

## LOW VOLTAGE ELECTRICAL STIMULATION AND WOUND HEALING IN RABBITS: EFFECT OF ALTERING FREQUENCY

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### ABSTRACT

The effect of electrical currents by different modalities on wound healing has been experimented. In this study a survey of the literature shows different results, and the mechanisms of action have not been elucidated for certain. The aim of this study was to compare the results obtained from low voltage-square wave stimulation of different frequencies. Results show that the healing period for the control group ( $14.9 \pm 0.58$  days) was reduced to  $11.28 \pm 0.52$  and  $10.37 \pm 0.7$  days for groups receiving 20 and 80 Hz stimulation respectively. This implies that wound closure as an index of wound healing was significantly increased in animals receiving 80 Hz stimulation. Tensile strength was increased from  $2013 \pm 192$  grams in controls to  $2589 \pm 235.1$  grams in the 80 Hz-receiving study group. We concluded that electrical stimulation can affect the process of healing and a stimulation of 80Hz is more effective than other frequencies.

**Keywords:** Electrical stimulation, Wound healing, Tensile strength.

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### INTRODUCTION

The effect of various forms of electrical current have been studied both in human and animal models. Some *in vivo* studies have demonstrated the effect of electrical fields on biological growth and tissue repair by different modalities of electrical stimulation (i.e., low and high voltage and frequencies).

Some investigators have shown that tensile strength and rate of healing increase by use of low voltage direct electrical stimulation.<sup>1,2</sup> Carley reported that when other methods

failed the use of electrotherapy provides beneficial and reproducible healing.<sup>5</sup>

Low intensity direct current has been used for a wide variety of wounds. It was also reported that direct current causes different histological responses beneath the anode and cathode and increase in wound tensile strength.<sup>2,4</sup>

On the other hand, high voltage stimulation (HVS) has been used in various studies. Kloth and Feedar<sup>12</sup> used HVS to enhance the rate and extent of wound tissue healing. They reported a mean healing rate of 44.8% per week and total healing of chronic wounds of patients. The HVS treatment time required to augment tissue healing did not need to exceed 60 minutes, five times a week, in their studies.

In spite of the use of electrical current for many purposes, the exact mechanisms of action are not clear. On the

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## Electrical Stimulation and Wound Healing

other hand, the characteristics of electrical stimulation used by various investigators have been different. Therefore the aim of this study was to investigate: 1) the effect of low voltage electrical stimulation on promotion of wound healing, and 2) the effects induced by square waves of different frequencies.

### MATERIALS AND METHODS

In this study 4-6 month old rabbits weighing between 1.7-2.3 kg were used. Animals were housed in standard cages specifically designed for rabbits in an approved animal facility. Food and water were freely available, and temperature was kept constant ( $20 \pm 2^\circ\text{C}$ ). Animals were divided into 4 groups: 3 experimental and one control group.

#### Skin wound

Animals were anesthetized by a 40 mg/kg intravenous injection of sodium pentobarbital. The skin on the dorsal aspect of the thoracic area was shaved. A full-thickness 35 mm incision was made on both sides of the vertebral column by a pair of sterile scissors. After the incision the length of the wound was measured and the wound cleaned by povidone iodine solution.

#### Treatment

Wound treatment was initiated on day 2 (post-incision time). Animals in experimental groups received two hours of low voltage stimulation (LVS) twice daily. The magnitude of voltage tolerated by each animal was different and ranged between 6 to 12 V. The pulse width was 500  $\mu\text{sec}$  with frequencies of 2, 20 and 80 Hz. Electrical stimulation (ES) was applied through carbon rubber surface electrodes mounted on both sides of the wounds. The voltage was set at an intensity inducing palpable contraction of skin. In the sham stimulation group the same procedure was performed except that electrical current was not applied.

#### Wound healing

The length of the wound was measured after injury and thereafter until wound closure occurred. The product of dividing the length of the wound at each day to the length of the wound at the time of incision showed the ratio of wound healing on that certain day. Subtracting the percentage of this ratio from 100 determined the percentage of healing. The surface area of the wound was also measured by tracing the wound on transparent plastic which would later be transferred to graded paper and calculated.

