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# Effects of Electrical Stimulation on Wound Closure in Mice with Experimental Diabetes Mellitus

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**Abstract:** The purpose of the present study was to examine the effect of electrical stimulation (ES) on the closure of full-thickness excisional wounds in mice with type-1 experimental diabetes mellitus (DM). Alloxan monohydrate (100mg/kg) was used to induce experimental DM in male CD-1 mice (n = 88). Full-thickness skin excisions (1cm<sup>2</sup>) in diabetic (urine glucose > 0) and non-diabetic (urine glucose = 0) mice were administered 1, 3, or 5 treatments of ES (200µs, 200 Hz) for 15 minutes, at 0 (sham), 5, 10, or 12.5 volts. Alloxan injection resulted in a positive urine glucose test in 48 mice yielding an induction rate for DM of 54.5 percent. All groups exhibited decreases in wound length, perimeter, and surface area between days 2 and 16 following the creation of wounds. Non-diabetic wounds treated with ES had the greatest percentage (60%) of closure. Diabetic wounds treated with ES had a greater percentage of closure (36%) compared with sham-treated diabetic animals (12.5%). Treatment of wounds with the highest voltage of ES (12.5V) produced significant (P < 0.01) decreases in the surface area, and significant (P < 0.01) changes in the shapes of wounds in both diabetic and non-diabetic animals compared with sham-treated animals. These results support the clinical use of this adjunctive therapy to accelerate the closure of ulcers due to DM.

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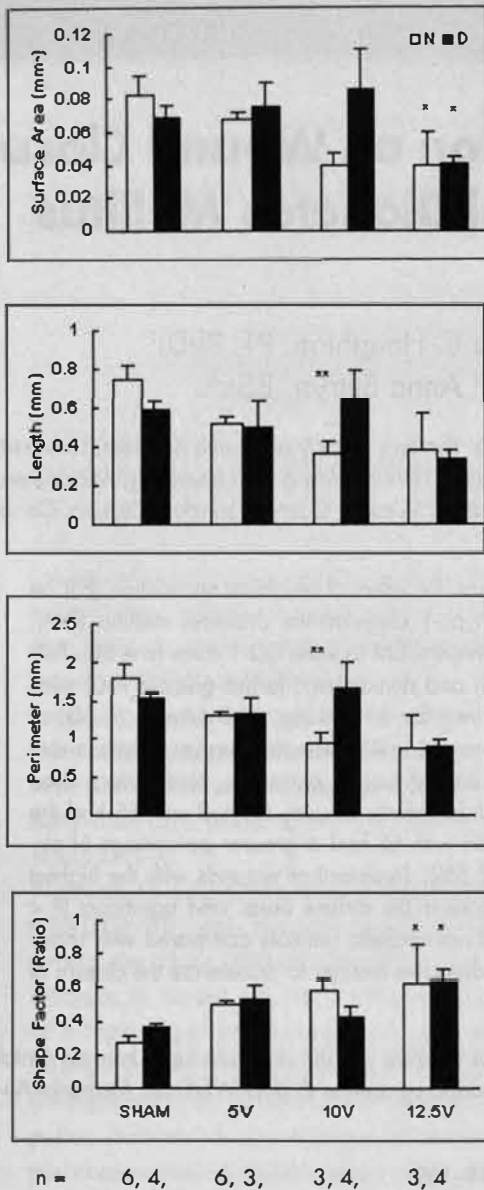
**T**he World Health Organization estimates the total number of people with diabetes mellitus (DM) in the population will double to 239 million by the year 2010. DM is a chronic disease characterized by atherosclerosis, neuropathy, and an increased incidence of infection. These sequelae of the disease process, compounded by the metabolic problems related to hyperglycemia, insulin deficiency, and/or insulin resistance contribute to impaired healing in individuals with DM. Foot ulceration and infection are leading causes of

hospitalization among individuals with DM.<sup>1</sup> Approximately 15 percent of all individuals with DM will develop foot or leg ulceration at some time during their lives contributing to one-half to two-thirds of all non-traumatic amputations in the United States.<sup>2,3</sup>

Numerous clinical reports including at least nine randomized controlled clinical trials have documented that ES accelerates wound closure in chronic wounds including diabetic ulcers.<sup>4-7</sup> Previous research studies have examined electrically-stimu-

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**Figure 1.** Bars represent the mean  $\pm$  the standard deviation of the mean (SEM) of the surface area, perimeter, length, and shape factor of wounds determined using computer image analysis. Measurements of wound dimensions were made from wounds placed in non-diabetic (N) and diabetic (D) animals that had been treated with electrical stimulation (ES) at an intensity of 0V, 5V, 10V, or 12.5V for 15 minutes on alternate days for a 16-day period. A single asterisk (\*) indicates significant differences between diabetic and non-diabetic animals treated with 12.5V of ES compared with sham-treated diabetic and non-diabetic animals. Double asterisks (\*\*) indicate significant differences between non-diabetic animals treated with 10V of ES and sham-treated non-diabetic animals. The number of animals (n) in each group is indicated at the bottom of the figure.

