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Injection Cryolipolysis: First-in-human Study

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Background: Injection cryolipolysis using an ice slurry has been hypothesized to be a novel method of reducing fat. The present first-in-human pilot study aims to investigate the feasibility, safety, and tolerability of ice slurry injection into human subcutaneous fat.

Methods: Preabdominoplasty subjects were recruited. Baseline measurements and serial follow-up visits following a single ice slurry injection procedure into tissue to be excised during abdominoplasty were performed. Melted ice slurry injection was used as control. Feasibility using standard injection techniques was assessed. Thermal imaging was used to determine cooling efficacy. Safety was assessed by adverse event monitoring. Tolerability was assessed by subject-reported pain score. Histology and ultrasound were monitored for structural changes associated with cryolipolysis.

Results: A single injection of ice slurry was feasible and sufficient to cool adipose below the target temperature (10C). There were no serious adverse events. The most common adverse events were bruising and erythema. The mean pain score for ice slurry-injected sites was 1.9/10 and 1.3/10 in control injection sites. Evidence of cryolipolysis was observed on ultrasound and tissue histology in ice slurry-injected sites.

Conclusions: Ice slurry injections are feasible, with an observed safety and tolerability profile comparable to topical cryolipolysis. The ice slurry can cool tissue to induce cryolipolysis, as observed by thermal imaging, ultrasound, and tissue histology, and is selective for ice-injected sites. No significant changes were observed in control sites. The ice slurry may be a promising candidate to enable more precise, effective, and customizable aesthetic fat reduction that warrants further investigation. *(Plast Reconstr Surg Glob Open 2021;9:e3818; doi: 10.1097/GOX.0000000000003818; Published online 23 September 2021.)*

INTRODUCTION

Cryolipolysis is a widespread and effective option for nonsurgical fat reduction.[1] Topical cryolipolysis relies on thermal diffusion through skin, limiting the depth, amount, anatomy, and precision of adipocyte targeting. Injection cryolipolysis using an ice slurry is hypothesized to remove the anatomic and thermal diffusion constraints of topical cryolipolysis, allowing for precise, customizable body contouring in any anatomic area.[2]

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Previous research demonstrated that 30 ml of ice slurry composed of 20%–40% ice content was sufficient to induce cryolipolysis and reduce subcutaneous fat in swine.[2] Preclinical research in a rodent model has also demonstrated that ice slurry injection into the subcutaneous adipose tissue in the neck was capable of inducing cryolipolysis.[3] Taken together, these data suggest that injection of an ice slurry into subcutaneous fat may be a safe, precise, and effective method for reducing subcutaneous fat.[2,3]

A sterile, biocompatible, injectable ice slurry, termed the investigational ice slurry device (Arctic Fox Biomedical, Inc., Cambridge, Mass.), was developed to enable direct

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injection of ice slurry into adipose tissue targeted for reduction in this first-in-human study. The goal of this feasibility study was to determine if precise injection of ice slurry was feasible, well tolerated, and could provide sufficient cooling capacity to perform cryolipolysis in abdominal subcutaneous fat in preabdominoplasty subjects.

Given the rheologic properties of phase-change materials, it was unknown if ice slurry could be injected into subcutaneous fat using standard injection techniques. Additionally, some reports associate cold temperatures with increased injection discomfort compared to physiologic temperatures,[4] whereas other reports indicate cold temperatures may have an anesthetic effect, termed cryoanalgesia.[5] Hence, a key objective was to determine the feasibility and tolerability of ice injections.

The safety and adverse effect profile of topical cryolipolysis has been well established.[1] Preclinical research has suggested that injection of ice slurry in swine resulted in transient erythema and swelling but did not note any additional side effects.[2] Hence, although it was hypothesized that injection cryolipolysis would have a similar safety profile to topical cryolipolysis, the characterization of the adverse effect profile of ice slurry injections into human adipose tissue was unknown. Furthermore, it has been speculated that injection ice slurry has the capacity to be a highly precise method of targeting subcutaneous fat,[2] yet research to date has not explored the effects of injection depth or dispersion of ice slurry in the human tissue.

