

NANO-CRYOSURGERY: A BASIC WAY TO ENHANCE FREEZING TREATMENT OF TUMOR

Jing Liu^{1,2}, Jing-Fu Yan², and Zhong-Shan Deng²

1. Department of Biomedical Engineering, School of Medicine, Tsinghua University,
Beijing 100084, P. R. China

2. Cryogenic Laboratory, P. O. Box 2711, Technical Institute of Physics and Chemistry,
Chinese Academy of Sciences, Beijing 100080, P. R. China
jliu@cl.cryo.ac.cn

ABSTRACT

Cryosurgery is a minimally invasive clinical technique with controlled destruction of target tissues through a specifically administrated freezing procedure. This method has now been used in a wide variety of clinical situations such as treatment of skin cancers, glaucoma, lung and prostate tumor etc. However, there still exist many bottle necks to impede the success of a cryosurgery. A most critical factor 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. Meanwhile, 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 this difficulty, we proposed an innovative strategy, termed as nano-cryosurgery, to significantly improve freezing efficiency of a conventional cryosurgical procedure. The basic principle of this protocol is to inject functional solution with nano particles into the target tissues, which then serves as 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. Meanwhile, introduction of nanoparticles during cryosurgery could also help better image the edge of a tumor as well as the margin of the iceball. Along this direction, several progresses have been made on mechanism interpretation, theoretical modeling, numerical prediction, conceptual experimental demonstration and treatment planning etc. in the authors' lab. This study is dedicated to present a preliminary outline on the nano-cryosurgery by summing up the aspects as mentioned above. The evident merits and shortcomings of the nano cryosurgery will be illustrated. Some potential feasibility, versatile applications and possible challenges when nanotechnology meets cryosurgery will be pointed out. It is expected that the concepts of nano-cryosurgery may suggest new opportunities for realizing a

highly safe, targeted and accurate freezing therapy in future tumor clinics.

INTRODUCTION

Cryosurgery is becoming popular for its many clinical advantages such as 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 it still can not be regarded as a routine cancer treatment way, the cryosurgery is developing rather rapidly as an alternative for traditional methods such as radiotherapy and chemotherapy. Owing to the advent of many modern imaging technologies, the field of cryosurgery has been significantly extended since its early stage [1]. A major concern involved in a cryosurgery is to maximize the freezing efficiency in killing target focus while minimize the irreversible damage to the surrounding normal tissues [4]. However, many clinical data revealed that only adopting a single freezing the diseased cells could not be effectively destroyed and there is always a high recurrence rate with follow-up surveys [3]. From the general view point of cryo medical engineering, a major reason lies in that the freezing rate is not appropriate enough which will not result in a massive ice nucleation in tumor cells [5], especially at the edge of tumor. It thus could not guarantee a complete lethal harm to the whole tumor regions.

In order to improve conventional cryotherapy and establish a flexible way to control the freezing rate as desirable as possible, we had suggested to use highly conductive nano particles to significantly enhance the freezing process of target tissues and very promising results were obtained [6-8]. The earliest effort in this topic can be dated back to the thesis work of Yu [9] and Deng [10], who were then supervised by the senior author of this paper. Recently, using nano particles to regulate a freezing or thawing process was further extended to

more cryobiology cases [11]. Considering that all these efforts fall in the category of using nano technology to innovate a conventional cryomedical practice, we finally come up to the technical concept which can be termed as nano-cryosurgery [12]. To the best knowledge of the authors, this may be the first time on defining such a physical therapy which may have generalized significance in future tumor clinics. Starting from this basic idea, some new modalities in cryosurgery can be possibly developed.

Clearly, the nano cryosurgery is closely related to the advanced nano-technologies. Its working principle is to introduce functional solution with nano particles into the target tissues (Fig. 1), which then serves as to either maximize freezing heat transfer, change ice-ball formation orientation or prevent healthy tissues from being frozen. The nano particles enabled cryosurgery takes full advantage of enhanced heat conduction effects and powerful performance to serve as nucleation seeds. Moreover, it can offer a beneficial hand to treat tumor by targeted delivery through angiogenesis or some antineoplastic drug accompanied by nano particles. In fact, the nano technology enabled medicines are becoming very hot topics. Among the many physical therapies thus appeared, the nano hyperthermia is especially receiving great attentions over the world [13, 14]. Many exciting progresses are kept being made which as a result further push forward the rapid growth of the nano medicine area. However, compared with the exploded efforts on the nano hyperthermia [15], only rather limited works are available on extending nano technology to enhance a conventional cryosurgery. Unlike using high temperature to thermally ablate tumor, the nano cryosurgery, which is based on a contradictory procedure, may be involved with more complex physical mechanisms, due to the phase change behavior it induced.

