

Nano-Cryosurgery: Advances and Challenges

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In clinics, the minimally invasive freezing therapy, commonly known as cryosurgery, has been increasingly used for the controlled destruction of tumor tissue. However, there are still many bottlenecks to impede the success of a cryosurgery. One of the most critical factors has been that insufficient or inappropriate freezing will not completely destroy the target tumor tissues, which as a result may lead to tumor regrowth and thus failure of treatment. In addition, the surrounding healthy tissues may suffer from serious freeze injury due to unavoidable release of a large amount of cold from the freezing probe. To resolve these challenges, we recently proposed a new strategy, termed as nano-cryosurgery, to improve freezing efficiency of the conventional cryosurgical procedure. The basic principle of this protocol is to deliver functional suspension of nanoparticles with favorable physical and/or chemical properties into the target tissues, which then serve as adjuvant or drug carrier either to maximize the freezing heat transfer process, regulate freezing scale, modify ice-ball formation orientation or prevent the surrounding healthy tissues from being frozen. In addition, introduction of nanoparticles during cryosurgery could also help better image the edge of a tumor as well as the margin of the iceball. The new therapy raised many critical fundamental as well as practical issues for solving. This review is dedicated to present a comprehensive review on multi-scale fundamental phase change heat transfer issues thus involved. Attention would span from micro-scale heat transfer in cellular scale to tissue level. Some related thermal physical effects of nanoparticles on the freezing process such as ice nucleation enhancement, water transport during freezing of a single cell will be discussed. Cryosurgical thermal management of using nanoparticles to modify thermal properties of the tissue-particle components, regulate the growth orientation and strength of an ice ball, enable a conformal tumor destruction in tissues with or without large blood vessels, etc. will be illustrated. Meanwhile, the fundamental issue for the transport of nanoparticle and its assisted drug delivery will be summarized. Theoretical modeling as well as experimental approaches for studying the micro/nano-scale heat transfer throughout the tissue or cell domain during nano-cryosurgery will be suggested. Some potential applications and possible challenges when nanotechnology meets cryosurgery will be outlined. The nano-cryosurgery is expected to help expand the boundary of the emerging frontier of nano-biomedical engineering.

Keywords: Nano-Cryosurgery, Nano-Technology, Nano-Medicine, Tumor Ablation, Conformal Treatment, Physical Therapy, Phase Change, Bioheat Transfer, Freezing Enhancement, Large Blood Vessel.

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1. INTRODUCTION

Cryosurgery, sometimes referred to as cryotherapy or cryoablation, is the use of extreme cold produced by cryogenic agents to destroy abnormal or diseased tissues. The application of cryosurgery covers treatment of many kinds of cancer and some noncancerous diseased tissues.¹ As an adjuvant therapy for benign or malignant tumors which are difficult or even impossible to be extirpated otherwise via conventional surgery, cryosurgery has produced many excellent outputs. Its attractive clinical advantages still include being less invasive than traditional surgical resection; minimizing pain, bleeding, and other complications of surgery; less expensive and requiring a much shorter recovery time and hospital stay.¹⁻³ Although cryosurgery still can not be regarded as a routine cancer treatment way, it is developing rather rapidly as an alternative for traditional radiotherapy or chemotherapy. Owing to the advent of many modern imaging technologies, the field of cryosurgery has been significantly extended since its early stage.¹ However, many clinical statistics imply that conventional cryosurgery may still not be very efficient in treating complex tumors. There is sometimes a high recurrence rate with follow-up surveys. For example, local recurrences at the site of ablation close to large blood vessels have been reported at rates from 5% to 44% or even higher.^{4,5} One challenging issue is that utilizing multiprobes during cryosurgery could hardly produce a strong enough freezing and desirable shape of an iceball to exactly enwrap the target especially when large volumes

and irregularly shaped tumors are to be tackled. Another major reason lies in that the freezing rate is not appropriate and could not produce a massive ice nucleation in tumor cells, especially at the edge of tumor.⁶ This fails to guarantee a complete lethal harm to the whole diseased regions. A major concern facing modern cryosurgery is to maximize its freezing efficiency in killing target focus while minimize the irreversible damage to the surrounding normal tissues.⁷

To improve the efficacy of a cryosurgery, several adjunctive therapies have been proposed so far. Among the approaches ever tried, the chemical adjuvants such as cancer chemo-therapeutic agents,⁸⁻¹⁰ antifreeze protein (AFP),¹¹⁻¹⁵ and salts¹⁶ were investigated. The concept of using chemo-therapeutic drugs with cryosurgery was tested by Ikekawa et al.,⁹ where two chemo-therapeutic agents, peplomycin and adriamycin, were introduced prior and posterior to cryosurgery, and improved cryosurgical outcome was found. Later, Clarke et al.⁸ reported that exposing PC-3 human prostate cancer cells *in vitro* on the chemo-therapeutic agent, 5-fluorouracil, could enhance PC-3 cells' cryoinjury by subsequent freezing. Mir and Rubinsky¹⁰ investigated another chemo-therapeutic agent, bleomycin, and observed promising improvement of cryoinjury to B16 F0 melanoma cells. Overall, the clinical use of chemo-therapeutic drugs as an adjuvant to cryosurgery is considered beneficial,^{17,18} although an optimal dose and timing of delivery is not well defined. The use of AFP is also an important adjuvant approach. AFPs have the abilities to enhance freezing injury by modifying the structure



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of ice crystals to needle like at high concentrations and protect cells from freezing injury by inhibiting ice formation at low concentrations.^{11,12} Han and Bischof¹⁶ investigated another effective method of improving cell and tissue injury in cryosurgery by use of eutectic freezing. The above studies have shown the feasibility of various chemicals as adjuvants of cryosurgery. However, experience gained so far by cryosurgery indicates that the possibilities of cryodestruction of sizable and complex pathological formations can still be possible.

Aiming to establish an ever efficient freezing treatment on tumor, Liu's group recently proposed to use nanoparticles to significantly enhance various freezing procedures.^{19–21} This new treatment modality, now termed as nano-cryosurgery, could not only be applied for flexibly controlling the freezing scale, but also help modify the direction of the iceball formation which is highly desirable for a successful cryosurgery for treating tumors with complex anatomical structure.^{22,23} The earliest effort in this topic can be dated back to the thesis work of Yu²⁴ and Deng,²⁵ who were then supervised by the senior author of this paper. Recently, using nanoparticles to regulate freezing or thawing processes was further extended to more cryobiology cases.^{26–32} Considering that all these efforts fall in the category of using nano-technology to innovate a conventional cryomedical practice, Liu et al. finally come up to the generalized concept which can be defined as nano-cryosurgery.³³ Such a physical therapy has unique significance in future tumor clinics.

Clearly, the nano-cryosurgery is closely rooted in the advanced nano-technologies. Its basic principle is to introduce functional solution with nanoparticles into the target tissues (see Fig. 1), which then serves as either maximize freezing heat transfer, change ice-ball formation orientation or prevent healthy tissues from being frozen. The nanoparticles enabled cryosurgery takes full advantage of enhanced heat conduction effects and powerful performance to serve as nucleation seeds. Moreover, it also offers a beneficial hand to treat tumor by targeted delivery through angiogenesis or some antineoplastic drug accompanied by nanoparticles. In fact, the nano-technology enabled medicines have already become rather hot topics. The emergence of nanotechnology over the past few years has had an immediate influence on modern medical therapy. Among the many physical therapies thus appeared, the nano-hyperthermia is especially receiving great attentions over the world.^{34–36} Many exciting progresses are kept being made which as a result further push forward the rapid growth of the nano-medicine area. In contrast to the heating strategy based on nano-technologies, the surgical concept of nano-cryosurgery was proposed until recently. Compared with the exploded efforts on investigating nano-hyperthermia, only rather limited works on nano-cryosurgery are available right now.³⁷ Unlike using high temperature to thermally ablate tumor, the nano-cryosurgery, which takes on a contradictory procedure,

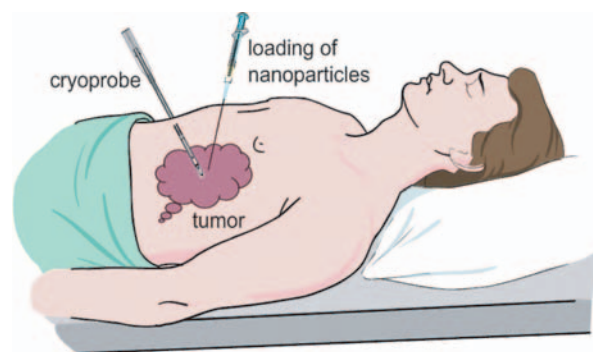


Fig. 1. Schematic for loading nanoparticle solution for administrating a nano-cryosurgery.

may be involved with more complex physical mechanisms, due to the phase change behavior it induced.

This paper is dedicated to present an overview on nano-cryosurgery by summarizing some of the latest advancements in experimental and theoretical works. Particularly, the thermal aspect will be paid with special attention. Some related effects of nanoparticles on the tissue or cell level freezing will briefly be discussed. This review is expected to help better understand the potential advantages brought about by the emerging nano-cryosurgical modality and thus inspire further investigations in this area.

2. BASIC FEATURE OF THE NANO-CRYOSURGERY

As is well known, freezing affects biological systems at both nanoscale (molecular) and microscale (cellular) levels, which may cause the change of structure, composition, water and fat content, and salinity of tissues.^{1–3} Clearly, introduction of nanoparticles would significantly modify such processes. For a conventional cryosurgery, there generally exists an optimum freezing rate for maximizing the killing effect on tumor tissues. However, this is often hard to achieve through out the whole region of interest. In this side, adoption of nanoparticles allows to administrate an accurate killing on the target via a more flexible and desirable way.³³

The basic capability of nano-cryosurgery can be summarized as follows: I. Enhance freezing and thus improve target killing efficiency; II. Avoid insufficient freezing between multiple cryoprobes during treating large scale tumor; III. Regulate growth direction and orientation of an ice ball and thus guarantee a conformal cryosurgery on complex tumor; IV. Weaken a freezing on healthy tissues and thus reduce injury there; V. Improve image contrast and offer a better image guidance for the cryosurgical operation; VI. Improve delivery efficiency of anti-cancer drug during nano-cryosurgical chemotherapy.

From the point of cell cryoinjury theory,³⁸ intracellular ice formation (IIF), which occurs due to insufficient time for water to escape the cells, is considered to be

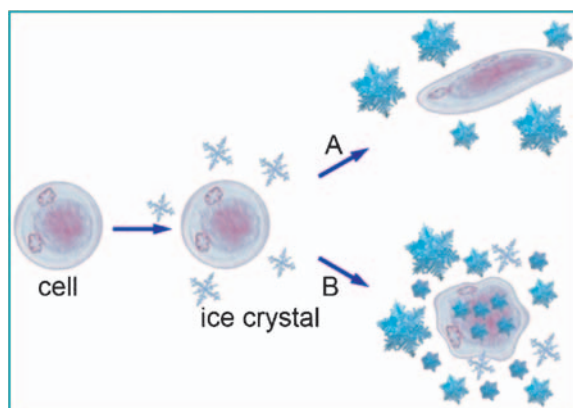


Fig. 2. Schematic for ice formation at cell level when subjected to freezing. A. Without loading nanoparticles; B. Loading with nanoparticles.

lethal reasons. IIF damages cytoskeleton, cell organelles and membranes leading to death which mainly depend on two important factors: one is cooling rates; the other is the probability of IIF (PIF). Once tumor cells contain some nanoparticles or a number of nanoparticles are immersed in intercellular area (see Fig. 2), such two factors can be significantly improved since the nanoparticles also serve well as the seeds of ice nucleation. As is realized, liquids containing nanometer sized metallic or non-metallic solid nanoparticles, show an increase in thermal conductivity compared with that of the base liquid. This can also be true when applied to nano-cryosurgery, where addition of metal or metal-oxide nanoparticles will significantly increase the tissue conductivity. Once nanoparticles with high thermal conductivity are injected into target area, the final temperature level could be significantly lowered, the maximum freezing rate will be increased and ice volume would be larger than that of no-particle case. Besides, the massive loading of nanoparticles is bound to result in an easier heterogeneous nucleation which to some extent guarantees a higher PIF (see Fig. 2). Therefore it can be expected that delivered nanoparticle will enhance tumor necrosis during cryosurgery. In addition, introduction of nanoparticles during cryosurgery could also help better image the edge of tumor as well as the margin of the iceball. This is very important in guaranteeing a successful cryosurgery. Such merits may lead to a highly “green” therapy on tumor. Meanwhile, as a natural antineoplastic agent carrier to kill tumor, the drug like nanoparticles would further improve the effective killing rate of tumor cell with the combination of cryosurgery, just as that performed before on nano-hyperthermia.³⁵

Since the particulate suspension can be locally injected and distributed into the region of interest as desired,¹⁹ it is possible to administrate an accurate killing on nano-scale by means of nano-cryosurgery. Although a series of studies have been published on the toxicological effect of nanoparticles,^{39–41} the potential toxicity to normal tissues with targeted particles injection could be prevented

through appropriate choice of particle type and well control of injection time, procedure and dose of particulate suspension. Up to now, it is clear that some candidate particles like the iron oxides magnetite (Fe_3O_4), and Au have perfect biological compatibility and have been widely used in clinics. Meanwhile, using nanoparticles to deliver antineoplastic drug or angiogenesis to damage target tumors is also proved feasible for tumor treatment. For instance, Bischof et al.³⁵ proposed a novel method using gold nanoparticle-assisted tumor necrosis factor- α delivery in combination with hyperthermia, which significantly delayed tumor growth, reduced tumor cell survival rate and tumor blood perfusion. All of these working medium and techniques can also possibly be used in nano-cryosurgery.

