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Non-invasive tumescent cryolipolysis using a new 4D handpiece: a comparative study with a porcine model

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Background/Purpose: The growing demand for a youthful appearance, including a favorable body shape, has motivated recent developments in noninvasive body contouring techniques. Our aim was to investigate the efficacy and safety of a new version of a 4D handpiece-mounted cooling device for cryolipolysis with or without tumescent injections.

Methods: We conducted a side-by-side comparative study using two female porcine models. Two areas of each pig's left abdomen were treated using a conventional device and the new cooling device, and two areas of the right abdomen were also treated using the conventional and new cooling device, but both were combined with tumescent-solution injections.

Results: The conventional method alone yielded a 75.25% reduction in skin thickness, while the new cooling device alone yielded a 81.63% reduction. When paired with tumescent injections, the conventional device yielded a 86.3% reduction in skin thickness and the cooling device yielded a 85.9% reduction. Using histological analysis with H&E, oil red O, and toluidine blue stain, we confirmed that selective cryolipolysis was able to induce selective apoptosis of fat cells.

Conclusion: This *in vivo* study presents a new 4D handpiece-assisted cooling device with tumescent anesthesia that is safe and effective for fat reduction.

Key words: cryolipolysis – fat – 4D handpiece – tumescent

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Variation in fat accumulation patterns leads to differential contour changes along the human body. These contour changes are related to a complex combination of factors, including lifestyle, age, gender, hormone levels, and genetic elements. Fat accumulation in the human body has dramatically increased in the past few decades and is associated with many diseases and various other health problems. Consequently, research efforts to develop a more effective and safe method for fat reduction are steadily increasing.

In recent years, there has been a strong focus on developing non-invasive methods as an alternative to liposuction; particularly promising emerging techniques include cryolipolysis, high-intensity focused thermal ultrasound, radiofrequency ablation, and low-level external laser therapy. Each technology employs a different mechanism to cause apoptosis or necrosis of targeted adipocytes. Cryolipolysis, a medical treatment used to destroy fat cells, relies on controlled cooling for non-invasive localized reduction of fat deposits, resulting in reshaped body contours. Cooling exposure is calibrated to cause cell death in subcutaneous fat tissue without damaging the overlying skin. In 2010, the US Food and Drug Administration approved the first cryolipolysis device and procedure (1). Initial animal and human studies have yielded significant reductions in superficial fat layer thickness, ranging from 20% to 80%, following a single cryolipolysis treatment. Decreases in fat thickness occur gradually over the first 3 months following treatment, and are most pronounced in patients with limited, discrete fat bulges (2). However, cryolipolysis is associated with local side effects, including transient redness, bruising, skin numbness, and uneven fat reduction. Therefore, improvements to cryolipolysis equipment for more effective and safe fat removal are necessary.

We performed this study to compare the efficacy and safety of a newly developed improved device (cool4D®; Classys Inc., Seoul, Korea), hereafter referred to as n-c, with a commercially available cooling device (CLATUU®; Classys Inc.), hereafter referred to as c-c (3).

Materials and Methods

Experimental animals

Pigs, an omnivorous non-rodent mammalian species, are often used to test in-development drugs or devices because their organ structures and metabolic processes are similar to those of humans (4, 5). This study was conducted using two female pigs (6–8 months of age; body weight, 110 ± 5 kg). All animals were obtained from the closed barrier unit at Medikinetics (MK, Pyeongtaek, Korea) and were housed individually under controlled environmental conditions (temperature, 18–22°C; relative air humidity, 30–70%; 15 air changes/h; 12 : 12-h light-dark cycle). Institutional approval for animal use was obtained prior to study initiation.

Study design

After confirming the adequacy and weight of the subjects, the pigs were pre-anesthetized with an intramuscular injection of 3 mL of a 6 : 4 mixed solution of Zoletil 50 (tiletamine hydrochloride + zolazepam hydrochloride; Virbac S.A, Carros, France) and Rompun™ (xylazine hydrochloride; Bayer, Shawnee Mission, KS, USA). We injected 2 mL of the same mixed solution after knocking on the operating table. We secured the animals' airways via laryngoscope and inserted an intubation tube 8.5. Respiratory anesthesia was induced by administering a 2 : 1-solution of mixed gas with Terrell™ (Isoflurane; Piramal Critical Care., Inc., Bethlehem, PA, USA) and oxygen.

