CHAPTER 1

Introduction

Application of low temperature to reduce pain during injury has long been recognised by mankind [149]. Cryotherapy is an advance application that utilises the therapeutic effects of low temperature in the area of cancer treatment. Rapid increment in the cancer cases globally and inability of conventional techniques to treat disease compelled researchers to seek options for cancer treatment, and it has given birth to modern day cryotherapy [61, 62, 87, 195]. Cryotherapy is further classified into two sections (i) cryosurgery, which deals with tumor occurring inside the body and (ii) cryospray, which deals with superficial tumor. Since its inception several modification have been incorporated to improve its applicability. In this perspective, following issues of cryspray are addressed in this chapter: history of cryotherapy, diversity in cryotherapy, modern cryotherapy, terminology of cryotherapy, advantages and limitations of existing method.

1.1 History of cryotherapy

Earliest recorded literature citing the use of ice as an anti-inflammatory agent date back to 3500 BC by Egyptians [22, 44]. It had laid the foundation of unique method of pain reduction in medical science. Later, therapeutic effects of cold was also reported by Hippocrates [146, 194] in 500 BC. Ice established itself as an analgesic and anesthetic agent by 1100 century. The quest to reduce temperature below 0 ^{0}C continues until Arontt [10] designed an equipment comprising of ice salt and water that can reach upto -20 ^{0}C. Arnott further stated that temperature lower than -20 ^{0}C can be used to arrest inflammation near the

Author	Year	Cooling agent
Ancient Egyptians	3500 BC	Ice
Arnott	1851	Ice/salt mix
Openchowski	1883	Ether
White	1899	Liquid air
Pusey	1907	Solid carbon dioxide
Hall	1942	Freon
Allington	1950	Liquid nitrogen
Amoils	1964	Nitrous oxide
Torre	1970	Argon

Table 1.1: Cooling agents used by researchers [170]

surface. After several decades, Cailletet [99] in 1877 introduced refrigerants to mankind through liquefaction of oxygen and carbon monoxide under high pressure. Linde [117] pushed this approach further and commercialised the production of liquid air. He utilised the principle of Joule-Thomson for continuous production of cryogen. Vacuum flask developed by Dewar [91] eased the storage and transportation of cryogen.

White [177] introduced refrigerants in the field of medical science and laid the foundation of modern cryotherapy. He stated that extreme cold produced by cryogens can be used as a necrotic agent (necrosis is a process when cell death occurs due to injury) rather than anti-inflammatory agent. He initiated the use of cryogen in the treatment of lupus erythematosus, herpes zoster, chancroid, naevi, warts, varicose leg ulcers, carbuncles and epitheliomas. Bowen and Towle [21] applied the similar approach in the treatment of skin lesions. The medical practice of treating carcinomas with cryogens got leverage with the design of spray bottle [177]. Researchers have employed various cryogens as per the requirement and availability such as ice (0 ^{0}C), ice-salt mixture (-20 ^{0}C), freon (-29.8 ^{0}C to -40.8 ^{0}C), carbon dioxide (-79 ^{0}C), liquid oxygen (-183 ^{0}C), and liquid air (-194.35 ^{0}C). The various cooling agents used by researchers are mentioned in table. 1.1. During the initial phase, liquid oxygen and liquid air gained popularity as cryogens in the field of cryotherapy. However, explosive nature of liquid oxygen and challenges of air liquefaction constrained their application. Other cryogens do not establish themselves in clinical practice due to the unsatisfactory results. Thus, after second world war, Allington [9] introduced the liquid nitrogen as cryogen in cryotherapy due to its superior properties and non hazardous nature. The properties of liquid nitrogen are listed in table. 1.2 . Cooper [43] encouraged the use of liquid nitrogen in cryotherapy and designed a probe that can achieve a temperature of -196 ^{0}C. He successfully treated the Perkinson's disease and freezed thalamus to treat movement disorders. It led to the eventual acceptance of cryotherapy as a standard treatment method. Zacarian [183, 184] developed the hand held device to spray

Thermal Property	Unit	Value
K	W/m.K	0.13
C	J/kg .K	$2.05x10^3$
ρ	kg /m^3	807.3
L	J/g	199.3
μ	Pa.s	$158x10^6$
T_l	^0C	-195.8

Table 1.2: Properties of liquid nitrogen

liquid nitrogen and popularised the equipment.