lated wound closure in healthy animal models using acute wounds.<sup>8-12</sup> However, one study examined the effect of ES in diabetic mice using a full-thickness incisional model.<sup>13</sup> Alloxan monohydrate, a drug known to induce diabetes by destruction of pancreatic islet beta cells by five days following intravenous injection,<sup>14</sup> was used to induce diabetes in these animals. Diabetic mice were treated with sham or ES daily for 15 minutes, with the negative electrode placed proximal to the wound. The study demonstrated diabetic animals treated with ES had greater tensile wound strength compared to sham-treated control animals.<sup>13</sup> Moreover, administration of ES to the wounds of diabetic animals resulted in an almost complete return of the wound histology to normal tissue appearance.

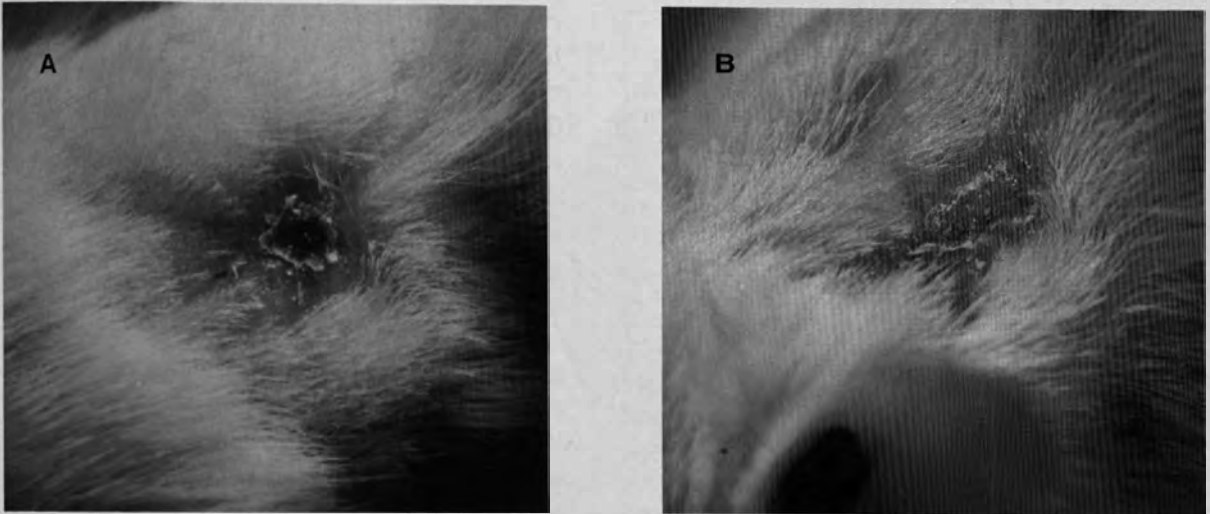
No studies to date have examined the effect of ES on the closure of full-thickness excisional wounds in diabetic mice. The purpose of this study was to identify a stimulus intensity of ES that produced the greatest healing of full-thickness excisional wounds in animals with DM. This optimal stimulus intensity of ES was then used in a second study to examine the effect of ES on the rate of healing of wounds in diabetic mice over time.

## Materials and Methods

**Animals.** Eighty-eight adult male CD-1 mice (Charles River Laboratories, St. Constant, QC, Canada) weighing between 22 and 28g were used for this study. Upon arrival, individual animals were identified with ear tags, placed into groups, and housed in an animal care facility approved by the Canadian Council on Animal Care, with controlled temperature (22°C) and light (12 hour light/12 hour dark cycle). Food and water were provided *ad libitum*.

## Diabetes Induction

Following five days of acclimatization, all animals were anesthetized with an intraperitoneal injection of ketamine/Xylazine (.03ml/10g of body weight), and given an intravenous injection of alloxan monohydrate (100mg/kg of body weight) via the tail vein. This agent has been used extensively to induce DM in experimental animals,<sup>15</sup> and is known to selectively destroy



**Figure 2.** Full-thickness excisional wounds were created dorsally in all animals. Wounds in diabetic animals treated with the highest voltage of ES (12.5V) appeared circular in shape (A) compared with sham-treated wounds in diabetic animals (B) which appeared more linear in shape by day 16.

insulin-producing pancreatic islet  $\beta$ -cells within seven days of intravenous administration.<sup>13,15-21</sup> This selective vulnerability of the islet  $\beta$ -cells compared with other cell types has been attributed to the rapid accumulation of the drug in the  $\beta$ -cells<sup>22</sup> and the high sensitivity of the  $\beta$ -cells toward peroxidases resulting from the metabolism of alloxan relative to other tissues.<sup>23-26</sup>