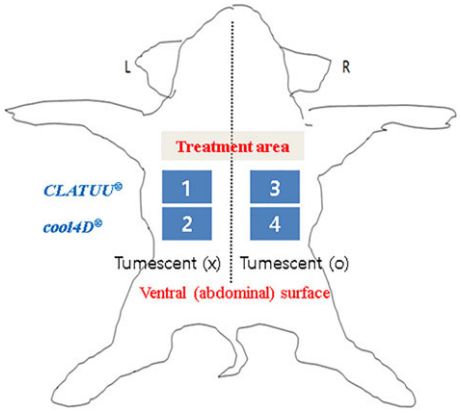


Fig. 1. The experimental design using a porcine model.

We treated two different areas of each pig's left abdomen with the c-c and n-c devices, and we also treated two different areas of the right abdomen using the c-c and n-c devices after injecting them with tumescent solution. The ventral skin of both pigs was separated into four areas as follows: area 1: c-c without tumescent injection; area 2: n-c without tumescent injection; area 3: c-c with tumescent injection; area 4: n-c with tumescent injection (Fig. 1).

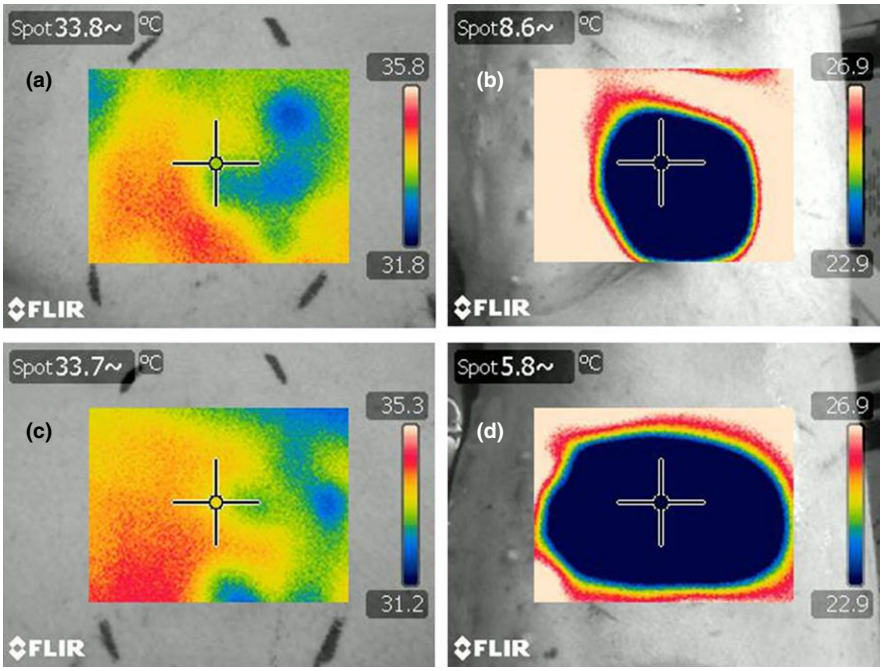


Fig. 2. Changes in porcine skin temperature for the two cooling devices: (a) Before c-c treatment, (b) immediately after c-c treatment, (c) before n-c treatment, (d) immediately after n-c treatment.

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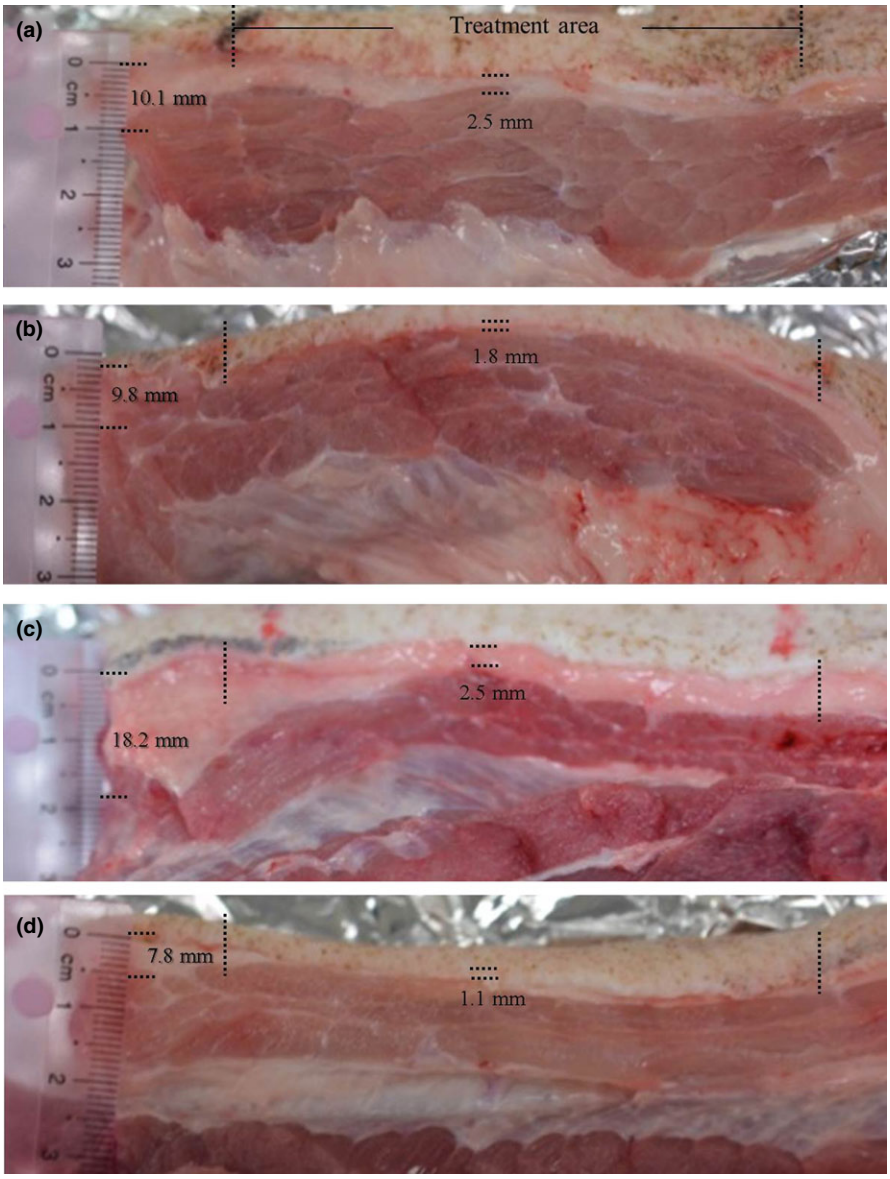