1.1.1 Terminologies of cryotherapy

Some common terminologies of cryotherapy:

Tumor: It can be described as an abnormal mass of tissue that results due to unconventional division of the cell or due to changes in the life cycle of the cell. They are further classified into two categories; (i) Benign and (ii) Malignant. Benign tumors are non cancerous and do not infiltrate to other parts of the body. Malignant tumors are cancerous and infiltrate to other parts of the body.

Lesion: A region in the tissue which has suffered damage through injury or disease, such as a wound, ulcer, abscess, or tumor.

Skin cancer: Our skin is the largest organ of the body. It protects our body from external pathogens and regulates its temperature. It consists of three layers: epidermis, dermis and hypodermis. Cancer triggers in skin due to abnormal growth of cell and forms tumor.

Necrosis: Artificial death of tissue due to injury caused through external factors.

Apoptosis: It is a process of programmed cell death.

Lethal temperature: The minimum temperature required to achieve necrosis. The most accepted range of lethal temperature varies from -20 ^{0}C to -40 ^{0}C [60, 63, 139].

Freeze cycle: The time duration of spray of cryogen is termed as freeze cycle.

Thaw cycle: The time provided to tissue after the freeze cycle is termed as thaw cycle.

Cooling rate: It is the ratio of change in temperature to the change in time at a particular location. It plays very important role in cryospray because the mechanism of cryoablation depends on it.

Intracellular ice formation: It occurs at a higher cooling rate when ice crystals forms inside the cell.

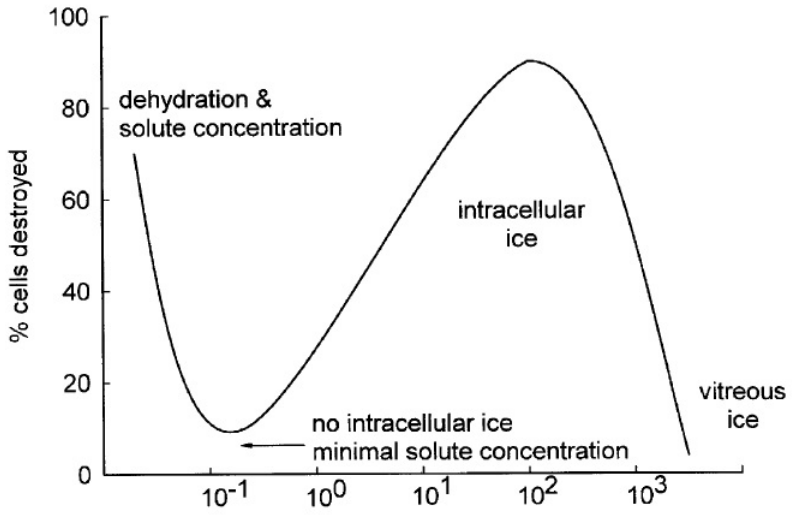


Figure 1.1: Influence of cooling rate on cell death [41]

Extracellular ice formation: It occurs at a lower cooling rate when ice crystals forms outside the cell.

Mechanism of tissue damage: Both the factors, Lethal temperature and cooling rate, are responsible for the cell damage. The lethal temperature guarantees the cell death but mechanism of cell death depends on the cooling rate. Cell death can also be observed above the lethal temperature. Researchers [74, 108] have shown the influence of cooling rate on cell damage. The role of cooling rate and lethal temperature on cell death is shown in fig. 1.2 and fig. 1.1. A cooling rate higher than 10 to several hundred degrees per minute and the temperature range from -20 to 40 ^{0}C cause the nucleation of ice within the cell before the process of osmotic dehydration. It causes the entrapment of water in the cell and ice crystals damage the cell walls during freezing. However, at a lower cooling rate, ice crystal forms in the extracellular spaces. It creates osmotic potential in intracellular and extracellular spaces resulting in the movement of water from the intracellular space to the extracellular space as shown in fig. 1.3. The imbalance caused due to this movement damages the enzyme system and damages the cell wall.

Cryogun: It is a portable device used to spray the cryogen on the lesion. Such a device procured from SMT Praha CS-1 cryogun is shown in fig. 1.4 . It comprises of the following components:

Dewar tank for holding nitrogen: It comes in variable volumes such as 0.2 liter, 0.3 liter and 0.5 liter.

Bypass valve: It is mounted on the top of the cryogun. It regulates the pressure inside the cryogun.