The presence of diabetes was confirmed using urine glucose test sticks (Chemstrip; Boehringer Mannheim, Canada) at five days post-alloxan injection and was repeated at the time of sacrifice. Body weights were also recorded using an electronic balance accurate to 1.0mg (ER-60A; Johns Scientific Inc., Canada) at the time of alloxan injection and prior to sacrifice. Animals that demonstrated symptoms typical of the experimental diabetic state such as glucosuria, polyuria, weight loss, and polydipsia were termed diabetic. A value greater than zero of the amount of glucose in the urine indicated the presence of DM. Animals that failed to demonstrate any level of glucose in the urine and that failed to demonstrate polyuria, weight loss, and polydipsia were termed non-diabetic.

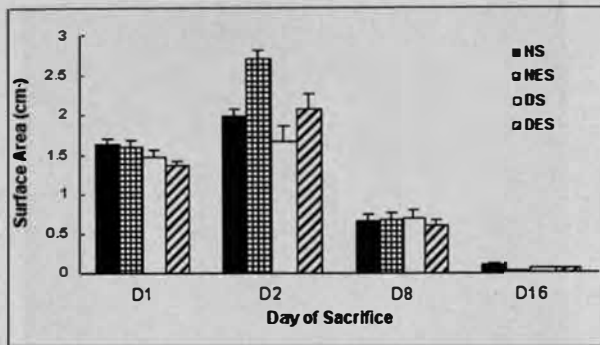
### Surgery and Recovery

On the seventh day following alloxan monohydrate injection, the area between the shoulder

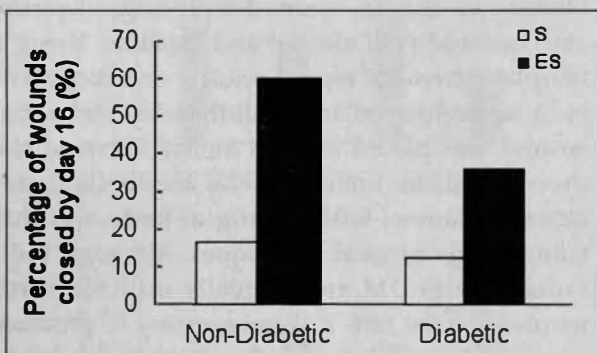
blades was shaved, washed with surgical scrub, and swabbed with alcohol and Betadine. Using a template to ensure reproducibility and consistency, a square-shaped 1cm<sup>2</sup> full-thickness excisional wound was placed in each animal between the shoulder blades under general anesthesia (ketamine/Xylazine; 0.03ml/10g of body weight) using sterile surgical techniques. Although individuals with DM are typically inflicted with wounds on the feet, it was necessary to produce wounds dorsally between the shoulder blades to minimize tampering of the wound by the animals. Since wound-dressing materials are not well tolerated by these animals, all wounds were left open for the duration of the study. However, the conducting gel that was used was retained on all wound sites following treatments in order to create a similar moist wound-healing environment. As a result, most animals developed a thin eschar over the wound site until complete epithelialization occurred.

### Treatment

Animals were then randomly assigned to receive either true ES or sham treatment. A Portmax 300 electrical stimulation device (Medelco Ltd., Mississauga, ON, Canada) was used to administer a monophasic, pulsed current with a pulse duration of 200  $\mu$ seconds, delivered at a frequency of 200



**Figure 3.** Bars represent the mean  $\pm$  SEM of wound surface area measured on day following surgery and prior to the onset of treatments (D1), and following 2 (D2), 8 (D8), and 16 days post surgery. Measurements are presented for non-diabetic animals that received either an electrical stimulus at an intensity of 12.5V (NES) or sham treatment (NS), and diabetic animals following administration of ES (12.5V) (DES) or sham treatment (DS).



**Figure 4.** Bars represent the percentage of the total wounds in each treatment group that had closed at the time of sacrifice (day 16 post surgery). Measurements are presented for non-diabetic animals that either received an electrical stimulus at an intensity of 12.5V (ES) or sham treatment (S) and diabetic animals following a 12.5V electrical stimulus (ES) or sham treatment (S).

