Final Report for the Protocol Titled:

Evaluation of the 800-810 nm Diode Laser System for a Comparison of Epidermal Protection Produced By Air Cooling Vs. Contact Cooling

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Purpose:

The purpose of the study was to evaluate the epidermal protection of the Nidek 2-chip cold window at 0 degrees C versus the Zimmer cold air unit. \

1.1 Objectives

Evaluation of Skin Hydration: The skin will be cooled with both devices and irradiated with the Nidek diode laser using a 3 mm spot size, and the square pattern with a 10% overlap. A piglet model will be used with the piglet skin cleaned, shaved and then thoroughly dried. This will be compared to skin that is left wet and skin that is hydrated with a collagen gel.

Evaluation of Post-Treatment Erythema. The animals will be followed for at least one month to allow adequate wound healing to occur. Digital photography will be used to record images that will then undergo computer analysis to quantify the amount of erythema present. This will allow precise comparison, as the wounds heal, between skin that is uncooled, cooled by contact cooling, and cooled by refrigerated air.

Evaluation of Wound Healing. Pretreatment biopsies will be taken from each treated area to use as controls, and serial biopsies will be collected for a period of four weeks after treatment. Histopathological evaluation will be performed, specifically evaluating for any latent damage to the epidermis and a return to the normal skin structure.

1.2 Justification for the Piglet Model

The piglet model was selected because of the similarities between pig skin and human skin. Both pigs and humans have sweat glands and regulate body temperature with blood flow to the skin (compared to regulating body temperature by panting). Therefore, the pig skin responds to the thermal damage of the laser similar to human skin.

2.0 Summary:

This study was performed at the Vanderbilt University Department of Otolaryngology, School of Medicine. The study started on 1 October, 2001, and was completed on 21 December, 2001.

A total of 7 animals were used for this study, and were divided into two study groups: Study Group 1 (6 animals), and Study Group 2 (1 animal). The purpose of Study Group 1 was to identify the acceptable range of fluence levels for each skin type based on gross tissue observation and histopathology immediately following treatment. The purpose of Study Group 2 was to perform additional wound healing evaluation (via histopathology) using the maximum fluence levels found in Group 1 for up to 28 days following treatment.

Brian Biesman, M.D. and Lou Reinisch, Ph.D. examined all of the histology slides from this study and found the following:

Cooling Methods Used:
1. Cooling with the Window at 0 C and waiting for thermal recovery between patterns
2. Cooling with the Blower at setting 6 and delivery through proto-typed nozzle

Epidermal Preservation

Based on histopathological examination of Group 1 and 2 animals and visual observation at the time of treatment, the maximum fluences used with no epidermal damage on *non-pigmented* skin were:

Dried Skin	
Nidek 2-chip window	300 J/sq. cm
Zimmer unit	300 J/sq. cm
Hydrated skin (water or gel)	
Nidek 2-chip window	>400 J/sq. cm
Zimmer unit	>400 J/sq. cm

3.0 Equipment and Materials

3.1 Equipment

EpiStar laser with 2-chip Optical Cooling Window (Nidek)
Cold Air Blower (Zimmer)
Zeiss Axioplan II light microscope (Carl Zeiss Inc., Thornwood, NY)
Carl Zeiss digital video camera (Model ZVS-3C75DE)

3.2 Materials

7 piglets, mixed breed farm piglets, 16-26 kg

4.0 Procedure

All studies were approved by the Vanderbilt University Animal Care Committee and the Animal use Sub-committee. All surgeries were without complications. The laser did not malfunction during the procedures. There were no adverse events and all animals survived to their harvest date in good health.

Six animals were assigned to the acute time group (Group 1), and 1 animal was used to follow the healing after the laser treatment (Group 2). Each animal was large enough to allow about 60 treatment areas. Areas were treated with 810 nm diode laser (EpiStar Laser, Nidek) 60 W, variable fluence and a scanned square pattern with 10% overlap. This laser was delivered by an optical fiber bundle and imaged to a 3 mm spot size. The spot was discretely stepped over an area about 12.5 mm x 10 mm. This laser was set to deliver a fluence of 10 - 600 J/cm^2 by varying the pulse length.

Animals were anesthetized with inhaled isofluorane vapor until there was no response to foot pad pinching. At this onset of anesthesia, the dorsal skin was cleaned and shaved and the animals were placed in the operating field. Each of the treatment areas were marked on the piglet using a template and an ink pen (see Figure 1 below). The perimeter of each treatment area was measured using a flexible plastic ruler.