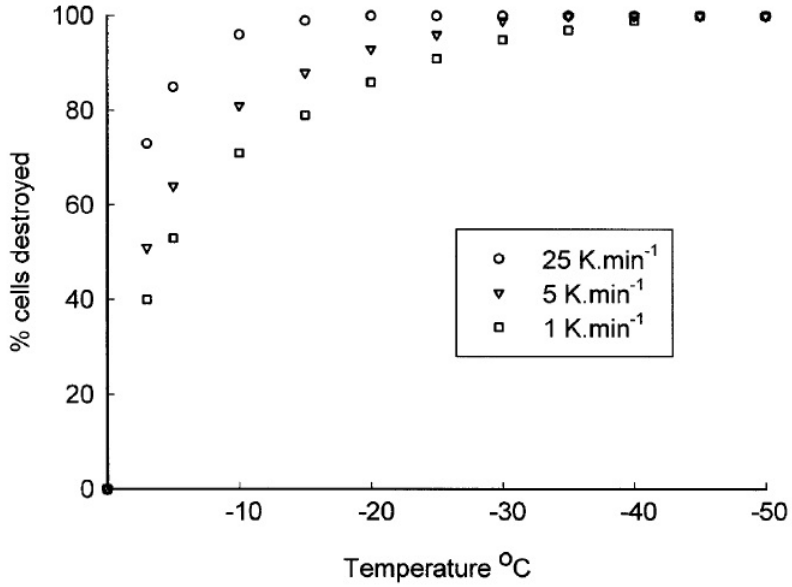


Figure 1.2: Influence of lethal temperature and cooling rate on cell death [41]

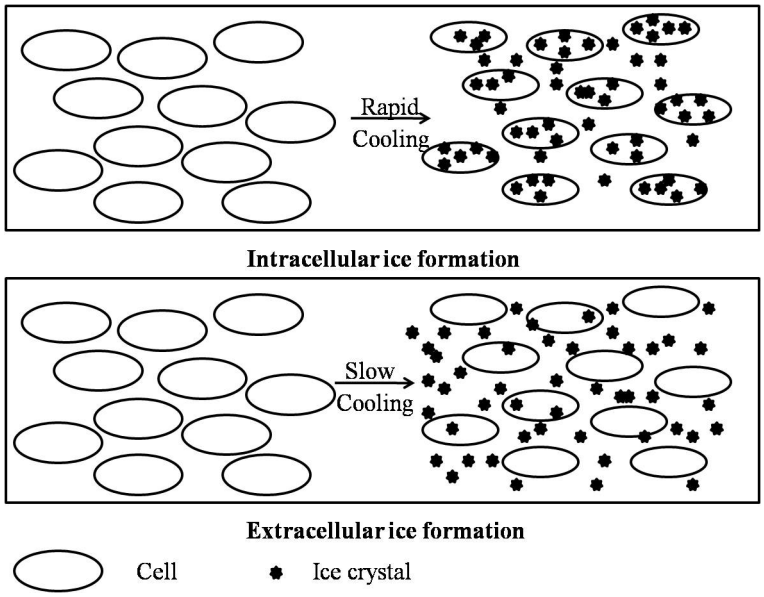


Figure 1.3: Intracellular and Extracellular Ice Formation

Figure 1.4: Cryogun

Spray nozzle: The hole diameter of spray nozzle depends on the size of lesion. Companies like Brymills and SMT Praha provide single hole nozzle ranging from 0.4 mm to 0.8 mm.

Trigger: It initiates and ends the cryogen spray.

Spraying distance: It is the distance between the nozzle exit to the lesion surface. The accepted range of spraying distance for single hole nozzle varies from 15 mm to 20 mm (refer fig. 1.9).

Probe and tips: Different probes are also used for spot freeze technique whereas tips are used to spray cryogen in delicate areas.

Phantom: It is used in in-vitro experiments and it mimics the property of lesion. Agarose powder is generally used for making the phantom. It is frequently used in the molecular biology applications.

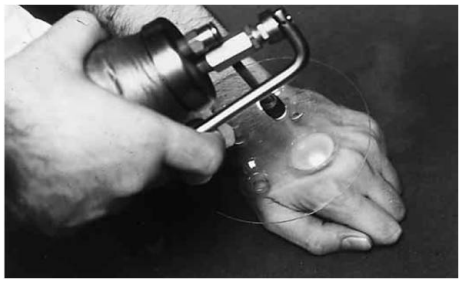
1.1.2 Diversity in cryotherapy

The scope of cryotherpay is quite diverse in terms of its application. It is used in the treatment of following diseases listed in table. 1.3.

