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ORIGINAL ARTICLE

Effect of skin surface temperature on skin pigmentation during contact and intralesional cryosurgery of keloids

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cryosurgery, intralesional cryosurgery, keloids, skin pigmentation, thermal history

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Abstract

Background This 15-month study was designed to compare the effect of skin surface temperature on skin pigmentation following a single intralesional or contact cryosurgical treatment of keloids.

Patients/methods Thirty Caucasian patients with 45 keloids present for more than 6 months were included in this study. Twenty-one keloids were treated by the contact method while the remaining 24 scars were managed using an intralesional cryosurgery technique. The skin surface temperature at the keloids was measured and recorded using a Ni/Cd thermocouple. Four variables of the thermal history were evaluated with the contact and the intralesional methods, namely cooling rate, hold time, end temperature and thawing rate. Assessment of the local hypopigmentation was performed 6 months after the treatment using a pigmentation scale.

Results Significantly slower cooling (6.09 ± 4.56 °C/min) and thawing rates (54.52 ± 32.17 °C/min) were recorded with the intralesional cryosurgery method when compared with the cooling rates (13.47 ± 9.04 °C/min) and thawing rates (89.00 ± 86.42 °C/min) of the contact method ($P < 0.000001$). The end temperature of the contact technique was significantly cooler (–46.77 ± 14.74 °C) when compared with that of the intralesional method (–15.55 ± 6.77 °C) ($P < 0.000001$). There was a trend for the hold time of intralesional cryosurgery to be longer (82.67 ± 138.03 s) than that of the contact method (16.86 ± 23.49 s) ($P < 0.059$). A significant difference in skin pigmentation was demonstrated between the two cryosurgical methods. In 91.7% of the keloids treated by the contact technique a significant hypopigmentation was noticed, while no marked hypopigmentation was detected in the skin surface of the keloids treated by the intralesional method ($P < 0.0001$).

Conclusion We hypothesize that the thermal history of the skin surface during the intralesional cryosurgery technique provides a better survival environment for the melanocytes than the contact method, thus producing a lower rate of permanent hypopigmentation and disfiguring.

Introduction

Keloids, which represent an abnormal healing response of injured skin, have been shown to be resistant to various surgical and non-surgical treatments such as silicone products, steroid injections, laser and surgical excision.[1] Cryosurgery using liquid nitrogen has been shown to be a safe and an effective method for the treatment of keloids, mostly with transient side-effects such as blister formation and local oedema, and with long-term hypopigmentation being the most significant long-term side-effect.[2–6] This persistent change of skin pigmentation may cause an unsatisfactory

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cosmetic result, which could affect self-body image and create social problems, despite the success of keloid treatment in flattening the scar and alleviating clinical symptoms.

Prospective studies have shown that the incidence of hypopigmentation following contact cryosurgery using liquid nitrogen has been up to 100%,[4–7] making cryosurgery difficult to use in dark-coloured skin.

Intralesional cryosurgery using cryoneedles was initially described by Weshahy[8] for the treatment of epidermal and dermal skin lesions. Recently, an intralesional cryosurgery method for the treatment of keloids was introduced.[4,7,9–12] This technique exhibits an increased efficacy in the treatment of keloids when compared with the contact method, due to the enhanced freezing area of deep scar tissue. However, its effect on skin pigmentation has not yet been evaluated. Therefore, this study was designed to compare the thermal history of skin surface temperature during the treatment of keloids using the contact and intralesional techniques and its effect on skin pigmentation.

Materials and methods

Patients

Thirty Caucasian patients with 45 keloids older than 6 months and recalcitrant to at least one other treatment modality were included in this study. Twenty-one keloids were treated by the contact method while the remaining 24 keloids were managed with intralesional cryosurgery. All scars prior to the cryosurgical treatment had no pigmentation changes. Previous scar treatments included surgical excisions, laser surgery, intralesional corticosteroid injections and local application of silicone ointments or sheeting. The hospital ethics committee approved this study and an informed consent was obtained from all participating patients.

The intralesional cryoneedle

A novel intralesional cryoprobe (CryoShape™, Etgar Group International Ltd, Kfar-Saba, Israel; Patented in the USA, Europe, Japan and others) was used in this study.[11,12] This probe consists of an elongated double-lumen, non-insulated needle with a safety vent and a sharp-cutting, sealed, distal tip, which enhances the penetration of the often hard, rubbery and dense keloid. The proximal end of the cryoprobe was attached to an adaptor, which was connected to a cryogen source. By forcing liquid nitrogen to circulate through the needle an ice ball around the cryoneedle developed, causing the abutted keloid tissue to be completely frozen.