Fig. 3. Changes in subcutaneous fat thickness at 90 days after application for the two cooling devices with/without tumescent injection: (a) area 1, (b) area 2, (c) area 3, (d) area 4.

Each treated site received a 1-h application with the c-c or n-c device. A cooling intensity factor (CIF) of 24.9 (−44.68 mW/cm^2), a quantitative value for calculating cold energy, was equally applied to each treated site. Thirty minutes into the procedure, the experimenter performed a 10-min massage with the device. Ambient room temperature was maintained at 23°C throughout the cooling and recovery periods.

The tumescent solution was prepared using Klein's formula (6), which includes normal saline solution (1000 mL), 1% lidocaine (50 mL), 1 : 1000 epinephrine (1 mL), and 8.4% sodium bicarbonate (12.5 mL). The recommended dosage for *in vivo* tumescent solution injection is 4–5% of body weight; thus, our tumescent-solution injections totaled 5 mL for each animal: 1 mL injections repeated five times during 30–60 min of treatment.

Assessments

The safety and efficacy of our study were evaluated immediately after treatment application and 1, 7, 15, 30, 60, and 90 days after treatment. We evaluated treatment effectiveness using standard photography (D5200; NIKON, Tokyo, Japan), diagnostic ultrasound (MU1V; Bionet, Seoul, Korea) and histological analysis. Histological sections were stained with hematoxylin

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and eosin (H&E), oil red O and toluidine blue. A DM2500 microscope (Leica, Gallen, Switzerland) was used for histological analysis and images were taken using a DFC-425-C Leica Digital camera. We also confirmed the skin surface temperature using a FLIR A-Series infrared camera (FLIR system Inc., Wilsonville, Oregon).

Results

Changes in skin surface temperature after treatment with two different cooling devices

We checked the pigs' surface temperatures using a heat camera to ensure that the treatment was proceeding correctly. The c-c yielded a skin-surface temperature of about 8.6°C immediately after treatment, while the n-c yielded a temperature of about 5.8°C. These results indicate that the n-c device passed cold energy to the skin surface more effectively than the c-c (Fig. 2). There were no temperature differences between the treatment areas according to whether they received a tumescent-solution injection or not.

Changes in subcutaneous fat after treatment with two different cooling devices with and without tumescent solution

At day 90, the full-thickness skin of the treated area was harvested, image-analyzed and the

