35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

New Treatment Strategy of Cryosurgery and Temperature Control

Xianjing Zou, Jingfeng Bai*, Aili Zhang, Ping Liu and Lisa X.Xu

Abstract—**Cryosurgery, recommended as an effective method for tumor treatment, has been widely used in clinics. However, it might lead to a high probability of tumor recurrence due to incomplete tumor damage. The treatment protocol for cryosurgery is essential to achieve the desired therapeutic effects. In this study, a new temperature fluctuation treatment method was proposed, and the fuzzy control method based on piecewise adjustment was developed for temperature control during the treatment. *Ex vivo* rat liver experiments were conducted and histopathology analysis used to study the therapeutic effects of the new treatment method.**

I. Introduction

Cryosurgery, as an efficient method utilizing ultra-low temperature to freeze and destroy pathological tissues, has got significant interest in tumor treatments. Compared with the traditional tumor treatments such as surgical resection, radiation therapy and chemotherapy, cryosurgery has some potential advantages of local treatment, minimal invasive to the surrounding normal tissue, minimal bleeding, less pain because of anesthetic effect and less cost [1, 2]. Cryosurgery could also lead to an augmented immune response [3, 4, 5] and rapid cooling was reported to generate a tumor-specific immune response [6]. Given these advantages, cryosurgery has been widely used in several kinds of tumor treatments, including skin carcinoma, prostate, liver, lung, breast and bone cancers [6].

Although cryosurgery can be used for localized treatment while causing less damage to the surrounding healthy tissues, it has encountered some problems. It might lead to a high probability of tumor recurrence due to incomplete tumor damage, especially at the tumor edge [7]. There are various factors that will have significant impact on the outcome of the cryosurgery procedure, i.e., the number of cryoprobes used, the choice of cryoprobes placement and the thermal parameters [8]. The key thermal factors for the tissue and cell damages during cryosurgery are the coldest tissue temperature, freezing time, cooling rate, thawing rate, freeze-thaw cycles [9]. Therefore, in order to achieve the desired therapeutic effect and prevent tumor recurrence, it is essential to choose the most appropriate treatment parameters to freeze target tumor sufficiently.

In common practice, the coldest temperature at the edge of target tumor is around -20°C, which is assumed lethal to cells, i.e. causing cell death [10, 11]. Mechanistically, the higher the cooling rate, the more likely the formation of the lethal intracellular ice crystals, and thus rapid cooling is more destructive [12, 14]. During thawing, recrystallization, which is a process of ice crystals melting to form large crystals, occurs usually when the temperature is warmer than -40°C (especially about -20°C to -25°C) [13]. When the speed of thawing is slower, it's more likely to increase solute effects and make ice crystals larger to produce more damages to tumor tissue [13, 14]. The repeated freeze-thaw cycles could be more destructive because the lethal intracellular ice crystals are more likely to form during repeated freeze-thaw cycles [15]. In summary, we should focus on the above factors and choose the most appropriate treatment protocol to cause lethal freezing damages to tumor and achieve the cryosurgery treatment goals.

In this study, we propose to utilize the fluctuated temperatures at the tumor edge to cause more complete damage. The related temperature control algorithm was developed to realize this cryosurgery treatment pattern. The therapeutic effects were also studied in *ex vivo* rat liver experiments and using histopathology analysis.

II. Methodology

A. Temperature Fluctuation Treatment Strategy

As has been said before, treatment parameters have significant influences on the therapeutic effects of cryosurgery. In order to achieve the desired cryosurgery treatment goals, it is of great significance to choose the most appropriate treatment protocol. At present, the common treatment protocol used of cryosurgery is usually freezing the whole tumor to a subzero temperature until the temperature at the edge tumor reaching the coldest target value -20°C, and then maintaining at the temperature for a period of fifteen minutes. Thus most studies on cryosurgery are researched based on this treatment strategy.

In this paper, the fluctuated temperature treatment strategy of cryosurgery was proposed. The fluctuated temperature was imposed at the tumor edge to cause more complete damages. During the treatment, the temperature at the edge tumor periodically fluctuated within a certain range. A mathematic model of the temperature fluctuation treatment strategy of cryosurgery was built, and five parameters used in the model were defined as: V_f, V_t, T_o, T_r and f.

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This research is partially supported by the Shanghai Science and Technology Research Fund (11DZ2211001).

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V_f: the initial cooling rate. It could be mainly evaluated by the time spent decreasing the initial temperature to the coldest temperature.

V_t: the thawing rate. It could be mainly evaluated by the time spent rising the coldest temperature to the fluctuation temperature point.