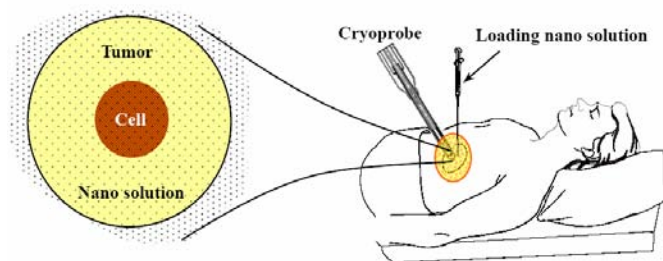


FIG. 1 Schematic for loading nano particle solution for administering A nano cryosurgery.

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, salinity of tissues [1-3]. Clearly, introduction of nano particles would significantly change 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 target. In this side, adoption of nano particles allows to administrate an accurate killing on nano scale, since the particles can be injected and distributed through out the region of interest as desired via a truly minimally invasive way [6]. Furthermore, if ice crystals form within the cell, they will probably disrupt the intracellular organelles as well as the

plasma membrane. Therefore, when nano technology meets cryosurgery, an improvement on the freezing treatment efficiency can be expected.

In fact, nano particles are now being intensely investigated in many branches of nano medicine. For example, great efforts have been made to use magnetic micro/nano particles for the concentrated energy deposition in hyperthermia at the target tissue inside the human body [13-15]. All these are owing to the progress made in recent nano technologies. The earliest effort to test the hyperthermia effect of magnetic materials dates back to 1957 when Gilchrist et al [16] heated various tissue samples with 20-100nm size particles of $\gamma\text{-Fe}_2\text{O}_3$ exposed to a 1.2MHz magnetic field. 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 [17-22]. 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.

This paper is dedicated to present a preliminary viewpoint on nano cryosurgery based on some of the existing experimental results and theoretical analysis. Particularly, the thermal aspect will be paid with special attention. Some related effects of nano particles on the tissue or cell freezing will briefly be discussed. It is expected to help better understand the potential advantages brought about by this emerging cryosurgical modality.

BASIC FEATURE OF NANO-CRYOSURGERY

Nano particles induced cryosurgery is superior to conventional cryosurgery in many respects. The basic capability it enabled can be summarized as follows: 1. Enhance freezing and thus improve target killing efficiency; 2. Avoid insufficient freezing between multiple cryoprobes during treating large scale tumor; 3. Regulate growth direction and orientation of an ice ball and thus guarantee a conformable cryosurgery on complex tumor; 4. Weaken a freezing on healthy tissues and thus reduce injury there. 5. Improve image contrast and offer a better image guidance for the cryosurgical operation.

The nano cryosurgery is deeply rooted in the latest advancement of nanotechnology, which has had an immediate influence on modern medical practices over the past few years. The nano-cryosurgery concept is just incubated in this stream of research frontier. Experiments and theoretical analysis indicate that, once nano particles are implanted into target area, not only the maximum freezing rate inside the target could be increased during cryosurgery compared with the conventional approach, but most importantly, the possibilities of ice

nucleation could also be significantly improved which would induce an enlarged death of tumor cells [6, 12]. Such innovation is quite beneficial to raise the curative effectiveness of conventional cryosurgery and decrease the recurrence rate of post-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 nano particles would further improve the effective killing rate of tumor cell with the combination of cryosurgery, just as that performed before on nano hyperthermia [14].

From the point of cell cryoinjury theory [23], intracellular ice formation (IIF), which occurs due to insufficient time for water to escape the cells, is considered to be 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 nano particles or a number of nano particles are immersed in intercellular area (Fig. 2), such two factors can be significantly improved since the nano particles could 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 nano metal particles will significantly increase the tissue conductivity. Besides, the massive loading of nanoparticles is bound to result in an easier heterogeneous nucleation which to some extent guarantees a higher PIF (Fig. 2). Therefore it can be expected that delivered nanoparticle will enhance tumor necrosis during cryosurgery.