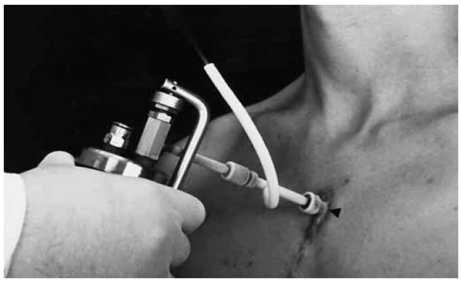
Table 1.3: Diversity in cryotherapy

Disease	Symptoms	Treatment methodology

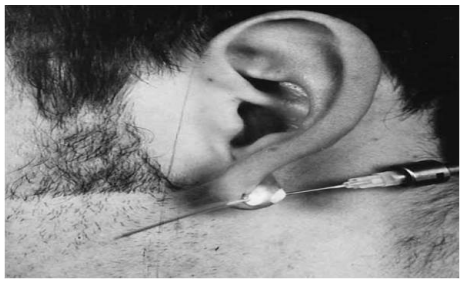
Port wine stain birthmark	It is a type of birthmark that occurs due to discoloration of the skin. It appears as a pink mark on the skin and later turns into maroon color. It is generally spotted on face, neck and hand	Milliseconds spurts of cryogen (R134a, R404a) are sprayed on the affected area followed by laser heating. Cryogen is sprayed to minimise the risk of epidermal damage during laser heating
Skin cancer	It occurs due to abnormal growth of skin tissue; often develops on the skin exposed to sun	10-30 s spurts of cryogen (liquid nitrogen) is applied on the affected area and cryoablation is achieved
Barretts esophagus	Often spotted as pink lining of the swallowing tube caused due to acid reflux	10-30 s spurts of cryogen (liquid nitrogen) are applied on the affected area and cryoablation is achieved
Rheumatoid arthritis	It is a chronic inflammatory disorder affecting joints of the body. It occurs due to malfunctioning of body's immune system	Whole body cryotherapy (with liquid nitrogen) is given to the patient for 3 minutes at -120 ^0C. Body is cooled above lethal temperature
Prostate Cancer	It is a gland responsible for the production of seminal fluid. Cancer occurs inside this gland generally after 50 years in male	A thin metal probe is inserted through skin into the prostate to freeze the tumor below lethal temperature
Osteoarthritis	It occurs when the cartilage that cushion the bone ends or deteriorates with time	Whole body cyotherapy, cold compress, cold sprays and ice message is given to the patient [46]
Obesity	Caused due to the excessive accumulation of fat in the body	Cryolipolysis, it is also known as fat freezing, in which cold temperatures are applied to reduce the fat deposits in the skin [107]



(a) Spray technique



(b) Spot freeze technique



(c) Intralesional technique

Figure 1.5: Method of cryotherapy for superficial lesions

1.1.3 Modern Cryotherapy

Commercial manufacturing of cryotherpay apparatus has started in 1960s due to its remarkable properties. Former methods such as cotton swab soaked in cryogen and copper disc soaked in cryogen became obsolete. The design of compact equipment increased the scope of cryotherapy (in terms of lesion diversity). In the present scenario, three techniques viz. spray technique, spot freeze technique and intralesional technique are quite popular in the treatment of superficial lesions. They are depicted in fig. 1.5. In the spot freeze technique and intralesional technique, the cryogen does not come in direct contact with the lesion, it circulates inside the cannula. Hence, the amount of cooling produced by these two techniques is less as compared to the spray technique. Spot freeze technique is suitable for the flat lesions whereas the intralesional technique is suitable for the protruding lesions. Moreover, chances of infections are also high with the intralesional technique because the lesion is punctured with a needle. Likewise, the apparatus need to be cleaned properly after every treatment cycle in spot freeze technique to reduce the chances of infection. Spray technique surpasses all these limitations of the former techniques. In this technique, cryogen comes in direct contact with the lesion; it causes more cooling of lesion which results in the greater destruction of lesion (more necrosis) in a short span of time. Further, the equipment does not come in direct contact with the lesion, so the chance of infection is less. Most important, in spot freeze and intralesional techniques,

Figure 1.6: Various spray methods [170]

the dimension of device is fixed so the modification in the equipment is required for different lesions depending upon their dimensions. Whereas in spray technique, the spray zone can be varied as per the lesion dimension and the amount of cooling can be manipulated.