Finally, although it was established that 30 ml of ice slurry of 20%–40% ice coefficient was able to provide sufficient cooling capacity to induce cryolipolysis in swine,[2] it was unknown if these cooling kinetics would be sufficient to induce cryolipolysis in human adipose tissue, which differs in lipid saturation and structure from swine adipose tissue. This first-in-human study sought to gain insight into the feasibility, tolerability, safety, and potential efficacy of a single injection of ice slurry in preabdominoplasty subjects.

METHODS

Study Design

The study was reviewed and approved as a nonsignificant risk study under IRB controls by WCG IRB. Twelve subjects were recruited at least 12 weeks before their scheduled abdominoplasty procedure. Subject recruitment was completed by collaborating with four board-certified plastic surgeons who provided information about the study to potential participants scheduled for abdominoplasty. If interested in study participation, potential subjects were referred to the study site. The study consisted of eligibility, screening, baseline assessments, a single treatment with the ice slurry device and follow-up visits at 1 day, 3 days, 2 weeks, 6 weeks, 12 weeks, and optionally 16 weeks postprocedure. All subjects provided written informed consent.

Ice Slurry Production

Sterile ice slurry of approximately 30% ice content was produced using a proprietary ice slurry production system and aseptically transferred to the ice slurry injection device. The ice slurry injection device was composed of a standard 10-ml syringe, a custom jacket to provide thermal insulation, and a 14G needle or cannula. The ice slurry device is pictured in Figure 1. Visualization of ice slurry injection ex vivo is available in the Video. (**See Video [online]**.)

Ice Slurry Injection Procedure

The surgical site to be excised during the scheduled abdominoplasty procedure was demarcated by the referring plastic surgeon. Then four 2 × 2 inch square injection sites were placed, using surgical tattoos at the corners, into this demarcated area. Three sites received ice slurry and one site received melted ice slurry solution. Melted solution has previously been used as a control comparator.[2] Injection condition for each site was determined by randomization. Lidocaine HCl 1% and epinephrine 1:100,000 was injected locally into the dermis to anesthetize the needle puncture site before injection. Supplemental anesthesia to the target subcutaneous tissue was not used.

To minimize potential risks to study subjects, a dose-escalation study design was employed for the first three study subjects (S01–S03). Ten milliliters of ice slurry or control solution was delivered to each subcutaneous tissue injection site in subject 1 (S01), 20 ml per site in subject 2 (S02), and 30 ml per site in S03, with pauses between each 10 ml injection to ensure tolerability. After completion of the 2-week follow-up of subject 03, in S04–S06, three 10-ml syringes of ice slurry or control solution were injected into each site in rapid succession. Ice slurry was deposited using a needle into a single cumulative depot within the center of each injection site at predetermined depths.

Six additional subjects (S07–S12) were recruited to test the feasibility of a variety of injection patterns, depths, techniques, and volumes. S07–S12 received variable ice slurry injection volumes using injection deposition methods such as fanning, linear threading, and delivery through a cannula (Table 1). Ice slurry was spread over a single 2 × 6 inch site or two 2 × 4 inch sites. The spread of the slurry throughout the injection site area in these subjects enabled analysis by histology when abdominoplasty specimens were able to be obtained postprocedure.

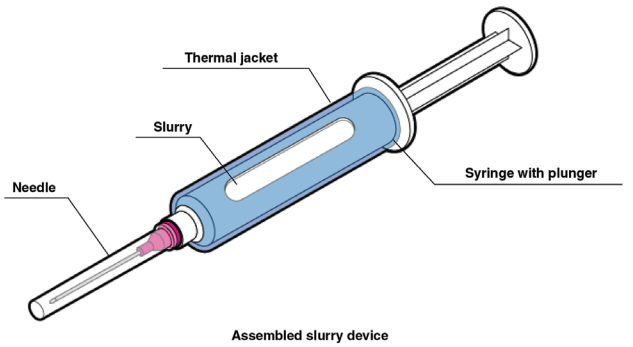

Fig. 1. The ice slurry device was composed of a standard needle, syringe, and custom thermal jacket. The syringe was filled with 10 ml of sterile ice slurry using aseptic technique before injection.

