A novel experimental model for studying efficacy of cryosurgery in keloids

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Abstract
Background Intralesional cryosurgery is effective in the treatment of keloids; however, clinical studies have presented diversified results.
Objective A novel, reproducible model for biophysical studies on intralesional cryosurgery of keloids is presented.
Methods Triplicate studies with a cryosurgical needle on 37°C-heated potatoes, which exhibit identical specific heat and similar heat conductivity with human skin, were performed.
Results No complete potato freezing resulted through a cryosurgical needle. The limited tissue damage achieved had a double concave form. The needle induced lower temperature and stronger tissue damage at the distal exit than the proximal entrance site. The concave form of tissue damage flattened with time at the area under the needle. Needle freezing with puncture distances of 0.5, 1.0 and 1.5 cm from the potato surface only revealed freezing temperatures within the 0.5 cm range. At any needle depth, tissue damage was detected at only an area to about 1 cm under the needle.
Conclusion Clinical extrapolation of these experimental findings indicates a proper needle positioning towards the keloid basis, shows keloid volume freezing limitations by a single needle and corroborates the observations of minor epidermal and deep dermal damage induced by intralesional cryosurgery.

Conflict of interest
This work was an investigator-initiated project. The authors declare no competing financial interests regarding both this manuscript and the research field. Overall, VAZ reports no conflict of interest. CCZ has received honoraria as an advisor and speaker for studies or lectures not associated with this study from AbbVie, Allergan, Almirall, Bayer Healthcare, Biogen, Celgene, Galderma, General Topics, GlaxoSmithKline, Idorsia, InflaRx, JenaPharm, Novartis, Pierre Fabre, PPM, Sobi and UCB. His department has received grants not associated with this study from AbbVie, AOTI, Astra Zeneca, Biogen, Celgene, Dr. Reddy’s, Galderma, Novartis and UCB for his participation as an investigator.

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Key message
A novel, reproducible biophysical model for intralesional cryosurgery was developed. The proper cryosurgical needle positioning, the tissue volume freezing limitations by a single needle and the minor epidermal and deep dermal damage induced were assessed.
Introduction

Keloids are benign tumours that can even occur after minimal skin injury. The probability of occurrence of hypertrophic scars or keloids after wounding depends on the duration of wound healing. Burn wounds have a 4% risk of developing into a hypertrophic scar after a healing period of maximum 10 days, but a 70% or greater risk when they take 21 days or more to heal.

Keloids can often induce a significant impairment. Cryosurgery has been proven to be a particularly effective treatment. Classical surgery exhibits recurrence rates of 45–100%, and radiation therapy is side-effect-rich. In contrast, the recurrence rate of cryosurgery and its side-effects and contraindications are considerably low. Among the different cryosurgical procedures, best results can be achieved by intralesional cryosurgery, a technique initially described by Weshahy for the treatment of epithelial lesions, such as warts, and been significantly modified by Har-Shai and Zouboulis to fulfil the requirements of dermal lesion and especially keloid treatment.

Experimental models for studying the cryosurgical treatment of keloids are missing. Interestingly, the specific heat of human skin and potatoes is identical, namely 3.39 KJ/kg. The specific heat capacity of the average tissue is 3.5 KJ/kg. Heat conductivity is also similar between human skin (approx. 0.38 W/m °C) and potatoes (approx. 0.57 W/m °C). These data have previously lead us and others to use potatoes in elucidating the therapeutic potency of cryosurgery on vessels and of contact cryotherapy on warts. The present study targeted the development of a relevant and reproducible cryosurgery model for elucidating the biophysical properties of intralesional cryosurgery in the treatment of keloids.

Materials and methods

The impingement of intralesional cryosurgery using a Cryoshape needle (Etgar Group, Kfar Saba, Israel) was studied on potatoes, a biological tissue with human skin-like biophysical properties. Potatoes were adapted to normal human body temperature by heating to 37°C before being exposed to cryosurgical treatment. All experiments were carried out thrice. The first set of experiments aimed to evaluate the duration of a complete potato freezing. For the second set of experiments, potatoes were treated for 5, 10 and 15 min with the cryosurgical needle, which was pierced at about 3/4 of the length through the short dimension of the potato. During the freezing procedure, the temperature at the two injection sites of the potato was measured with a GTF 300 NiCr-Ni thermocouple type K (temperature drift of 0.01%; Greisinger, Regenstauf, Germany). Subsequently, the potato was divided in two equal parts by a knife and the spread of cell destruction (frostbite) was recorded. The third set of experiments targeted the detection of possible differences in the frozen area, at various puncture depths. For this purpose, the needle was pierced into the potato at 0.5, 1.0 and 1.5 cm distance from its surface and the temperature was measured at the potato surface. Tissue damage caused by the treatment with the needle has been documented by a photographic device (A6000; Sony, Tokyo, Japan) standardized for distance under similar light conditions. A computer simulation of the frostbite spread around the needle was modelled using the photographs. In addition, a comparison of the temperatures at the proximal entrance and distal exit sites of the needle was carried out.