The contact cryoprobe

A flat cryoprobe, 1 cm in diameter, (Brymill Cryogenic Systems, Ellington, CT, USA) was used to perform the contact cryosurgery treatment by employing liquid nitrogen as a refrigerant. The metallic probe was placed directly on the keloid surface so that it was in contact with the lesion without injuring the surrounding healthy skin.

Clinical trial protocol

This study, which extended over a 15-month period, evaluated post-treatment hypopigmentation following a single intralesional or contact cryosurgical session. Assessment of hypopigmentation was carried out 6 months after the treatment, by comparing skin pigmentation on the keloid surface with the healthy surrounding skin in a scale from 0 to 2: 0 = treated skin colour without significant pigment changes; 1 = treated skin colour with hypopigmentation and pigment islets; 2 = treated skin colour with hypopigmentation without pigment islets.

During the cryosurgical treatment the skin surface thermal history was recorded using a digital Ni/Cd sensor thermocouple. Four parameters, which constitute the thermal history, were evaluated, i.e. cooling rate, end temperature, hold time and thawing rate.

Cryosurgery treatments

Intralesional cryosurgery method

With the patient lying at a supine position, the skin surface of the keloid was cleansed with disinfecting solution and draped. The area of penetration into the scar and the underlying subcutaneous tissue was anaesthetized locally, using an intralesional approach, with bupivacaine hydrochloride 0.5% (marcaine). Thereafter, the sterile cryoprobe was forced into the long axis of the scar in a forward awl-like rotary movement, parallel to the skin surface. The cryoneedle was inserted at the core of the scar, approximately 5–6 mm deep from the skin surface of the keloid. The scar itself was grasped between the index finger and thumb of the other hand, until the sharp tip of the needle penetrated the opposite distal edge of the scar, thus maximizing the volume of scar tissue to be frozen. Attention was taken to prevent any penetration of the cryoprobe into uninvolved healthy surrounding skin. Sterile gauzes were placed under the proximal and distal parts of the cryoprobe and care was taken to make sure that the vent nostril was positioned away from the patient to prevent accidental freezing of adjacent skin or tissue. The proximal part of the probe was connected via a luer lock elongation tube to the cryogun (Brymill Cryogenic

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Systems), which was filled about 10–15 min beforehand with liquid nitrogen in order to allow a sufficient pressure to build up inside it (about 0.7 ATM/10 psi). The cryogun was placed on a steady surface with no direct contact with the patient's body. By activating the cryogun trigger, the pressure valve was opened and the cryogen entered the cryoneedle, thereby freezing the keloid. A forced steam of the liquid nitrogen gas flowed out from the vent nostril during the entire freezing process. The strength of the steam flow indicated the efficacy of the freezing procedure. Two ice balls appeared shortly at the two cryoprobe penetration sites and with time they gradually spread towards each other until a complete freezing of the scar is achieved clinically. Following the complete freezing of the keloid, regardless of the duration of the cryosurgery process, the cryogun trigger was released to stop the freezing process and the cryoprobe was left to thaw for 1–2 min and was then withdrawn in a reverse awl-like rotary movement. After a complete thawing of the keloid is clinically observed, slight bleeding from the penetration points of the probe requires the application of a sterile dressing. The patients were instructed to wash the treated keloid daily and to apply an antibiotic ointment until full recovery of the keloid was achieved.

Contact cryotreatment

With the patient sitting or lying at a supine position, the skin surface of the keloid was cleansed with disinfecting solution. No use of local anaesthesia was needed. A 1-cm-diameter contact metallic probe was applied on to the keloid surface to cover maximal keloid area without harming the surrounding healthy skin. The proximal part of the probe was connected to the cryogun (Brymill Cryogenic Systems, Ellington, CT, USA). The activation of the cryogen was identical to that of the intralesional method described above. During treatment ice formation appeared on the keloid surface, which spread radially. Treatment was ceased when ice formation reached the keloid border. After the frozen surface of the scar had completely thawed a sterile dressing was applied. The patients were instructed to wash the treated scar daily and to apply an antibiotic ointment until full healing of the scar was achieved.

