International Journal of Thermal Sciences 132 (2018) 296–308

Contents lists available at ScienceDirect

International Journal of Thermal Sciences

journal homepage: www.elsevier.com/locate/ijts

Adjuvant-perfluorocarbon based approach for improving the effectiveness of cryosurgery in gel phantoms

K.K. Ramajayam[a], A. Kumar[b], S.K. Sarangi[b], A. Thirugnanam[a,*]

[a] Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, Odisha 769008, India
[b] Department of Mechanical Engineering, National Institute of Technology, Rourkela, Odisha 769008, India

ARTICLE INFO

Keywords:
Cryosurgery
Alumina
Gel phantoms
Perfluorohexane
Low thermal conductivity
Glycine

ABSTRACT

The current study suggests novel methods to enhance freezing inside the tissue mimicking gel phantoms during the cryosurgery process. In this study, freezing of gel phantoms is carried out by a cryosurgery setup and the temperature is measured in real time using a data acquisition system. Alumina is used as an adjuvant and the variation of the alumina concentration in the gel phantoms results in a decrease of end temperature achieved after cryosurgery. However, the lowest end temperature is attained when the alumina concentration in the gel phantom is 1% (w/v) and for further studies this optimal concentration is utilised. It is observed that with the increase in insertion depth of the cryoprobe (from 1 to 1.5 cm), there is a decrease in end temperature at each thermocouple location in these alumina gel phantoms. The cooling of these alumina containing gel phantoms in presence of a perfluorohexane layer reveals that there is an inhibition of freezing due to this low thermal conductivity barrier (the axial ice ball depth is reduced from 2 cm to 1.5 cm). Furthermore, with the addition of glycine (i.e. 1% (w/v) alumina 5% (w/v) glycine gel phantom), a substantial end temperature decrease is observed at the thermocouples placed nearer to the cryoprobe, thus indicating the usefulness of this strategy in enhancing localised freezing. In conclusion, this study provides various new approaches that enhance the efficacy of cryosurgery immensely.

1. Introduction

With the rapid rise in the number of cancer cases globally, surgeons are shifting from routine surgical ablation to other alternative methods of cancer treatment like chemotherapy and looking for other alternative methods of cancer treatment like chemotherapy, radiotherapy, laser therapy and cryosurgery [1]. When compared to other available treatment modalities mentioned earlier, cryosurgery is comparatively a safer option. This technique uses cryogen like liquid nitrogen to provide low temperature for destruction of tumours. Due to the coolant (liquid nitrogen, $-196°C$), the cryosurgical probe cools down to a low temperature which initiates the cooling of the tumour around the probe. The fabrication of the first and crude form of modern cryosurgery device was first demonstrated by Cooper et al. [2,3]. This cryosurgery system could freeze the brain tissue and cause damage to it. Since then, phenomenal advancements have been made in the field of cryosurgery [4]. Rapid technological improvements in the field of imaging have helped in performing cryosurgeries with ease [5,6]. In the same context, medical and industrial computed tomography (CT) techniques are currently available for imaging the cryosurgery process with an isotropic spatial resolution of 3 mm. This imaging capability can further be enhanced to 10 μm with the help of micro CT [7]. In another work, Bischof et al. have utilised CT to image the ice ball during cryosurgical tumour ablation in presence of adjuvants [8]. Further, the use of semi-automated methods have aided the researchers in developing a prostate model from a transrectal ultrasound image [9]. The cellular destruction during cryosurgery occurs due to extracellular and intracellular ice formation. A lower cooling rate leads to the formation of extracellular ice while in case of higher cooling rates water molecules are unable to move across the cell membrane leading to a perturbation in the osmotic equilibrium. Hence, the ice formation that occurs inside the cells aids in the maintenance of the osmotic equilibrium [10]. Apart from the damage induced by extracellular or intracellular ice formation, there are other cellular mechanisms that cause cellular injury through immunological and vascular mechanisms [11].