3. EXPERIMENTAL PHENOMENA ON NANO-CRYOSURGERY

3.1. Tissue Level Freezing Enhancement

What presented in Figure 3 are typical temperature response curves of pork tissue either with or without loaded metal nanoparticles (aluminum), respectively.¹⁹ It can be found that, the lowest temperature for the case of injecting nanoparticles can reach $-115\text{ }^\circ\text{C}$ at the position with a distance of 5 mm from the probe wall. This magnitude is much lower than the lowest temperature of its counterpart case of no injection. The late one only achieves a lowest $-75\text{ }^\circ\text{C}$ at the same tissue position when subjected to the same freezing condition as above. This was owing to the enhanced heat conduction due to addition of metal nanoparticles into tissues. To one’s surprise, the improvement of freezing due to injection of nanoparticles is rather significant.

Except for adopting the highly conductive nanomaterials, nanoparticles with lowered thermal conductivity can also have unique virtue in cryosurgery. However,

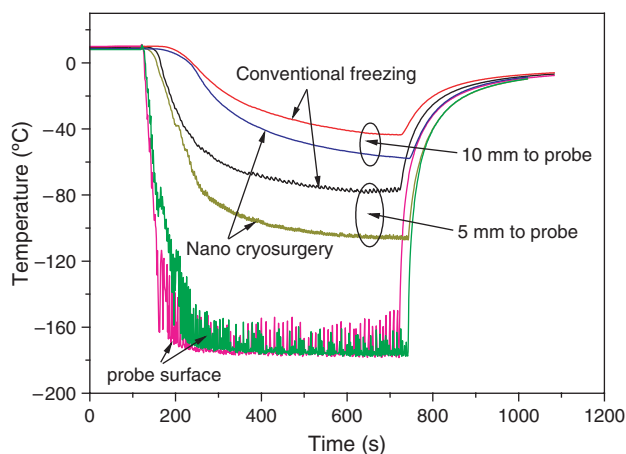


Fig. 3. Comparison between freezing temperature responses of pork tissues either with or without injected nanoparticles. Reprinted with permission from [19], T. H. Yu et al., *Cryobiology* 50, 174 (2005). © 2005.

its role is not to enhance but to weaken heat transfer at a specific tissue region. In this way, it can help prevent over freezing on the healthy tissues surrounding the target tumors during cryosurgery. Besides, if such nanoparticles, most probably non-metal materials, are injected into the edge of solid tumor, the normal tissues can be possibly protected. Overall, the nano-cryosurgery provides a flexible way for controlling the process of a cryosurgery and avoiding damaging normal tissue.

3.2. Regulation of Ice Formation

The iceball growth during cryosurgery can be artificially controlled via asymmetrically loading nanoparticle suspension to the targeted tissues. This makes cryosurgery more flexible for tumor treatment. Generally, the conventional cryosurgical technique is often hard to produce an optimal cryolesion area due to the irregularly shaped tumor. However, by virtue of injected nanoparticles, the growth direction and orientation of an iceball can be well modified as desired, which guarantee a conformal cryosurgery.

To evaluate the capacity of controlling the size, shape and orientation of the iceball formation via injecting nanoparticle suspensions with specific thermal properties into the target tissues, Yan et al. had ever adopted a medical infrared thermometer to map the temperature profile over the whole surface above the freezing area.²³ The cryosurgical procedure was performed using a minimally invasive cryoprobe cooled by liquid nitrogen in order to obtain a deep regional freezing. The obtained infrared image was applied to monitor and evaluate the ice ball formation process. Simulation experiments on biological tissues (fresh pork and liver) were performed *in vitro* and four different liquids were injected into the test materials, which were distilled water, an aqueous suspension of aluminum nanoparticles in water, ethanol and a 10% solution of the cryoprotective agent dimethylsulfoxide (Me₂SO), respectively. It was clearly demonstrated that the localized injection of an appropriate functional solution could effectively regulate the tumor-killing area via directional freezing.

In a typical test as shown by Figure 4, different volumes for the nanoparticle suspension were considered.²³ Yan et al. injected symmetrically aqueous suspension of aluminum nanoparticles in water via a clockwise direction from sign 1 to sign 4 at the same distance from the cryoprobe. The volume for the injection is respectively 1 ml, 2 ml, 3 ml and 4 ml. Clearly, as the thermal images indicated, different volumes of injecting solution have resulted in varied or asymmetrical magnitudes on iceball formation. The more volume for the solution to be injected, the more possible the iceball will grow fast toward that direction.

Further, Yan et al. also performed visual experiments using both digital camera (optical measurement) and medical infrared thermometer (functional measurement) to

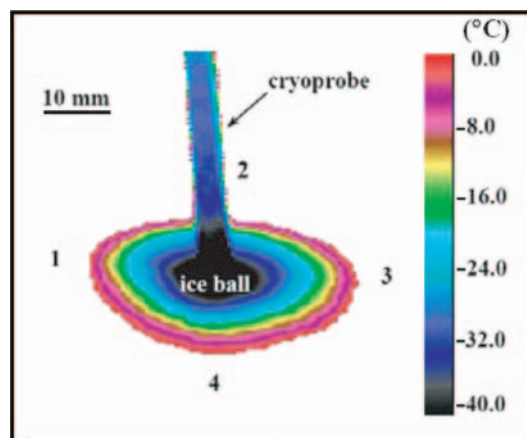


Fig. 4. Thermal image for temperature distribution on pork tissues injected with 1 ml, 2 ml, 3 ml and 4 ml (from sign 1 to sign 4) aqueous suspension of aluminum nanoparticles in water, respectively. Modified with permission from [23], J. F. Yan et al. *The 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS)*, Shanghai, China, September (2005). © 2005.

monitor the iceball volume.⁴² The cryosurgical procedure was performed using one or two minimally invasive cryoprobes cooled by liquid nitrogen in phantom gel and biological tissues (fresh pork and liver). According to the experimental measurements on gel, the maximum freezing rate and iceball volume in carbon nanotube and gel mixture could respectively increase to 67.6% and 40%, compared to that in pure gel. The similar results were also obtained in the tissue experiments that 6%–10% increasing difference of iceball covered area in none-nanoparticle and nanoparticle injected zone could be found under the same condition.

In summary, localized injection with nanoparticle suspensions during cryosurgery was feasible and could serve as an effective supplement to the conventional cryosurgery. The variation in kinds and dosages of nanoparticle solutions could play different roles in the freezing process and lead to different freezing effects. Therefore, election of nanoparticles and optimization of nanoparticle suspension dose were important issues to address in future research.

3.3. Cellular Level Injury by Nano-Cryosurgery

Deng et al. investigated effective cryosurgical adjuvants using nanoparticles to enhance freezing injury of brain tumor cells.⁴³ In their experiments, human glioma cell line SF763, was cultured in standard medium consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in a humidified 95% air and 5% CO₂ atmosphere. Before experiments, cells were detached from the culture flask and washed with 0.25% Trypsin solution and phosphate buffered saline (PBS). The cells suspended in PBS were then divided into 3 groups. Group 1 was the control group, in which no nanoparticles were loaded.

Group 2 and group 3 were treated with carbon nanotube (CNT) and silver nanoparticles (SNP), respectively. After this, cells in group 2 were suspended in a solution of 2 mg/ml SNP, and cells in group 3 were suspended in a solution of 2 mg/ml CNT.

The freezing experiments on cell suspensions were carried out by using a liquid-nitrogen-based cryosurgical system with a 5 mm diameter cryoprobe.^{44,45} The general protocol of the freezing experiment was as follows. First, a sample was cooled for 8 minutes by the cryoprobe. Second, freezing was temporarily stopped. After 7 minutes thawing naturally in the air, the sample was cooled once again for 5 minutes. Freezing was then stopped, and the frozen sample was thawed in an isothermal water bath. Finally, cell viability of the sample after two freeze-thaw cycles was assessed by typan blue dye exclusion under a biological microscope (DM-IRB, Leica). The viability was calculated from the numbers of all cells appeared with intact membrane integrity. Cell death rate was defined by the ratio between the viability difference (before and after experiments) and the viability before the experiments.

Figure 5 shows the temperature responses and temperature changing rates of the cellular suspensions for three groups during freezing and thawing, in which group 1 is for the case without introducing nanoparticles, and group 2 denotes the case of adding CNT into cell suspension while group 3 is the case of treating the cell suspension with SNP. The temperature measurement positions for all cases were at a distance of 1mm from the tip of cryoprobe. In Figure 5, the data of temperature changing rates were obtained by differentiating the polynomial segment fitting functions of temperature curves. For the three different cases, it can be easily found that the temperatures of cell suspensions for the cases of treating with nanoparticles (group 2 and group 3) dropped more quickly than that of the case without treating with nanoparticles (group 1) during freezing, and that the temperature for the case treating with SNP dropped the most quickly among the

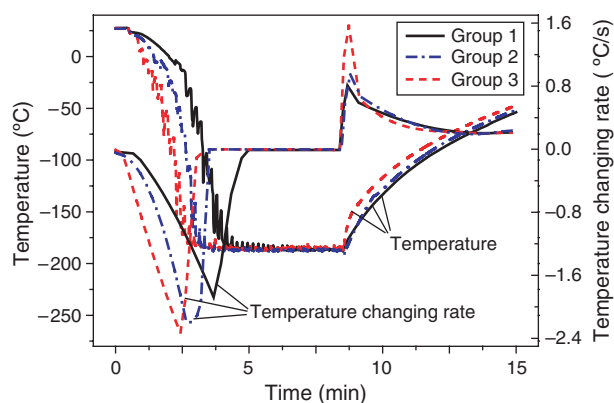


Fig. 5. Temperature responses and temperature changing rates of the cellular suspensions for different groups during freezing and thawing. Modified with permission from [43], Z. S. Deng et al., *International Congress of Refrigeration*, Beijing, China, August (2007). © 2007.

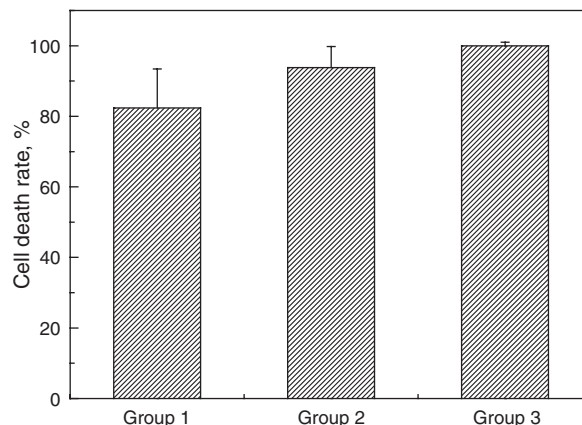


Fig. 6. Percentage of cells destroyed by freezing under different conditions. Reprinted with permission from [43], Z. S. Deng et al., *International Congress of Refrigeration*, Beijing, China, August (2007). © 2007.

three groups. It indicates that from the point view of heat transfer mechanism, introducing nanoparticles with high thermal conductivities to improve the freezing effect is feasible, as had been demonstrated in tissue level study.^{19,20}

Figure 6 depicts the percentage of dead cells respectively for the three different cases, following freezing by the same cryoprobe with same freezing power. An obvious difference in cell death rates can be found between the cases with and without introducing nanoparticles into the cellular suspensions. The results show that in the absence of nanoparticles, a significant percentage (about 18%) of tumor cells survive freezing. Only about 6% of cells survive when the sample was treated with CNT, and all cells were completely destroyed when treated with SNP. It indicates that by introducing nanoparticles, freezing injury to brain tumor cells has been significantly increased, and that using nanoparticles as an adjuvant to cryosurgery could result in complete destruction to tumor cell. However, if no measure has been adopted to improve the cryosurgical injury of cells, tumor recurrence may be resulted from the surviving tumor cells, although all tumor tissue was totally frozen during cryosurgical treatment.

It is well known that the two main mechanisms of cell destruction during cryosurgical procedure are associated with extracellular and intracellular ice crystallization, respectively.¹ When nanoparticles are introduced into cell suspension, they may probably be permeated everywhere within the suspension (both extracellular and intracellular). Consequently, more ice crystals will be formed in both extracellular and intracellular mediums due to the nucleation effect of nanoparticles during freezing. This may be the mechanism of the enhancement of cryosurgical injury to tumor cells by use of nanoparticle adjuvants.