T_o: the coldest temperature of cryosurgery.

T_r: the range of temperature fluctuation.

f: the fluctuation frequency.

The coldest tissue temperature, freezing time, cooling rate, thawing rate and freeze-thaw cycles are all key thermal factors for the tissue and cell damages during cryosurgery. We propose to use the temperature fluctuation treatment method of cryosurgery to cause more complete damages. Besides, there might be an optimal choice for these five parameters to produce the most damages, which need to be validated by experimental studies.

At present, the coldest treatment temperature at the tumor edge is normally around -20^{o}C in practice. During cryosurgery, it is necessary to minimize the damages to the surrounding healthy tissue. Therefore, in this paper, the following studies about the realization and the therapeutic effects of the temperature fluctuation treatment strategy of cryosurgery, were mainly studied under the condition that the temperature at the edge of target tumor fluctuates between -15^{o}C and -25^{o}C, i.e. T_o = -25^{o}C and T_r = 10^{o}C.

B. Alternate of Cooling and Heating Treatment System

All the following researches in this paper were based on the alternate of cooling and heating treatment system which was developed in our laboratory [16]. As can be seen from the Fig.1, the system is mainly composed of the following six parts: the control system, the data acquisition system, the radio-frequency generator, the vapor-liquid separator, the liquid nitrogen Dewar and the cryo-heat probe. The alternate of cooling and heating treatment system can be used to perform cryosurgery and hyperthermia treatment, but in this paper we only used the function of cryosurgery. During the cryosurgery operation, after opening the main-pass valve, liquid nitrogen flows out from the liquid nitrogen Dewar. It flows through the vapor-liquid separator, and then flows into the inner tube of the probe. Finally liquid nitrogen exhausts through the channel between the inner tube and the outer tube of the probe as shown in Fig.2 [17]. Liquid nitrogen circulates in the probe to freezing tissue. The vapor-liquid separator is designed by our collaborators, which can change the proportion of liquid nitrogen and gas nitrogen to adjust the cooling capability [18]. When the by-pass valve is opened, gas nitrogen discharges from the pipe so that the proportion of liquid nitrogen reaches its maximum and the cooling capability is also strongest.

C. Fuzzy Control Method Based On Piecewise Adjustment

During the cryosurgery treatment, the temperature at the tumor edge is detected by thermocouple. Generally speaking, in order to achieve the best therapeutic effects, it is usually required to drop quickly to the coldest target value. The overshoot of the temperature is also controlled as small as possible which is best less than 1^{o}C. According to the above introduction of the treatment system, we know that during the cryosurgery operation, the temperature control is mainly realized by adjusting the frequency of opening and closing the main-pass and by-pass valve. The two valves cannot be opened or closed too frequently. It is also difficult to know exactly the proportion of liquid nitrogen and gas nitrogen of the flow to determine how to better control temperature. The heat transfer is based on heat conduct. It needs time to be transferred so that the temperature is very prone to exceed the target value. Therefore, it will not satisfy the requirements of the temperature control. All the above factors make it difficult to establish a precise mathematical model of cryosurgery, and conventional control methods are unlikely of satisfying the basic control requirements.

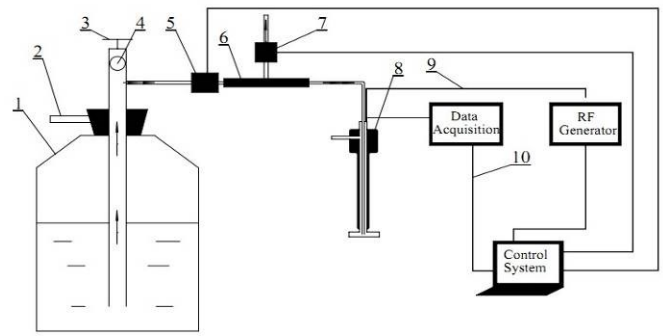


Figure 1. The schematic diagram of the treatment system.
1, liquid nitrogen Dewar. 2, safety valve. 3, switch. 4, pressure meter. 5, main-pass valve. 6, liquid-gas separator. 7, by-pass valve. 8, cryo-heat probe. 9, single conductor shielded wire. 10, data cable.

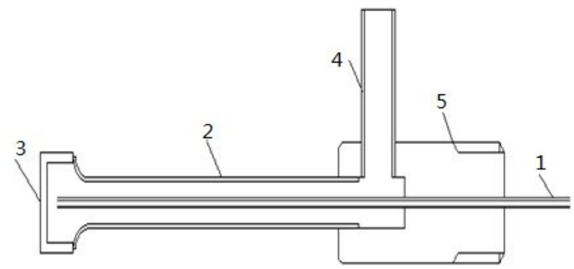


Figure 2. The schematic diagram of the probe.
1, inner tube. 2, outer tube. 3, treating face. 4, exhausting pipe. 5, T type pipe.