Thermal history

In our recent publication,[12] the thermal history of the contact and intralesional treatment was evaluated in an *ex vivo* swine model. During the intralesional cryosurgery process the cryoneedle was located at a depth of 6 mm and an abutting thermocouple measured the thermal history. Another two thermocouples were located at a depth of 3 mm and on the muscle surface. A significant difference in the thermal history was recorded between the 6-mm deep, 3-mm deep and the keloid surface as well as significant differences between the surface temperature and thermal history of the two cryosurgical methods. Therefore, on the basis of the *ex vivo* work this clinical study was carried out to evaluate the thermal history of the two methods and its effect on the melanocyte viability and the possible aetiology of post cryosurgical skin hypopigmentation. The keloid surface thermal history was measured and recorded for further analysis using a Ni/Cd thermocouple GMH 3250 (Greisinger Electronic GmbH, Regenstauf, Germany) capable of measuring temperature at fixed intervals (1 s) and with a precision of 0.001 °C. The memorized data were transferred to a personal computer and were analysed using the Excel program. During the contact treatment, the thermocouple probe was placed between the skin surface of the treated keloid and the undersurface of the probe (fig. 1). During the intralesional cryosurgery treatment the thermocouple was placed on the skin surface of the keloid just above the cryoneedle, which was inserted beforehand into the core of the scar some 5–6 mm deep. There was no contact between the cryoneedle and the thermocouple (fig. 2).

Thermal history measurements

Four phases, which comprise the thermal history, were measured during the freezing process (fig. 3): (1) cooling rate – the ratio between the initial room temperature and the lowest freezing temperature reached in the time interval $[T_1 - T_2]/[t_2 - t_1]$; (2) end temperature – the lowest temperature reached during freezing $[T_5]$; (3) hold time – the time in which the freezing process retained its lowest temperature $[t_3 - t_2]$; (4) Thawing rate – the ratio between the time elapsed since the cessation of the freezing process and the tissue temperature difference (up to 0 °C) $[T_4 - T_3]/[t_4 - t_3]$.

Statistical analysis

The comparison of the four variables of the thermal history between the contact and intralesional methods (cooling rate, hold time, end temperature and thawing rate) was executed using the two-tailed *t*-test. Comparison of the pre- and post treatment hypopigmentation of the keloid surface was analysed using the Mann–Whitney test.

Results

Thermal history

The comparison of the surface thermal histories of the two cryosurgery techniques revealed a significant difference

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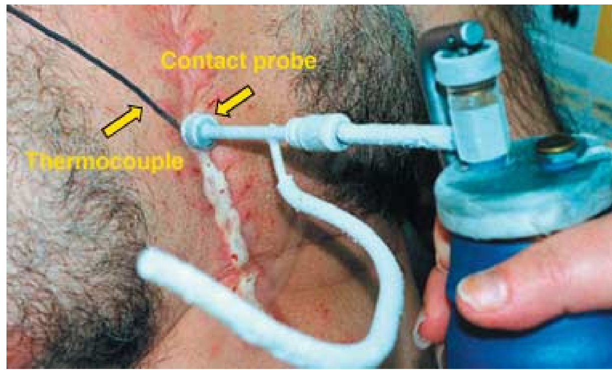

fig. 1 Thermal history measurements during the contact cryosurgery technique. The thermocouple probe was placed between the skin surface of the treated scar and the undersurface of the probe.

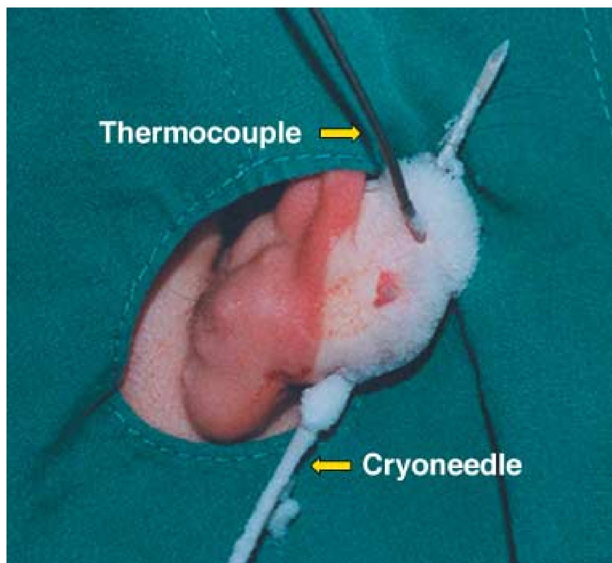

fig. 2 Thermal history measurements during the intralesional cryosurgery method. The thermocouple was placed on the skin surface of the scar just above the cryoneedle, which had been inserted beforehand into the core of the scar to a depth of 5–6 mm.