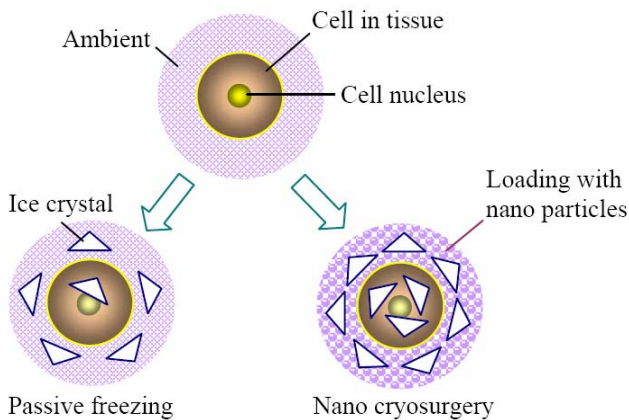


Fig. 2 Ice formation at cell level when subjected to freezing.

EXPERIMENTAL PHENOMENA

What presented in Fig. 3 are typical temperature response curves of pork tissue either with or without loaded metal nanoparticles (aluminum), respectively [6]. It can be found that, the lowest temperature for the case of injecting nano particles can reach -115°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°C at the same tissue position when subjected to the same freezing condition as above. Besides, a larger freezing scale, i.e. the size of ice ball, has been observed. This was owing to the enhanced heat conduction due to addition of nano metal particles into tissues. To one’s surprise, the improvement of freezing due to injection of nano particles is rather significant.

Except for adopting the highly conductive nano materials, nano particles with lowered thermal conductivity can also have unique virtue in cryosurgery. However, 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 in the healthy tissues surrounding the target tumors during cryosurgery. Besides, if such nano particles, 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 very flexible way for controlling the process of a cryosurgery and avoiding damaging normal tissue.

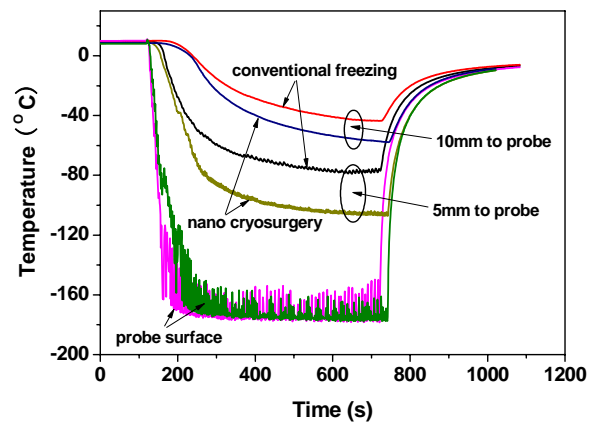


FIG. 3 Comparison between freezing temperature responses of pork tissues either with or without injected nano-particles

To evaluate the capacity of controlling the size, shape and orientation of the iceball formation by injecting nano solutions with specific thermal properties into the target tissues, we had ever adopted a medical infrared thermometer to map the temperature profile over the whole surface above the freezing area [24]. 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, (Me_2SO), respectively. It was clearly demonstrated that the localized injection of an appropriate solution could effectively regulate the tumor-killing area via directional freezing.

In a typical test as shown by Fig. 4, different volumes for the nano solution were considered. We injected symmetrically aqueous suspension of aluminum nano-particles 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 1ml, 2ml, 3ml and 4ml. Clearly, as the thermal images indicated, different volumes of injecting solution have resulted in varied magnitudes on iceball formation. The more volume for the solution to be injected, the more possible the iceball will grow fast toward that direction.

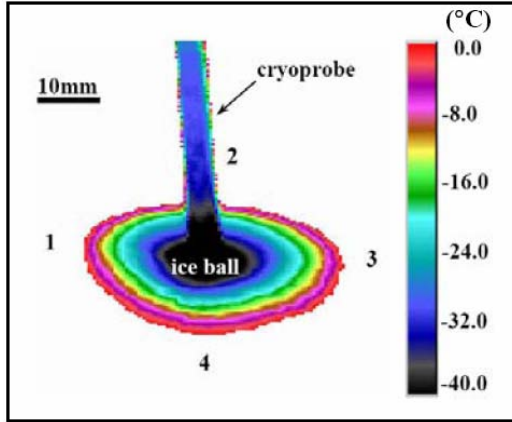


Fig. 4 Thermal image for temperature distribution on pork tissues injected with 1ml, 2ml, 3ml and 4ml (from sign 1 to sign 4) aqueous suspension of aluminum nano-particles in water, respectively.