In an interesting study, Deng et al. have reported that the injection of solutions of high thermal conductivity results in a better clinical outcome during cryosurgery [12]. To improve the efficacy of the cryosurgical procedure, Rossi et al. have suggested a method of computerised planning for prostate cryosurgery that advocates variable

* Corresponding author.
E-mail address: thirugnanam.a@nitrkl.ac.in (A. Thirugnanam).

https://doi.org/10.1016/j.ijthermalsci.2018.05.037
Received 7 April 2017; Received in revised form 11 April 2018; Accepted 23 May 2018

insertion depth of cryoprobes [13]. Researchers have recently used mathematical tools to reconstruct the temperature field for wireless temperature sensors. The results demonstrate that this technique can help in determining the lethal front temperature to a significant degree of certainty [14]. In another study, research has been carried out to recreate a database of prostate models that can later help in computerised planning and training of cryosurgical procedures [15]. Further, a cryosurgery tutoring aid has been developed that helps in optimising a geometrical constraint like cryoprobe placement and predicting temperature distribution [16]. A new potential field analogy method (PFAM) is combined with the temperature field reconstruction method (TFRM) to determine the temperature profile in the frozen area. The results of this integrated approach (PFAM and TFRM) suggest that this is an efficient tool to calculate the thermal history in the frozen region during ultrasound-assisted cryosurgery [17]. Recent studies have evaluated the effects of the structure of blood vessel and injected nanoparticles on the cryo-freezing of a clinically extracted vascular tissue. The results of these studies showed that the nanoparticles enhanced the cryofreezing region [18,19].

A lot of in-vivo and in-vitro experiments have been done to improve the efficiency of the cryosurgical protocol [20,21]. Researchers have showed that injecting antifreeze protein type-I at higher concentration of 10 mg/ml causes cell death and acts as an adjuvant to the cryosurgery process [22]. Several chemotherapy adjuvants like adriamycin and peplomycin have been used in combination with cryosurgery and have shown better therepeutic outcomes [23]. In a study, researchers have experimentally investigated the thermal effects of large vessels during cryosurgery [24]. Further, in the case of eutectic freezing, it is seen that presence of salts also enhances the tissue destruction during cryosurgery [25]. In a significant study, experimental results suggest that injection of water, aluminium oxide nanoparticles in water, 10% solution of dimethyl sulfoxide (DMSO) and ethanol increased the tumour damage during cryosurgical process without causing any variation in the freezing process [26]. In an another critical study, Rabin et al. [27] fabricated a device that limited the destruction to the desired location. Further, studies have also been carried out to investigate the role of vasculature in enhancing cryosurgical cell death. Pre-conditioning of the tumour prior to cryosurgery using TNF-α, which is a vascular cytokine, has shown good results. The cryolesion diameter increased substantially when the cryosurgery is performed after its injection [28]. Thus, showing that even vascular adjuvants increase the efficiency of the cryosurgery process significantly. In addition, Chua and Chou have suggested that employing more number of freeze-thaw cycles results in enhanced cellular damage within the tissue [29]. Recent numerical studies carried by our research group have proposed a novel strategy for improvement of cryosurgical outcome. In these studies, numerical modelling of temperature distribution has been carried out during cryosurgery in a tumour in presence of a layer of perfluorocarbon around the tumour interface. Further, these studies also suggest the practical approach that can be adopted to layer perfluorocarbon liquid at the tumour interface using antigen-antibody reaction and specific linker molecule (like avidin and biotin) [30,31]. Although numerical studies have been done with this novel approach, experiments need to be performed to ascertain the efficacy of this method in a real time scenario. Therefore, this study presents experimental study on cooling of adjuvant containing gel phantoms in presence of perfluorocarbon (perfluorohexane) layer. Perfluorohexane has a low thermal conductivity of 0.091 W/m.K. However, this unique aspect of perfluorocarbon has not been used for improving the outcome of cryosurgery. These compounds are non toxic, chemically inert and find numerous applications in the field of biomedical engineering [32]. Perfluorocarbons are highly fluorinated chemicals that have the ability of dissolving a large volume of respiratory gases like oxygen andcarbon dioxide [33,34]. Researchers have showed that perfluorocarbon nanoparticles can be used for molecular imaging during magnetic resonance imaging (MRI) [35]. In another work, Barnett et al. have performed the non-invasive imaging of human cadaveric islets using perfluorocarbon nanoparticles. The results of the study demonstrate that both perfluoropolyether and perfluorooctyl bromide are fluorinated efficiently for F-MR (magnetic resonance) imaging [36]. Apart from these applications, perfluorocarbons have also been utilised for supporting liquid breathing and optical imaging of cells [37,38].