Table 1. Subject Demographics, Injection Parameters, Pain Scores, and Estimated Minimum Cooling Durations of Surface Temperature below 10°C Are Depicted

	Age, y	BMI	Injection Volume, ml	Injection Pattern	Injection Area	Injection Method	Injection Feasible (>90% Target Volume)	Mean Pain VAS: Ice Slurry (0–10)	Minimum Cooling Durations and Corresponding Surface Temperature	Histology
S01	51	32.4	10	Depot	3 sites, 2 × 2 inches	Needle	Yes	0.7	Did not achieve (<10°C)	NA
S02	55	32.8	20	Depot	3 sites, 2 × 2 inches	Needle	Yes	2.7	19 min (7.9°C)	NA
S03	55	29.0	30	Depot	3 sites, 2 × 2 inches	Needle	Yes	1	19 min (9.5°C)	NA
S04	50	28.0	30	Depot	3 sites, 2 × 2 inches	Needle	Yes	0.7	14 min (5.6°C)	NA
S05	48	26.0	30	Depot	3 sites, 2 × 2 inches	Needle	Yes	1.3	18 min (9.8°C)	NA
S06	63	29.0	30	Depot	3 sites, 2 × 2 inches	Needle	Yes	1.7	9 min (2.4°C)	NA
S07	42	28.7	240	Linear thread, deep	1 site, 2 × 6 inches	Cannula	Yes	0	34 min (9.8°C)	Yes
S08	47	30.7	240	Linear thread, combo	1 site, 2 × 6 inches	Cannula	Yes	4	26 min (9.6°C)	Not obtained
S09	62	29.2	240	Linear thread, superficial	1 site, 2 × 6 inches	Cannula	Yes	3	40 min (5.1°C)	Not obtained
S10	48	27.7	240	Linear thread, deep	1 site, 2 × 6 inches	Cannula	Yes	2	33 min (6.3°C)	Not obtained
S11	44	28.3	80/160	Fan, superficial	2 sites, 2 × 4 inches	Needle	Yes	1	46 min (10.2°C)	Yes
S12	49	25.7	80/160	Fan, superficial	2 sites, 2 × 4 inches	Needle	Yes	5	18 min (1.1°C)	Not obtained

Ultrasound guidance was used during ice slurry injections (Sonosite M-Turbo Ultrasound System; Bothell WA& Sonosite HFL38x/ 13-6 MHz, Bothell, Wash., linear transducer) to confirm placement of the ice slurry or control solution either[1] subdermal and superficial to the most superficial fascial layer[6] of subcutaneous fat (termed "superficial placement"),[2] inferior to the superficial fascial plane in the subcutaneous fat (termed "deep placement"), or a[3] "combination" of injection into both the subdermal and deeper compartments.

Thermal Assessment

Thermal imaging (FLIR C3 Camera; FLIR Systems, Wilsonville, Ore.) of the treatment area was performed to determine the surface temperature and cooled surface area postinjection of the ice slurry device. The estimated temperature for intracellular lipid crystallization in human adipocytes and subsequent cryolipolysis is +10°C.[7] Hence, a surface temperature of +10°C was selected as a conservative target temperature, as given thermal diffusion, the subcutaneous fat at the injection site would be colder.