THEORETICAL INTERPRETATION

Modification of tissue properties by loading nano particles

Without any doubt, a detailed understanding on the mechanisms for the modified bioheat transfer process in tissues due to injected nano particles is still not available. But such issues can be preliminarily interpreted using existing phenomenological models. Generally, the thermal properties for the composite consisting of cancerous tissue and injected nano particles depends on the particle concentration and the amount of injected solution [25]. If assuming a homogeneity within the target region, the mean value for the specific heat C , density ρ and thermal conductivity K of cancerous tissues embedded with nano particles can be approximated by a serial arrangement of the two materials with the respective volume proportions [26], i.e.

$$\rho = (1-\eta)\rho_1 + \eta\rho_2 \quad (1)$$

$$C = (1-\eta)C_1 + \eta C_2 \quad (2)$$

$$\frac{1}{K} = \frac{(1-\eta)}{K_1} + \frac{\eta}{K_2} \quad (3)$$

where, $\eta = \frac{4}{3}n\pi r^3$ stands for the volume concentration of particles inside the sphere, r the radius of the micro/nano particles, n the concentration of the micro/nano particles in tissue; the subscript 1, 2 refers to the tissue and nano solution,

respectively. Such equation can be approximately applied to evaluate whether loading nano solutions (material 2) into tissue (material 1) will modify the target as desired. For example, if an enhanced heat conduction is required, one can have $K/K_1 > 1$, which would easily lead to $K_2/K_1 > 1$. The more conductive the nano solution, the more enhanced heat transfer performance will be achieved, and vice versa.

Similar analysis can also be performed on other thermal properties such as specific heat, density and latent heat of the tissue-particle composite material.

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 is expected to 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 [7]. In the calculations, the functional solution is assumed uniformly distributed through out the target domain after injection. The functional solution with high thermal conductivity can be made up by mixing nano-particles of metaloxide such as Fe_3O_4 nano-particles with water, which have been widely used in RF-magnetic hyperthermia. Presented in Fig. 5 is a comparison of the temperature responses between two cases: no injection and injection. The transient temperatures are shown for 3 positions, 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 nano functional solution. 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 Fig. 3 [6].

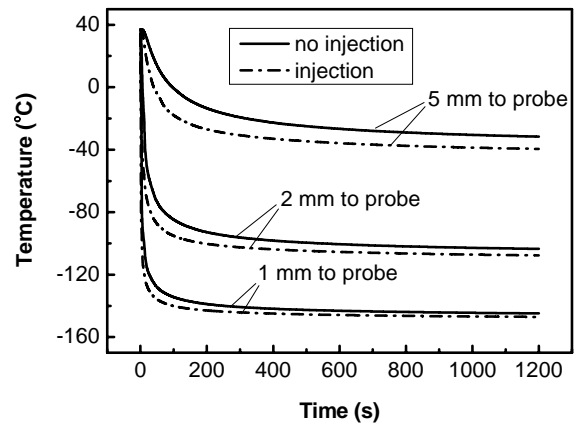


Fig. 5 Comparison of temperature responses between the cases of injecting solution with high thermal conductivity and that without injecting solution.

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 nano solution into the tumor was numerically investigated [7]. The tissue calculation domain for a trans-dermal cryosurgery as depicted by Fig.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 nano solutions 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 Fig. 6. It can be seen that the ice ball grows along the tissue permeated with nano solution, and thus the position and scale of the ice ball is successfully altered, compared with the case of no injection. Readers are referred to [7] for more details. Clearly, artificially controlling the iceball growth through injecting highly conductive nano solution 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.

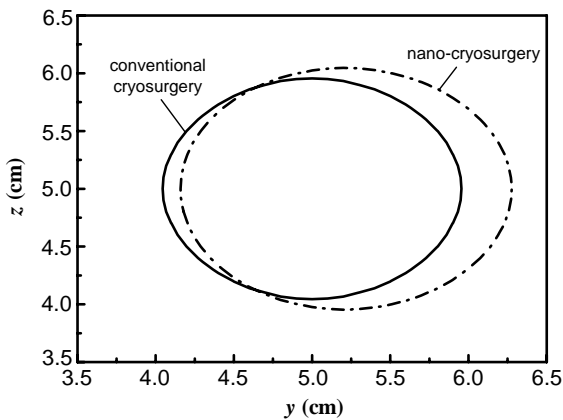


Fig. 6 Comparison of typical ice ball interfaces between the cases of injecting solution with high thermal conductivity and that without injecting solution.