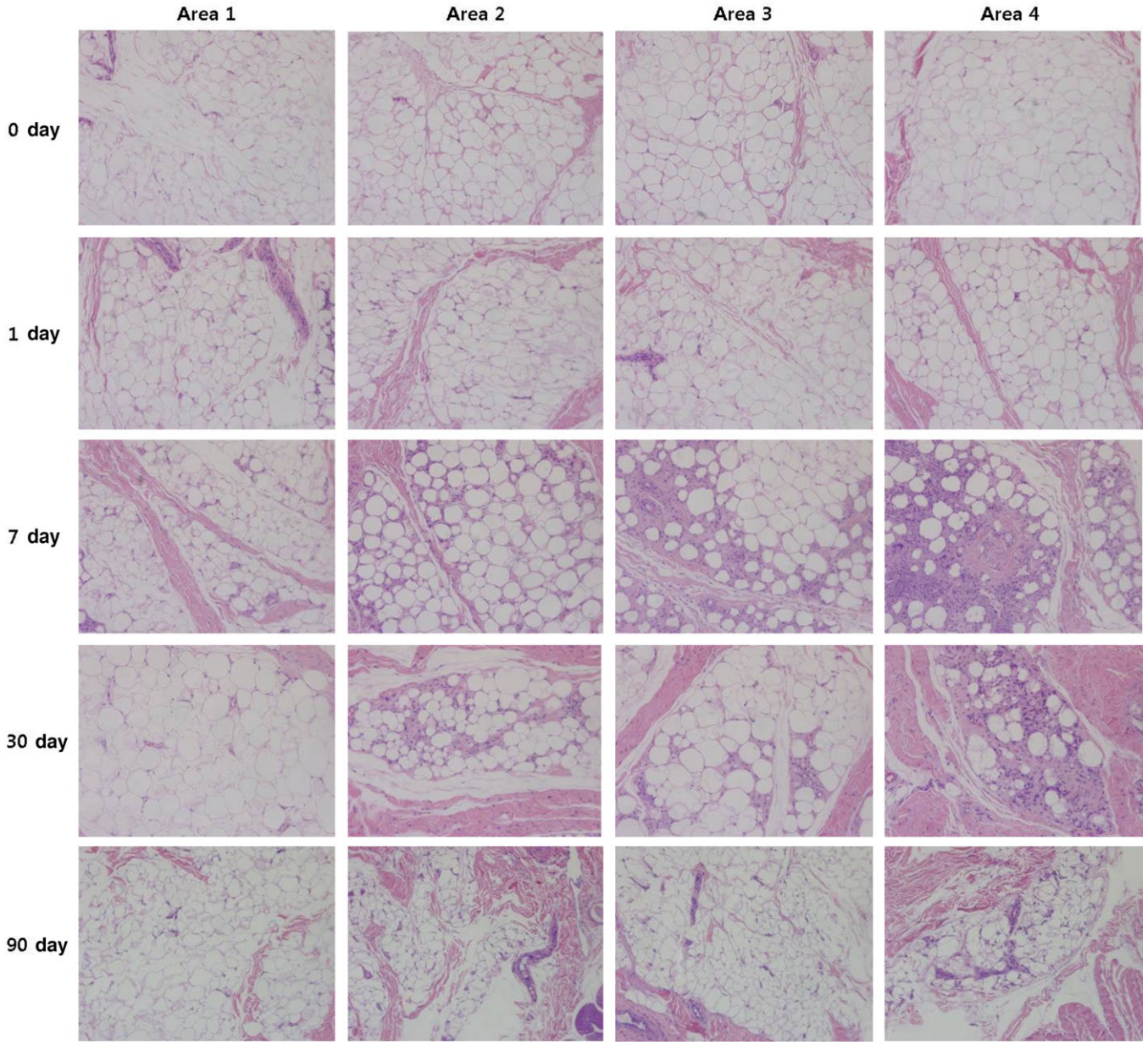


Fig. 4. Histological changes after treatment: (a) area 1, (b) area 2, (c) area 3, (d) area 4 (H&E, ×200).

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actual thickness was checked. The thickness of area 1 was reduced from 10.1 mm to 2.5 mm after treatment, a 75.25% reduction rate (Fig. 3a). In comparison, area 2 showed a 81.63% of reduction rate, the thickness decreased from 9.8 mm before treatment to 1.8 mm after treatment (Fig. 3b).

After subcutaneous injection with tumescent solution, area 3 showed a reduction rate of 86.3% (18.2–2.5 mm after treatment) (Fig. 3c) and area 4 had a reduction rate of 85.9% (7.8–1.1 mm after treatment) (Fig. 3d).

Histological changes after treatment with two different cooling devices with and without tumescent solution

We obtained cutaneous tissues for histopathological examination at several time points: prior to treatment and 1, 7, 30, and 90 days after treatment. The collected samples were fixed in 10% formalin and were mounted in paraffin blocks. H & E staining revealed the presence or absence of skin inflammation or other adverse reactions. We also confirmed that targeted cryolipolysis could induce selective apoptosis of fat cells. We observed the most significant