(Table 1). Intralesional cryosurgery had significantly slower cooling (6.09 ± 4.56 °C/min) and thawing (13.47 ± 9.04 °C/min) rates when compared with the cooling (54.52 ± 32.17 °C/min) and thawing (89.00 ± 86.42 °C/min) rates of the contact method ($P < 0.000001$). The end temperature of the contact technique (–46.77 ± 14.74 °C) was significantly cooler when compared with that of the intralesional method (–15.55 ± 6.77 °C) ($P < 0.000001$). There was a trend for the hold time of intralesional cryosurgery (82.67 ± 138.03 s) to be longer than the hold time of the contact method (16.86 ± 23.49 s) ($P < 0.059$).

Pigmentation changes of treated skin lesions

Both groups of the treated keloids were examined (by the authors Y.H.S. and E.D.) 6 months following the cryosurgery treatments. Twelve patients with 12 keloids treated by the contact method (80%) and 14 patients with 22 keloids treated by the intralesional technique (93.3%) were evaluated for pigmentation changes following a single freezing cycle. Hypopigmentation grading scores were evaluated (Table 2). The results revealed a significant difference in skin pigmentation between the two cryosurgical methods. In 91.7% of the keloids treated by the contact technique a significant hypopigmentation (score 1 and 2) was noticed while the skin surface of the keloids treated by the intralesional method did not exhibit marked hypopigmentation (i.e 0% score 1 and 2) ($P < 0.0001$) (fig. 4).

Discussion

The sensitivity of melanocytes to cold has been documented experimentally and clinically.[13,14] Skin hypopigmentation following cryosurgery can be caused by the destruction of melanocytes, a reduction in melanosomes synthesis or a block in melanosome transfer into the keratinocytes.[15] Burge *et al.*[14] investigated post cryosurgery hypopigmentation and hyperpigmentation on tricolour guinea pigs after standard freeze contact cryosurgery. This animal skin

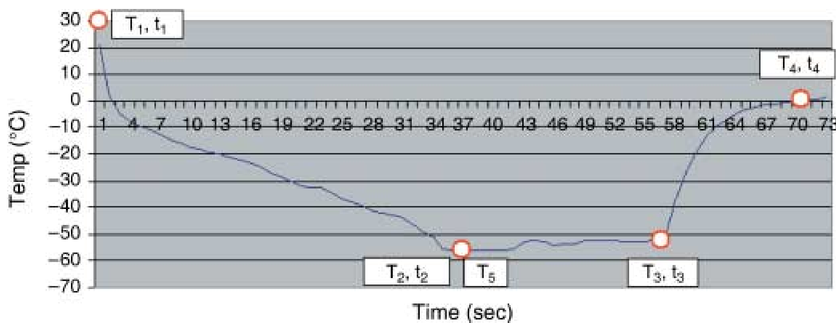

fig. 3 Schematic representation of thermal history measurements during a cryosurgical process (contact/intralesional), which includes four phases: cooling rate $[T_1 - T_2]/[t_2 - t_1]$, end temperature $[T_5]$, hold time $[t_3 - t_2]$ and thawing rate $[T_4 - T_3]/[t_4 - t_3]$.

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Table 1 Measurements of the four phases of the thermal history recorded during contact and intralesional cryosurgery

	Cooling rate* (°C/min)	End temperature* (°C)	Hold time* (s)	Thawing rate* (°C/min)
Contact	54.52 ± 32.17	–46.77 ± 14.74	16.86 ± 23.49	89 ± 86.42
Intralesional	6.09 ± 4.56	–15.55 ± 6.77	82.67 ± 138.03	13.47 ± 9.04
P-value**	0	0	0.05949	0

*Mean value ± STD; ** *t*-test (two-tailed).