Even after having several advantages over its analogous techniques, spray method has several challenges as well. The dimension of lesion varies from case to case, but the dimension of spraying nozzle is fixed. Hence, surgeons have to opt various spraying techniques to cover the whole lesion. These techniques are shown in fig. 1.6. The selection of these techniques depends on the dimension of lesion; if the lesion is small (< 5 mm) then direct spray technique is used whereas circular and paint brush technique is used for lesions upto a dimension of 5-15 mm. For the treatment of larger lesions, surgeons have to use overlapping spray technique as shown in fig. 1.7. In this technique, surface area of lesion is divided into several parts. Selective freezing is carried out on that limited part of lesion and it continues until the whole lesion is covered. Since the whole process is governed manually so there are chances of annihilation of healthy tissue due to over penetration of cryogen. Neoprene cones and cryoplates are used to deal with such situations (refer fig. 1.8). Their selection and application depend on the type (nodular, flat etc.) and orientation (in terms of the body part) of the lesion.

The spraying nozzles used in cryogen spray cooling are circular so the conical pattern of spray is obtained from them. In order to obtain maximum cooling, cryogen should be sprayed perpendicular to the skin surface. There are several factors that govern the efficacy of cryospray process such as cooling rate, thermophysical properties of the lesion and its orientation, duration of spray, spraying distance, nozzle geometry etcetra. The schematic diagram of cryospray process is shown in fig. 1.9.

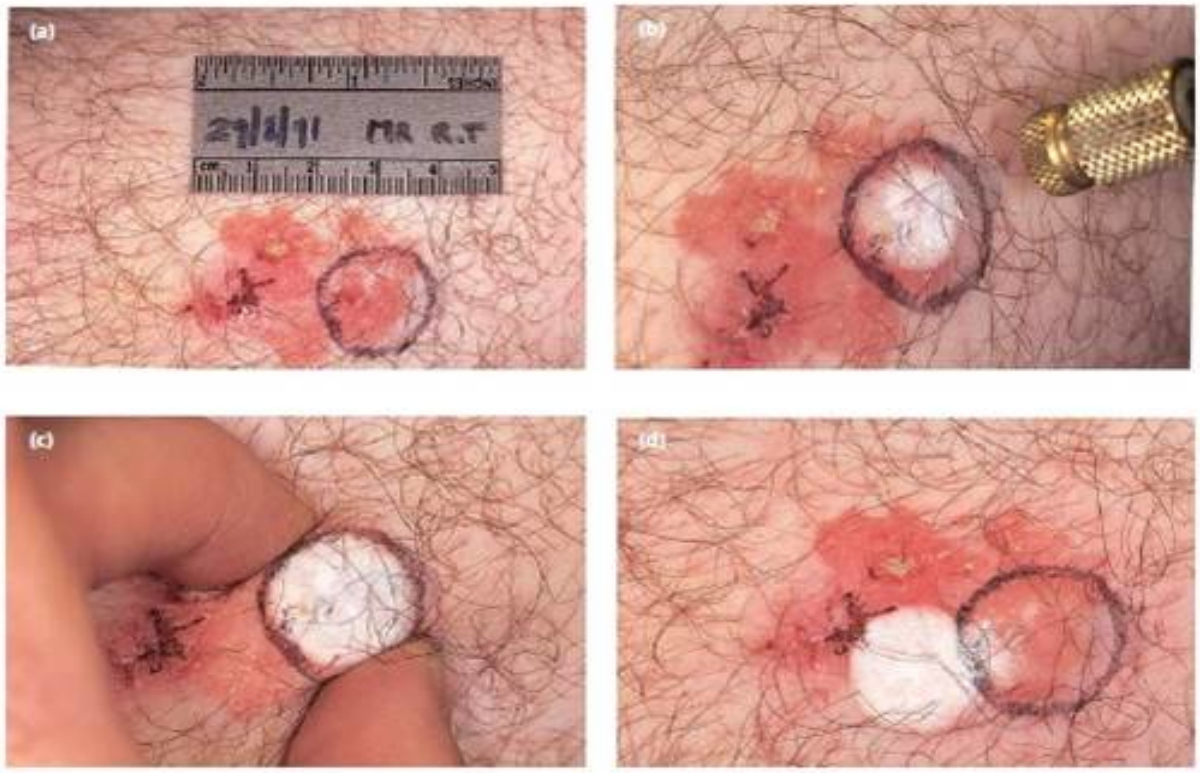


Figure 1.7: Overlapping spray technique [170]