pulses per second. Throughout the study, negative polarity was maintained at the treatment electrode (cathode), which was placed directly over the wound site. Treatments were initiated 24 hours following the infliction of wounds. To verify the voltage delivered, the electrical stimulator was calibrated at each treatment voltage using an oscilloscope (HM 408, HAMEG GmbH, D-6000 Frankfurt am Main 71, West Germany). Immediately prior to treatment, animals were placed in individual restraining devices in the form of narrow cones fab-

ricated of a flexible fiberglass mesh. Consistent electrode attachment was maintained during the 15-minute treatment sessions. Sterile conducting gel (DuoDerm; ConvaTec, Princeton, NJ) was applied to cover the wound site, directly over which the 2cm<sup>2</sup> treatment electrode (cathode) was then secured. The size of the treatment electrode was greater than that of the wound to ensure complete coverage of the wound. A larger non-treatment electrode (anode; 4cm<sup>2</sup>) was wrapped around each animal's tail to complete the circuit. Electrodes were cut out of Metaline gauze (Bohmann GmbH & Co., KG, Germany) and were moistened in sterile saline solution prior to application. Sham-treated non-diabetic and diabetic mice were treated identically, but the electrical stimulator was not activated. All procedures were in accordance with guidelines of the Canadian Council for Animal Care and were approved by the Animal Care Committee at the University of Western Ontario.

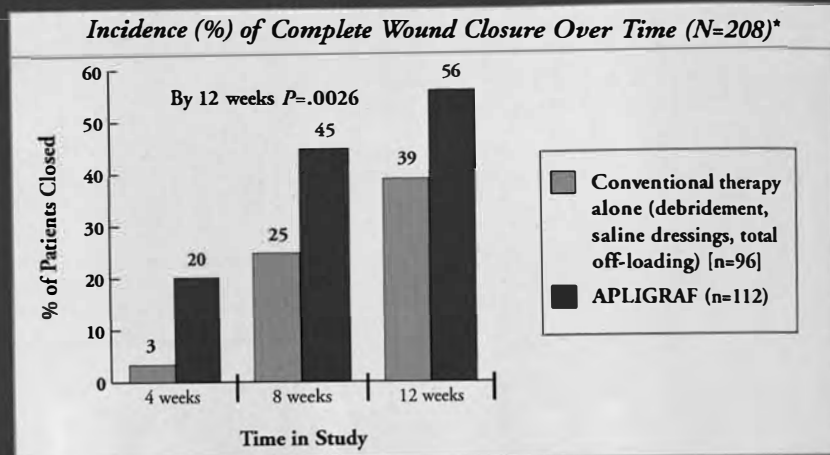
## Study Design

In the first study, animals (diabetic [n = 15] and non-diabetic [n = 18]) received either ES at one of the following intensities (5.0, 10.0, 12.5V) or sham treatment. After the first electrical treatment, it was apparent that some animals were not tolerating the higher intensities of electrical current (20V and 40V). Data derived from these animals were eliminated from the study. The remaining animals that were originally allocated to the 20V and 40V groups were combined with animals in the 12.5V group for the remaining treatments and for subsequent data analysis. At the end of the 16-day treatment period, wound size was measured using planimetric (PLANIX 7; Digital Planimeter; Koizumi Sokki Mfg. Co. Ltd., Nagaoka-Shi, Japan) and computerized image analysis (Northern Exposure; Empix Imaging, Mississauga, ON, Canada) techniques.

The second study was designed to examine the time course of the changes that occurred in wounds treated with electrical stimulation. To do so, wounded animals (diabetic [n = 33] and non-diabetic [n = 22]) received treatments of either electrical current at the optimal intensity determined during the first stage of this study (12.5V), or sham treatment. The animals were randomly divided into two groups, and each group was treated on alternate

Continued on page 164

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\*Estimated frequency of complete diabetic foot ulcer wound closure as a function of time by Kaplan-Meier analysis.

†The persistence of APLIGRAF cells on the wound and the safety of this device in venous ulcer patients beyond 1 year and in diabetic foot ulcer patients beyond 6 months have not been evaluated.

### INTENDED USE/INDICATION

Apligraf is indicated for use with standard therapeutic compression for the treatment of noninfected partial- and full-thickness skin ulcers due to venous insufficiency of greater than 1 month in duration and that have not adequately responded to conventional ulcer therapy.

Apligraf is also indicated for use with standard diabetic foot ulcer care for the treatment of full-thickness neuropathic diabetic foot ulcers of greater than 3 weeks' duration which have not adequately responded to conventional ulcer therapy and which extend through the dermis but without tendon, muscle, capsule or bone exposure.

### CONTRAINDICATIONS

Apligraf is contraindicated for use on clinically infected wounds and in patients with known allergies to bovine collagen or hypersensitivity to the components of the shipping medium: agarose, L-glutamine, hydrocortisone/bovine serum albumin, bovine insulin, human transferrin, triiodothyronine, ethanolamine, O-phosphorylethanolamine, adenine, selenious acid, DMEM powder, HAM's F-12 powder.

### WARNINGS AND PRECAUTIONS

Do not open and do not use Apligraf after the expiration date or if the pH is not within the acceptable range (6.8-7.7).

A clinical determination of wound infection should be made based on all of the signs and symptoms of infection.