Table 2 Post cryosurgery scar surface hypopigmentation following contact and intralesional cryosurgery. A high score grade indicated a more pronounced change of hypopigmentation

Hypopigmentation	Contact	Intralesional
0	1 (8.3%)	22 (100%)
1	9 (75%)	0 (0%)
2	2 (16.7%)	0 (0%)
Total	12 (100%)	22 (100%)

$P < 0.0001$ (Mann–Whitney test). 0: treated skin colour without significant pigment changes; 1: treated skin colour with hypopigmentation and pigment islets; 2: treated skin colour with hypopimentation without pigment islets.

model has been used widely in the investigation of wound healing due to the similar melanosome distribution pattern when compared with human skin. Melanocytes, which are DOPA-positive, could not be detected 1 month after cryosurgical treatment. Gradual melanocyte migration into the frozen lesion was detected and during the following 3 months most lesions were entirely recolonized with melanocytes, which were distributed evenly throughout the diffusely hyperpigmented skin. This melanocyte distribution in the hyperpigmented lesion was pathological when compared with the adjacent normal skin in which melanocytes were concentrated around hair follicles or along epidermal ridges. Burge *et al.*[13] have also shown a characteristic central hypopigmentation and a peripheral hyperpigmentation rim in the area treated with spray cryosurgery on the flexor surface of the forearm of healthy adult volunteers. These findings have persisted for months and even years after the freezing procedure. Repigmentation of deeply frozen lesions is rare, although it may be possible to demonstrate melanocytes in the wound. Gage *et al.*[16] have carried out a controlled freezing injury using the contact method on the skin of adult mongrel dogs. The tested end temperatures were between 0 and 40 °C. Biopsies of the various treated sites were obtained and evaluated by light and electron microscopy. The results of this study revealed that viable melanocytes with melanin were identified in the freezing range of 0 to –4 °C. In the range between –4 to –7 °C, lysis of pigment granules or enzymatic melanin digestion within melanocytes and keratinocytes were noticed in the deeper layer of the frozen epithelium. Between the ranges of –7 to –30 °C, no melanocytes or melanin were demonstrated. The authors concluded that selective destruction of melanocytes could be achieved in the temperature range –4 to –20 °C, while repigmentation was clearly temperature-dependent and did not occur at temperatures colder than –30 °C.

In our study, after 6 months follow-up 91.7% of contact cryosurgery lesions revealed a 1 to 2-grade hypopigmentation score, which indicates a high degree of melanocyte malfunction or loss. The follow-up of the intralesional cryosurgery lesions revealed no severe hypopigmentation, which indicates an almost normal distribution and function of melanocytes compared with the adjacent healthy skin. In the majority of cases a significant repigmentation could be seen 6 months following the treatments; however, this follow-up period may be different depending on the skin type of the population studied.

These results can be explained by the significant difference of the thermal history between the two cryosurgical techniques. Contact cryosurgery exhibited fast freezing and thawing rates and a profound subzero end temperature when compared with intralesional cryosurgery. Contact cryosurgery applies an exterior metallic probe, composed of brass, with a high thermal conductivity, which ensures negligible temperature loss between the probe and the cryogen. Therefore, the temperature of the keloid surface depends on two variables: (a) heat removal by the cryogen (liquid nitrogen) and (b) skin tissue thermal conduction property, which is affected in part by the subdermal plexus of blood supply that removes or, as in our study, provides thermal energy (fig. 5).

The intralesional cryoneedle, composed of stainless steel, has the same high thermal conductivity as the contact cryoprobe and uses the same cryogen, but the methods differ regarding the position of the cryoprobe. The cryoneedle is inserted into the keloid core so that tissue heat removal takes place at some distance from the skin surface (fig. 6). Due to thermal conductivity, the tissue temperature, as a function of the distance from the cryoneedle, is radially reduced and therefore skin surface