The outcome of cryosurgery can be improved susbstantially by using adjuvants. In the same context, this study proposes novel adjuvants like alumina and glycine for decreasing the end temperature during cryosurgery. Alumina is a well known biocompatible material that has been used extensively in tissue engineering applications [39,40]. Glycine, on the other hand, is an amino acid found naturally in the human physiology and therefore non toxic to the cells. In a study carried out by Wang et al. the eutectic solidification of glycine has been used to augment cryosurgery in MCF-7 cells [41]. Till date, alumina has not been used for increasing the thermal conductivity of gel phantoms for the betterment of cryosurgical outcome. Hence, in the current study, this new approach is utilised to enhance the freezing inside the agarose gel phantoms. The concentration of alumina in the emulsions is varied to obtain the optimal emulsion that has higher thermal conductivity. There is an urgent need for a combinative treatment where the freezing in increased in a localised region but does not affect the region immediately neighbouring it. Therefore, the ability of a perflurocarbon (perfluorohexane) to insulate the agarose gel phantom with alumina as adjuvant (alumina gel phantom) is studied exhaustively. A parametric study is also carried out to visualise the effect of change in the insertion depth on the cooling of gels with alumina as adjuvant. In addition, experiments have also been performed to verify the effectiveness of perfluorohexane as an insulation with the increase in insertion depth of the cryoprobe. Cryosurgical cooling has also been carried out on the alumina gel phantoms that contain glycine in a varied concentration. The presence of glycine in alumina gel phantom would result in a two fold effect: a) The higher thermal conductivity of alumina particle would result in a faster diffusion of heat and, b) During cryosurgery, the addition of glycine in alumina gel phantoms causes eutectic freezing lowering the end temperature even more further. Hence, this study also proposes a new method of combined freezing of gel phantoms containing alumina and glycine that causes a lower end temperature in comparison to the earlier studied alumina gel phantoms.

For this approach to be successful in the clinics, first, the tumour interface needs to be identified using the current day imaging technique like Magnetic resonance imaging (MRI), X-Ray. Subsequently, a thin space can be created around the tumour interface by a surgeon using laparoscopy which is a minimally invasive technique. This has to be followed by the injection of perfluorocarbon in the created space around the tumour. The perfluorocarbon (PFC) will not enter the tumour because it is hydrophobic and the inner environment is hydrophilic, thereby, preventing the mixing of PFC inside the tumour tissue. Secondly, the mass diffusivity of PFC is $5 \times 10^{-5}\ cm^2/s$ which is very low and this is very favourable for the proposed application as once the PFC is filled in that space, owing to meagre diffusivity, it would not enter the tumour tissue. One more reason which would make this perfluorocarbon layer approach successful it the time needed to complete the procedure, generally, a complete cycle of ablation takes 10–20 min and the PFC layer filled in that space surrounding the tumour needs to be maintained till that particular time. Once the freezing cycle is completed, PFC can be drained out in a minimally invasive way. After surgery, the removal of PFC layer will give a measure of the ablated zone. The avidin-biotin interaction for attaching PFC at the tumour interface can be made reversible. Modified versions of avidin resins and modified forms of biotin labeling reagents are commercially available which can make the reaction readily reversible. Therefore, once the cryosurgery process is complete, the bound avidin-biotin and PFC can be removed and drained out using minimally invasive techniques.