3.4. Effects of Tissue Types on Nano-Cryosurgery

The aim of the nano-cryosurgery is to exactly destroy all tumor cells within the target region. However, the amounts

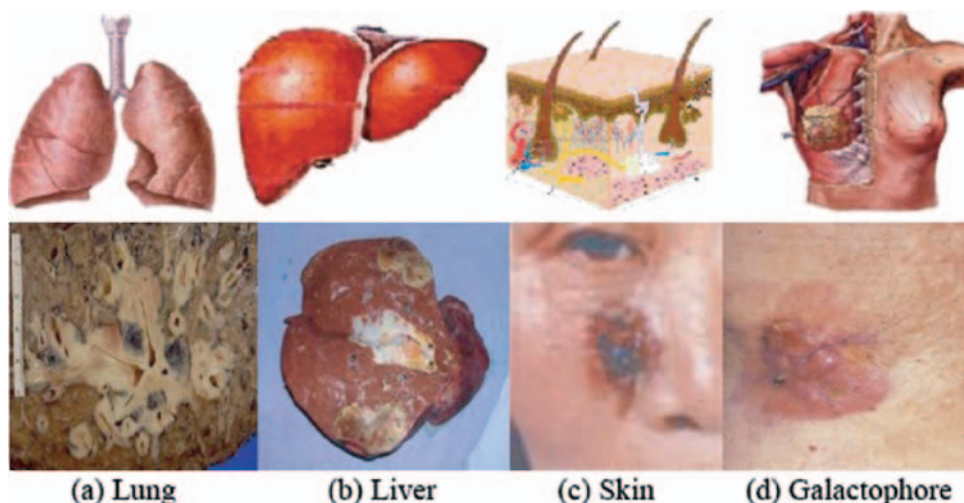


Fig. 7. Anatomical structures for typical tissue types and tumors developed inside them. Modified with permission from [46], Z. Q. Sun et al., *The 3rd IEEE International Conference on Nano/Micro Engineered and Molecular Systems*, Sanya, China, January (2008). © 2008.

of nanoparticles could be loaded generally differ significantly due to variation of tissue structures. This is particularly true for tumors situated at or surrounding some important organs. Since the lethal freezing temperature to tumor cells is heavily tissue-dependent and generally ranges from -20 to -70 °C,³ the structures of each organ are very much different which calls for a specific administration of nano-cryosurgery on each individual tissue. Sun et al. evaluated the temperature responses of different tissue types when subjected to the same cryosurgery by adjunct use of nanoparticles.⁴⁶

Figure 7 illustrates an overview on tumor types developed in corresponding organs and tissue via a dissimilar modality.⁴⁶ Clearly, it is not wise to neglect their variation when performing a treatment planning on nano-cryosurgery. In fact, each tissue has its unique anatomical structure, shape, micro or bulk properties. Generally, distribution of blood vessels, endocrine pipeline, and cell organizations are very different for such tissues.

Knowing the temperature changing behavior of tissues due to particularities in porosity, water content, density, blood perfusion, thermal conductivity, specific heat and other property is critical for the clinicians to determine the operation scheme, and plan for surgical process such as amount, duration time and place to load nanoparticles, or the way to inject it.

Sun et al. selected three kinds of typical tissues, such as pork, porcine heart and liver.⁴⁶ Skin cancer and liver cancer are frequently encountered in clinics. Focus was placed on the temperature responses due to loading of nanoparticles to the dissimilar tissue structure. In the first experiment, three kinds of fresh organs were classified as three groups to simulate nano-cryosurgeries. The way of loading nanoparticles was based on needle injection followed by free spread. And 5% Fe_3O_4 nanoparticle aqueous

was adopted and LN2 cryoprobe system was used for the freezing.

As shown in Figure 8(a), the pork size was about $10\text{ cm} \times 10\text{ cm} \times 6\text{ cm}$. The active part of the cryoprobe was inserted into the center of the tissue, at a distance of about 2.5 cm from the surface. Three thermocouples were adopted to record the temperature data at three points. There were five injected points: one is center, four points are located equidistantly from the center (about 2 cm). For each point, the injection amount of nanoparticle suspension was 2 ml.

The positions for inserting the cryosurgical probes and thermocouples were also illustrated in Figure 8. At the same time, Figure 8(b) also showed the positions of the thermocouples (TC) which were arranged one by one. Thermocouples were inserted into tissues via an angle of 25° with the horizontal level, with measuring points equidistant. As one can see, the first detected point was the temperature of tissue at the probe tip point, with the second one 1.5 cm away from and the third one 3 cm. The nanoparticles were injected into defined area from the five injecting points. Similar experiments were also carried out in more tissue types.

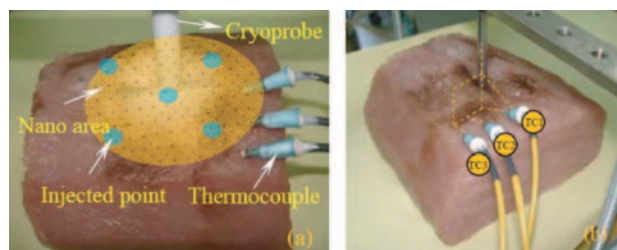


Fig. 8. Sketch for nano-cryosurgery on pork tissue (TC means thermocouple). Modified with permission from [46], Z. Q. Sun et al., *The 3rd IEEE International Conference on Nano/Micro Engineered and Molecular Systems*, Sanya, China, January (2008). © 2008.

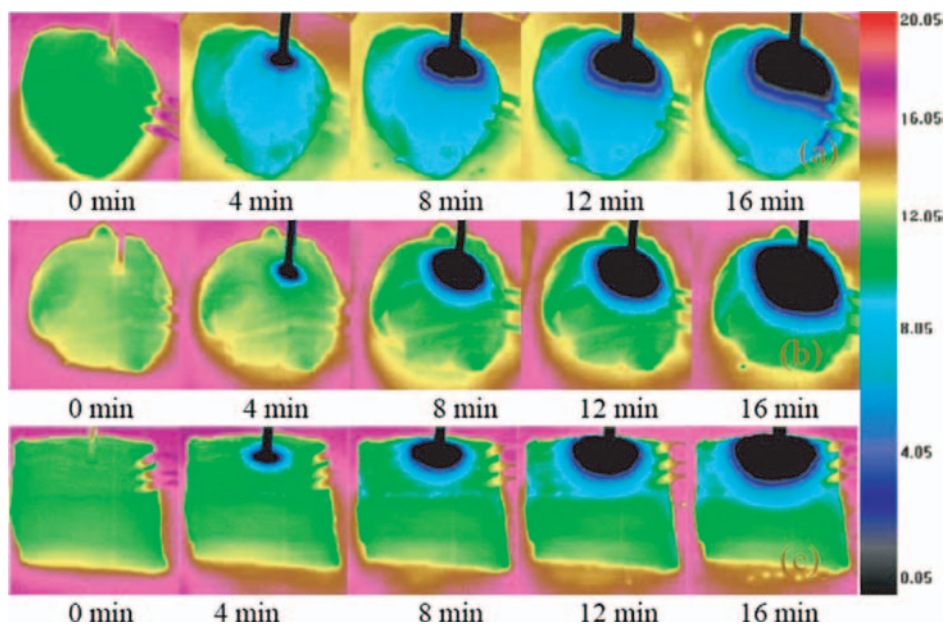


Fig. 9. The infrared images of different tissues: (a) Heart; (b) Liver; (c) Pork. Modified with permission from [46], Z. Q. Sun et al., *The 3rd IEEE International Conference on Nano/Micro Engineered and Molecular Systems*, Sanya, China, January (2008). © 2008.

According to the measurement, one could see that, for different tissues, the temperatures significantly vary between each other under the same freezing. For TC1 which is the nearest to the cryoprobe, there is a nearly 10-degree temperature difference, so did the other two TCs. Taking the type of tissues into account, one can draw a conclusion that the tissue which has a high conductivity would lead to a lower temperature. The TCs in pork were proved to have the lowest temperatures. Clearly, the dense structure in the pork allows having a greater thermal conductivity when loaded with nanoparticles.

As indicated by Figure 9, the size of iceballs becomes larger and larger with the time passing by. The exact size of the iceball can thus be measured. The sizes of iceballs appear diverse because of the dissimilar tissue constructions which illuminate that cryosurgery should vary with the actual situation.

Overall, due to different enhancement by the nanoparticles, pork realized a stronger freezing than liver and heart. If one wishes to realize the same iceball size in dissimilar tissues, different amounts of nanoparticles are required, and a less quantity is needed in pork while a little more is requested in heart. All in all, for each organ, individuation must be considered for controlling the cooling dose in a nano-cryosurgery in order to avoiding negative effect. Pork, liver and heart have diverse properties such as porosity, water content, density, blood perfusion, thermal conductivity, specific heat, and so on. Therefore the amount of nanoparticles which would be injected into tissue should be preliminarily predicted in advance. This will help realize a high quality conformal ablation on target tumor tissues in future clinics. It should be pointed out

that the mechanisms coming from the diverse porosity, water content, density, blood perfusion, thermal conductivity, specific heat, and so on for each tissue need further clarification.

3.5. Nano-Cryosurgery on Tissues Embedded with Large Vessels

In the presence of large blood vessels, special attention should be paid during cryosurgical treatment of tumors because the heat source/sink nature of a large vessel in the cooled tissue may result in insufficient freezing and thus tumor residual or recurrence. From the viewpoint of heat transfer, a large blood vessel (also termed as thermally significant vessel) denotes a vessel larger than 0.5 mm in diameter.⁴⁷ Anatomically, tumors are often situated close to or embedded with large blood vessels, since a tumor's quick growth ultimately depends on nutrients supplied by its blood vessel network. As is well known, the blood flowing through large blood vessels acts as a heat source or heat sink and plays an important role in affecting temperature profiles of cooled or heated tissues.^{48–52} During cryosurgery, the blood flow inside a large vessel represents a source which heats the nearby frozen tissues and, thereby, limits freezing lesions during cryosurgery. Under this condition, a part of the vital tumor cells may remain in the cryolesion and lead to recurrence of tumors after cryosurgical treatment. More specifically, tumor cell survival in the vicinity of large blood vessels is often correlated with tumor recurrence after treatment.⁵³ Consequently, it is difficult to implement an effective cryosurgery when a tumor is contiguous to a large

blood vessel. In fact, the heating effects of large blood vessels on the surrounding tumor tissues during cryosurgery can be eliminated by vascular exclusion in which vascular inflow occlusion is performed by clamping the entrance of the large vessels.^{54,55} However, the vascular occlusion requires a major surgical procedure which will exclude one of the main merits of minimally invasive percutaneous cryosurgery.

Up to now, simple but effective approaches to totally destroy tumor cells in the vicinity of large blood vessels are still not available. To resolve this difficulty, Deng et al. recently proposed to perform cryosurgical treatment for tumor embedded with large vessels through adjuvant introduction of nanoparticles.⁵⁶ The key point of the method lies in that, high thermal-conductive nanoparticle suspension (loaded into tumor tissue before cryosurgery) would significantly enhance the freezing effect of cryoprobes to tumor tissues surrounding large vessels and help completely destroy them.

To illustrate the effectiveness of nano-cryosurgery in the treatment of tumors embedded with large blood vessels, experiments were investigated through simulated experiments in phantom gels and *in vitro* porcine liver tissues.⁵⁶ For all experiments, the large blood vessels were simulated by a 1 mm OD/0.8 mm ID Teflon tube. Water (25 °C) was used to simulate the blood for conceptual illustration. But for practical purpose, the simulating fluid could also take a glycerol solution containing glycerol at a specific concentration and distilled water as the blood analog fluid, which is routinely adopted in previous blood simulation. The flow velocity of water can be calculated from the flow rate, and the value was about 10 cm/s. A liquid nitrogen based cryosurgical system was applied to supply the cooling power, in which two cryoprobes with five mm diameters were selected to perform the simulated cryosurgery.⁴⁴ The total freezing time was 20 minutes. Then, the freezing was stopped and thawing commenced at room temperature.

3.5.1. Phantom Case

The medium used to simulate the biological tissue was a semitransparent gel phantom composed of 10 percent of gelatin and 90 percent of water by weight, which was contained in a cubical organic glass box (the inner dimensions were 16 × 16 × 5 cm). The Teflon tube passed through 1-mm-diameter holes drilled on the two opposite panels of the organic glass box. For all phantom experiments, the distance between two cryoprobes was 25 mm, and the insertion depth of cryoprobes was 35 mm into gel phantom. The vertical distances between blood vessel and the two cryoprobes were equal each other.

Four different cases were investigated for the simulated experiments with tissue phantom, which were: the case of a single large blood vessel without introduced

nanoparticles, the case of a single large blood vessel with adjuvantly introduced nanoparticles, the case of parallel counter-current vessel pairs without loaded nanoparticles, and the case of parallel counter-current vessel pairs with adjuvantly loaded nanoparticles, respectively. Photographs for one experimental setup are shown in Figure 10. For the case of parallel counter-current vessel pairs, the distance between the artery and the back surface of phantom was 20 mm, and the distance between the vein and the back surface of phantom was 10 mm.

