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ORIGINAL RESEARCH

# A Novel Mouse Model for Frostbite Injury

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**Background.—**Frostbite injury occurs when exposure to cold results in frozen tissue. To screen drugs and other field therapies that might improve the outcome for a frostbite victim, it would be helpful to have a reliable and cost-effective preclinical in vivo model.

**Objective.—**We sought to create a novel mouse skin model of induced frostbite injury. This model would allow quantification of the surface area of involved skin, histology of the wound, rate of wound healing, and skin loss in a standardized fashion after the frostbite injury.

**Methods.—**Thirty-six mice were studied. Standardized 2.9-cm diameter circles were tattooed on the mouse dorsum. Magnets frozen in dry ice (−78.5°C) were used to create a frostbite injury on skin within the circle, either as a continuous 5-minute freeze or as 3 repeated freeze (1-minute) and thaw (3-minute) cycles. Appearance, healing rate, skin surface area loss, and histology were recorded until the wounds were healed.

**Results.—**The amount of skin surface area loss was approximately 50% for both freeze methods. Although the time to surface skin healing was similar for both freeze methods, the initial healing rate was significantly ($P = .001$) slower in mice exposed to the freeze-thaw cycles compared with the continuous freeze model. Histopathology reflected inflammatory changes, cell death, and necrosis.

**Conclusions.—**This novel in vivo mouse model for frostbite allows quantification of affected skin surface area, histology, healing rate, and skin loss and has the potential of being utilized to screen future treatment modalities.

*Key words*: frostbite, cold injury, wound healing, mouse model, murine model

## Introduction

Frostbite occurs when exposure to cold external temperature results in freezing of tissue, sometimes leading to extensive tissue loss and disability. High altitude mountaineers, soldiers deployed to frigid environments, and homeless persons exposed to cold temperatures are examples of persons who have frostbite injury.[1–3] Frostbite is most commonly observed in the fingers/hands, toes/feet, nose, cheeks, and ears because they are exposed, peripheral, and difficult to protect. Exposing tissue to freezing temperature causes vasoconstriction, decreased blood flow, and eventually, microvascular thrombosis. At the cellular level, intracellular ice crystal formation causes protein changes and results in membrane damage.[4] In situations associated with slower tissue cooling, extracellular water is crystallized, which dehydrates cells. Because of this effect, electrolyte concentrations within cells are altered, resulting in modified protein structures.[5] Cellular dehydration and microvascular occlusion cause progressive tissue ischemia, leading to necrosis, severe injury, and disability, including limb loss.[6–8] Injury is likely compounded by reperfusion effects after the thaw.[9]

Other than rapid rewarming, there has not been a dramatic advance in out-of-hospital clinical therapy for frostbite in nearly 50 years.[7] Current primary treatment modalities to minimize frostbite-induced tissue damage are amenable to improvement. One impediment to progress is lack of a preclinical (nonhuman animal) screening method. This situation might be improved by a convenient, reliable, and affordable preclinical method with

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which to screen new therapeutics. An animal model for screening that efficiently allows quantification of tissue histology, rate of wound healing, and tissue loss after a frostbite event might accelerate research efforts and encourage innovation for prevention and treatment. It could also be used to evaluate current methods to establish or refute efficacy. Finally, a preclinical model might provide a way to expand understanding of the pathophysiology, such as the presence of apoptosis or reactive oxygen species, of frostbite.

For these reasons, we set out to invent a novel mouse skin model of induced frostbite injury. This model would allow quantification of the surface area of involved skin, histology of the wound, rate of wound healing, and skin loss in a standardized fashion after the frostbite injury. Our experiments would also identify the possibilities for refinement and further expansion of data acquisition within our chosen model. Because there is thought to be an increased severity of injury in humans associated with a freeze-thaw-freeze (FTF) event, as compared with a single continuous freeze (CF) event, we included both situations in our experiments. Our goal, then, was to create a novel model of frostbite in the mouse.

## Methods

### FROSTBITE MODEL

Thirty-six 8- to 12-week-old C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME) were used in a study approved by the Stanford Institutional Review Board and in accordance with Stanford University Institutional Animal Care and Use Committee Guidelines. The animals were housed 5 per cage before and after surgery in a temperature-controlled facility under a 12-hour light/dark cycle.

### SKIN PREPARATION

Twenty-four hours before day 0, mice were individually anesthetized using isoflurane (Baxter, Deerfield, IL). The dorsal skin surface from the base of the neck to the top of the rear haunches was shaved with an electric clipper, after which a depilatory cream (Nair, Church & Dwight, Princeton, NJ) was applied for 2 minutes to remove any remaining hair (Figure 1A). After depilation, the skin was cleansed with a 70% isopropyl alcohol swab. A circle of uniform diameter, 2.9 cm, was gently traced on the skin with a permanent parker using a silicone sheet template (Invitrogen, Grand Island, NY) as a guide (Figure 1B). The marked circle was then permanently darkened by using an animal tattoo machine (Animal Identification and Marking Systems, Hornell, NY [Figure 1C]).

### CONTINUOUS FREEZE METHOD

Eighteen mice were studied using the CF method. Twenty-four hours after skin preparation, ceramic (ferrite) magnets (diameter 0.5 inches, thickness 0.219 inches, weight 3.5 g) were placed in crushed dry ice (−78.5°C) and allowed to cool for 15 minutes. The center of the tattooed circle was marked to determine precisely the placement of magnets. Using fingers, the back skin of the mouse was lifted into a skin fold, and then, 2 frozen magnets were placed so that they adhered from opposite sides of the intervening skin fold with the mark at the fold's apex center location. A silicone barrier was then slid underneath the magnets as a thermal shield to limit the mouse's body temperature decline (Figure 1D). Sets of 2 cooled magnets were left in place for 1 minute, then removed to allow new magnets to be immediately placed in the same location against the frozen tissue. The magnet exchange occurred in less than 5 seconds, which did not allow any thaw to occur. The magnet exchange was repeated for a total of 5 applications, resulting in a freeze time slightly longer than 5 minutes.

Injury was intentionally inflicted on a lifted skin fold rather than by applying topical pressure and freezing the tissue in its normal position directly underneath the magnet to simplify the method and avoid between-mouse variability, confine the injury to the skin, create a precise injury, limit systemic hypothermia, and maximize survival. It was not the intention to create a wound below the dermis.

Core temperature was monitored using an infrared thermometer (ThermoWorks, Alpine, UT) applied to the abdomen of the mouse. After 5 magnet placements and removals, the skin was allowed to completely thaw (Figure 1E). Mice were given subcutaneous injections of buprenorphine (0.05 mg/kg) for analgesia after the thaw. No dressings were applied to the wounds.

### FREEZE-THAW-FREEZE METHOD

Eighteen mice were studied using the FTF method. Twenty-four hours after skin preparation, as for the CF method, magnets were cooled and placed for 1 minute. However, after 1 minute of application, the magnets were removed, and the skin was allowed to thaw for 3 minutes, achieving a complete thaw with pliable tissue. After the 3-minute thaw, cold magnets were reapplied in the same location. That was done to accomplish 2 additional cycles of freezing (refreezing) for 1 minute and thawing for 3 minutes, for a total of 3 full freeze-thaw cycles. Mice were given buprenorphine (0.05 mg/kg) for analgesia after the final thaw. No dressings were applied to the wounds.