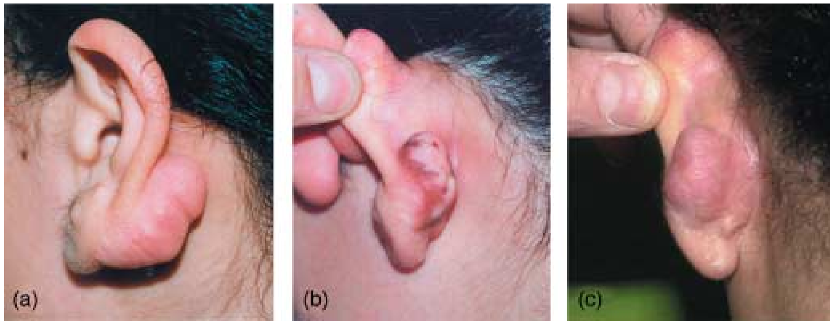

fig. 4 A clinical case demonstrating the sequence in pigmentation changes following the intralesional cryosurgery method which was executed on a keloid located at the posterior aspect of the lobule. (a) Pre-treatment view, (b) 2 months following intralesional cryotreatment (demonstrated in fig. 2). Note the hypopigmentation and appearance of pigment islets. (c) Complete recovery of pigmentation 6 months following treatment.

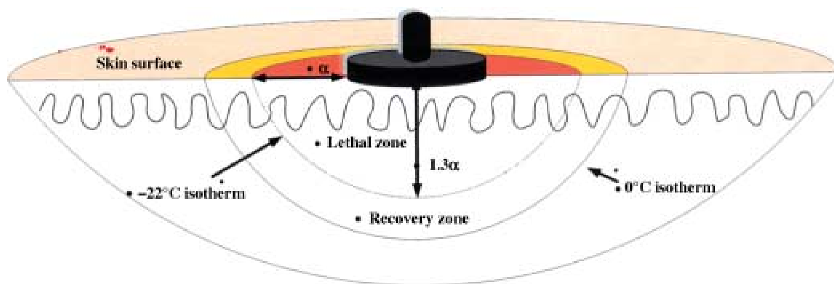

fig. 5 Ice ball induced by the contact cryoprobe. The interface between the ice ball and unfrozen tissue represents the 0 °C isotherm. The volume of tissue located between the –22 °C isotherm and contact probe is the lethal zone in which cells undergo cryonecrosis. Cells situated in the warmer region between –22 °C isotherm and the 0 °C isotherm (recovery zone) generally survive the freeze.

is relatively warmer and the end temperature is significantly higher than with the contact cryotechnique. Therefore, melanocytes, which are only located approximately 0.12 mm from the skin surface, are found at a depth where the difference in temperature compared to the skin surface is negligible.

Apoptosis during freezing injury might be the mechanism by which melanocyte cells die. Hanai *et al.*[17] have identified that human colon carcinoma cells were susceptible to entering the apoptotic state up to 8 h after thawing following exposure to modest freezing temperatures (–6 to −36 °C). Similar findings were reported by Yang *et al.*[18] Furthermore, apoptotic cells after freezing are found primarily in the peripheral zone of the cryogenic lesion, where the temperature was not sufficiently cold to kill all the cells. This study has shown that a significant difference of skin surface temperature (end temperature) exists between the intralesional and contact cryosurgery methods, i.e. −15.55 ± 6.77 °C and −46.77 ± 14.74 °C, respectively ($P < 0.000001$). Therefore, the combination of sensitivity to subzero temperatures and proximity to skin surface can explain the high rate of post contact cryosurgery hypopigmentation which has been clinically demonstrated, when compared with that of the intralesional method. In addition, the skin surface temperature achieved during the intralesional cryosurgery process (−15.55 ± 6.77 °C), which causes a selective destruction (apoptosis) of melanocytes, as was postulated by Baust and Gage,[19] Hanai *et al.*[17] and Yang *et al.*,[18] may permit the migration and recolonization of the melanocytes into the surface of the cryo-injured keloid, thus causing a significant reduction in permanent hypopigmentation.

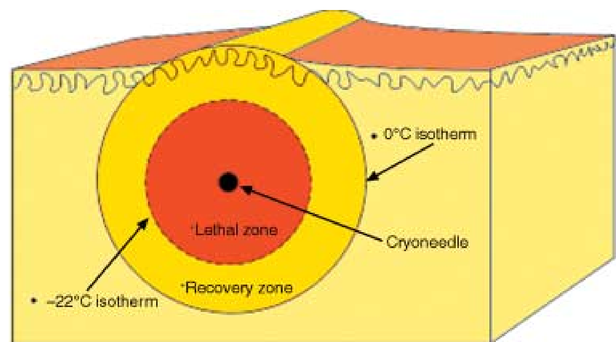

fig. 6 Ice ball induced by the intralesional cryoneedle. The interface between the ice ball and unfrozen tissue represents the 0 °C isotherm. The volume of tissue located between the –22 °C isotherm and contact probe is the lethal zone in which cells undergo cryonecrosis. Cells situated in the warmer region between –22 °C isotherm and the 0 °C isotherm (recovery zone) generally survive the freeze.