Allergic reactions to products containing components present in the Apligraf agarose shipping medium (see CONTRAINDICATIONS section) or bovine collagen

(a component of Apligraf) have been reported. Discontinue product use if a patient shows evidence of an immunological reaction. Patients should notify their physician of any symptoms of an allergic reaction. In evaluating over 1000 patients no allergic reactions to Apligraf were reported.

The persistence of Apligraf cells on the wound and the safety of this device in venous leg ulcers beyond one year and in diabetic foot ulcer patients beyond 6 months has not been evaluated. Testing to date has not revealed a tumorigenic potential of the cells contained in the device. However, the long term potential of skin cancers from these cells is unknown.

### ADVERSE EVENTS

In the controlled clinical study conducted in patients with ulcers due to venous insufficiency of greater than 1 month in duration, the incidence of adverse events was comparable between the 2 study groups, with the exception of suspected infection, which was reported more frequently in Apligraf-treated (29.2%) than control patients (14.0%). There were 1 life-threatening and 3 severe infections in the Apligraf group and none in the control arm. Of these, two severe infections were considered related to treatment, however, one occurred one month after the last application of Apligraf and the other occurred following application on a pre-existing *Pseudomonas* infection. While the overall incidence of wound infection was

higher in the Apligraf arm, the incidence of wound closure was 72/130 (55.4%) and 54/110 (49.1%) for Apligraf and control-treated patients, respectively.

In the controlled clinical study conducted in patients with ulcers due to diabetic foot ulcers of at least 3 weeks' duration, the most common adverse events at the study site were suspected wound infection (10.7% vs 13.5%), cellulitis (8.9% vs 8.3%), and osteomyelitis (2.7% vs 10.4%) in the Apligraf and control arm, respectively. The incidence of wound closure was 63/112 (56%) and 36/96 (39%) for Apligraf in control-treated patients, respectively.

### HOW SUPPLIED

Apligraf is supplied in a heavy gauge polyethylene bag with a 10% CO<sub>2</sub>/air atmosphere and agarose nutrient medium, ready for single use. To maintain cell viability, Apligraf should be kept in the sealed bag at 20°C to 31°C (68°F to 88°F) until use. Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick.

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**Table 1.** Urine glucose (% = g/dl) and weight (g) on the day of surgery and at sacrifice of non-diabetic (NS) and diabetic (DS) animals that received sham treatment, and non-diabetic (NES) and diabetic (DES) animals that were treated with ES

Group	N	Surgery	Sacrifice	Surgery	Sacrifice
		Urine Glucose (% = g/dl)		Body Weight (%)	
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
NS	18	0±0	0±0	29.30±0.86	30.91±0.56
NES	22	0±0	0±0	29.00±0.68	29.89±0.56
DS	19	*3.82±0.43	3.52±0.31	28.54±0.61	**27.69±0.90
DES	29	*3.34±0.34	3.18±0.26	28.58±0.62	**27.81±0.64

N refers to the number of animals that were actually sacrificed.

\*NS and NES significantly ( $P < 0.01$ ) different from both DS and DES.

\*\*NS significantly ( $P < 0.01$ ) different from both DS and DES.

days. This treatment schedule was chosen to facilitate handling of the large number of animals in the present study. All treatments were administered for 15 minutes over a period of either 2, 8, or 16 days following the creation of wounds. At each of these time points (2, 8, 16 days), animals were randomly selected to be sacrificed and wound size was determined using computer image analysis.

### Documentation of Wound Size

All animals were sacrificed using the introduction of carbon dioxide into a Plexiglas chamber. Each animal's body weight and urine glucose was reassessed at this time (Table 1). Following the removal of eschar, the perimeter of each wound was traced onto a transparent acetate film. Wound tracings were repeated three times for each animal. Wounds were also photographed using a WILD Lietz stereomicroscope at 6.5X (Leica, Germany), and digitized on a 930 D.C. RGB Sony video camera. An investigator that remained blinded to the treatment groups performed all documentation.

### Determination of Wound Size

The surface area, perimeter, shape factor, and length of each wound were determined

using computer image analysis of the wound perimeter tracings. This computer image analysis performs measurements on a digitized image obtained by a CCD camera, thus permitting rapid quantification of multiple morphometric features of the wound. The digitized image is then transformed into a binary image using a process called monochrome or grey-level thresholding, which permits automatic detection of the boundary of the image by a boundary tracing.<sup>27</sup> The surface area of the image is then calculated using the pixel counting method, which sums the pixels enclosed

by and including the image boundary, multiplied by the calibrated area of each pixel.<sup>27</sup> Other morphometric values, such as the perimeter and shape factor, are derived using standard algorithms.<sup>27</sup> The shape factor for a perfect circle is 1.0. As the shape of the object moves toward a line, the value of the shape factor approaches zero.