Outcome Measurements

Injection feasibility was defined as the ability to deliver more than 90% of target injection volume using standard injection technique. Tolerability of the injection was assessed by average subject-reported pain using an analog score (0–10) at the time of the injection procedure, with 0 representing no pain and 10 representing the worst possible pain.[8] Safety was assessed as the frequency and severity of adverse effects at each follow-up visit. Adverse effects were classified as "expected" or "unexpected" and graded on severity of "mild," "moderate," or "severe" by the investigator. Safety was also assessed by the presence or absence of any observed clinically significant trends via lipid panel obtained by finger prick (Cholestech LDX System, Abbott, Chicago, Ill.). Achievement of target temperature was defined as a surface temperature of less than 10°C as assessed by thermal imaging. Thermal imaging was also used to estimate minimum cooling duration, defined as the time the surface temperature overlying the ice slurry injection remained below 10°C.

Ultrasound images of treatment areas were obtained by an experienced ultrasonographer with a linear transducer (Sonosite HFL38x/ 13-6 MHz) and captured using an ultrasound system (Sonosite M-Turbo Ultrasound System). Ultrasound was used to visualize injection precision at time of injection, subsequent cold-induced panniculitis at follow-up, as well as to observe potential changes in the treated fat layer thickness. Although the data are limited, focal areas of increased echogenicity have been previously described as sonographic findings associated with panniculitis of the subcutaneous fat.[9] Similarly, ultrasound has previously been used to quantitate normalized changes in fat layer thickness in cryolipolysis.[10]

Tissue biopsies were able to be obtained from[1] the center of the ice slurry treated area,[2] center of the control site, and[3] adjacent, untreated, abdominal tissue in two gross specimens which was made available postabdominoplasty for histology analysis. Biopsies underwent blinded processing and analysis by a board-certified dermatopathologist.

RESULTS

Feasibility

The target ice slurry injection volume was feasible in all subjects using standard injection techniques. Ice slurry was capable of being injected across a range of volumes, injection patterns, methods, and depths (Table 1).

Thermal Imaging

The minimum surface temperature of the lowest injection volume of ice slurry, 10 ml, was 12.5°C. All other ice slurry injection volumes were sufficient to achieve a skin surface temperature of less than 10°C. The minimum surface temperature achieved with 20 ml of ice slurry was 4.7°C and for 30 ml was 2.4°C. Larger ice slurry volumes were able to achieve subzero temperatures such as –1.7°C observed in injection of 160 ml of ice slurry. Estimated

durations of cooling obtained from time stamped thermal images revealed that ice slurry injections of 20 ml and greater were able to maintain a temperature below 10°C for longer than 15 minutes, and in larger slurry injection volumes lasted as long as 40 minutes (Table 1). Furthermore, thermal imaging reveals that diffusion of cooling was limited and remained mainly confined to the injection site areas. (Fig. 2) (SDC 1). (**See figure, Supplemental Digital Content 1,** which displays the sites postinjection of ice slurry, **http://links.lww.com/PRSGO/B784**.)

Safety and Tolerability

Subjects rated the pain (0–10 VAS scale), with a mean score for the ice slurry injection procedure as 1.9/10 (STDEV: 1.5) compared to a 1.3/10 (STDEV: 1.2) in sites receiving injection of control solution. There was no statistically significant difference between groups.

There were no serious adverse effects observed in the study. Adverse effect profiles were similar between control and ice slurry treated sites. The most common adverse effects were bruising (mild-to-moderate), erythema (mild), edema (mild), pain (mild), and induration (mild). Most adverse effects resolved by the 2-week visit and all resolved by the conclusion of the study. There were no clinically significant trends in serum lipids posttreatment. The frequency and severity of adverse effects are depicted in Table 2.

Ultrasound Imaging

Ultrasound guidance was used to confirm injection depth of the ice slurry. Ultrasound image capture immediately postinjection demonstrated that ice slurry can be placed into subcutaneous fat with a high degree of precision (Fig. 3). The most superficial layer of ice crystals can be visualized on ultrasound with an inferior acoustic shadow.

At time of follow-up focal areas of increased echogenicity were observed in ice slurry-injected sites, but not in sites receiving control solution. Figure 4 illustrates this observation on ultrasound images obtained 2 weeks postinjection. This sonographic finding persisted through the 12-week follow-up time point but became progressively less marked at each follow-up visit.