In clinics, a complex-shaped iceball which conformally enwraps the tumor with complex geometry is usually obtained by combined use of multiple cryoprobes [27]. 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 [27].

Presented in Fig. 7 is a comparison between iceballs produced by 3 cryoprobes for the cases with and without introducing nano particles. Details for the particle injection

position and the corresponding information are referred to [27]. It was observed that the volume and geometry of iceball for the case of without injection of nano particles are similar to that of the injection case. However, it clearly indicated by the blue arrows that there exist some “dead regions” representing insufficient freezing areas between cryoprobes for the case of without loading nano particles. Introduction of the nano solution significantly improve such adverse situation. Therefore, adjuvant use of nano particles with high thermal conductivity to perform conformal cryosurgery is rather useful.

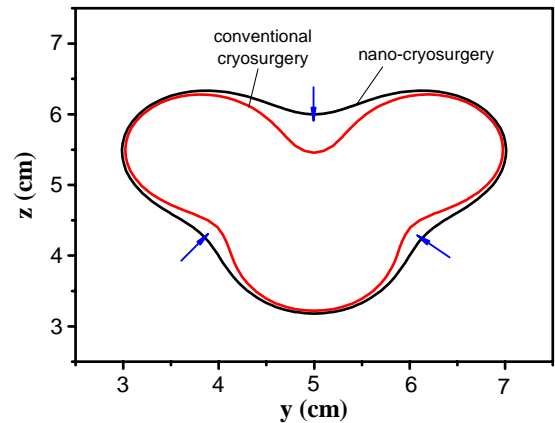


Fig. 7 Numerical prediction on lethal areas produced by 3 cryoprobes.

Cellular level ice nucleation

Besides its effects on freezing speed, nano particle also plays an important role in inducing ice nucleation which is critical in determining the final cell damage. As has been theoretically interpreted in our previous work [12], using nano particles as seeds, heterogeneous nucleation rate could be significantly improved. It can be seen that 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 nano particles will often become frozen earlier than that in a normal tissue when subjected to the same freezing condition (see Fig. 3). This is just partially caused by the particle induced ice nucleation.

Except for the thermal aspect of using nano particles to enhance cryodestruction, there are still many other biological and physico-chemical reasons that need to be explored as well. For example, nano particles 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]. So far, there is still no experimental evidence to directly show that nano particles will enhance intracellular ice formation. Such intriguing challenging issue needs further clarification in the near future.

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.

DISCUSSION

Candidates for nano particles and their delivery

According to the purpose of enhancing or reducing the freezing strength at a target tissue, the nano particles 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 micro/nano particles have been applied in several ways in the form of glass ceramics, microcapsules, or suspensions of magnetic micro/nano particles [15]. The amount of material required to produce the desired temperatures depends to a large extent on the method of administration [25], such as mainline, arterial injection, hyodermic injection, and direct injection. Due to blood circulation, the nano particles loaded to the tissue can be either absorbed or expelled. That means, the nano particles 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.

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 $10\mu\text{m}$ 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 nano particle 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 nano particles 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 [11]. Recently, it was also discovered that, the nano particles may not always do good for healthy tissues. In other words, some of them can be toxic [28-30]. For the minimally invasive nano cryosurgical practice, certain types of nano particle/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 needs tremendous efforts in the near future.

From the view of imaging techniques, introduction of nano particles 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 [31]. 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 , 20-30nm 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 [32]. Such progress would aid further cryosurgical monitoring in the near future.

Biomedical device innovation enabled by nano cryosurgery

Introduction of nano technologies into the area of cryobiology offers many opportunities for medical device innovation. For example, if metal nano particles were injected into the target tumor, a nano-cryosurgery can be followed up by a nano hyperthermia. For this purpose, a minimally invasive needle with both freezing and heating capability can be designed, just as that made before in our lab [33]. For example, the freezing can be realized by ventilating liquid nitrogen through the channel of the needle, while the heating can be done by incorporating 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 approaches or more can be pushed forward.