### Validation of Wound Size Measurements

All measurements of wound surface area, perimeter, and length in this study were obtained using an internal scale calibrated to a ruler of known length (1mm) under each magnification and had a resolution of one micron. To ensure accuracy of this new method of wound size detection using computer image analysis, the surface area of 10 wounds of known size was determined on two different occasions and compared against measurements obtained using planimetry. The planimetry method has previously been shown to have an intrarater and inter-rater reliability of 0.99.<sup>28-30</sup>

To assess the intrarater reliability of each measure of wound size using the planimetry and computer image analysis methods, the intraclass correlation coefficient ( $ICC_{(0,1)}$ ) was determined. The agreement of the measurements from each method was

evaluated using an  $ICC_{(2,1)}$ . A one-way repeated measures ANOVA was used to obtain the variance components for calculating both the ICCs.<sup>30</sup>

## Data Analysis

This was a stratified, block-randomized, placebo-controlled study, comparing the effects of DM and ES on the wound size of full-thickness excisional cutaneous wounds in animals termed diabetic (urine glucose > 0) and non-diabetic (urine glucose = 0). The following dependent variables were assessed in the first stage of the study using a one-way analysis of variance (ANOVA) test ( $\alpha = 0.01$ ): surface area, perimeter, length, and shape factor of wounds. The data was assessed for evidence of a dose dependent relationship between the voltage of ES delivered and the size of wounds. To assess for an effect of ES on the size of wounds over time, data from both studies was combined into the following experimental groups: non-diabetic (NS) and diabetic (DS) animals administered sham ES, and non-diabetic (NES) and diabetic (DES) animals administered true ES. The percent of wounds that closed by the end of the experimental period (day 16) in each group were calculated by dividing the actual number of wounds that closed in a group by the total number of wounds in that group. The surface area of wounds in each treatment group (NS, NES, DS, DES) on each day of sacrifice (day 2, 8, 16) was assessed using a one-way ANOVA test ( $\alpha = 0.01$ ). The Tukey HSD method of multiple comparisons was used to compare group means ( $\alpha = .05$ ). To further assess the effects of ES on wound closure, all wounds (diabetic and non-diabetic) were separated into two groups: those administered sham or true ES. A two-tailed t-test for independent samples was then used to identify significant differences ( $\alpha = 0.01$ ) in surface area between sham and ES-treated groups. Differences between diabetic and non-diabetic animals were assessed in a similar manner. The urine glucose and body weights of animals from both stages of the study on the days of sacrifice and surgery were also combined and assessed using a one-way ANOVA. All results are expressed as the mean  $\pm$  the standard error of the mean (SEM).

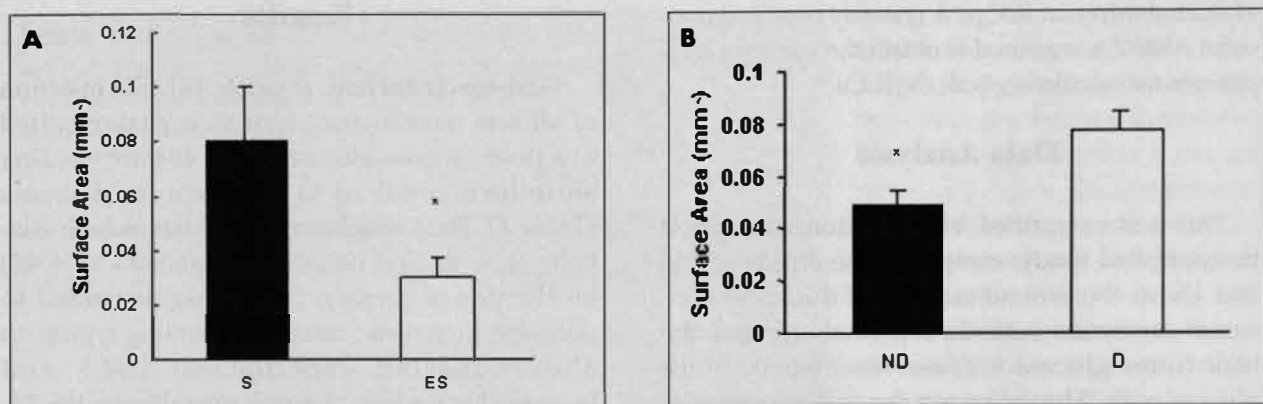
## Results

**Diabetes induction.** A single tail vein injection of alloxan monohydrate into 88 animals resulted in a positive urine glucose test in 48 mice yielding an induction rate of 54.5 percent for diabetes (Table 1). Body weights were similar in both diabetic ( $n = 48$ ) and non-diabetic animals ( $n = 40$ ) on the day of surgery. Body weights tended to decrease in diabetic animals, a finding typical in alloxan-induced experimental DM,<sup>31</sup> and increased in the non-diabetic animals over the 16-day experimental period. Non-diabetic animals treated with sham ES had significantly ( $P < 0.01$ ) greater body weights compared to diabetic animals treated with either sham or true ES over the time between surgery and sacrifice (Table 1).

