# Abstract

Cryospray is a process in which liquid nitrogen is sprayed on a superficial cancerous lesion to achieve necrosis. In order to achieve necrosis through cryospray a combination of particular cooling rate and lethal temperature is required. The small aperture of single hole nozzle (SHN) and less mass flow rate associated with it reduce the rate of heat transfer from the lesion. Moreover, the low thermal conductivity of lesion further reduces the rate of energy diffusion inside it. These limitations associated with the existing method of cryospray fail to provide complete necrosis in the lesions larger than 15 mm in diameter.

Moreover, a distinctive trend is always observed in the geometry of skin cancer, i.e. more lateral spread on superficial skin as compared to the penetration depth into the skin. Cryospray techniques followed in current scenario compels surgeons to use single hole nozzle to treat skin cancer. The SHN provides less lateral spread compared to penetration depth, which ultimately lingers the process and the chances of healthy tissue destruction also increase due to over penetration of cryogen. In this perspective, six multihole nozzles (MHN) are designed and fabricated to study their influence on cryoablation. It has been observed through in-vitro study that these customised MHNs can rule out the existing limitations of cryospray. The designed MHNs are capable enough to treat larger lesions in terms of lateral spread in one sitting as compared to the existing methods that require more than one sitting, resulting in a fair amount of discomfort to patients. Several governing factors of cryoablation like spraying distance, number of holes in the nozzle, margin among the holes and spray duration are optimised for the MHN. The optimum range of cooling rate and lethal temperature required for the intracellular ice formation is obtained upto 15 mm from the centre of spray (on the surface of gel) when MHNs are employed to spray cryogen. However, commercial SHN fails to provide such outcomes.

These nozzles (MHNs) are tested under the in-vivo conditions as well. An in-vivo experiment is performed on healthy male rats (Charles Foster rats) weighing about 150–200 g while the in-vitro experiment is performed on tissue phantom. Single freeze–thaw cycle (freezing 120 s and thawing 130 s) with a spraying distance of 18 mm is selected for both the experiments. A comparative study between customised MHN (with 5 holes and margin 2 mm) and commercial SHN is conducted to analyze the impact of number of holes on cryoablation in in-vivo conditions. It is an attempt to explore the difference between in-vivo and in-vitro experiments. The data extracted through thermocouples advocates that biological factors have negligible impact on cryoablation. However, histopathological results suggest that in-vivo necrotic zone is larger than the in-vitro necrotic zone; natural thawing is responsible for such behavior. The area of cryoablation on the surface of rat skin is 50 % larger when cryogen is sprayed through MHN as compared to SHN.

As mentioned above, a combination of particular cooling rate and lethal temperature is required to achieve necrosis through cryospray. MHNs used in the study fulfill that requirement upto a depth of 2 mm below the gel surface and in a radius of 10 mm from the centre of spray when the spraying distance is taken as 18 mm and the duration of spray is kept constant as 120 s for all the cases. The dimensions of necrotic zone for the same spray duration and the spraying distance can further be improved with the introduction of adjuvant in the lesion. Thus, in-vitro experiment to quantify the role of adjuvant in cryoablation with MHNs is also carried out in the study. A comparative study between nano-phantom and normal-phantom is conducted to examine the influence of adjuvant in cryoablation. The most promising adjuvant and MHN for efficient cryoablation are obtained through the study. It is found that Magnesium Oxide nanoparticle provides the most optimised result with MHN having 5 holes and margin 1.5 mm in terms of cryoablation. The proposed approach is employed with SHN as well. It has been observed that lesions with a surface area of 7.5 $cm^2$ and a penetration depth of 2 mm can easily be treated through the administration of MgO nanoparticles with SHN. On contrary, conventional technique of cryospray (without administration of adjuvant) can treat lesions with a surface area less than 3.14 $cm^2$ with a penetration depth of 2 mm.

The amount of cooling produced by cryogen during the spray depends on its interaction with the surrounding. Cryogen sprays are different from liquid sprays in which mechanical forces cause the atomisation of liquid. The saturation temperature of cryogens is much lower than the ambient temperature, therefore flashing occurs in the cryogen as they interact with the surrounding. Flashing causes primary atomisation of the droplets. Also, secondary break up occurs due to the surface tension of the droplets and velocity difference between the two phases. Thus, during its (cryogen) flight from nozzle exit to the cooling surface, cryogen exchanges heat and mass with the surrounding. The experimental and numerical studies conducted so far in the field of laser dermatology have enhanced the understanding of this phenomenon but absorbing its full potential in cryospray requires more research. In this perspective, an Eulerian- Lagrangian mathematical model is developed to simulate the behavior of cryogen spray.The effect of spraying distance on necrosis is quantified. The multi-phase flow of cryogen emanating from commercial cryospray nozzle is validated against the experimental result. The axial depth and radial spread of ice ball are found to increase by 15 % and 25 % respectively when the spraying distance is changed from 27 mm to 9 mm. It is also noticed that spray dispersion increases with the duration of spray. It causes a larger necrotic zone in radial direction than that in the axial direction with respect to time. It has been observed that the spraying distance of 18 mm is providing the most optimised necrotic zone among the three spraying distances considered in the study.

***Keywords:*** **Thermal Imaging Camera; Cryospray; Multihole nozzle; Singlehole nozzle; Necrotic zone; Nanoparticle; Flashing;**