In order to solve this problem and realize the temperature fluctuation treatment strategy of cryosurgery as well as possible, the fuzzy control method was adopted in this paper. Fuzzy control method is widely used in the experienced operators implementing instead of having a precise mathematic model [19]. Fuzzy control algorithm is a summary of a lot of experience of operators, and there are three important stages for us to design fuzzy control, the first stage is having an understanding of base design, the second stage is defining control tuning parameters and the last stage is establishing membership functions [20].

When fuzzy control method is applied to cryosurgery of this treatment system, the input parameters are the size of tumor, e(t) and Δe(t) while the output parameter is the frequency of the opening and closing the main-pass and by-pass valve, e(t) is the difference between the current and

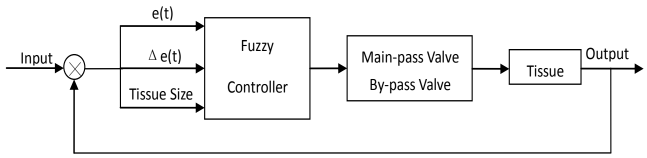


Figure 3. The fuzzy control system.

the target temperature at the edge of target tumor, and Δ e(t) is the slope of the temperature change. The fuzzy control system can be described as Fig.3.

According to the mathematic model of the new treatment strategy of cryosurgery proposed in this paper, the temperature curve obviously can be divided to the following typical three sections: the initial cooling section, the thawing section and the cooling section during the fluctuation of temperature. Based on these characteristics, we should adopt piecewise adjustment to realize the treatment method. Specifically, during the initial cooling period, both the main-pass valve and by-pass valve are opened so that the cooling ability of the system is strongest and the freezing rate is fastest. At the same time, we should determine when to close these valves in order to avoid large overshoot. During the thawing period, both the valves are closed so that the temperature goes up gradually. Finally, during the latter cooling period, the opening time and the opening frequency of valves are needed more precise to avoid a large overshoot. Through fuzzy control method based on piecewise adjustment to realize the new treatment strategy as well as meet the requirements of temperature control.

D. Ex Vivo Experiment

For the purpose of realizing the temperature fluctuation treatment strategy of cryosurgery and meeting the related control requirements, *ex vivo* experiments were conducted. In this study, *ex vivo* pork experiments were done to observe the freezing curve and adjust the control parameters so that the new treatment method with a fast response speed and a small overshoot could be realized through accumulating a large amount of experience. In addition, the therapeutic effects of the new treatment strategy were studied by *ex vivo* rat liver experiment and using histopathology analysis by Hematoxylin-Eosin (H&E) staining to view the tissue's damages.

III. RESULTS

At present, the temperature curve of the common cryosurgery treatment was achieved as shown in Fig.4. During the process of the temperature fluctuation treatment method, we adjusted related control parameters after summing up the experience through lots of *ex vivo* pork experiments, and eventually achieved a satisfactory temperature curve which was described in Fig. 5. As shown in Fig.4 and Fig. 5, we can see that the tissue was frozen to the coldest temperature T_o within about 90 seconds and the overshoot within 2^{o}C. The temperature curve met the requirement of rapid response and small overshoot.

Then the therapeutic effects of the new treatment strategy of cryosurgery were researched and Hematoxylin-Eosin

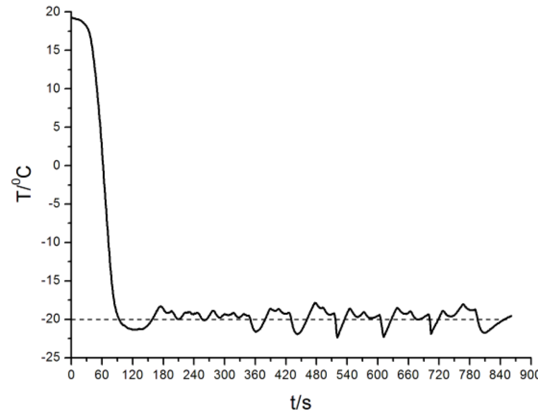


Figure 4. Temperature curve of the common treatment strategy.