For the cases with adjuvantly loaded nanoparticles, a 20 mini-liter (ml) aqueous suspension of Fe₃O₄ nanoparticles (20% wt) was first introduced into the target area before the phantom gel solidified in the cubical organic glass box. Then, the solid mixtures of phantom gel and Fe₃O₄ nanoparticles were formed at the corresponding region, i.e., the black area as shown in Figure 10(b).

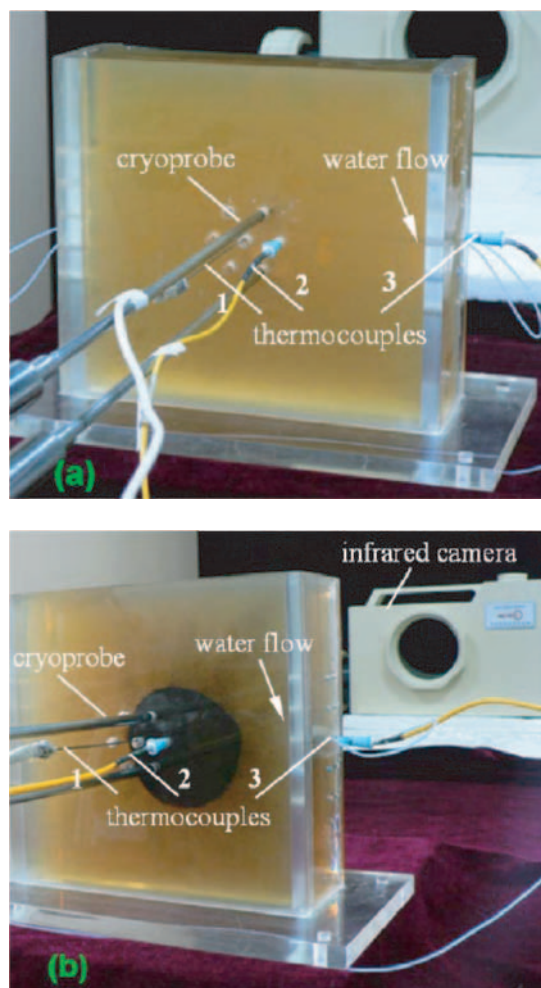


Fig. 10. The experimental setup for the tissue phantom with parallel counter-current vessel pairs in which (a) is for the case without loaded nanoparticles, and (b) is for the case with adjuvantly loaded nanoparticles. Modified with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

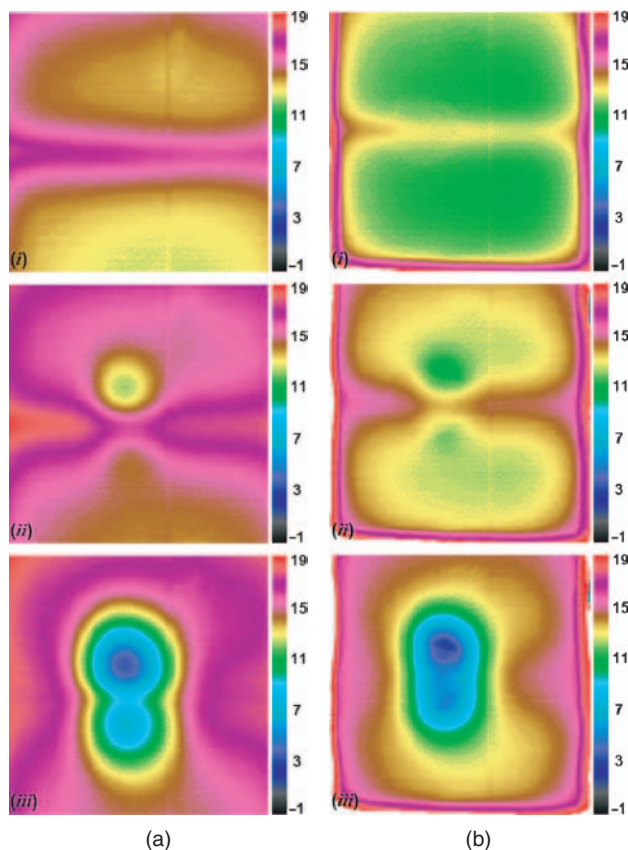


Fig. 11. Infrared thermographs for the case of parallel counter-current vessel pairs in phantom experiments: (a) without introduced nanoparticles; (b) with adjvantly introduced nanoparticles. Note: (i) before freezing, (ii) after 10 minutes of freezing, and (iii) after 20 minutes of freezing. Modified with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

In Figure 10, the U-type Teflon tube plays both roles of artery and vein. When the water flowed through the cubic box for the first time, it served as the arterial blood. When the water re-flowed through the box, it was regarded as venous blood. The infrared thermographs for the above cases are shown in Figures 11. Similar results for the cases of a single large vessel had been obtained. Since the water flow in counter-current vessel pairs provides more heat energy than does a single large vessel, the tissue phantom surrounding the vessels was harder to freeze compared to the case of single large vessel. The concave shape of the isotherm or iceball became more evident, and finally took on the shape of “8.” For the case of parallel counter-current vessel pairs without loaded nanoparticles, the 8-shape isotherm in the phantom tissue did not disappear since it formed about 15 minutes after freezing (as shown in Fig. 11(a)), due to the heating effect resulting from uninterrupted water flow in the large vessel. For the case of parallel counter-current vessel pairs with adjvantly introduced nanoparticles, the 8-shape isotherm also formed about after 15 minutes of freezing (as shown in Fig. 11(b)). However, it was found that the water flow

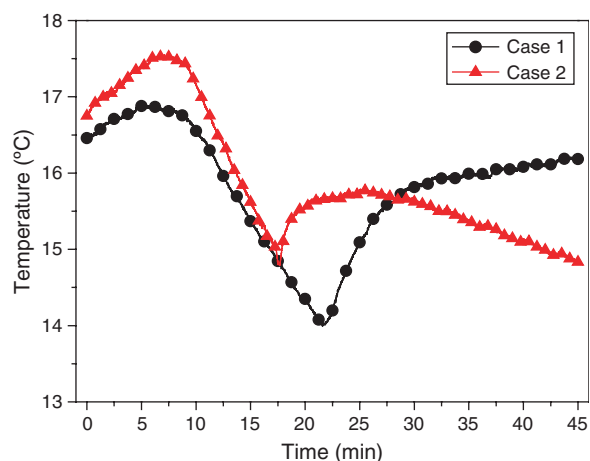


Fig. 12. Transient temperatures of thermocouple 3 for two different cases (phantom experiments) in which case 1 denotes the case of parallel counter-current vessel pairs without introduced nanoparticles, case 2 denotes the case of parallel counter-current vessel pairs with adjvantly introduced nanoparticles, and the sites of thermocouple 3 are shown in Figures 10(a) and (b) for the above two cases, respectively. Reprinted with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

stopped after about 16.5 minutes of freezing. Then, the 8-shape isotherm disappeared after about three more minutes of freezing since the water flow stopped (as shown in Fig. 11(b)).

Figure 12 shows that, the temperature curve for the case with loaded nanoparticles has an inflexion point. Different from the case of a single vessel with nanoparticles, the temperature for this case (shown in Fig. 12, case 2) starts to increase after the inflexion point occurred. The reason for this phenomenon lies in that the location of thermocouple 3 for this case was close to the inlet of a vein, whereas for the case of a single vessel, the thermocouple was close to the inlet of an artery. At the inlet of a vein, the local temperature of the phantom tissue, which almost had not been affected by the freezing of cryoprobes, could be cooled by the water flow in the vein (flowing from the cooled section of artery). After the vessels were frozen, the cooling effect did not exist any more, and then the temperature in the vicinity of the vein inlet could increase. Moreover, it can also be seen from Figure 12 that during the first several minutes after the freezing started, the temperature of the phantom tissue had slightly increased since the phantom had just been taken out of the cooling room of a refrigerator 10 minutes before the experiments, and the temperature of the phantom tissue was lower than the temperature of water flow at that time. The above results can be attributed to the higher thermal conductivity of the metallic oxide nanoparticles. These results imply that nanoparticles with high thermal conductivity can serve as effective adjuvants for enhancing the efficacy of cryosurgical treatment of tumors with embedded large blood vessels.

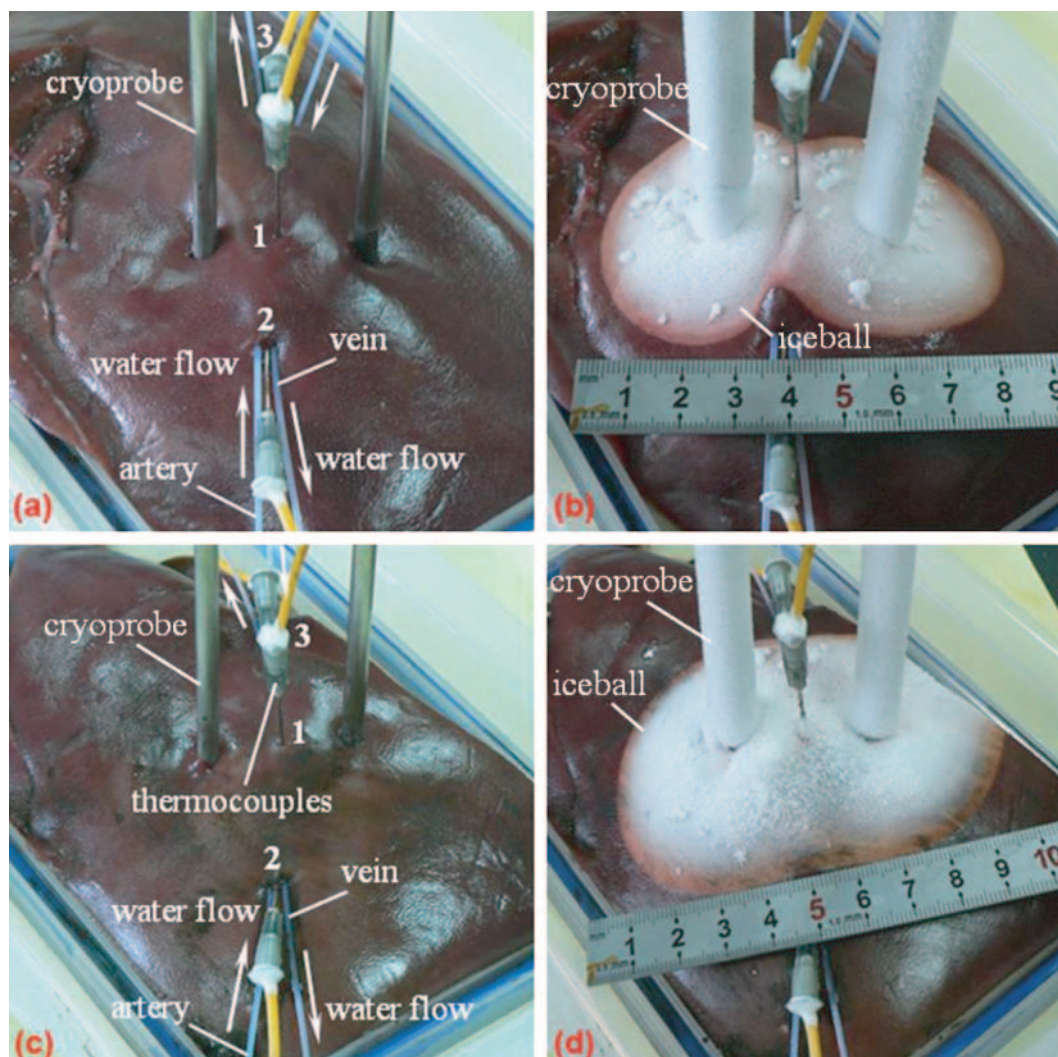


Fig. 13. The experimental setup for *in vitro* porcine liver tissue with parallel counter-current vessel pairs in which (a) and (b) are for the case without introduced nanoparticles, and (b) shows the iceball formed after 20 minutes of freezing. Parts (c) and (d) are for the case with adjvantly introduced nanoparticles, and (d) shows the iceball formed after 20 minutes of freezing. Modified with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

3.5.2. *In vitro* Tissue Case

In order to more realistically simulate the thermal effect of a large blood vessel during an actual cryosurgery, Deng et al. also perform similar simulated experiments inside *in vitro* porcine liver tissues. The distance between two cryoprobes was 35 mm, and the insertion depths of cryoprobes were about 25 mm in liver tissues. The depths of large blood vessels were taken as 5 mm from the surface of liver tissues. The vertical distances between the blood vessel and the two cryoprobes were equal each other.

Photographs for one experimental setup are shown in Figure 13. For the case of parallel counter-current vessel pairs, the locations of thermocouples 1, 2, and 3 are shown in Figures 13(a) and (c), in which thermocouples 2 and 3 are inserted along the direction of the blood vessels at the same depth as the blood vessels. The insertion

depth of thermocouple 1 was also 5 mm, and the vertical distance between thermocouple 1 and the vein vessel (shown in Figs. 13(a) and (c)) was about 2 mm. For the cases with adjvantly loaded nanoparticles, 10 ml aqueous suspension of Fe_3O_4 nanoparticles (20% wt) was injected into the target area of the liver tissue before freezing.