Given the pilot nature of the study and varied injection parameters, the study was not adequately powered to quantify mean reductions in fat layer thickness. However, the consistency of injection pattern, deposition method, and volume (30 ml) in subjects 04–06 (n = 3) enabled limited quantification of normalized mean changes in treated fat layer thickness, as assessed by ultrasound, which resulted in reductions of fat layer thickness up to −29.0%.

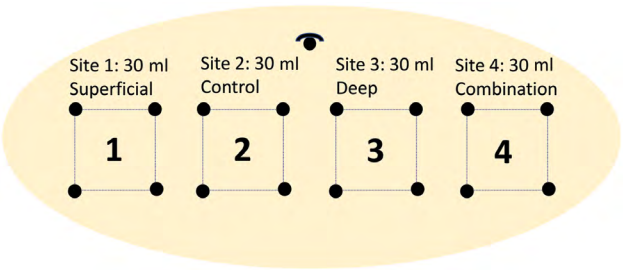

Fig. 2. Thermal image obtained postinjection of 30 ml of ice slurry or control solution in each injection site. The schematic details the injection conditions of each site.

Histologic Analysis

Two specimens from the higher volume injection conditions were obtained at approximately 15 and 20 weeks post ice slurry injection, respectively. Histologic evaluation was consistent between both samples and did not demonstrate significant changes to the epidermal or dermal layer in sites receiving ice slurry or control solution. No significant changes were noted in the fat of control solution sites; however, evidence of marked histologic changes characteristic of cryolipolysis were observed in sites receiving ice slurry injections, including thickening of fibrous septae within fat tissue, shrinkage of fat lobules, marked variability in adipocyte size, and the appearance of foam cells (Fig. 5).

DISCUSSION

Given the rheologic properties of phase-change materials, it was unknown if ice particles could be delivered to the subcutaneous tissue using standard injection techniques in the clinic. This study demonstrated that ice slurry could easily and reliably be injected into subcutaneous fat across a range of injection volumes, patterns and methods. Ultrasound guidance demonstrated ice slurry can be delivered with a high degree of precision to the target tissue at any depth within the subcutaneous fat. In contrast, topical cryolipolysis is limited in depth and precision of fat targeting by thermal diffusion and the low spatial resolution of a topical cooling applicator.

Furthermore, it was unknown if the injection of ice into tissue would cause excessive discomfort. This study demonstrated that ice slurry injections were well tolerated.

The results of this study are consistent with preclinical swine data demonstrating that 30 ml of ice slurry of 20%–40% ice content is sufficient to induce cryolipolysis. First, cryolipolysis is characterized by a predominantly lobular panniculitis that peaks 2–4 weeks post cooling treatment and may persist for up to 3 months posttreatment.[11] The sonographic findings of lobular panniculitis in subcutaneous fat have previously been described as focal areas of increased echogenicity.[9] Sonographic data obtained at follow-up in this study demonstrated focal panniculitis peaking at 2 weeks, and gradually decreasing through subsequent study visits. This finding was observed in ice slurry-injected sites at all injection volumes, including the lowest dose of 10 ml of ice slurry.

Second, thermal data revealed that injection volumes of 20 ml of ice slurry were sufficient to achieve a surface temperature of 10°C, with increasing injection volumes correlated to colder temperature, in some cases subzero, and longer estimated cooling durations. Furthermore, 20 ml of ice slurry was sufficient to maintain the surface

Table 2. The Nature, Frequency, and Severity of Adverse Effects across All Study Subjects