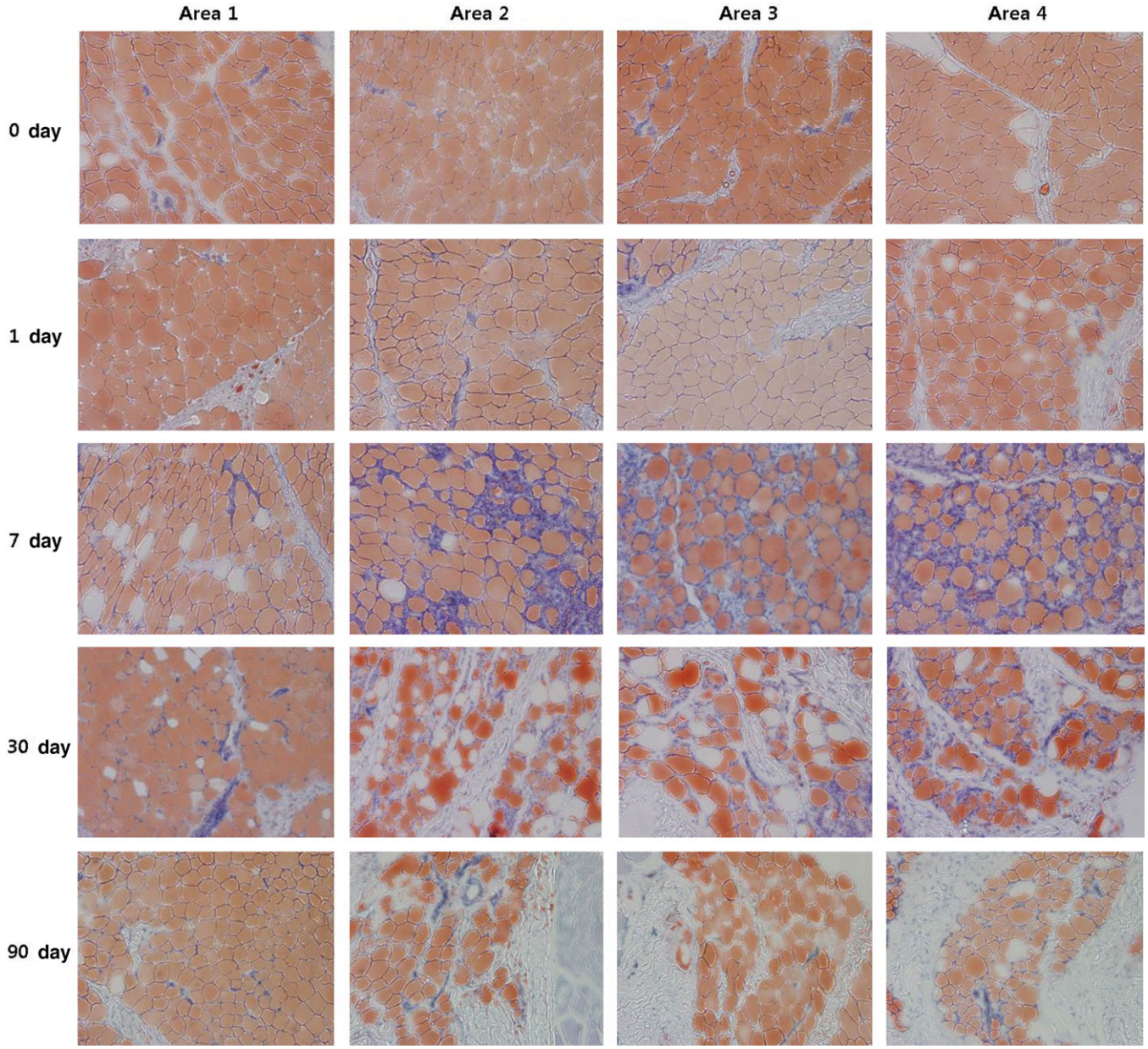


Fig. 5. Fat tissues decreased after 30 days: (a) area 1, (b) area 2, (c) area 3, (d) area 4 (oil red O staining, ×200).

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inflammation levels in tissues collected on day 7. By 90 days post-treatment, inflammation was nearly completely resolved. The areas that received tumescent injections had more severe inflammation than the no-injection areas, and the n-c treatment area had more severe inflammation than the c-c treatment area (Fig. 4). The fat tissues that stained red after oil red O staining decreased in the treated tissues after 30 days compared with normal tissue, and the size of individual fat cells also decreased (Fig. 5). Toluidine blue staining showed increasing macrophage infiltration up to 30 days after treatment; this pattern was consistent with patterns of mechanistic pathways of cell death. After 60 days, adipocytes began showing patterns consistent with a recovery process from cell death. The tumescent injection areas had greater macrophage infiltration than the no-injection areas, with the n-c treatment area having more macrophage infiltration than the c-c treatment area (Fig. 6).