**Wound closure: Reliability of measurement of wound dimensions.** Intrarater reliability, determined using the  $ICC_{(1,1)}$ , was high using the computer image analysis method of calculating wound surface area (0.98), perimeter (0.99), shape factor (0.97), and length (0.98). A high degree of agreement was found between measurements of wound surface area obtained using the computer image analysis and planimetry methods ( $ICC_{(2,1)} = .96$ ).

**Effect of ES: Dose response.** Results presented in Figure 1 show that ES reduced the surface area, perimeter, and length and elevated the shape factor of wounds in both diabetic and non-diabetic animals compared with sham ES. Analysis of the effects of ES on diabetic and non-diabetic animals within each treatment group revealed decreases in the area, perimeter, and length of wounds in both diabetic and non-diabetic animals with increasing intensities of ES (Figure 1). The perimeter and length of wounds in non-diabetic animals treated with 10.0V of ES decreased significantly ( $P < 0.01$ ) compared with wounds in sham-treated non-diabetic animals (Figure 1). Although the surface area of these wounds also tended to decrease, the differences were not statistically significant. In diabetic animals, a higher voltage of ES (12.5V) was required to induce a significant ( $P < 0.01$ ) change in the dimensions of wounds compared to sham-treated controls. Administration of the highest dose of ES (12.5V) to both diabetic and non-diabetic animals produced significant change in the shape of the wounds (Figure 1). This change in the





**Figure 5.** Bars represent the mean  $\pm$  SEM of the surface area measured in a) animals treated with 12.5V of electrical current (ES;  $n = 16$ ) or sham treatment (S;  $n = 20$ ) for 16 days post surgery; and b) wounds in diabetic animals treated with either sham or real ES treatment (D;  $n = 48$ ) compared to those in non-diabetic animals (ND;  $n = 40$ ).

shape factor of the wounds indicated that wounds treated with ES were more rounded in shape compared to the linearly shaped wounds of animals treated with sham ES (Figure 2). Although not statistically significant, we found that the mean of the diabetic animals treated with 10V of ES did not follow the expected decrease but was consistently greater than the mean for sham-treated diabetic animals, indicating that diabetic animals treated with 10V of ES had larger and more linearly shaped wounds compared with sham-treated diabetic animals.

**Effect of ES over time.** All wounds, diabetic and non-diabetic, increased in surface area between surgery and day 2 (Figure 3), a finding reported previously, and due to the retraction of the margins of the skin.<sup>32</sup> Both diabetic and non-diabetic wounds administered ES showed a greater increase in wound size on day 2 compared to their sham controls (Figure 3). However, wounds in all groups decreased in surface area between day 2 and either day 8 or 16 (Figure 3).

Of the wounds that were treated with 12.5V for 14 to 16 days ( $n = 36$ ), those that were non-diabetic and treated with ES had markedly higher percentage (60%) of closed wounds compared with sham-treated non-diabetic animals (16.7%) (Figure 4). Diabetic wounds treated with ES also had a greater percentage of closed wounds (36%) compared with sham-treated diabetic animals (12.5%) by day 16 (Figure 4). As well, diabetic and non-diabetic animals treated with 12.5V for 16 days ( $n = 16$ ) had significantly ( $P < 0.01$ ) smaller wound surface area

measurements ( $0.04 \pm 0.01\text{mm}^2$ ) compared with animals that received sham treatments over the same time period ( $0.08 \pm 0.02\text{mm}^2$ ;  $n = 20$ ; Figure 5a).

**Effect of diabetes.** Although the measurements of wound dimensions tended to be greater in non-diabetic compared to diabetic animals at each intensity of ES administered, no statistically significant differences were found between diabetic and non-diabetic animals (Figure 1). However, when wound surface area measurements made in all diabetic animals ( $n = 48$ ), regardless of the intensity of the electrical stimulus and the number of days of treatment, were compared to surface area measurements made in non-diabetic animals ( $n = 40$ ), it was found that wound size was significantly ( $P < 0.01$ ) smaller in non-diabetic animals ( $0.05 \pm 0.01\text{mm}^2$ ) compared with diabetic ( $0.08 \pm .03\text{mm}^2$ ) animals (Figure 5b).