4.1 Group 1 Study

The acute animals were treated at the fluences given in Table 1. The epidermis was protected by the optical window at 0˚C. Alternately, the epidermis was protected with the cooled air and a setting on the blower of 6. The blower nozzle was fixed at 1-2 cm from the skin surface.

Table 1 : Fluences and Spot Sizes used on the Group 1 piglets (acute study)

3 mm spot size (J/cm^2)
10
20
30
40
50
60
100
200
300
400

Animals in the Group 1 study were sacrificed following laser treatment (Day 0) and tissue samples were collected for histologic analysis using the techniques described in Section 4.2 below.

4.2 Group 2 Study

A single piglet was treated as follows. Each area to be treated was marked with permanent ink using a plastic template. The laser was applied in a single scanned pattern with 10% overlap as described above. The 3 mm spot size was used. The Nidek 2-chip Optical Cooling Window was used at 0˚C with the gel hydration. The blower was only used on a setting of 6 and with the gel cooling.

The piglet was treated on 4 separated days (each separated by 7 days). So that when the piglet was sacrificed and the skin harvested, there would be samples treated 7, 14, 21, and 28 days prior to harvest.

Anesthetic gas was withdrawn and the piglet was observed while it recovered. The animal was then housed in a cage with food and water ad libitum. Each piglet was anesthetized with isofluorane and treated at additional on the designated days.

On the day of harvest, the piglet was sacrificed and the skin sectioned. The individual sections were fixed in 10% normal buffered formalin solution, embedded in paraffin, and sectioned. Using standard procedures, tissue sections were stained with either hematoxylin and eosin or Gomori's trichrome stains.

At each time point, the histologic features of the healing incisions were examined at 40-100X magnification using a Zeiss Axioplan II light microscope (Carl Zeiss Inc., Thornwood, NY).

5.0 Results

5.1 Group 1 Study Results

The optimal cooling was done in conjunction with wetting the skin surface with water or with a collagen gel. The hydration of the skin surface was essential for the highest level of protection. As shown in Fig. 1, obvious damage could be observed when the surface was not hydrated before treatment.

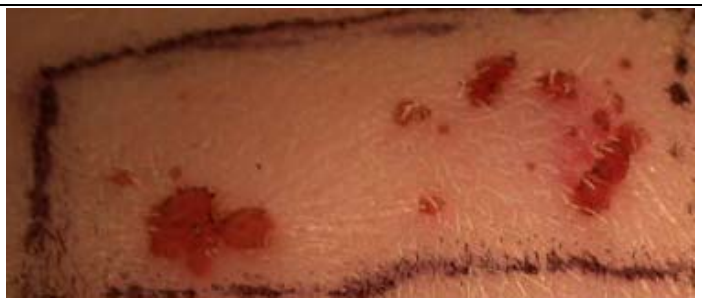

Figure 1a: Piglet skin treated with the diode laser at 400 J/sq. cm and the Zimmer cooling unit on a setting of 6 with no previous hydration of the skin.

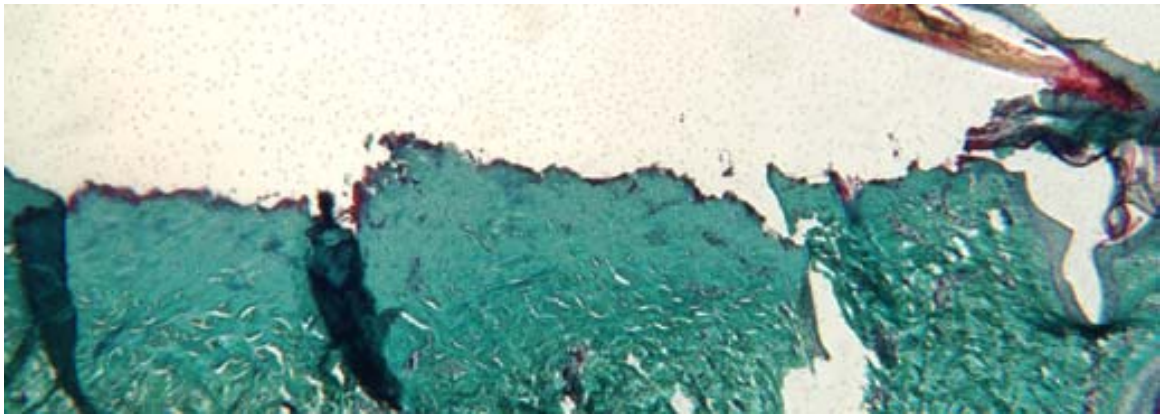

Figure 1b: Histology of piglet skin treated with diode laser at 400 J/sq. cm and the Zimmer blower at a setting of 6. The skin was not wet before the treatment. The epidermis is missing and a wide band of coagulated collegen is seen. The slide is stained with Guomori's trichrome.