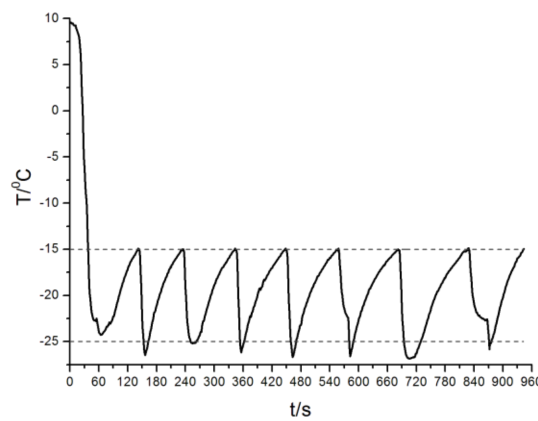
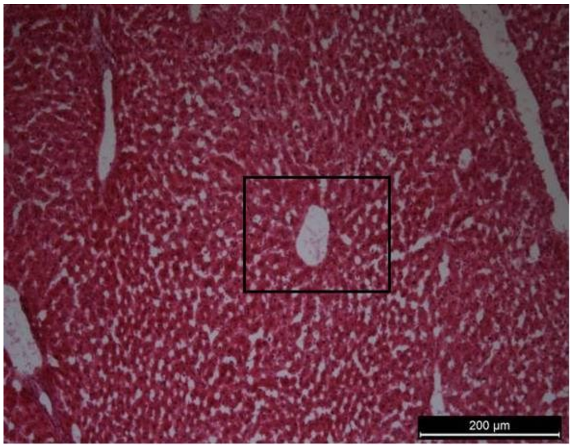
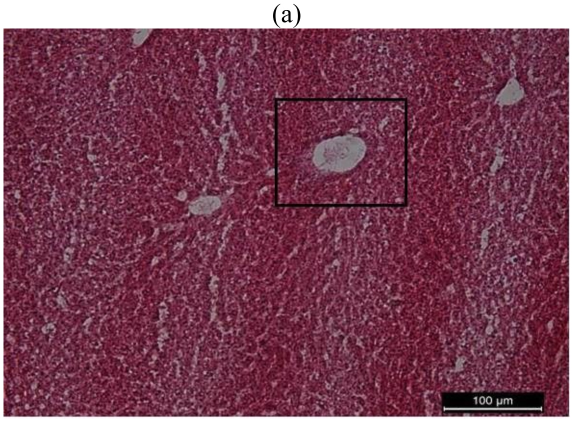


Figure 5. Temperature curve of the new treatment strategy.

(H&E) staining results of *ex vivo* rat liver were analyzed. *Ex vivo* rat liver experiments were conducted with the temperature fluctuation treatment strategy, while the temperature at the edge tumor fluctuated around -20^{o}C up and down within a range of 10^{o}C, the coldest temperature T_o was -25^{o}C and the freezing time was fifteen minutes. The H&E staining results shown in Fig.6 were obtained at the bottom of rat livers. As shown in Fig. 6, by microscope observation, we found that after cryosurgery, rat liver was damaged and the damages were mainly evaluated by observing the destruction of hepatic sinusoid around central vein. In normal liver, hepatic sinusoid locates radially around the central vein, as is shown in Fig. 6 (a). However, the hepatic sinusoid in Fig.6 (b) and Fig.6 (c) were clearly different from in Fig. 6 (a), one could clearly find that the radial distribution of hepatic sinusoid around the central vein was damaged in Fig. 6 (b) and in Fig. 6 (c). In addition, hepatic sinusoid in Fig. 6(c) was found to be more destruction in comparison with hepatic sinusoid in Fig. 6(b).



(a)



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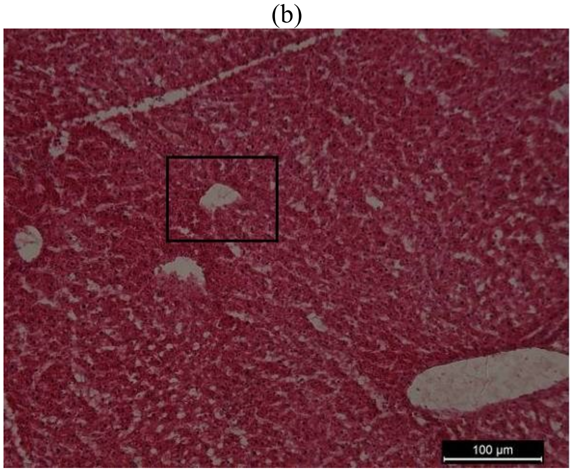

(c)

Figure 6. Hematoxylin-Eosin (H&E) staining results of rat liver. (a) Normal rat liver H&E graph (original magnification ×10). (b) Rat liver with common cryosurgery treatment H&E graph (original magnification ×10). (c) Rat liver with temperature fluctuation cryosurgery treatment H&E graph (original magnification ×10).