The experimental results for *in vitro* study on porcine liver tissues are shown in Figure 14. The infrared thermographs show representative results for illustrative purposes. For the case with adjvantly introduced nanoparticles, it was found during the experiment that the water flow stopped after about 14 minutes of freezing.

Figure 15 shows the transient temperatures of thermocouples 1, 2, and 3 for the cases of parallel counter-current vessel pairs without and with introduced nanoparticles, respectively. The sites of thermocouples 1, 2, and 3 were

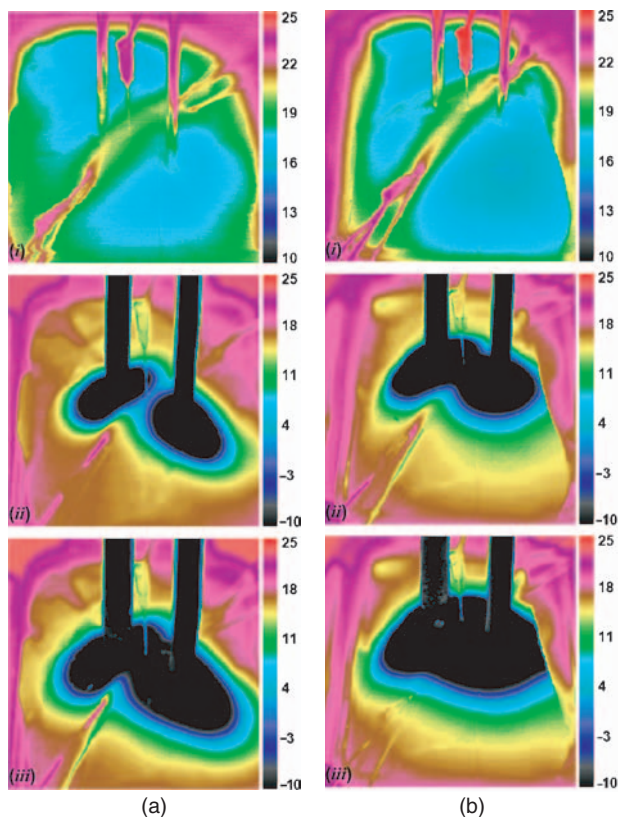


Fig. 14. Infrared thermographs for the case of parallel counter-current vessel pairs in *in vitro* tissue experiments: (a) without introduced nanoparticles; (b) with adjvantly introduced nanoparticles. Note: (i) before freezing, (ii) after 10 minutes of freezing, and (iii) after 20 minutes of freezing. Modified with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

shown in Figure 13. It can be found from case 1 in Figure 15 that for the case without introduced nanoparticles, the temperature curves appear to be relatively smooth. For the case with adjvantly introduced nanoparticles, a jump in the cooling rate of liver tissue is observed (as shown in case 2 in Fig. 15). The time for this jump to occur was at about 15 min, i.e., about 14 minutes after freezing was started, which agrees with the time when water flow in the large vessels stopped.

The aforementioned experimental results indicated that when cryosurgical treatment is performed for tumors embedded with large vessel(s), the rapid flow of blood through the large vessel(s) will cause a heating effect on the target tissues, and such heating prevents freezing of the large vessel(s) and the surrounding tissues. With adjvantly introduced nanoparticles, nano-cryosurgery can significantly enhance the freezing efficacy of tissues and totally freeze the tumor tissues in the vicinity of large vessel(s). It is expected to serve as an attractive modality for treatment of tumors embedded with large blood vessel(s), due to its convenience in operation and excellent performance in disabling the thermal effect of large vessel(s).

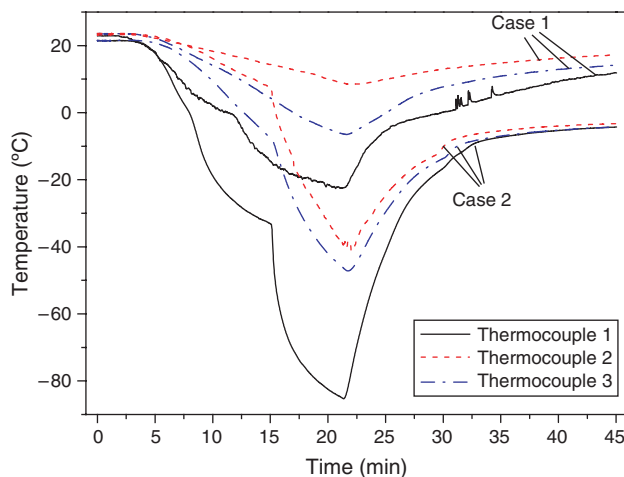


Fig. 15. Transient temperatures at three different sites for *in vitro* tissue experiments. Case 1 denotes the case of parallel counter-current vessel pairs without introduced nanoparticles, in which the sites of thermocouples 1, 2, and 3 are shown in Figure 13(a); and case 2 denotes the case of parallel counter-current vessel pairs with adjvantly introduced nanoparticles, in which the sites of thermocouples 1, 2, and 3 are shown in Figure 13(c). Modified with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

4. THEORETICAL MODELING ON NANO-CRYOSURGERY

4.1. Modification of Tissue Properties via Nanoparticles

So far, a detailed understanding on the mechanisms for the modified bioheat transfer process in tissues due to injected nanoparticles is still rather limited. But interpreting such issues can be started by extending existing phenomenological models. Generally, the thermal properties for the composite consisting of cancerous tissue and injected nanoparticles depends on the particle concentration and the amount of injected suspension.³⁶ If assuming homogeneity within the target region, the mean value for the specific heat C , density ρ and thermal conductivity K of cancerous tissues embedded with nanoparticles can be approximated by a serial arrangement of the two materials with the respective volume proportions.³⁷

$$\rho = (1 - \eta)\rho_1 + \eta\rho_2 \tag{1}$$

$$C = (1 - \eta)C_1 + \eta C_2 \tag{2}$$

$$\frac{1}{k} = \frac{(1 - \eta)}{k_1} + \frac{\eta}{k_2} \tag{3}$$

where, $\eta = (4/3)n\pi r^3$ stands for the volume concentration of particles, r the radius of the nanoparticles, n the number of the nanoparticles in per unit volume of tissue; the subscript 1, 2 refers to the tissue and nanoparticle suspension, respectively. Such equation can be approximately applied to evaluate whether loading nanoparticle suspensions (material 2) into tissue (material 1) will modify the target as desired.

Except for the above simple expression, the classical Hamilton-Crosser (H-C) model can also be adopted to predict thermal conductivity of the nanoparticle suspension which could effectively describe the macroscale thermal conductivity of nanoparticle-tissue mixtures without considering size effect of nanoparticles. For example, in frozen area and unfrozen area, the thermal conductivity for the treated object can be depicted, respectively, as follows:

$$k_f = k_{ft} \cdot \frac{k_p + 2k_{ft} - 2\eta(k_{ft} - k_p)}{k_p + 2k_{ft} + \eta(k_{ft} - k_p)} \quad (4)$$

$$k_u = k_{ut} \cdot \frac{k_p + 2k_{ut} - 2\eta(k_{ut} - k_p)}{k_p + 2k_{ut} + \eta(k_{ut} - k_p)} \quad (5)$$

where, subscript *ft* and *ut* mean frozen and unfrozen pure tissues, respectively. Subscript *p* stands for the loaded nanoparticles.

If including the effects of nanolayer thickness, nanoparticle size, volume fraction, and different kinds of nanoparticles, the final expression of effective thermal conductivity of unfrozen particle embedded tissue can also be further expressed as:

$$k_u = \left(3\Theta\alpha_T + \frac{3\Theta^2\alpha_T^2}{1 - \Theta\alpha_T} + 1 \right) k_{ut} \quad (6)$$

with

$$\Theta = \frac{\beta_{lf}[(1 + \gamma)^3 - \beta_{pl}/\beta_{fl}]}{(1 + \gamma)^3 + 2\beta_{lf}\beta_{pl}} \quad (7)$$

where, α_T is the total volume fraction of the original nanoparticle and nanolayer; γ is the ratio of the nanolayer thickness to the original particle radius; $\beta_{lf}, \beta_{fl}, \beta_{pl}$ are, respectively, the ratio coefficient of thermal conductivities of the nanofluid, nanolayer, and nanoparticles. For brevity, we will not discuss this model here. Readers are referred to Ref. [58] for more details.

4.2. Tissue Level Heat Transfer

Thermal conductivity affects significantly the phase change heat transfer process. If the thermal conductivity of target tissue is increased, the temperature there would decrease faster during freezing, and thus induce a larger cryolesion scale. To theoretically evaluate the freezing enhancement by injecting solution with high thermal conductivity, a detailed numerical simulation has been performed before by Deng and Liu.²⁰ In the calculations, the solution was assumed uniformly distributed throughout the target domain after injection. The solution with high thermal conductivity can be made up by mixing nanoparticles of metaloxide such as Fe_3O_4 nanoparticles with water, which have been widely used in RF-magnetic hyperthermia.

Presented in Figure 16 is a comparison of the temperature responses between two cases: no injection and injection. The transient temperatures are shown for 3 positions,

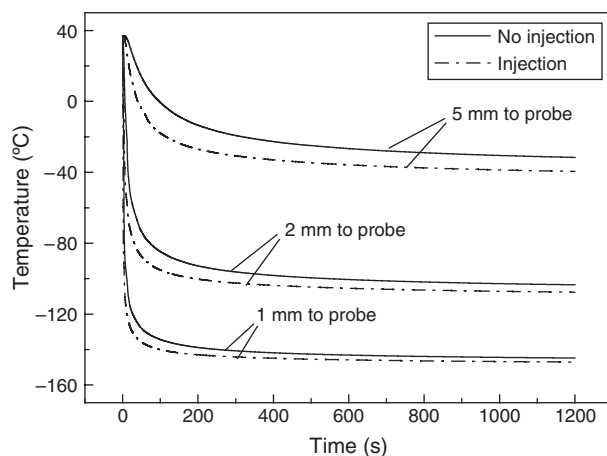


Fig. 16. Comparison of temperature responses between the cases of injecting solution with high thermal conductivity and that without injecting solution. Reprinted with permission from [20], Z. S. Deng and J. Liu, *Cryobiology* 50, 183 (2005). © 2005.

which are at the distances of 1 mm, 2 mm, and 5 mm from the probe wall, respectively. It can be seen that the temperature at the same spot greatly decreases due to injection of nanoparticle suspension. The maximum temperature difference between the cases of injection and no injection reaches about 15 °C at the spot which is away from the probe by 5 mm. These results show that injecting solution with high thermal conductivity into the target tissues to improve the freezing effect is highly feasible, as has already been demonstrated experimentally in Figure 3.¹⁹

4.3. Cell Level Response of Nano-Cryosurgery

Nano-cryosurgery can also produce a predictable temperature response on the target cell. Yan and Liu evaluated the freezing effect of a single tumor cell when different kinds of nanoparticles have already been injected in or outside the cell membranes. The computational domain was simplified and divided into two parts: one is intracellular area, the other is extracellular area.³³ And a spherical coordinate system in one dimension was used. Calculations of heat transfer are based on the widely accepted Pennes bioheat model.

Presented in Figures 17 are the temperature responses and freezing rates at the core of cell during freezing for the situations loading with different kinds of nanoparticles when their volume fraction are $\eta = 1\%$ in cell and $\eta = 10\%$ outside the cell, respectively. It can be seen that different kinds and concentrations of nanoparticles produce different influence on the freezing rate in the cell. Large volume fraction of nanoparticles with high thermal conductivity could evidently increase the freezing rate of a cell. As shown in Figure 17, at the same volume fraction, polytetrafluoroethylene (PTFE) and diamond play a much more significant role in affecting the freezing rate than other candidate particles. This can be attributed

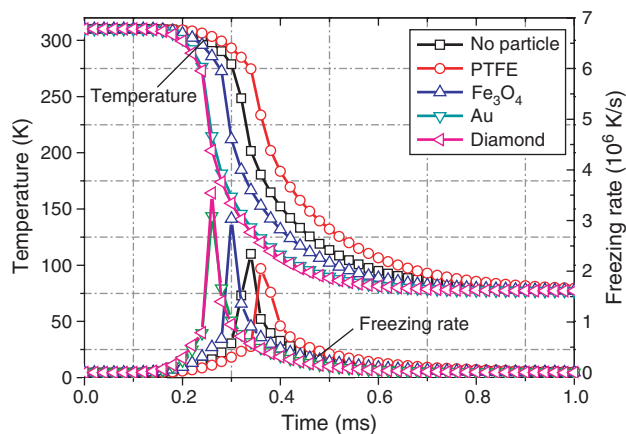


Fig. 17. Transient temperature and freezing rate at the core of the studied cell for different nanoparticle loading situations where the volume fraction of particles is distributed uniformly by $\eta = 1\%$ within the cell interior and outside the cell by $\eta = 10\%$. Modified with permission from [33], J. F. Yan and J. Liu, *Nanomedicine: Nanotechnology, Biology, and Medicine* 4, 79 (2008). © 2008.

to their lowest and highest thermal conductivity, respectively. However, calculations also show that at one volume fraction, there would be limitation of increasing freezing rate by way of using particles with larger thermal conductivity. That is, concentrations have close correlations with thermal conductivities of particles on the contribution to the freezing enhancement. For instance, although diamond has a thermal conductivity exceeding that of Au by 5 fold, it is noted that if its volumetric loads are not high enough there is almost no differential cooling effect with nano-Au. Therefore, choosing an optimal concentration with appropriate particles is crucial to maximize the effects of cryosurgery with minimum cost. In addition, with the increase of volume fractions in tumor cells, the influence induced by particles becomes stronger and more apparent.