The added protection of the water or gel with both the Nidek two chip cold window and the Zimmer blower is best shown in Figure 2. The skin showed obvious damage when only protected by the cold air or the blower unit. However, when the skin was first

hydrated with water or gel, no epidermal was noted. Both methods of hydrating the skin surface and both cooling units showed the same effect.

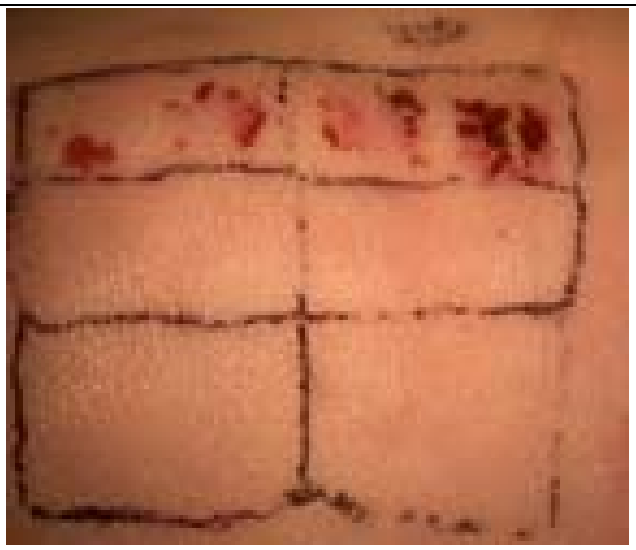

Figure 2: Piglet skin and all six areas were treated with the diode laser at 400 J/sq. cm. The three boxes on the left were protected with the Zimmer cold air at a setting of 6, the three boxes on the right were protected with the Nidek 2 chip cold window. The boxes at the top of the figure had only the cold air or cold window cooling. The middle boxes were treated the same, except the skin was hydrated with water before treatment. The bottom boxes were also treat the same, except the skin was hydrated with a collagen gel.

In Figure 3, we plot the percent epidermal preservation as a function of the laser fluence. This is for the cooling units with not hydration of the skin. We can fit the data to a two-state model (shown as lines). However, since we were unable to treat the skin at fluences greater than 400 J/sq. cm., these fits are very uncertain. We found for the Nidek 2-chip window that 50% of the epidermis was preserved at 525 +/- 250 J/sq. cm. We found for the Zimmer cold air unit that 50% of the epidermis was preserved at 425 +/- 80 J/sq. cm. These values are identical within the accuracy of the experiment.

When the skin was hydrated with water or gel, the epidermis was fully preserved for both methods of cooling.

