields has been used. The mechanisms that bring about cell death through apoptosis and necrosis have also been well described in the literature. The 2 main processes involved are mechanical destruction through the development of intracellular ice crystals and decreased blood supply by disrupting the local vasculature.1-3 It has been extensively proven that repeated cycles of freezing and thawing enhance the efficacy and effective area of cryotherapy. This is thought to be related to multiple intrinsic and extrinsic variables, including the increased size of ice crystal development with subsequent freeze-thaw cycles, the changes of the innate thermal conductive properties with local cell death, and the inability of the vasculature to maintain proper thermoregulation secondary to damage.3-6

Ways to optimize the destruction of a multitude of various types of cells and biological tissues have also been described fairly comprehensively. As previously mentioned, manipulation of the freeze-thaw cycle and its individual components can have a profound effect on the efficacy of cryotherapy. In general, a fast freeze with a slow thaw seems to be most effective for the majority of tissue types.5-9 The minimal freezing temperature required, however, varies from tissue to tissue and is at times controversial.

Regarding bone, injury has been demonstrated to begin at approximately -2°C; this injury quickly becomes irreversible once temperatures drop below -5°C.5 Few studies, however, have described the thermal conductivity of human bone at subphysiological temperatures. In addition, there appear to be no studies describing how bone thermal conductivity is relevant in the treatment of primary bone tumors with cryoprobe therapy.

The aim of this literature review is to summarize the current information regarding the basic science behind cell death with cryotherapy and the relevant principles of thermodynamics. In addition, current cryotherapeutic applications as related to the management of primary bone tumors are discussed with a focus on the potential of cryotherapy through cryoprobe use.

Basic Science

Although cryotherapy is a relatively new modality for
the treatment of tumors, many aspects of its mechanism of action have been well described. There are 2 primary mechanisms at work that synergistically adversely affect the cells exposed to extreme cold temperatures. The first is a direct cellular injury caused principally by the formation of intracellular ice crystals, which results in mechanical destruction.\textsuperscript{3,5,6} In addition, a dehydration effect is seen, resulting in destruction secondary to an extreme increase in intracellular osmotic concentration. These ice crystals have been shown to increase in size with each subsequent freeze-thaw cycle, resulting in further mechanical destruction with each cycle.\textsuperscript{6,7,9}

The second mechanism is related to the vascular supply to the cells. Freezing results in endothelial damage, which, combined with the direct cellular injury of local blood cells, has been shown to increase platelet activation, resulting in an increasing sludging effect leading to coagulation formation.\textsuperscript{9,11} Subsequently, cells are susceptible to necrosis through permanent loss of blood supply to the area or, on resolution of the coagulation, injury secondary to reperfusion.\textsuperscript{12} In addition, the loss of blood flow to the area results in a decreased ability for thermoregulation through vasodilation, which can lead to an increased area of necrosis on subsequent freeze-thaw cycles.\textsuperscript{3,5,12}

There is much debate surrounding the manipulation of the freeze-thaw cycle, which largely depends on the goals and time constraints of the physician in specific clinical scenarios. Generally, a fast freeze with a slow thaw has been shown to have a greater destructive effect through the direct damage effect described above.\textsuperscript{6-9} Most observers consider a temperature of -40°C to -60°C sufficiently lethal to all cells.\textsuperscript{9} However, particulars of the freeze-thaw cycle become relevant to cells in warmer temperature ranges (-10°C to -30°C). Specifically, a fast freeze held for 1 to 20 minutes, depending on the cell type, with a slow thaw seems to cause the largest amount of destruction to cells in this zone of freezing.

A third process involved in the destruction of abnormal cells is related to a delayed immune response. This is an antibody-mediated mechanism that occurs in response to antigens that develop during cellular destruction and necrosis described by the previous 2 mechanisms. Breitbart\textsuperscript{13} eloquently described the effect of the delayed immune response in a study where he showed a decrease in the size of all metastatic melanoma nodules after a single nodule was treated with cryotherapy.

**Thermodynamics**

The use of cryotherapy in clinical settings has seemingly expanded more quickly than the ability of basic science, thermodynamics, and computer science to accurately describe and predict the utility of cryotherapy in complex biological systems. Using basic thermodynamics as a foundation, many formulas have been developed to accurately describe temperature changes in biological tissues at specific distances from a cryoprobe. The goal, clinically, of these models is to assist clinicians in developing strategic plans for the management of undesirable cells when using stereotactic cryotherapy.

Starting first with conduction, the movement or flow of heat through stationary materials, the heat flow can be described for 1-dimensional systems at steady-state with the equation shown in Figure 1.

Convection, the heat transferred secondary to the fluid flow, is another form of heat transfer that is relevant in biological systems and described by the equation shown in Figure 2.

In Penne’s bioheat equation, a combination of these 2 heat transfer equations with the intrinsic heat created through metabolic activity is seen (Figure 3). The equation shown in Figure 3 describes 1-dimensional systems. However, in cryoprobe therapy, the equation can be expanded to describe a 3-dimensional system (Figure 4). These equations make it clear that there are many variables. The values of these variables can differ drastically depending on the tissue type and the individual. To further complicate the ability to create an accurate model, some of the values can be altered with each subsequent freeze-thaw cycle. Blood perfusion, for example, is altered by the disruption of vascular integrity seen after the first freeze-thaw cycle. Furthermore, studies have shown that the intrinsic and extrinsic properties of the tissues can be strategically manipulated to increase the effective volume of cryotherapy.\textsuperscript{14}

Despite all of these challenges, mathematical models are becoming more accurate at predicting the effective freeze/


\[ \rho_t C_{pt} \frac{\delta T_t}{\delta t} = k_t \left( \frac{\delta^2 T_t}{\delta x^2} \right) + \rho_b C_{pb} \dot{V} (T_A - T_V) + Q_m \]

Figure 3: Penne’s bioheat equation.

\[ = k_t \left( \frac{\delta^2 T_t}{\delta x^2} \right) + \rho_b C_{pb} \dot{V} (T_A - T_V) + Q_m \]

Figure 4: Generic 3-dimensional expansion of Penne’s bioheat equation. The equation’s 3-dimensional components can be altered to represent a multitude of volumetric shapes, including a sphere or a cylinder.