## Discussion

ES facilitated wound closure in both diabetic and non-diabetic animals. This was reflected by a decrease in the dimensions of wounds in both diabetic and non-diabetic animals compared with sham-treated controls. Although a dose-dependent relationship between ES and wound size was not apparent, the highest voltage of ES (12.5V) produced the greatest decrease in the dimensions of wounds in both diabetic and non-diabetic animals. Our findings suggest ES may have affected the process of wound contraction. In the present study, we found that both diabetic

and non-diabetic wounds treated with ES appeared more circular in shape. These circular wounds also appeared more contracted, resulting in the formation of a slightly elevated scar. This pattern of wound contraction was most obvious in the group that received the highest voltage of ES (12.5V). Wound contraction is known to contribute significantly to the closure of excisional wounds in loose-skinned animals.<sup>33,34</sup> Furthermore, Weiss, et al.,<sup>35</sup> reported a similar observation following ES treatment using positive polarity of surgically-induced partial-thickness wounds. Further studies are needed to examine the effects of ES on the myofibroblast and to elucidate the mechanisms underlying the effects of ES on wound contraction.

A full-thickness cutaneous excisional wound model was utilized in the present study. This model has several advantages over a linear incisional wound model. First, an excisional wound model results in an open wound that requires considerable fibroplasia to fill the defect. Therefore, such a model provides more wound tissue for the quantification of connective tissue metabolism and collagen.<sup>36</sup> This is important for subsequent studies aimed at elucidating the mechanisms underlying electrically enhanced wound closure. An excisional wound also more closely resembles the chronic, open wounds encountered in clinical situations. Few studies have examined the effects of ES on excisional wounds. Assimakopoulos<sup>37</sup> in 1968 and Alvarez, et al.,<sup>8</sup> showed that low-intensity direct current delivered with a negatively active electrode produced an acceleration in the healing of excisional wounds in rabbits and domestic pigs, respectively. Both these studies were performed in young healthy animals.

Alloxan monohydrate was used to induce DM in healthy, adult male mice. This agent has been widely used to induce DM in experimental animals. The levels of glucosuria measured in our study were relatively consistent between diabetic animals that received true or sham ES treatment. Animals that received an alloxan injection but did not exhibit glucosuria were termed non-diabetic and served as the control group. These animals failed to show glucosuria at the end of the study (three weeks post-alloxan injection) and did not exhibit symptoms typical of alloxan-induced

experimental DM such as polyuria, polydipsia, or weight loss. Furthermore, the selective vulnerability of the insulin-producing islet  $\beta$ -cells compared with other cell types has been attributed to a high rate of uptake of alloxan in the  $\beta$ -cells<sup>22</sup> and the high sensitivity of the  $\beta$ -cell toward peroxidases relative to other tissues.<sup>23-26</sup> No effects of alloxan on other islet cell types have been reported.<sup>38</sup> Studies also indicate that alloxan does not appear to irreversibly affect lymphoid function and the functions critical to the generation of an immune response,<sup>25</sup> nor does it affect collagenase activity.<sup>24</sup> Therefore, it is unlikely that animals in the present study that were termed non-diabetic could have been diabetic without detectable urine glucose. Animals identified as having glucose in the urine were termed diabetic. These animals also exhibited typical symptoms of alloxan-induced experimental DM such as polyuria, polydipsia, and weight loss by five days following alloxan injection. Alloxan produced DM in 54.5 percent of animals injected. This relatively low diabetic induction rate is probably reflective of the technical difficulty of performing tail vein injections in such a small animal species.

Smith, et al.,<sup>13</sup> reported pathologic changes in the epidermis and scar of mice following five days of alloxan injection and a subsequent two weeks of treatment with ES. Therefore, although the three-week period may not be long enough to generate complications of DM such as atherosclerosis, neuropathy, and retinopathy, it is sufficient to produce cutaneous changes characteristic of DM. Using this alloxan-induced diabetic model, we have also reported that lower amounts of collagen were recorded in the superficial and deep scar of wounded and adjacent unwounded tissues in diabetic animals compared with non-diabetic animals.<sup>39,40</sup> This result is in agreement with earlier reports that also document a decrease in the repair of deeper wounds that require collagen formation in diabetic animals.<sup>41</sup>

Computer image analysis was used to document the dimensions of wounds. This method uses pixel counting to calculate wound dimensions. This study showed that the computer image analysis and planimetry methods provided measurements of wound dimensions that demonstrated equivalent accuracy and intrarater reliability.

bility. The planimetry method has previously been shown to have an intrarater and interrater reliability of 0.99.<sup>28,29,42</sup> The planimetry method is considered to be the gold standard for the documentation of wound dimensions.<sup>42,43</sup> This computer analysis method may be used to accurately and reliably document change in wound dimensions.

Using reliable and valid computerized image analysis techniques, we have demonstrated that ES significantly reduced the dimensions of wounds, and significantly altered the shape of wounds in both non-diabetic and diabetic mice. Further studies are needed to examine the mechanisms underlying this electrically induced wound closure and change in shape seen in diabetic animals.

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