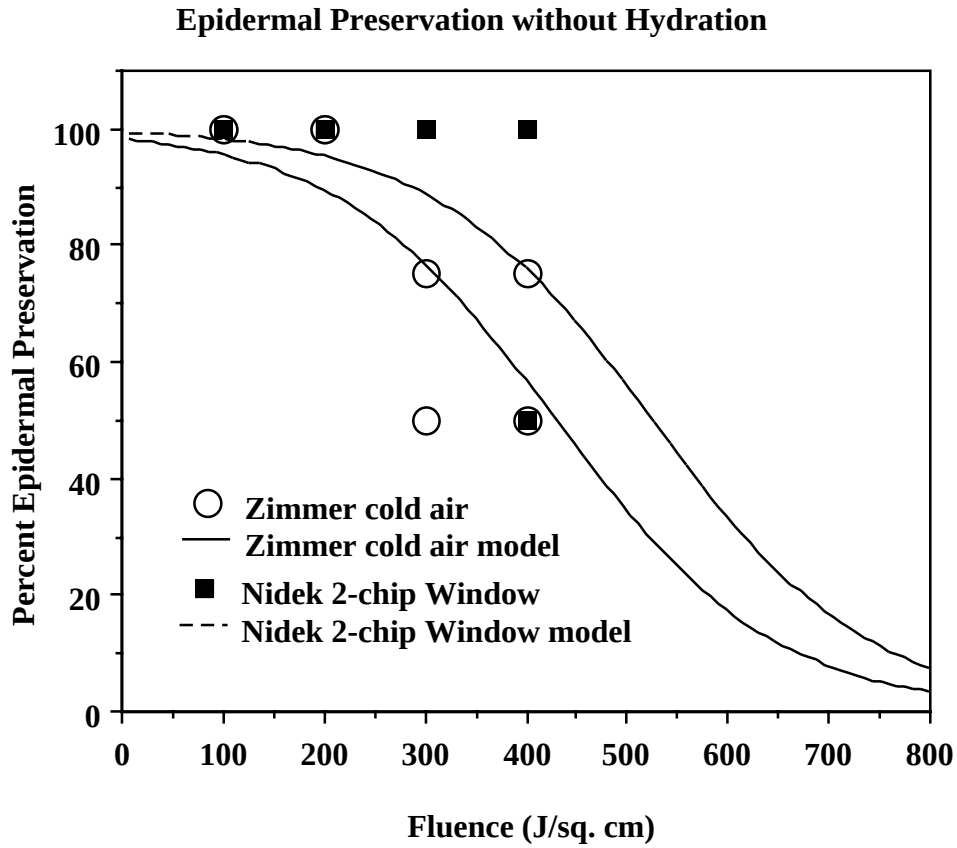

Figure 3: The percent epidermal preservation as a function of the fluence for both the Zimmer cold air and the Nidek 2-chip window on light colored piglet skin with no hydration. The lines are fits of the data to a two-state model. The two lines are identical within the accuracy of the data.

Dark Pigmented Piglet Model

Various fluences tested adjusted in increments of 10 J/sq.cm. The piglet was pigment to a Type III+ skin with black, course hair. The epidermal damage was dependent upon the density of the hair. The hair was closely shaved before the experiments.

The epidermal preservation is summarized in Table 2.

Table 2: Maximum fluence in J/sq. cm for epidermal preservation

No protection	20
Gel only	25
Water only	25
Zimmer cold air	30
Zimmer cold air + water	40
Zimmer cold air + gel	50

Nidek window	45
Nidek window + water	55
Nidek window + gel	55

5.2 Group 1 Study Comments & Concerns

Thermal damage thresholds vary dependent upon the skin color and hair color. Variations for the threshold of damage to the epidermis was noted to vary according to skin thickness. We attempted to used skin from different sites on the piglets for all measurements.

It is also possible to preserve the epidermis but create full thickness burns below the surface at very high fluences (above 400 J/sq. cm). One must monitor for this damage which is evident by stark blanching of the skin and severe edema.

5.3 Group 2 (Wound Healing) Study Results

At 400 J/sq. cm with both cooling systems, there is a small amount of collagen damage in the mid-dermis. The epidermis is preserved during the wound healing process. After 28 days the collagen structure returns to a native appearing state.

6.0 Discussion

It is the opinion of Brian Biesman, M.D. and Lou Reinisch, Ph.D. that the results of these investigations indicate that fluences above 50 J/cm^2 can be safe to treat very lightly pigmented patients with the 810 nm diode laser. However, when working at fluences of this level great care must be taken to protect the epidermis. It is of note that there were no cases in which the epidermis appeared to be left intact immediately after treatment and then later sloughed. The cooling of the skin should always be supplemented with skin hydration.

In this phase of the study we demonstrated that adequate epidermal protection is imparted by a rapid flow of cooled air or a 2-chip cooled optical window with a fast recovery from thermal loads. The maximum fluence tolerated by fully hydrated skin ranged from 50 J/cm^2 on pigmented animals (similar to Fitzpatrick Type III+) to more than 400 J/cm^2 on non-pigmented animals (similar to Fitzpatrick Type I). While it is not surprising that the threshold for epidermal injury decreased with increasing amounts of cutaneous pigment, we were impressed by the very high fluences that could be delivered without epidermal injury in the presence of hydration and epidermal cooling. It is therefore apparent

that if great care and careful judgment are employed, the 810 nm diode laser may be used to treat patients with pigmented skin.

7.0 Conclusions

The EpiStar Laser performed well during these trials. It is the opinion of Brian Biesman, M.D. and Lou Reinisch, Ph.D. that the use of the Nidek EpiStar Laser used at fluence levels up to 400 J/ cm^2 is acceptable for consideration for human use. The maximum fluence used on a subject should depend upon the skin color of the subject. Fluences up to 400 J/cm^2 should be safe on a subject with Fitzpatrick Type I skin combined with hydration and cooling.

8.0 References

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