For developing the image guidance device for cryosurgical operation, nano particles with specific physical properties can be particularly chosen as image enhancer for better image 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 [31]. Besides, it should be small in size in order to flow through capillaries. The latest available agents have been found as microbubbles, perfluorocarbon emulsion nanoparticles etc. As for the magnetic resonance agents, the superparamagnetic iron oxide nanoparticles can be a good candidate. For optical imaging, the agents with appropriate optical contrast properties are desired. In fact, a perfect nano particle 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 following treatment.

CONCLUSION

The nano cryosurgery may lead to an advancement in solid tumor therapy by increasing treatment efficacy through a carefully planned combination of a minimally invasive cryosurgical procedure and nano particle 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 mention that, what has been presented as above is never conclusive. It is just a beginning to investigate the nano cryosurgery. In fact, many complex factors lie behind the phenomena. For example, it is still not clear for the relation between the nano particle 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 view on the nano-cryosurgery is still not clear so far, this paper has preliminarily outlined the promising future of this emerging physical therapy. Such surgical protocol could favorably 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 be obtained. Future efforts should be made on investigating both the fundamental mechanisms as well as practical issues of the nano-cryosurgery.

ACKNOWLEDGMENTS

This work was partially supported by Dr. Shun-De Wu's Foundation of Tsinghua University and the NSFC Grants 50575219, 50436030.

REFERENCES

- [1] Rubinsky, B., 2000, "Cryosurgery," Annual Review of Biomedical Engineering, **2**, pp. 157-187.
- [2] Bischof, J. C., 2000, "Quantitative measurement and prediction of biophysical response during freezing in tissues," Annual Review of Biomedical Engineering, **2**, pp. 257-288.
- [3] Gage, A. A., and Baust, J. G., 1998, "Mechanisms of tissue injury in cryosurgery," Cryobiology, **37**, pp. 171-186.
- [4] Rabin, Y., and Shitzer, A., 1998, "Numerical solution of the multidimensional freezing problem during cryosurgery," ASME Journal of Biomech Eng., **120**, pp. 32-37.
- [5] Karlsson, J. O., and Toner, M., 1996, "Long-term storage of tissues by cryopreservation: critical issues," Biomaterials, **17**, pp. 243-256.
- [6] Yu, T. H., Liu J., and Zhou Y. X., 2005, "Selective freezing of target biological tissues after injection of solutions with specific thermal properties," Cryobiology, **50**, pp. 174-182.
- [7] Deng, Z. S., and Liu, J., 2005, "Numerical simulation of selective freezing of target biological tissues following injection of solutions with specific thermal properties," Cryobiology, **50**, pp. 183-192.
- [8] Deng Z. S., Liu J., 2004, "Numerical simulation of selectively freezing the target biological tissues by injecting 'functional solution'," Abstract of a conference presentation, Cryobiology, **49**: 306.
- [9] Yu, T. H., 2004, "Study on the combined freezing and heating therapy for Minimally invasive tumor treatment," Master Degree Thesis of the Technical Institute of Physics and Chemistry, the Chinese Academy of Sciences, Beijing, July 2004.
- [10] Deng, Z. S., 2005, "Study and application of complex thermo-physical problems during tumor hyperthermia and cryosurgery," Ph.D. Thesis of the Technical Institute of Physics and Chemistry, the Chinese Academy of Sciences, Beijing, December, 2004.
- [11] Thirumala, S., Forman, J. M., Monroe, W. T., and Devireddy, R. V., 2007, "Freezing and post-thaw apoptotic behaviour of cells in the presence of palmitoyl nanogold particles," Nanotechnology, **18**, 195104 (12pp) doi:10.1088/0957-4484/18/19/195104
- [12] Yan, J. F., and Liu, J., 2007, "Nano-cryosurgery and its mechanisms on enhancing freezing treatment of tumor," submitted.
- [13] Kalambur, V. S., Han, B., Hammer, B. E., Shield, T. W., and Bischof, J. C., 2005, "In vitro characterization of movement, heating and visualization of magnetic nanoparticles for biomedical applications," Nanotechnology, **16**, pp. 1221-1233.
- [14] Visaria, R. K., Griffin, R. J., Williams, B. W., Ebbini, E. S., Paciotti, G. F., Song, C. W., and Bischof, J. C., 2006, "Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor- α delivery," Mol Cancer Ther, **5**, pp.1014-1020.
- [15] Lv, Y. G., Liu, J., 2004, "Research advancement in applying magnetic micro/nano particles for tumor hyperthermia," (in Chinese) Micronanoelectronic Technology, **41**, pp. 22-28.
- [16] Gilchrist, R. K., Medal, R., Shorey, W. D., Hanselman, R. C., Parrott, J. C., and Taylor, C. B., 1957, "Selective inductive heating of lymph nodes," Annals of Surgery, **146**, pp. 596-606.
- [17] Gordon, R. T., Hines, J. R., and Gordon, D., 1979, "Intracellular hyperthermia: a biophysical approach to cancer treatment via intracellular temperature and biophysical alterations," Medical Hypotheses, **5**, pp. 83-102.
- [18] Rand, R. W., Snow, H. D., Elliott, D. G., and Snyder, M., 1981, "Thermomagnetic surgery for cancer," Applied Biochemistry and Biotechnology, **6**, pp. 265-272.
- [19] Chan, D. C. F., Kirpotin, D. B., and Jr. Bunn, P. A., 1993, "Synthesis and evaluation of colloidal magnetic iron oxides for the site-specific radiofrequency-induced hyperthermia of cancer," Journal of Magnetism and Magnetic Materials, **122**, pp. 374-378.
- [20] Matsuki, H., and Yanada, T., 1994, "Temperature-sensitive amorphous magnetic flakes for intra tissue