IV. Discussions and Conclusions

In this paper, the new treatment method of cryosurgery using the temperature fluctuation treatment strategy was proposed. The temperature control method was researched and the fuzzy control method based on piecewise adjustment was adopted to realize the temperature fluctuation treatment strategy of cryosurgery. Moreover, the therapeutic effects of the new treatment method were studied primarily through histopathology analysis. Through histological analysis, we discovered that the new treatment strategy of cryosurgery could produce severe damages to tissue. At the same time, the primary results showed that the damages were more destructive with the new treatment method as compared with the constant temperature cryosurgery. However, there is still need more experimental studies to optimize the parameters used to achieve the best outcome of this new method. Further, research of different treatment patterns could be also very meaningful in various clinical applications.

References

[1] G. Onik, "Cryosurgery," *Critical reviews in oncology/hematology,* vol. 23, p. 1, 1996.

[2] E. Li, *et al.*, "An efficient algorithm for phase change problem in tumor treatment using αFEM," *International Journal of Thermal Sciences,* vol. 49, pp. 1954-1967, 2010.

[3] S. Tanaka, "Immunological aspects of cryosurgery in general surgery," *Cryobiology,* vol. 19, pp. 247-262, 1982.

[4] H. B. Neel III, *et al.*, "Experimental evaluation of in situ oncocide for primary tumor therapy: Comparison of tumor - specific immunity after complete excision, cryonecrosis and ligation," *The Laryngoscope,* vol. 83, pp. 376-387, 1973.

[5] C. Blackwood and I. Cooper, "Response of experimental tumor systems to cryosurgery," *Cryobiology,* vol. 9, pp. 508-515, 1972.

[6] M. S. Sabel, *et al.*, "Rate of freeze alters the immunologic response after cryoablation of breast cancer," *Annals of surgical oncology,* vol. 17, pp. 1187-1193, 2010.

[7] J. F. Langenhuijsen, *et al.*, "Cryosurgery for prostate cancer: an update on clinical results of modern cryotechnology," *european urology,* vol. 55, pp. 76-86, 2009.

[8] R. Baissalov, *et al.*, "Simultaneous optimization of cryoprobe placement and thermal protocol for cryosurgery," *Physics in medicine and biology,* vol. 46, p. 1799, 2001.

[9] P. Mazur, "Cryobiology: the freezing of biological systems," *Science (New York, NY),* vol. 168, p. 939, 1970.

[10] J. R. Chandler, "CRyosurgery for recurrent carcinoma of the oral cavity," *Archives of Otolaryngology,* vol. 97, pp. 319-321, 1973.

[11] W. Gill and W. Long, "A critical look at cryosurgery," *Plastic and Reconstructive Surgery,* vol. 50, p. 100, 1972.

[12] J. Farrant and C. Walter, "The cryobiological basis for cryosurgery," *J Dermatol Surg Oncol,* vol. 3, pp. 403-407, 1977.

[13] P. Mazur, "Freezing of living cells: mechanisms and implications," *American Journal of Physiology-Cell Physiology,* vol. 247, pp. C125-C142, 1984.

[14] A. A. Gage and J. Baust, "Mechanisms of tissue injury in cryosurgery," *Cryobiology,* vol. 37, pp. 171-186, 1998.

[15] D. Whittaker, "Ice crystals formed in tissue during cryosurgery: II. Electron microscopy," *Cryobiology,* vol. 11, pp. 202-217, 1974.

[16] J. Sun, *et al.*, "A new thermal system for tumor treatment," in *Engineering in Medicine and Biology Society, 2005. IEEE-EMBS 2005. 27th Annual International Conference of the*, 2006, pp. 474-477.

[17] C. Zhanghao, *et al.*, "Design of a new probe for tumor treatment in the alternate thermal system based on numerical simulation," in *Engineering in Medicine and Biology Society,EMBC, 2011 Annual International Conference of the IEEE*, 2011, pp. 6874-6877.

[18] S. L. Qi, *et al.*, "Development and performance test of a cryoprobe with heat transfer enhancement configuration," *Cryogenics,* vol. 46, pp. 881-887, 2006.

[19] S. Tzafeseas, "Fuzzy systems and fuzzy expert control: an overview," *Knowledge Engineering Review,* vol. 9, pp. 229-229, 1994.

[20] Z. W. Woo, *et al.*, "A PID type fuzzy controller with self-tuning scaling factors," *Fuzzy Sets and Systems,* vol. 115, pp. 321-326, 2000.