Accurate estimation and description of the biological properties of bone is vital if one expects to make an accurate and precise mathematical model predictions of the affected areas for better intraoperative and postoperative management of bone tumors.

**CURRENT CRYOTHERAPY MODALITIES IN ORTHOPEDIC ONCOLOGY**

A multitude of modalities have been established for cryotherapy since the discovery that rapid freezing could be used for the selective necrotic effect on certain cells. For instance, in dermatology, the selective destruction of specific superficial cells is often desired, the “spray freeze” method is often used. This is an “open” method that involves spraying liquid nitrogen directly on the affected area. At times, a “closed” method can be used in which liquid nitrogen is circulated to freeze a metal probe that is in turn applied directly to the skin.

Variations of these 2 methods have commonly been used in orthopedic oncology for the treatment of various types of bone tumors. Marcove and Miller described the first known instance of cryotherapy in orthopedic oncology when they used an open system method called the “direct pour” to treat a metastatic bone tumor that remained refractory to other types of therapy. As the name implies, the direct pour method involves creating a bone window for access to the tumor-containing cavity and then pouring liquid nitrogen directly into said cavity. This method has proved to be an invaluable adjuvant therapy to mechanical methods, such as curettage and burr drilling, in reducing the local recurrence of bone tumors.

In some specific cases, a closed method involving a metal probe with circulating liquid nitrogen can be used. This technique, however, is most suitable for small, regularly shaped cavities. Bone tumors in large or irregularly shaped cavities have typically been treated more effectively with the direct pour method.

Neither modality is without consequence and adverse effects, however. Both methods require a large cortical bone window of which the minimum size should be equivalent to the largest diameter of the tumor for sufficient exposure. Additionally, they both expose a significant portion of the surrounding bone to adverse temperatures that can lead to eventual devitalization as described above. As such, the structural integrity of the affected bone is severely compromised and in many cases requires extensive reconstruction for prophylaxis of future injury such as pathologic fracture. Another complication observed more commonly in the direct pour method is damage to surrounding structures (i.e., nerves, skin, cartilage).

**POTENTIAL FOR CRYOPROBE THERAPY IN ORTHOPEDICS**

Recently, cryoprobes have been used extensively by urologists for the stereotactic destruction of cells. This minimally invasive technique allows for the maximal destruction of cancerous cells while causing minimal damage to appropriately functioning cells and tissues. Thus, much of the literature associated with innovations in this area involves urological studies. However, many of these concepts can be transferred to orthopedics.

The first step in instituting cryoprobe therapy as a clinical tool in orthopedics involves describing the intrinsic properties of the tissues an orthopedic surgeon may commonly encounter. Specifically, the thermal conductivity and specific heat of bone, cartilage, and common primary bone tumors can be calculated using empirical data. Unfortunately, the current literature lacks an accurate mathematical model for use in orthopedic oncology.

Popken et al. studied the variations in temperature en vivo in goat tibias and femurs at specific intervals from the cryoprobe application site. In this study, a steady-state temperature was achieved at approximately 17 minutes. At steady-state, a temperature drop of greater than 40°C was measured 1 cm from the cryoprobe in metaphyseal bone and greater than 35°C
in metadiaphyseal bone. In addition, on histological examination, necrosis was noted to occur in areas where temperatures were measured to be -10°C or higher. A method for measuring empiric data in cadaveric bone to calculate intrinsic constants related to thermodynamics is shown in Figure 5.

Although some studies have been conducted to illustrate the sensitivity of bone at various temperatures below zero, this area also appears to be underinvestigated. The literature shows that osteocytes are susceptible to irreversible damage at relatively high temperatures when compared with other types of cells. Temperatures ranging from -5°C to -2°C have been shown to be detrimental to osteocytes.9

Furthermore, by examining tetracycline uptake in bone after freezing, Lind- que9 showed that there is an extension of the affected bone beyond what could be speculated based on gross observations during the freeze-thaw cycle (Figure 6).

Further characterizing what factors affect the zone of necrosis in musculoskeletal tissues is an essential second step in the use of cryoprobe therapy in orthopedics. Of additional importance is characterizing the effects of multiple freeze-thaw cycles on the structural integrity of bone in the acute to subacute setting.

Once sufficient data are obtained, mathematical models can be developed and applied to predict the necrotic zone that can be expected through cryoprobe application. The ultimate goal would be to decrease morbidity by minimizing damage to healthy tissue and bone while effectively treating oncological conditions.

Additional clinical applications in orthopedics include the freezing of aggressive benign tumors or metastatic bone tumors, such as renal cell carcinoma, melanoma, or metastatic disease, resistant to other treatment modalities. Promising results (Figure 7) have been achieved with percutaneous cryotherapy for small metastatic lesions confined to bone. For example, intramedullary freezing of metastatic skip lesions has the advantages of not spilling live tumor, avoiding full-thickness freezing of the entire cortical diameter, and maintaining some structural integrity of the humeral diaphysis.

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