By 90 days after treatment with the c-c and n-c devices, fat cell size was reduced by a rate of 46.8% and 63.1%, respectively, compared to baseline. Distribution of fat cells decreased by

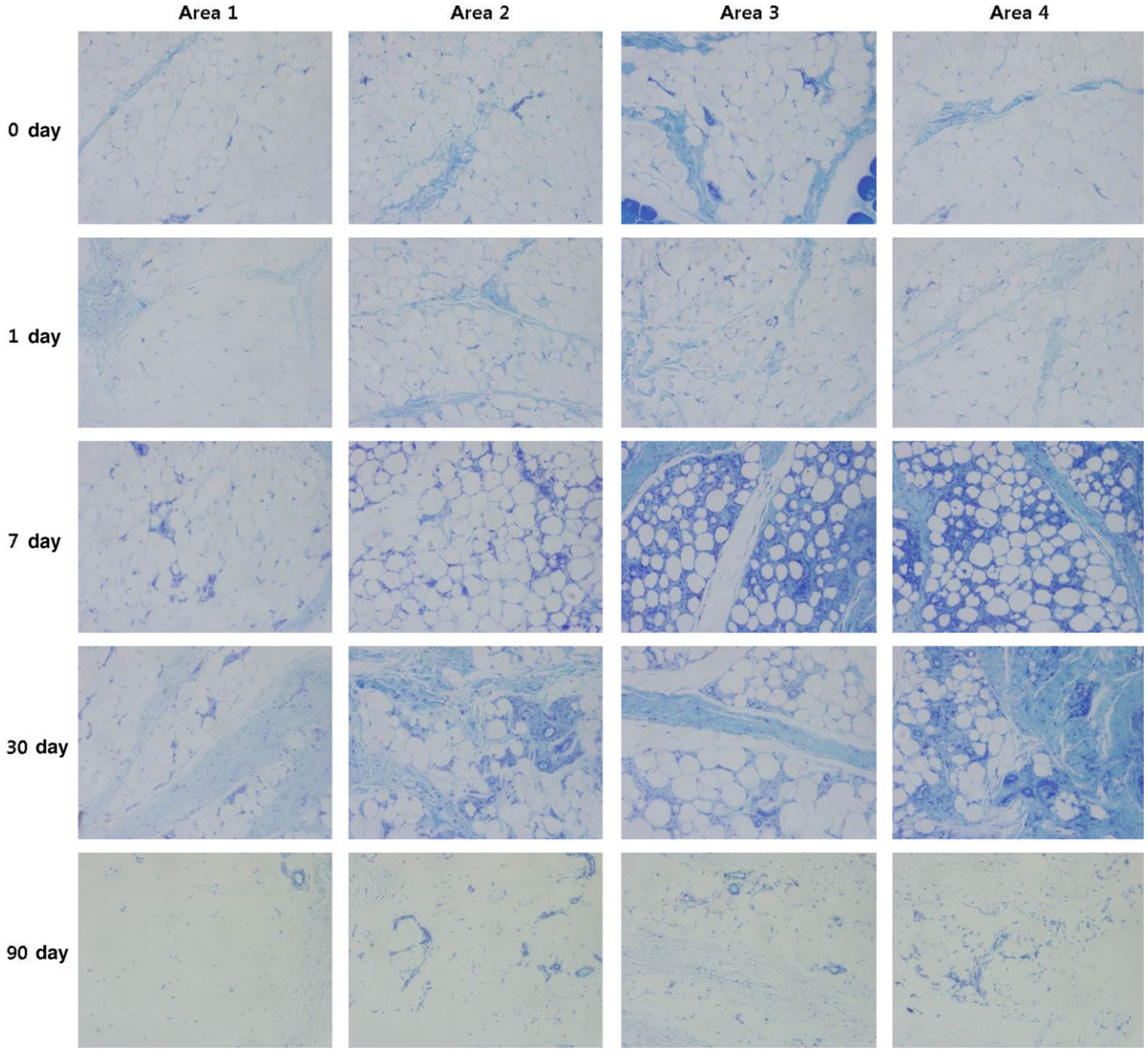


Fig. 6. Macrophage infiltration increased significantly by 30 days after treatment, and adipocytes showed signs of recovery by day 60: (a) area,1 (b) area 2, (c) area 3, (d) area 4 (toluidine blue stain, ×200).