Statistical evaluation

For the second set of experiments, the mean values and the standard deviation of the different runs were assessed and compared among the different treatment durations by Student’s t-test. In the third set of experiments, the mean values of the different time points, the standard deviation and error of the individual test series were calculated and statistically compared by ANOVA test. Differences were considered to be significant when P < 0.05.

Results

In the first set of experiments, no complete freezing of a potato could be achieved over long time periods. The detected potato tissue damage occurred in a double concave form (Fig. 1a). The concave shape of the frozen tissue flattened below the needle with freezing time (Fig. 1b). However, the tissue damage below

![Figure 1](image1.png)

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the cryosurgical needle did not exceed the distance of approximately 1.0 cm independently of the freezing duration.

In the second set of experiments, a marked (but not statistically significant) difference of temperature drop was recorded between the proximal entrance and the distal exit sites of the needle (Fig. 2a), as also visualized by the tissue damage (Fig. 2b). In order to explain this finding, a temperature measurement was carried out directly on the cryosurgical needle. After a short time, distal exit site temperatures of \( \leq -65^\circ C \) were assessed (with \(-65^\circ C \) being the lower limit of the temperature measuring instrument), while the proximal entrance site only reached temperatures around \(-47^\circ C \).

A complete freezing of the potato tissue over the freezing needle in the third set of experiments was only achieved at a needle position of 0.5 cm under the potato surface (Fig. 3a), while at 1.0 and 1.5 cm distances, the potato surface temperatures were only reduced to 5–10\( ^\circ C \), at which the temperature curve almost flattened after 25 min of freezing (\( P < 0.0001 \); Fig. 3b).

**Discussion**

Although intrallesional cryosurgery has been reported to be effective in the treatment of keloids,\(^6,8,15–17\) reports also exist, which dispute this efficacy.\(^18\) Moreover, the result of a cryosurgical treatment depends on several factors, including the technique and parameters used as well as the experience of the treating physician.\(^16\) In order to obtain objective information on the proper technical procedure of intrallesional cryosurgery, we have developed and present here a novel biophysical model, which imitates the *in vivo* procedure. The most important information obtained from our experiments is as follows: (i) the frozen tissue shape is that of a concave, whereas the frozen part below the cryosurgical needle flattens with time and does not exceed 1 cm from the needle. (ii) The freezing effect is not equally distributed at the proximal entrance and distal exit sites of the needle, whereas the distal needle exit site induces a stronger freezing effect. Therefore, and in order to maximize effectiveness, two needles could be used for large keloids arranged one over the other at distances of maximally 2 cm and entering the tissue from contralateral sites (parallel or crossed). (iii) The tissue over the cryosurgical needle was entirely frozen only at a needle position of 0.5 cm from the tissue surface but not at deeper positions. Therefore, a needle positioning deeper than 1.0 cm from the surface protects the surface from freezing, a finding that confirms the low rates of epidermal damage and permanent depigmentation of intrallesional cryosurgery reported compared with surface cryosurgical methods.\(^5,7\) On the other hand, it can be concluded that a single cryosurgical needle can provide

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**Figure 2** (a) Temperature measurements at the proximal entrance site and the distal exit site of the cryosurgical needle during potato freezing. A marked, but not statistically significant, temperature difference between the proximal entrance site and the distal exit site was recorded, whereas the temperatures at the distal exit site were markedly (but not significantly) lower at 5 and 10 min of freezing. Mean values and standard deviation of three independent experiments are presented. (b) Potato tissue damage after freezing.

**Figure 3** (a) Complete freezing of the potato tissue over the cryosurgical needle at a needle position of 0.5 cm below the potato surface. (b) Temperature of the potato surface with freezing time after positioning the cryosurgical needle 0.5, 1.0 and 1.5 cm below the potato surface. The surface temperatures at the different depth position of the cryosurgical needle were significantly different through the time (\( P < 0.0001 \)).
satisfactory freezing to only small keloids, and for larger and vascularized ones, several needles have to be simultaneously administered. A single needle should be positioned at approx. 1 cm from the keloid basis for optimum treatment results. The aggregated knowledge from our biophysical experiments represents the basis for extrapolation at a clinical setting through future clinical studies, whereas subsequent vascular thrombosis and immunological sequelae may potentiate the clinical result.

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Author contributions
VAZ and CCZ designed the study; VAZ performed the experiments; VAZ and CCZ were involved in statistical analysis; VAZ prepared the manuscript; and VAZ and CCZ revised the manuscript.

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