- hyperthermia,” *Materials Science and Engineering*, **A181-182**, pp. 1366-1368.
- [21] Jordan, A., Scholz, R., Wust, P., Fahling, H., Krause, J., Wlodarczyk, W., Sander, B., Vogl, T., and Felix, R., 1997, “Effects of magnetic fluid hyperthermia (MFH) on C₃H mammary carcinoma in vivo,” *International Journal of Hyperthermia*, **13**, pp. 587-605.
- [22] Hilger, I., Fruhauf, K., Andra, W., Hiergeist, R., Hergt, R., and Kaiser, W., 2002, “Heating potential of iron oxides for therapeutic purposes in interventional radiology,” *Academic Radiology*, **9**, pp. 198-202.
- [23] Toner, M., 1993, *Advances in Low-Temperature Biology* (London: JAI Press), **2**, pp. 1-51.
- [24] Yan, J. F., Liu, J., and Zhou, Y. X., 2005, “Infrared image to evaluate the selective (directional) freezing due to localized injection of thermally important solutions,” 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS). Shanghai, China.
- [25] Lv, Y. G., Deng, Z. S., and Liu, J., 2005, “Study on the induced heating effects of embedded micro/nano particles on human body subject to external medical electromagnetic field,” *IEEE. Trans. Nanobiosci.*, **4**, pp. 284-294.
- [26] d’Ambly, A., Andra, C. G., Hergt, R., Hilger, I., and Kaiser, W. A., 1999, “Temperature distribution as function of time around a small spherical heat source of local magnetic hyperthermia,” *Journal of Magnetism and Magnetic Materials*, **194**, pp. 197-203.
- [27] Deng, Z. S., and Liu, J., 2007, “Conformal cryosurgical treatment of tumor by use of nanoparticles: feasibility study,” 1st Annual IEEE Int. Conf. on Nano/Molecular Medicine and Engineering, Aug. 6-9, Macau, China.
- [28] Hoet, P., Nemmar, A., and Nemery, B., 2004, “Health impact of nanomaterials?” *Nature Biotechnology*, **22**, pp. 19.
- [29] Colvin, V. C., 2003, “The potential environmental impact of engineered nanomaterials,” *Nature Biotechnology*, **21**, pp. 1166 - 1170.
- [30] Mullins, J., 2004, “Safety concerns over injectable quantum dots,” *New Scientist*, **181**, pp. 10.
- [31] Wickline, S. A., and Lanza, G. M., 2003, “Nanotechnology for molecular imaging and targeted therapy,” *Circulation*, **107**, pp. 1092-1095.
- [32] Amy, O., Farah, T., Kenneth, S., Alexander, W., and Stephen, B., 2005, “Magnetomotive contrast for in vivo optical coherence tomography,” *Opt. Express*, **13**, pp. 6597-6614.
- [33] Liu, J., Zhou, Y. X., Yu, T. H., Gui, L., Deng, Z. S., and Lv, Y. G., 2004, “Minimally invasive system capable of performing both cryosurgery and hyperthermia treatment,” *Minimally Invasive Therapy and Allied Technologies*, **13**, pp. 47-57.