		Subject Incidence						
Adverse Effect	**Severity**	**2 h**	**1 d**	**3 d**	**2 wks**	**6 wks**	**12 wks**	**16 wks**
Numbness	Mild	1, 4	1, 5	1				
	Moderate	5						
Bruising	Mild	3, 6, 7, 8, 9, 10, 11	1, 2, 3, 4, 5, 6, 7,8, 9, 10, 11	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12				
	Moderate	3	4, 7, 9,	4, 8, 11				
Erythema	Mild	3, 7, 9, 10	1, 2, 3, 4, 5, 6,11	2				
	Moderate		3					
Bleeding	Mild	3, 11		11				
	Moderate	3						
Induration	Mild	3, 9, 11	3		5, 6			
	Moderate							
Edema/Swelling	Mild	7, 8, 9, 10, 11	3, 9, 11	11	5			
	Moderate	3	3					
Pain	Mild	11	2, 7, 12	2, 6				
	Moderate							
Pruritus	Mild			5				
	Moderate							
Hematoma	Mild				12			
Hyperpigmentation	Mild						11	

temperature below 10°C for a minimum estimated cooling duration of 19 minutes. Research from topical cryolipolysis has suggested that the treatment time at which the fat should be at target temperature may be in the range of 10–25 minutes.[12]

Third, data from subjects S04–S06 demonstrated up to 29% normalized reduction in treated fat layer thickness. Although a small number of subjects, this preliminary data is suggestive of quantitative reductions in fat layer thickness, which necessitates further investigation in future research in the target subject population for nonsurgical fat reduction.

Fourth, histologically cryolipolysis has been characterized by thickening of the fibrous septae, shrinkage of fat lobules, and clearance of apoptotic fat cells by macrophages.[11] These significant findings were observed in ice slurry-injected sites, but not in control sites or adjacent tissue. Taken together, the data from this study demonstrated the feasibility of inducing cryolipolysis with a single injection of ice slurry.

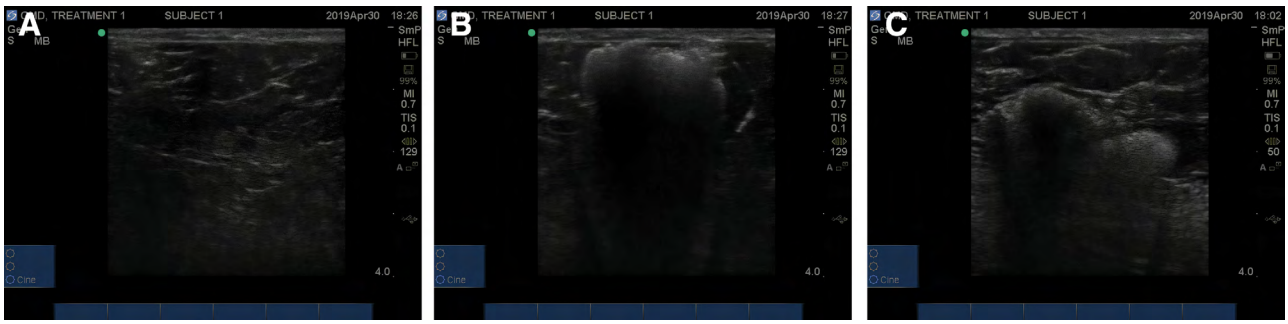

Fig. 3. Ultrasound images obtained immediately postinjection of a single 10 ml depot of control solution (A) ice slurry injected below the dermis and superficial to the superficial fascia ("superficial") (B), and ice slurry injected deep into the superficial fascia ("deep") (C). The hyperechoic boundary in (B) and (C) is the most superior layer of ice particles with inferior shadowing.