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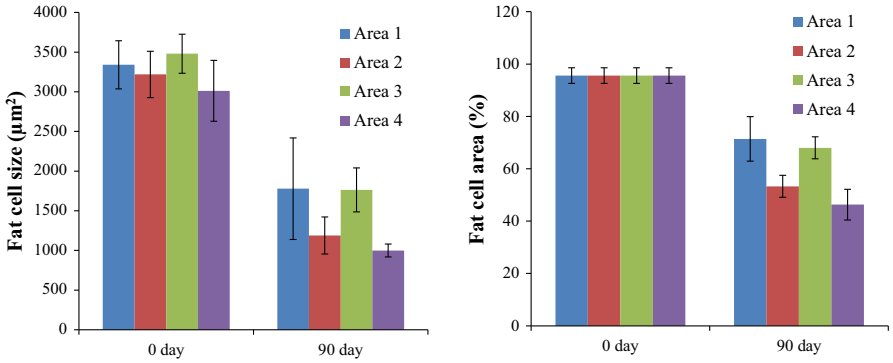


Fig. 7. Changes in fat-cell size and number after treatment with the two cooling devices with/without tumescent solution at day 0 vs. day 90.

24.2% after c-c treatment and by 42.3% after n-c treatment. By 90 days after receiving a tumescent solution injection, fat cell size decreased by 49.3% in the c-c-treated site and by 66.8% in the n-c-treated site. Treatment with the c-c device resulted in a 27.6% reduction in fat-cell distribution and treatment with an n-c device led to a 49.3% reduction (Fig. 7).

Safety assessment via skin-surface photography

Experts performed a visual safety assessment to check for changes or damage to the skin surface. We evaluated for skin changes on day 0 (immediately after treatment), 1, 7, 30 and 90; our final evaluation was completed on day 90 (Fig. 8). Although we noted some erythema immediately after applying the cooling devices, the animals recovered within 2–3 days after treatment completion. We did not observe any significant side effects in the tumescent solution-treated sites.

Discussion

According to the American Society for Aesthetic Plastic Surgery, by 2013, liposuction had replaced breast augmentation as the most popular surgical procedure, with 363,912 procedures performed. Its popularity has grown considerably because it offers esthetic improvement and numerous metabolic benefits (7, 8). Despite its popularity, liposuction is associated with rare but significant risks that can sometimes be fatal, including complications from anesthesia and infections (9). Currently available nonsurgical liposuction procedures employ mechanical vacuum massage, lasers, radiofrequency, ultrasound, or low-level energy infrared light, but these techniques are not intended for the removal of large volumes of fat (10).

Current cryolipolysis procedures are performed with either small or large vacuum-pressure applicators that are capable of extracting heat from both sides of a fold and reducing blood flow via tissue compression and cold-induced vasoconstriction (11–13). Two mechanisms of fat-cell loss have been described in the literature (dedifferentiation and apoptosis), and the results we present here are consistent with the conclusion that exposure to cold induces fat-cell apoptosis (14).

Conventional cryolipolysis equipment contains a cooling-plate-mounted applicator, but the newly developed cool4D® has an applicator that acts as its own cooling plate. It has the world's first 360° cooling panel that can deliver cooling energy more effectively than the conventional two-sided model (Fig. 9).

The advantages of this new device are its higher cooling conductivity and that it delivers uniform cooling energy to the skin better than the conventional applicator. Its cooling effects are not focused on limited areas and it can effectively reduce subcutaneous fat. A rare side effect of cryolipolysis is frostbite. Improved technology allows it to maintain a stable cooling temperature so that it can provide enhanced treatment results in a shorter time period compared with conventional methods. Furthermore, we found a stronger decreasing effect for subcutaneous fat by 90 days after treatment with the n-c compared with the c-c.

Klein's introduction of the tumescent liposuction technique in 1987 revolutionized the field of cosmetic body-fat sculpting. Some debates about the use of tumescent solution