Rupp *et al.*[20] has shown on a porcine kidney model that the main mechanism of cell cryo-injury is direct temperature damage and not post cryosurgical vascular stasis. Destruction of cells by freezing not only depends on the end temperature achieved but also on cooling and thawing rates and the hold time. The relationship between cell survival percentage and cooling rate has the shape of an inverted parabola (∩), which represents low survival at slow and fast cooling rates and a higher survival rate at

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moderate cooling rates of the same end temperature. The reason for these variable survival rates is the two main mechanisms, which are involved in cell freezing damage. The first occurs during slow cooling rates when extra cellular ice is formed and cell dehydration is encountered. The second one is expressed during fast cooling rates in which intracellular ice is formed causing direct cellular destruction and cell death.[21,22] An *in vitro* study using an epidermoid carcinoma cell line (A–431) demonstrated a different cell survival rate for the same target end temperature. Two cooling rates of 1 °C/min and 10 °C/min with variable end temperatures were tested. It was evident that the 1 °C/min cooling rate was more lethal to the cultured cells at the same end temperature.[21] Gage *et al.*[23] conducted a study which evaluated the effect of varying freezing and thawing rates in experimental cryosurgery. It was found that a long hold time and slow thawing rate would maximize tissue destruction, especially in cancer cryosurgery.

In our study, moderate cooling and thawing rates (6.09 ± 4.56 and 13.47 ± 9.04 °C/min, respectively) where recorded during the intralesional cryosurgery process when compared with the fast cooling and thawing rates of the contact technique (54.52 ± 32.17 and 89.00 ± 86.42 °C/min) ($P < 0.000001$). We assume that these moderate cooling and thawing rates, which are measured on the skin surface, are more 'friendly' for melanocyte survival. Experimental studies using other cell lines such as AT-1 rat prostate tumour cells and LNCaP Pro5 human prostate tumour cells[21] have shown similar characteristics of death of cells by freezing, but there are no data available on cell behaviour of non-tumourous human skin during freezing.

The trend for a longer hold time demonstrated during the intralesional cryosurgery process when compared with the contact method (82.67 ± 138.03 and 16.86 ± 23.49 s, respectively, $P < 0.059$), which is solely related to the scar volume i.e. a larger scar volume needs a longer freezing/hold time, may explain the superior efficacy of the intralesional technique in the treatment of keloids.[11,12] Lower temperatures are present around the needle; this increases the freezing area of the core of the keloid tissue, which is abutting the cryoneedle, causing cryonecrosis. The long hold time allows time for solute effects, ice-crystal formation and recrystallization effects. Furthermore, in a long duration of freezing, the biochemical changes and the growth of ice crystals are enhanced, increasing the rate of cell death.[19] Therefore, the intralesional technique is more effective than that of the contact method which requires more cryosurgical sessions (between one and 20 sessions),[7] to obtain keloid flattening. On the other hand, the contact method causes a severe superficial cryodestruction harming mainly the epidermis, including the melanocytes, and upper dermis. Although, the lethal zone during the intralesional or contact cryosurgical treatment was not investigated in this study, previous *ex vivo* studies[12] have demonstrated the creation of an ice ball around the cryoneedle at an end temperature, which was lethal for the fibroblasts i.e. −30 °C. Therefore, more cryosurgical sessions (between one and 20 sessions)[7] are required to obtain keloid flattening. Support for this assumption is found in Gage *et al.*'s[23] work, which evaluated the effect of varying freezing and thawing rates in experimental cryosurgery. It was found that a long hold time and slow thawing rate maximized tissue destruction, especially in cancer cryosurgery.

According to our unpublished data, keloids of darker/black-coloured skin exhibits less depigmentation following intralesional cryosurgery when compared with that following the contact method. These findings may encourage the use of intralesional cryosurgery for dark-skinned individuals suffering from keloids.

In conclusion, we suggest that the thermal history of the skin surface during the intralesional cryosurgery technique may provide a better survival environment for melanocytes compared with that of the contact method, thus producing lower permanent hypopigmentation and disfigurement.

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