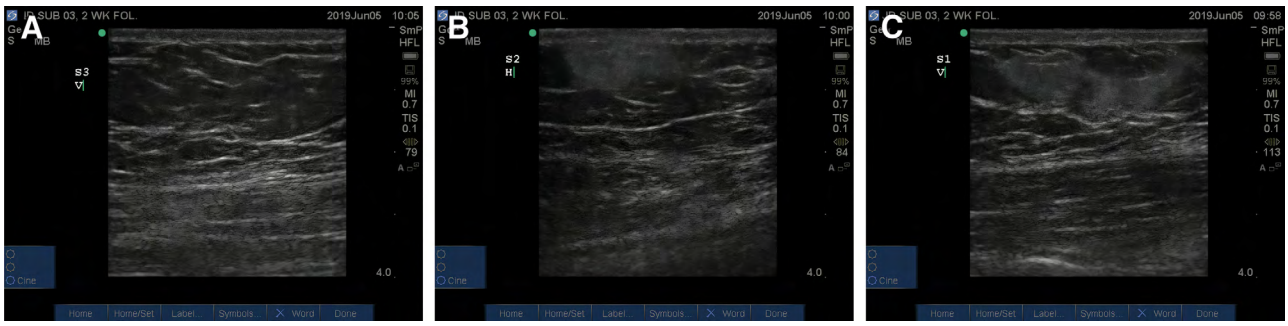

Fig. 4. Focal areas of increased echogenicity observed within the subcutaneous fat at 2 weeks postinjection with 30 ml of depot ice slurry (B and C). This finding was not observed in sites receiving control solution (A).

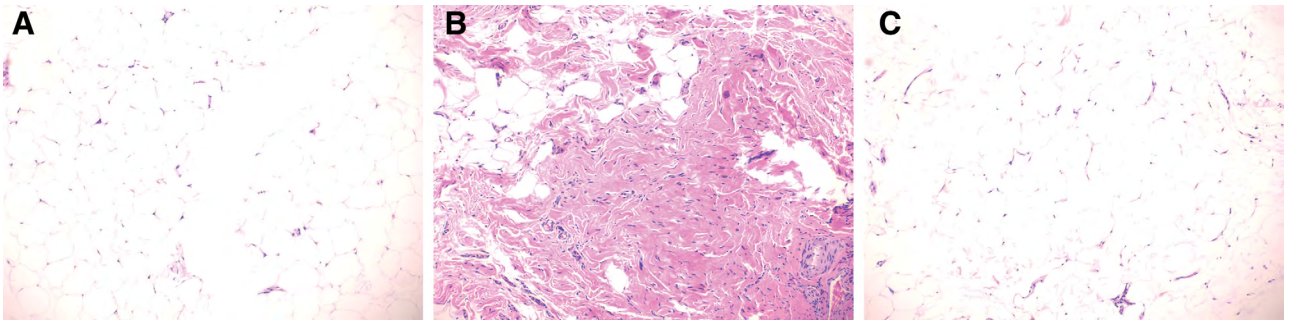

Fig. 5. Histology of adipose tissue at 100× magnification obtained 20 weeks postinjection procedure. No significant changes are observed in specimens obtained from the injection site receiving melted control solution (A) or the untreated site (C). Marked changes are noted in specimens collected receiving the ice slurry injection (B), including marked thickening of fibrous septae within fat tissue and shrinkage of fat lobules.

As an initial feasibility study, the data is limited by its small sample size and exploratory design. Furthermore, to minimize potential risks in this first-in-human study, preabdominoplasty subjects were recruited so that the ice slurry injections were only performed in tissue that was subsequently excised during surgery. A significant limitation of this subject population is excessive skin laxity and adiposity of the injected tissue. These tissue characteristics limit the ability to assess the potential aesthetic efficacy of the ice slurry. Future research will be directed toward further characterization of safety and tolerability of ice slurry injection in a larger subject population, as well as investigation of quantitative and aesthetic efficacy in subjects who are candidates for cryolipolysis.

CONCLUSIONS

The data from this first-in-human study suggest that ice slurry injections are feasible and well tolerated. Thermal imaging, ultrasound imaging, and tissue histology are consistent with preclinical data, indicating a single, 20 ml injection of approximately 30% ice provides sufficient cooling capacity to selectively target and reduce subcutaneous fat via induction of cryolipolysis. Ultrasound imaging revealed that ice slurry can be injected with a high degree of precision and depth, suggesting that in addition to a role in generalized nonsurgical fat reduction, ice slurry may also have potential as a nonsurgical alternative to very personalized and highly precise body contouring and sculpting.[13] In conclusion, ice slurry injections represent a promising new method of nonsurgical body contouring that warrants further investigation.

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