An average temperature decreasing rate at the core of cell can be defined as $\bar{B} = -(1/\tau) \int_0^\tau (\partial T/\partial t) dt$, where τ is the freezing time. Here the total freezing time is calculated as 1 ms since the freezing procedure tends to be relatively stable after that. An interesting result can be found in Figure 17 that at the same concentration of particles, the time of maximum freezing rate occurs in accordance with the order of thermal conductivity. The diamond with largest thermal conductivity results in the earliest maximum freezing rate while the PTFE results in the last one. However, it seems that the value of maximum freezing rate has no clear rule with the kinds and volume fractions of particles. Meanwhile, it can also be seen that when $\eta = 0.2$, the best thermal conductivity particles (diamond) does not necessarily guarantee reaching a maximum freezing rate. Therefore, one can come to the conclusion that the maximum freezing rate not only depends on the thermal conductivities but also relies on the volume fraction

and other thermal properties such as density, heat capacity, and latent heat etc. As for the average freezing rate, it can be found that the better thermal conductivity and the larger volume fraction it has, the higher value of average freezing rate it could reach. It can be found that the maximum average freezing rate reaches about 2.33×10^5 K/s when diamond is used and $\eta = 20\%$ outside the cell, and the increasing magnitude attains about 2.5% compared with PTFE state. Likewise, the maximum magnitude of maximum freezing rate can reach 76% when Au is used and $\eta = 20\%$ outside the cell compared with no-particle case. Therefore, from the above discussion, it is clear that nano-cryosurgery could produce stronger freezing effects than that of conventional ones especially on the maximum freezing rate. Such influence is very important in a large extent to enhance killing of tumor tissues during cryosurgery.

4.4. Nucleation Mechanism of Nano-Cryosurgery

Besides its effects on freezing speed, nanoparticle also plays an important role in inducing ice nucleation which is critical in determining the final cell damage. As has been theoretically interpreted in Yan and Liu's work, using nanoparticles as seeds, heterogeneous nucleation rate could be significantly improved.³³ When nanoparticles are introduced into cells, they could help significantly increase the possibility of nucleation there, which in turn would result in a high killing effect. It is such factor that results in a higher PIF leading to a lethal killing of tumor cells. Experimentally, when doing a cryosurgery on a biological tissue, the tissues with loaded nanoparticles will often become frozen earlier than that in a normal tissue when subjected to the same freezing condition (see Fig. 17). This is just partially caused by the particle induced ice nucleation.

Except for the thermal aspect of using nanoparticles to enhance cryodestruction, there are still many other biological and physico-chemical reasons that need to be explored as well. For example, nanoparticles might change the structure/shape of ice crystals formed during a freezing process. Previously, as had been reported by Rubinsky and Colleagues, some forms of ice crystals are more damaging than others.¹ In addition, considering that some nanoparticles could deliver antineoplastic drug and induce ice formation in cell during freezing, such multichannel comprehensive tumor killing factor is bound to increase the curative rate of tumor and decrease the recurrence rate of cryosurgery. Clearly, it is worth of investigation on an animal or clinical basis along this direction in the future.

Figure 18 quantitatively depicts the influence of particle size and volume fractions upon nucleation effectiveness. It is easy to see that once nanoparticles are loaded in cell, heterogeneous nucleation rate would be significantly high at the beginning of freezing process in comparison with

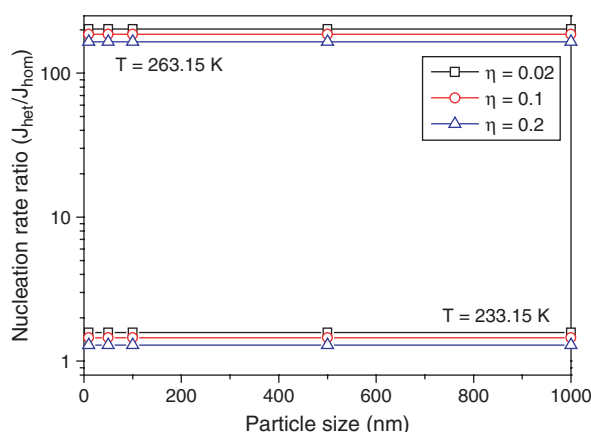


Fig. 18. The influence of particle size and volume fractions upon nucleation effectiveness at two different temperature levels. Modified with permission from [33], J. F. Yan and J. Liu, *Nanomedicine: Nanotechnology, Biology, and Medicine* 4, 79 (2008). © 2008.

no-particle case which implies that nano-cryosurgery could result in cell death at a relatively high temperature threshold. In addition, if particle size exceeds the radius of the critical cluster, its influence on ice nucleation keeps the same. However, it is interesting to note that particles with low volume fraction can increase the nucleation rate much higher than the ones with high volume fraction. This is a contradiction when involving with freezing enhancement. Consequently, the optimal relationship between appropriate particle size, concentration and freezing process, ice nucleation to maximize the cell death should be further studied.

Figure 19 shows the effect of various kinds of nanoparticles on cell (M5) nucleation rate during freezing process.⁵⁸ The case of nano-Ag has the shortest time (650 s) to reach maximum nucleation rate ($5.82 \times 10^{31} \text{ m}^{-3} \text{ s}^{-1}$) while the case of no particles has the longest one (1150 s). Therefore, it can be seen that, at the same position, intracellular ice formation happens earlier in the cell with nanoparticles than the one without nanoparticles. In addition, various kinds of nanoparticles have different influence on the IIF of the target cell. From Figure 19, it can be observed that IIF is mainly dependant on the thermal history of the cell. The cell with nano-Ag which results in the fastest freezing process leads to the earliest IIF. However, a faster freezing process does not necessarily leads to an earlier IIF. When comparing nano- Fe_3O_4 case with nano-Au case, the cell with nano- Fe_3O_4 results in an earlier IIF than the one with nano-Au, though the freezing rate of the later case is higher than the former one. The reason lies in that nano- Fe_3O_4 has a high ν_p which has more influence on the nucleation rate than the characteristic temperature T . Therefore, it can be said that some characteristic features of nanoparticles can also play important role along with characteristic temperature T to influence the IIF which is commonly believed the major factor leading to irreversible damage to the cell. If proper characteristic

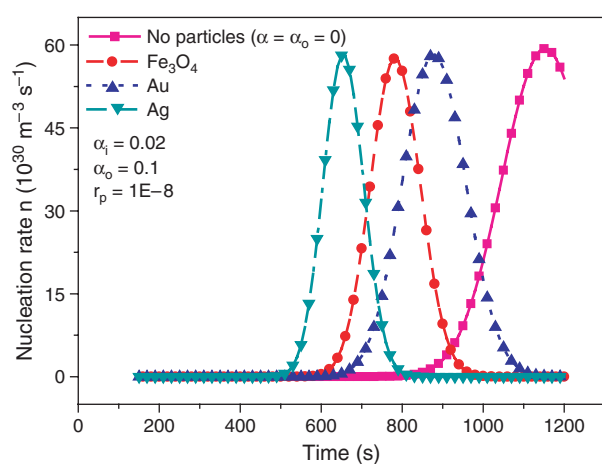


Fig. 19. Effect of various kinds of nanoparticles on cell nucleation rate during freezing procedure. Reprinted with permission from [58], J. F. Yan and J. Liu, *J. Appl. Phys.* 103, 084311 (2008). © 2008.

features of nanoparticles are taken in cryosurgery, it is possible to realize the maximum killing effect.

4.5. Cellular Level Transport Process in Nano-Cryosurgery

At present, the most commonly used model predicting the cell volumetric change is developed by Mazur.⁵⁹ Based on this classical model, the well known two factor hypotheses were proposed to describe the biophysical responses coupled to tissue injury during freezing process. Cell dehydration and intracellular ice formation (IIF) are two major factors which have been proved and recognized as an important mechanism to explain cell injury as a result of the freezing process.^{60–65} However, up to now, no existing model can be directly adopted to predict the dynamics of both the thermal conditions inside frozen tissue and cellular-level biophysical events when nanoparticles are loaded into tissues and cells. For such reason, a modified Mazur's model was developed by Yan and Liu to probe into the biophysical events such as water transport (or cell volumetric change) and IIF, experienced by cells with nanoparticles during freezing.⁵⁸ The Pennes bio-heat equation using effective heat capacity as governing equation was applied to describe the phase change process of tissues during cryosurgery.⁶⁶ For unfrozen particle embedded tissues, a model of the effective thermal conductivity of nanoparticle-fluid mixture which considers the effects of nanolayer thickness, nanoparticle size and volume fraction was adopted.⁶⁷ On the other hand, the Hamilton-Crosser model was applied to calculate the effective thermal conductivity of frozen particle embedded tissues.⁶⁸ The modified model and numerical results are expected to provide valuable information to optimize the protocol of nano-cryosurgery.

Figure 20 shows the final cell volume at four measurement points (M2 to M5) with different volume fraction of

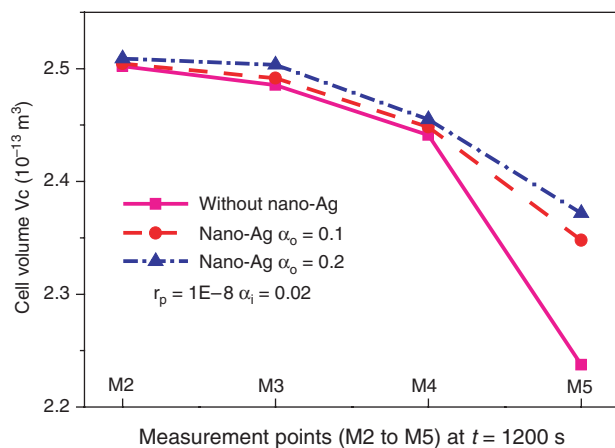


Fig. 20. The final cell volume at each measurement points (M2 to M5) with different volume fraction α_o of nano-Ag at $t = 1200$ s ($r_p = 1 \times 10^{-8}$ m, $\alpha_i = 2\%$). Reprinted with permission from [58], J. F. Yan and J. Liu, *J. Appl. Phys.* 103, 084311 (2008). © 2008.

nano-Ag at $t = 1200$ s. It is clearly seen that once the volume fraction of nano-Ag increased, its influence on the target cell far from the probe could be stronger than the one near the probe and this could be useful to improve killing effects of the cells far away from the probe.

Figure 21 reflects the influence of various kinds of nanoparticles on the thermal history of the cell (M5) and its corresponding volume response during freezing process. It is easy to see that various kinds of nanoparticles result in different transient temperature profile which leads to different water transport process. The reason lies in that various kinds of nanoparticles lead to different thermal conductivities of particle-tissue mixtures which create different thermal dynamics. Consequently, the whole water transport process which has a close relationship with the characteristic temperature T would be different. The higher value of thermal conductivity of nanoparticles is the higher value of final cell volume will be. From Figure 21(b), it can be concluded that the nano-Ag which has the highest thermal conductivity value produces much more influence on freezing as well as water transport process of target cell than that by nano-Au or nano- Fe_3O_4 .

All in all, nano-cryosurgery has stronger freezing effects than that of conventional one. By taking full use of this advantage, some insufficient freezing area usually occurred when treating large tumors in a conventional cryosurgery could be prevented. In addition, it can be found that when nanoparticles are introduced into tumor cells, they would result in an earlier IIF which could accelerate the death of the target cells. On the other hand, using nanoparticles as seeds, heterogeneous nucleation rate could be significantly improved. This will help dramatically increase the possibility of nucleation in cells, which in turn results in a higher probability of IIF (PIF) leading to a lethal killing of tumor cells.