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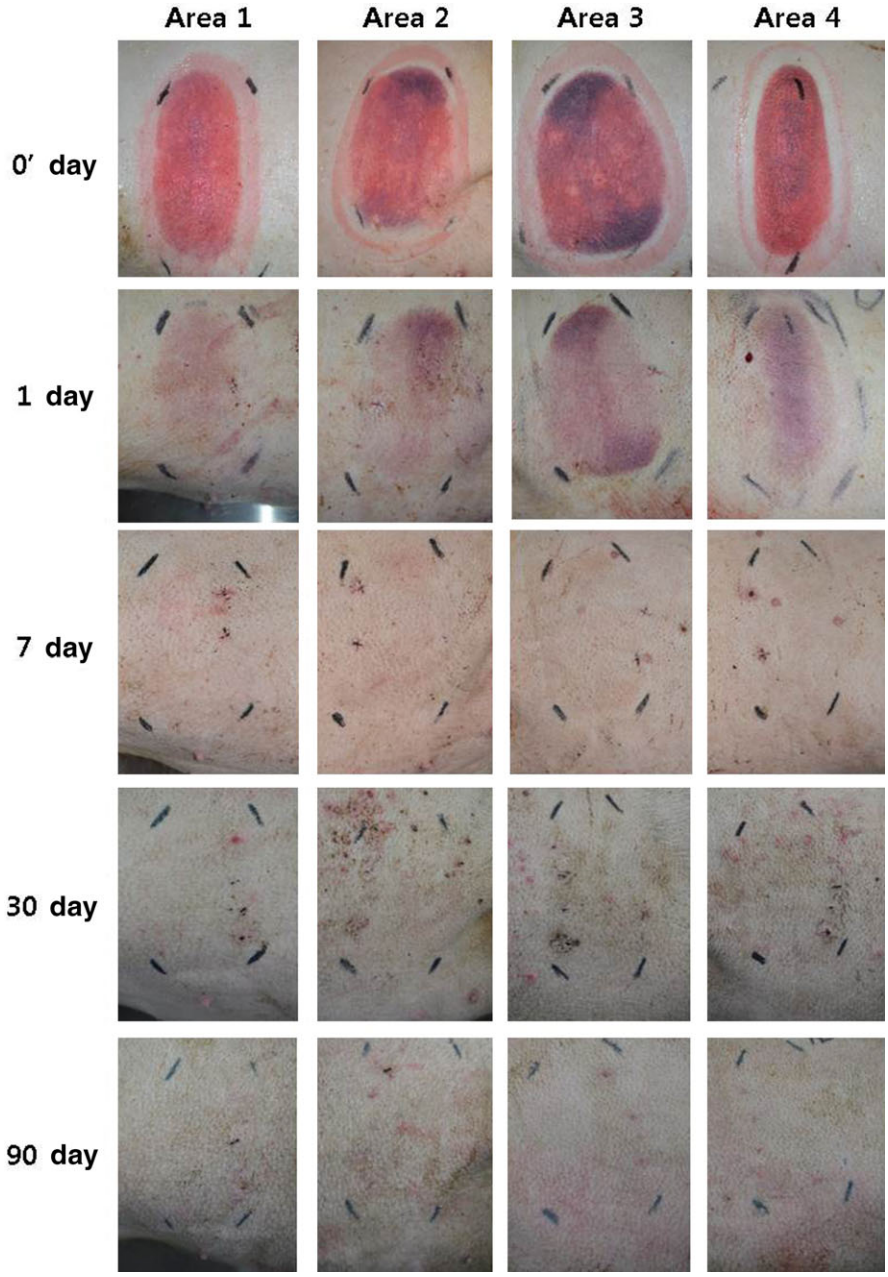


Fig. 8. Skin-surface assessments by photography.

persist; however, liposuction with tumescent local anesthesia facilitates the removal of large volumes of fat with minimal blood loss or postoperative morbidity, and is associated with a low infection rate, excellent esthetic results, and a remarkably superior safety profile compared to procedures requiring general anesthesia (15). Our study demonstrated that fat reduction efficacy was greater when combined with tumescent-solution injection; the effect was present for both cooling devices. We argue that the positive effect of tumescent lipolysis was a result of the effect of local

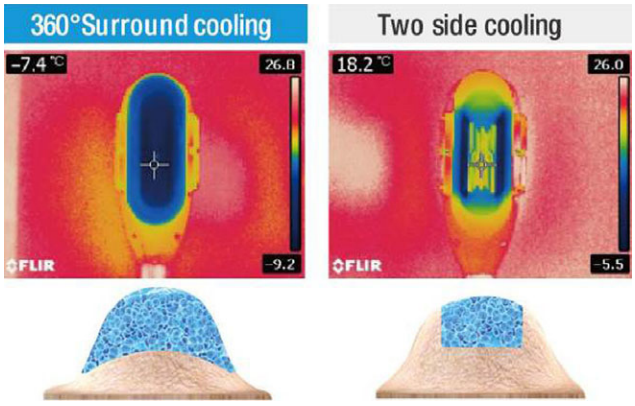


Fig. 9. n-c has a 360° cooling panel that delivers cooling energy more effectively than the conventional two-sided model.

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anesthetics on adipocytes. Keck et al. reported that several local anesthetics, including lidocaine, markedly impaired adipocyte differentiation. They observed that there were significant differences in the viability of preadipocytes under the influence of various local anesthetics (16).

Although the exact mechanism of cryolipolysis remains unclear, our investigational results confirmed that non-invasive fat cooling results in adipocyte cell death and apoptosis over time. This selective non-invasive treatment for localized excess fat was associated with a noticeable reduction in subcutaneous fat in our study and did not cause skin surface damage. This *in vivo* study suggests the newly developed cryolipolysis device with a 4D handpiece, when combined with tumescent anesthesia, is an effective and safe treatment for fat reduction. We hope this work will encourage additional studies of fat reduction techniques and safer delivery of cryolipolysis.

Funding sources

None.

Conflicts of interest

None declared.

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