(a) Neoprene cone (b) Cryoplate

Figure 1.8: Neoprene cones and cryoplates [170]

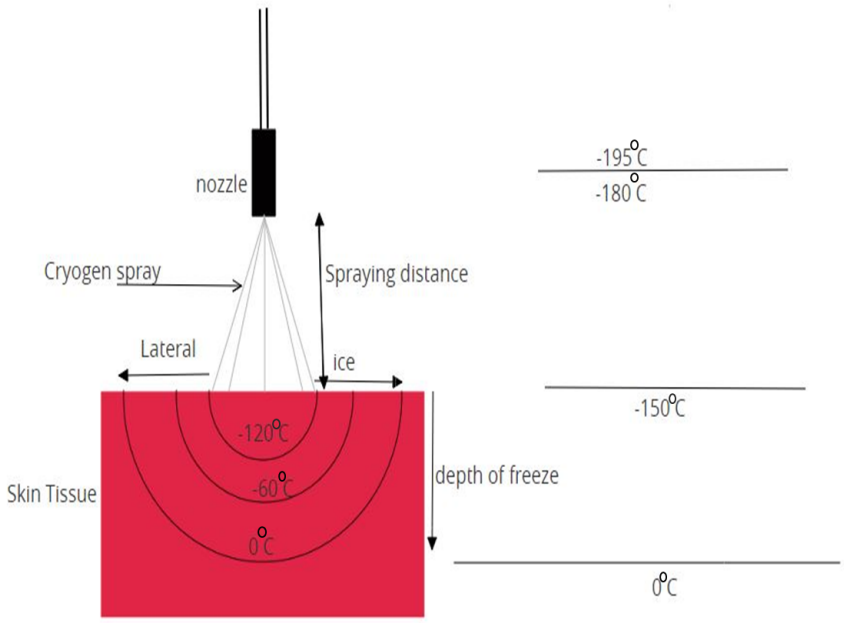


Figure 1.9: The schematic representation of cryospray

1.1.4 Pros and cons of cryotherapy

1.1.4.1 Pros

- Optional anesthesia: The treatment procedure of cryospray completes within a minute and the extreme cold produced due to spray numb the tissue. Hence, the use of anesthesia is optional in this modality. However, for special cases ledocaine (an anesthetic gel) can be used.

- Minimal wound care: Bleeding does not occurs during the process, so the chances of infection are quite less. Moreover, there is no need to cover the treated area.

- No need of suture removal: Cryospray is a non-invasive process as it deals with the superficial lesions.

- Useful in pregnancy: Medication is not required for the treatment. Thus, it can be used with the pregnant women.

- Economical: The whole cryospray setup costs around $ 2000 with only liquid nitrogen (cryogen) as consumable part. Liquid nitrogen is easily available across the globe at a reasonable price, so the process is quite economical.

1.1.4.2 Cons

- In the present scenario, real time imaging of the cryospray process is a challenge task. The present imaging techniques like interaoperative ultrasound requires surface contact which is not possible in cryospray process because it deals with superficial lesion. Moreover, imaging techniques like magnetic resonance imaging requires sophisticated environment which adds in the complexity of the system. Hence, the estimation of necrotic zone depends on the experience of the medical practitioner only.
- The outcomes of the cryospray process are operator dependent, because cryogen is sprayed manually on the lesion.
- Optimal planned protocol in terms of lesion dimension is required to be introduced in cryospray which can predict the exact thermal history of lesion with respect to time.

1.1.5 Thesis structure

The present thesis is divided into six chapters. The evolution of cryospray in a chronological order is discussed in the first chapter along with the different terminology of the cryospray process. Diverse applications of cryospray are also mentioned in the chapter. Chapter 2 presents the literature survey in the field of cryospray. The chapter acknowledges the studies conducted to analyse the physics of cryoablation as well as the innovation in the equipment to increase the efficacy of cryotherapy and cryospray. The novel concept of increasing the efficacy of cryospray process through the application of multihole nozzles is introduced in Chapter 3. The chapter deals with the in-vitro study conducted to optimise the parameters of multihole nozzle. Chapter 4 represents the comparative analysis of cryoablation in in-vivo and in-vitro experiments. The influence of adjuvants on cryoablation is discussed in chapter 5. A numerical study to investigate the role of spraying distance in cryospray process is presented in chapter 6.