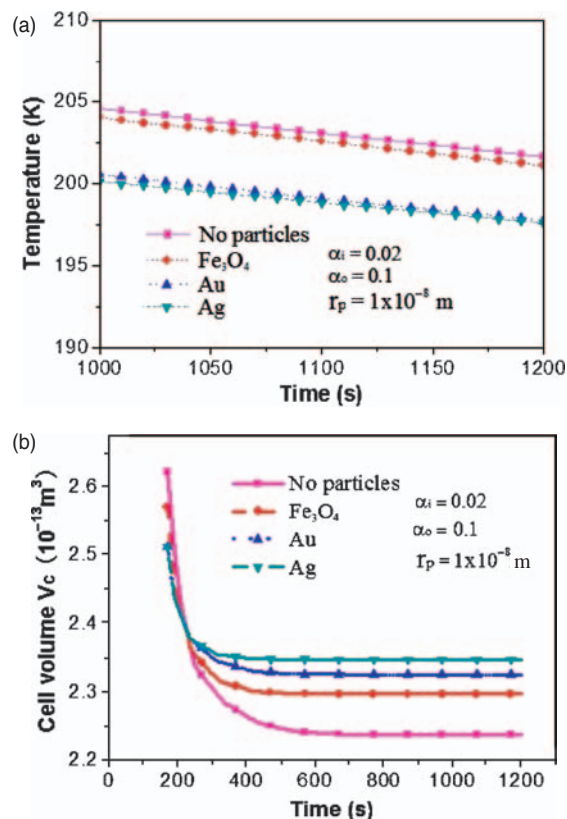


Fig. 21. The transient temperatures (a) and volume responses (b) of cell (M5) during freezing procedure for different kinds of nanoparticles with same radius $r_p = 1 \times 10^{-8}$ m when the intracellular volume friction of particles is $\alpha_i = 2\%$ and outside-cell one is $\alpha_o = 10\%$, respectively. Reprinted with permission from [58], J. F. Yan and J. Liu, *J. Appl. Phys.* 103, 084311 (2008). © 2008.

4.6. Modelling on Tissues Embedded with Large Vessels

Deng et al.⁵⁶ adopted two typical vascular models to simulate the phase-change heat transfer of biological tissues embedded with large blood vessels, which include: (a) a model with a single artery passing through the tumor, SATT; (b) a model with counter-current vessel pairs passing through the tumor, CVTT (shown in Fig. 22). The boundary conditions are a constant temperature of 37°C on all surfaces of the parallelepiped. Similar models and boundary conditions were first developed to study the effects of large blood vessels on temperature distributions during hyperthermia treatment by Chen and Roemer.⁶⁹

Figure 23 gives a comparison of numerical results for the SATT and CVTT models, in which the curves depict the temperature distribution at $t = 1200$ s in the artery at $x = 0.05$ m, $y = 0.05$ m. It is clearly shown that for the case with introduced nanoparticles, the large blood vessel(s) had been frozen for both SATT and CVTT models, whereas the large vessel(s) could not be frozen for the case without introduced nanoparticles even using the SATT model (in which only one large vessel was present).

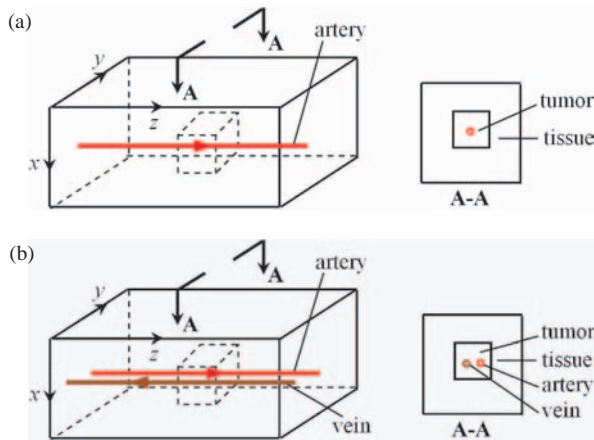


Fig. 22. Illustration of two typical vascular models (not to scale): (a) a model with a single artery transiting the tumor, SATT; (b) a model with counter-current vessel pairs transiting the tumor, CVTT.

The foregoing numerical results indicate that the heating effect created by blood flow in large vessel(s) can produce steep temperature gradients and lead to inadequate cooling of the surrounding tumor tissues. Therefore, they may seriously contribute to failed-killing of tumors during cryosurgery. These results also imply that highly conductive nanoparticles can serve as effective adjuvants for enhancing the efficacy of cryosurgical treatment of tumors embedded with large vessels, and this feature enhances the possibility to totally destroy tumor tissues surrounding large vessel(s) by nano-cryosurgery. However, it should be pointed out that when performing nano-cryosurgery for treatment of a tumor embedded with some major vessel(s) (which has important function for tissues or organs at other region), it must be seriously considered whether freezing such vessel(s) will result in severe postoperative complications.

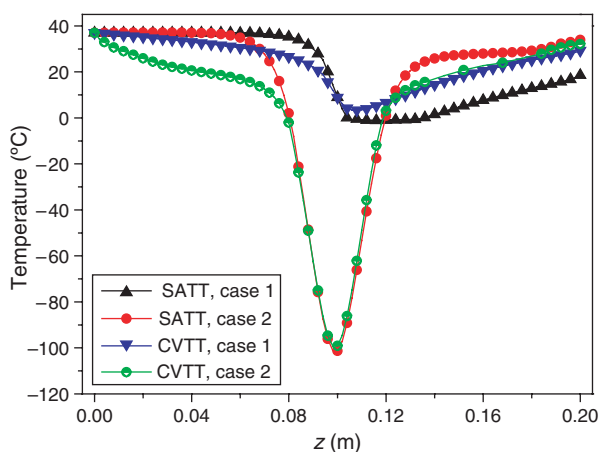


Fig. 23. Comparison of numerical results for both SATT and CVTT models, in which case 1 denotes the case without introduced nanoparticles, and case 2 denotes the case with adjvantly introduced nanoparticles. Reprinted with permission from [56], Z. S. Deng et al., *Advances in Numerical Heat Transfer*, In press. ©

4.7. Sensitivity Analysis of Model Parameters

It should be pointed out that the predicted results are sensitive to the values of some important model parameters of nanoparticles. Besides, the nanoparticles that could be loaded inside the target cells may depend on the particle type and cellular environment. To address such issue, Yan and Liu performed a sensitivity analysis to identify and rank the most influential variables in the model.⁵⁸

According to the compilation, it was found that most of those parameters of nanoparticles influence the thermal conductivity. Extracellular volume fraction α_o , as one of the input parameters, has the highest impact on the model predictions. Another important output parameter which reflects the effect of nanoparticles on freezing dynamics is the nucleation rate. Considering the high complexity and apparent non-linear behavior of the nucleation rate as output parameter, only some typical input parameters were considered to calculate the sensitivity measure for the characteristic interval but not the entire input–output range. Since the input parameters are highly dependent of each other, for simplicity, Yan and Liu define the range of temperature T and cell volume V_c according to the freezing process of cell (M5) with nano-Ag.⁵⁸ Theoretical evaluations predict that T has the highest sensitivity value, the next is α_i , h' , V_c and κ' are the input parameters to which the ice nucleation rate is less sensitive. Overall, the parameters of nanoparticles which are related to thermodynamics are more influential to the model than other parameters of nanoparticles. Further cellular level experimental justifications of the temperature and concentration dependence of these parameters are crucial to improve model accuracy.

4.8. Nano-Cryosurgery Enabled Conformal Freezing

Physiologically, tumors usually have very irregular shape which significantly increases the difficulty for the conventional cryosurgical technique to realize an optimal cryolesion. If the iceball growth during cryosurgery can be artificially controlled, such trouble will be easily solved, and the treatment effect of cryosurgery will be improved. In order to prove the feasibility of controlling the iceball growth, a conceptual problem by asymmetrically injecting nanoparticle suspension into the tumor was numerically investigated.²⁰ The tissue calculation domain for a trans-dermal cryosurgery as depicted by Figure 1 is prescribed in a rectangular space, $5 \times 10 \times 10$ cm along the x , y , and z directions, respectively. The tissue regions permeated with injected nanoparticle suspensions is in the range of $(0.01 \text{ m} \leq x \leq 0.03 \text{ m}, 0.045 \text{ m} \leq y \leq 0.075 \text{ m}, 0.045 \text{ m} \leq z \leq 0.055 \text{ m})$. One typical calculation result is depicted in Figure 24. It can be seen that the ice ball grows along the tissue permeated with nanoparticle suspension, and thus the position and scale of the ice ball is successfully altered, compared with the case of no injection. Readers are referred to Ref. [20] for more details. Clearly,

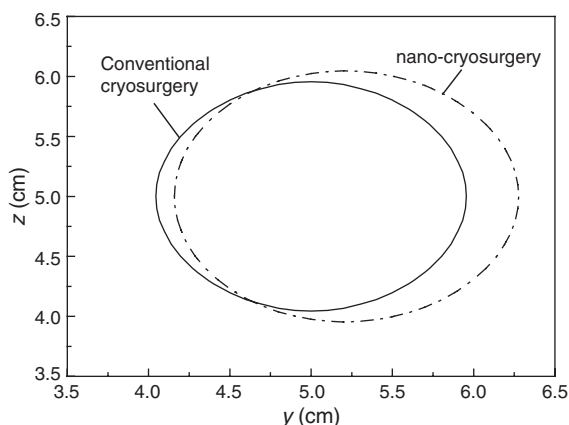


Fig. 24. Comparison of typical ice ball interfaces between the cases of injecting solution with high thermal conductivity and that without injecting solution. Modified with permission from [20], Z. S. Deng and J. Liu, *Cryobiology* 50, 183 (2005). © 2005.

artificially controlling the iceball growth through injecting highly conductive nanoparticle suspension is rather convenient. This may be used as a flexible approach to guide the direction of iceball formation during cryosurgery. Such feature is very useful in tumor treatment, because it can ensure physicians to focus cryosurgical treatment on a limited area to avoid destruction of nearby healthy tissue, and thus maximize tumor killing and minimize normal tissue injury at the same time.

In clinics, a complex-shaped iceball which conformally wraps the tumor with complex geometry is usually obtained by combined use of multiple cryoprobes.⁷⁰ However, since the lethal freezing temperature to tumor cells is tissue-dependent and generally ranges from -20 to -70 °C, “dead region” (where the freezing is insufficient) thus often occurs at the interstitial spaces between multiple cryoprobes. Therefore, one of the most important issues for a conformal cryosurgical treatment is to avoid “dead region” within iceball produced by multiple cryoprobes as could as possible. This important issue was recently addressed by our theoretical research.⁷⁰

Presented in Figure 25 is a comparison between iceballs produced by 3 cryoprobes for the cases with and without introducing nanoparticles. Details for the particle injection position and the corresponding information are referred to Ref. [70]. It was observed that the volume and geometry of iceball for the case of without injection of nanoparticles are similar to that of the injection case. However, it clearly indicated by the arrows that there exist some “dead regions” representing insufficient freezing areas between cryoprobes for the case of without loading nanoparticles. Introduction of the nanoparticle suspension significantly improves such adverse situation. Therefore, adjuvant use of nanoparticles with high thermal conductivity to perform conformal cryosurgery is rather useful.

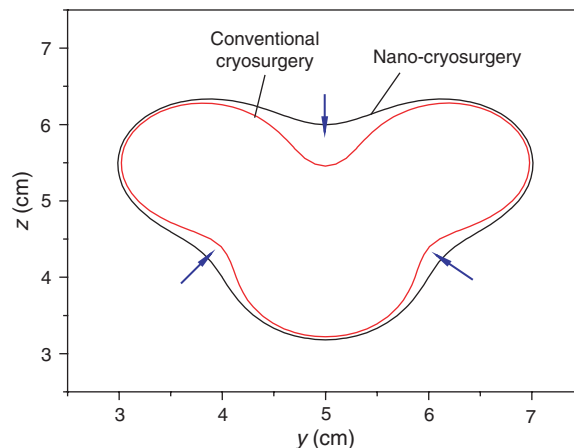


Fig. 25. Numerical prediction on lethal areas produced by 3 cryoprobes. Modified with permission from [70], Z. S. Deng and J. Liu, *The 1st Annual IEEE Int. Conf. on Nano/Molecular Medicine and Engineering*, Macau, China, August (2007). © 2007.

4.9. Uncertainty Prediction on Nano-Cryosurgery

The ultimate aim of cryosurgery is to exactly kill all tumor cells within a closely defined region. The conformal cryosurgical treatment of tumors by adjuvant use of nanoparticles opens possibilities for high performance surgery. However, as clarified from the above sensitivity analysis, it is clear that theoretical results cannot always reflect the reality. Certain significant reasons may come from the uncertainties implied in all sorts of parameters in heat transfer model used for predicting the tissue temperature.⁷¹ In the last several years, there has been increasing attention directed toward critically assessing the accuracy and reliability of computational simulations. Recently, the uncertainties for the predicted temperatures of tissues in nano-cryosurgery due to approximate parameters were addressed by Deng and Liu.⁷² Contributions of uncertainties from the tissue area permeated with nanoparticles, the concentration of nanoparticles, and the thermal parameters of tissues were respectively analyzed, and the uncertainty limits for temperature distributions in these cases were also estimated.

In general, based on the heat transfer model, the tissue temperature during nano-cryosurgery can be expressed by the following function:

$$T = f(w_1, w_2, \dots, w_N) \quad (8)$$

where w_1, w_2, \dots, w_N are N independent parameters such as the probe size, probe temperature, probe location, blood perfusion and metabolic heat generation rate of tissue, heat capacity and the thermal conductivities of the frozen and unfrozen phases, tissue area permeated with nanoparticles and the concentration of nanoparticles etc. Using the root sum-of-the-squares method, the overall uncertainty of the

tissue temperature can be given by

$$\Delta T = \sqrt{\left(\frac{\partial f}{\partial w_1} \Delta w_1\right)^2 + \left(\frac{\partial f}{\partial w_2} \Delta w_2\right)^2 + \dots + \left(\frac{\partial f}{\partial w_N} \Delta w_N\right)^2} \quad (9)$$

where $\partial f/\partial w$ and Δw are the sensitivity coefficient and uncertainty for parameter w . Equation (9) is a rigorous description of the uncertainty, which has been widely used for uncertainty analysis.

Based on the numerical simulation of three-dimensional (3-D) transient temperature response in biological tissues, Deng and Liu studied the uncertainties for the predicted temperatures of tissues in nano-cryosurgery due to approximate parameters.⁷² The uncertainty contributions from the tissue area permeated with nanoparticles, the concentration of nanoparticles, and the blood perfusion of tissue were shown in Figure 26. It is indicated that the tissue temperature uncertainties due to the approximate parameters cannot be ignored. Therefore, the uncertainty analysis is expected to serve as a significant guide for performing a highly efficient and also completely safe nano-cryosurgical treatment.

5. CHALLENGES AND FUTURE DIRECTIONS

5.1. Nano-Cryosurgery Enabled Innovation on Biomedical Device

Introduction of nano-technologies into the area of cryobiology offers many opportunities for medical device innovation. For example, the combined nano-cryosurgery and nano-hyperthermia has been identified as a good way to improve treatment efficiency far better than each of them alone.²² For this purpose, the metal nanoparticles can be first injected into the target tumor for a nano-cryosurgery, which can then be followed up by a nano-hyperthermia or vice versa. Belonging to the same category of physical therapy, modern hyperthermia combined with advanced nano-technique presents a good example for such endeavors. The newly developed magnetic nano-hyperthermia which takes full advantage of electromagnetic heating effects and powerful thermo-osmosis offer some attractive possibilities in tumor therapy. Moreover ability to treat cancer by targeted delivery through angiogenesis or some antineoplastic drug is also reported and shows a good treatment effect.

The new modalities offer many chances for treating tumor. For example, a minimally invasive needle with both freezing and heating capability can be designed for a combined surgery. The freezing can be realized by ventilating liquid nitrogen through the channel of the needle, while the heating can be done by incorporating any heating strategies such as radio frequency antenna or laser fiber etc. into the freezing probe. Along this direction, a group of different combinations between nano-freezing and nano-heating

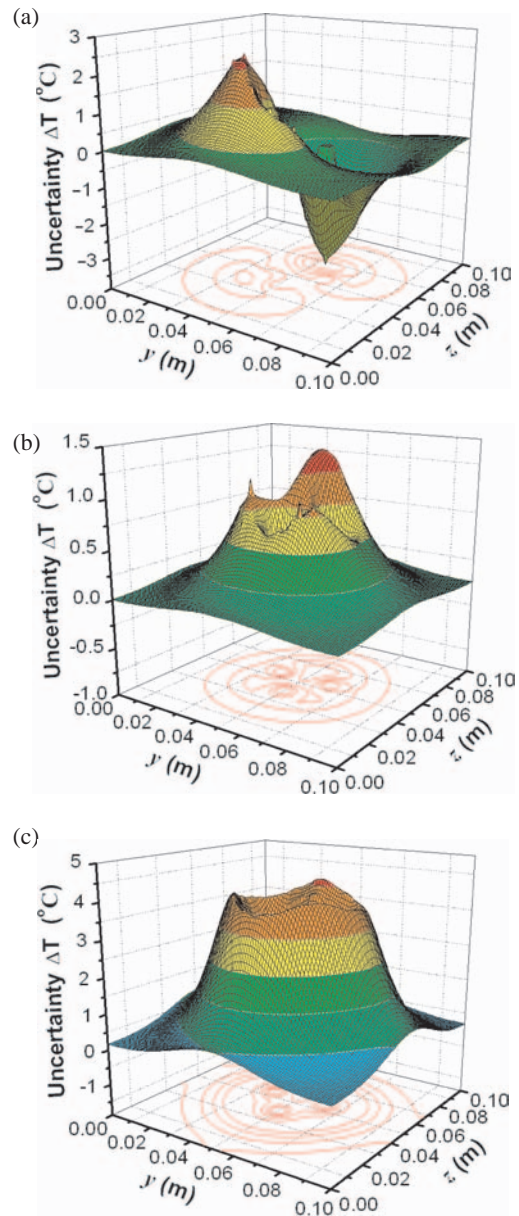


Fig. 26. Predicted temperature variational scale during nano-cryosurgery by 3 cryoprobes due to uncertain model parameters: (a) effect of uncertain area permeated with nanoparticles; (b) effect of uncertain concentration of nanoparticles; and (c) effect of uncertain blood perfusion of tissue. Reprinted with permission from [72], Z. S. Deng and J. Liu, *The 2nd ASME International Conference on Integration and Commercialization of Micro and Nano-systems*, Clear Water Bay, Kowloon, Hong Kong, June (2008). © 2008.

approaches or more can be pushed forward. The corresponding tumor treatment efficiencies are expected to be significantly improved.

5.2. Nanoparticle Enhanced Imaging on Cryosurgery

For developing the image guidance device for cryosurgical operation, nanoparticles with specific physical properties can be particularly chosen as image enhancer for

better detection by MRI, Ultrasound, X-CT, and PET etc. For example, in an ultrasound imaging, the contrast agents generally should have high echogenicity so as to realize a strong acoustic scattering.⁷³ Besides, it should be small in size in order to flow through capillaries. The latest available agents have been found as microbubbles, per-fluorocarbon emulsion nanoparticles etc. As for the magnetic resonance agents, the superparamagnetic iron oxide nanoparticles can be good candidates. For optical imaging, the agents with appropriate optical contrast properties are desired. In fact, a perfect nanoparticle medium could even have both freezing and image enhancement capability. Therefore, after finishing their complementary surgical role, the particles also serves as mediums either for pre- or post-diagnostics on the treatment output of a cryosurgery and help plan for the subsequent treatment.

From the view of imaging techniques, introduction of nanoparticles into target tissues are especially beneficial in helping increase curative accuracy of minimally invasive cryosurgery. Many fluorescent nanoparticles for imaging applications have been reported in the literature.⁷³ Such materials include but are not limited to semiconductor quantum dots, fluorescent silica nanoparticles, silica coated fluorescent polymer particles, dye-loaded latex nanobeads, fluorescent polystyrene particles and fluorochrome conjugated iron oxide nanoparticles etc. Nowadays researchers are trying to develop a thermometry system to image and monitor thermal lesion of tissue during thermal therapies in order to guarantee an accurate treatment. For a cryosurgery one can also possibly choose to use fluorescent nanometer size particles which are temperature dependent as imaging probes to detect temperature distributions within the iceball. Moreover, *in vivo* imaging of the nanoparticle-tissue interaction reveals processes which will obviously aid in the improvement of disease-specific markers. Some imaging magnetic nanoparticles such as Fe_3O_4 , 20–30 nm diameter, has been found beneficial to increase the resolution and contrast of some commonly used imaging techniques in minimally invasive therapy such as Magnetomotive Optical Coherence Tomography (MM-OCT) or MRI.⁷⁴ Such characteristics of nanoparticles offer a good hand to increase the curative effect of tumor and decrease local recurrence rate as well. Therefore, when nano-technology meets cryosurgery, the treatment efficiency of conventional cryosurgery is expected to be significantly improved. Progress in this direction would aid further cryosurgical monitoring in the near future.

5.3. Material Aspects for Nanoparticle Candidates

In fact, nanoparticles are now being intensely investigated in many branches of nano-medicine. For example, great efforts have been made to use magnetic nanoparticles for the concentrated energy deposition in hyperthermia at the target tissue inside the human body.^{34, 35, 37} All these are

owing to the progress made in recent nano-technologies. In fact, the earliest effort to test the hyperthermia effect of magnetic materials dates back to 1957 when Gilchrist et al. heated various tissue samples with 20–100 nm size particles of $\gamma\text{-Fe}_2\text{O}_3$ exposed to a 1.2 MHz magnetic field.⁷⁵ Since then many empirical work has been reported, with attentions on a variety of schemes using different types of magnetic materials, different field strengths and frequencies and different methods of encapsulation and delivery of particles by performing experiments with animals or using cancerous cell cultures.^{76–81} However, routine medical procedures to follow in clinics are still not available until now. There is a strong lack of profound understanding of the events occurring inside the tissues due to external field induction, which is however critical to render a reliable tumor therapy. Clearly, with phase change as its additional feature, the nano-cryosurgery is very different from the nano-hyperthermia and appears much complicated since it is involved with more physical or chemical processes. At the present stage, the nano-hyperthermia has been developed to be more mature. Therefore, experiences gathered there can be borrowed to investigate the future nano-cryosurgery.

According to the purpose of enhancing or reducing the freezing strength at a target tissue, the nanoparticles can be made from a wide variety of materials such as biodegradable polymer, liposome, micelle, drug and semiconductor etc, not only the metal ones. Previously in hyperthermia, magnetic nanoparticles have been applied in several ways in the form of glass ceramics, microcapsules, or suspensions of magnetic nanoparticles.³⁷ The amount of material required to produce the desired temperatures depends to a large extent on the method of administration,³⁶ such as mainline, arterial injection, hyodermic injection, and direct injection. Due to blood circulation, the nanoparticles loaded to the tissue can be either absorbed or expelled. That means, the nanoparticles may not always be contained in the region of interest. Their migration or transport within the tissue is important for the high quality cryosurgery. Uncertainty thus caused should be treated with caution in future tumor clinics. In addition, during the freezing process, the tumor vessels dilate and increase in permeability sharply and quickly, hence the particulate suspension would be concentrated in the tumor but not the normal tissue. Such mechanism has been successfully applied in cryochemotherapy which can also be applied in nano-cryosurgery. Then the toxicity of particles resulted from high concentration is mainly lethal to targeted tumors but not normal tissues if the particle injection time, procedure and dose of particulate suspension are artificially controlled well.

Regarding the choice of particle for enhancing freezing, the iron oxides magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) are perhaps the most popular and appropriate ones because of their good biological compatibility. Particle sizes less than 10 μm are normally considered small

enough to enable effective delivery to the site of the tumor, either via encapsulation in a larger moiety or suspension in some sort of carrier fluid. Clearly, the particles used for cryosurgery should be extremely small. It is known that the conductive properties of powders may vary significantly depending on their grain sizes and the particle microstructure. In this side, the nano-cryosurgery can be made better by the advent of many advanced nano-materials. Due to introduction of nanoparticle into the target, it would effectively reduce the temperature of the entire tumor-bearing region or improve the killing temperature. In this case, a cryoprobe with only a moderate freezing capability may work well for treating the tumor. And the resulting temperature pattern may be more uniform than that produced by only using passive conventional freezing approach.

It is worth mentioning that, effect of nanoparticles on cell freezing is a rather complex issue. The final output depends heavily on the material type, its affinity with the biological sample, particle configuration, shape, size, concentration, and the physical especially thermal properties, etc. Therefore, various experimental designing may even lead to contradictory result on freezing output.²⁶ Recently, it was also discovered that, the nanoparticles may not always do good for healthy tissues. In other words, some of them can be toxic.^{82–84} For the minimally invasive nano-cryosurgical practice, certain types of nanoparticle/cell interactions might be harmful while others may not be. Therefore, a comprehensive evaluation in advance on the nano-materials used for cryo-medicine is very necessary. As a new direction in the bioheat transfer area, there is still a long distance to completely grasping the physical, chemical and biological pictures of the nano-cryosurgery. This calls for tremendous efforts in the near future.

6. CONCLUDING REMARKS

The nano-cryosurgery opens many new possibilities for solid tumor therapy by increasing treatment efficacy through a carefully planned combination of a minimally invasive cryosurgical procedure and nanoparticle loading. This surgical modality appears very simple, flexible, quick and relatively conformable. However, a comprehensive understanding of nano-cryosurgery still needs tremendous work in the near future. We should particularly mention that, what has been presented as above has never been conclusive. It is just a beginning to develop the nano-cryosurgery. In fact, many complex factors lie behind the phenomena. It is still not clear for the relation between the nanoparticle size or shape and ice crystal formation and the biological damage effect it caused. And the tissue fabric has to be studied before the loading and the position of the injection should be carefully chosen to avoid damaging some important inner structure of the tissue such as blood vessels, connective tissues and so on.

Although a complete understanding of the nano-cryosurgery is still not available at the present stage, this

review has outlined the promising future of the new therapy. Such surgical protocol could break the limitation of conventional cryosurgery in many respects and offers a much higher maximum freezing rate as well as possibilities of ice nucleation. It is also quite helpful and flexible for an adaptive tumor treatment as well as *in vivo* medical imaging. In this way, an idealistic minimally invasive and safe freezing therapy can possibly be guaranteed. Future efforts should be placed on both the fundamental mechanisms as well as clinical issues of the nano-cryosurgery.

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