#### Tensile strength

After wound closure, an area of skin containing the scar was removed. This piece of skin, 5 cm in length and 1 cm in width, was attached to two clamps, the upper one fixed, and

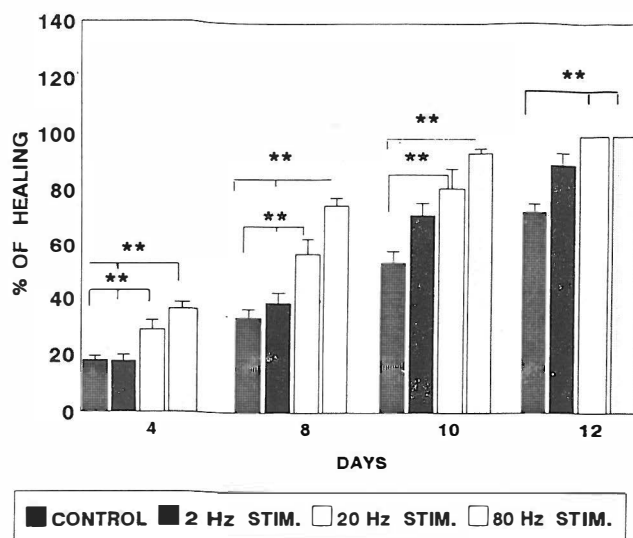


Fig. 1. Comparison of the percentage of wound healing at different frequencies of low-voltage electrical stimulation. Percentage of healing is 100% on day 12 for 20 & 80 Hz stimulation groups.

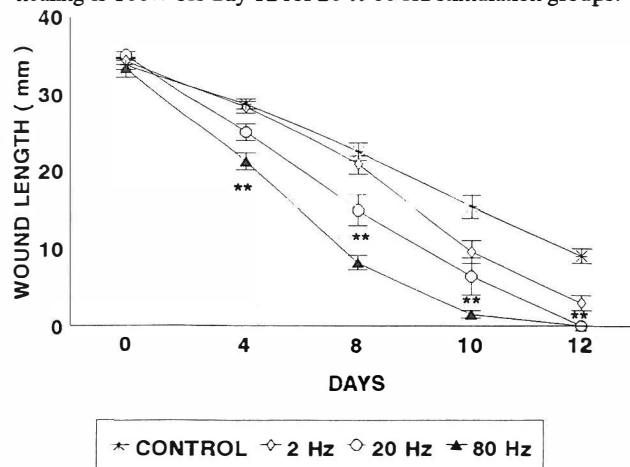


Fig. 2. Comparison of wound size changes at different frequencies of electrical stimulation. Wound size on day 10 for the 80 Hz group is significantly less than others.

the lower one attached to a light-weight plastic bag. The weight of these items was 480 grams. Then water was added to the bag at a rate of 20 mL/sec for 5 sec. A 10-second watch period followed. If the scar had not separated, water would again be added in the same manner and the scar re-inspected. This procedure was repeated until the scar underwent dehiscence.

#### Data analysis

Newman-Keuls' analysis of variance (ANOVA), and student's t-test were used. The figures represent mean  $\pm$  SEM and values of  $P < 0.05$  were considered statistically significant.

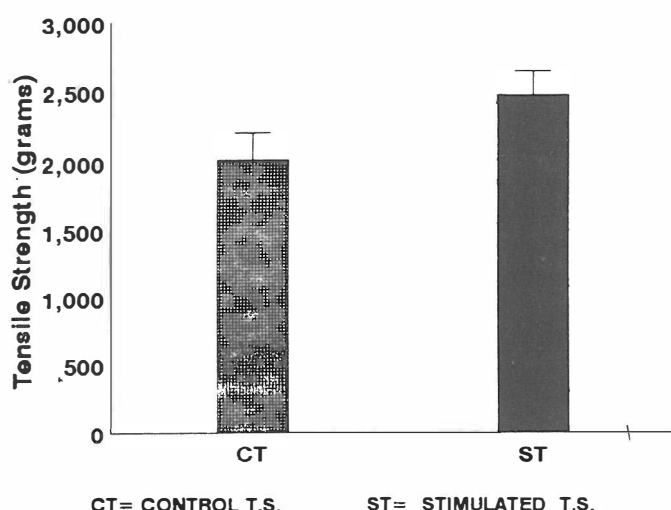


Fig. 3. Comparison of tensile strength (T.S.) in control and 80 Hz stimulation groups. CT= control T.S., ST= stimulation T.S.

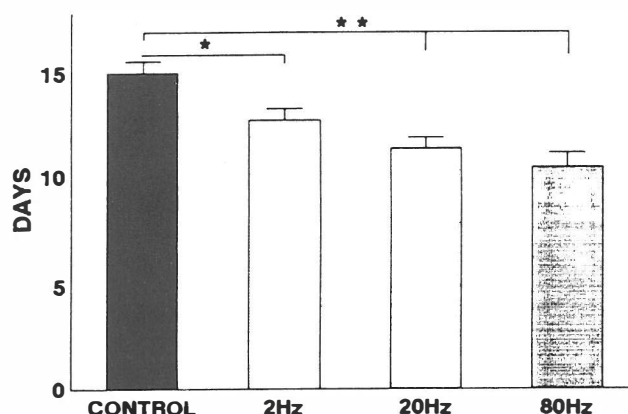


Fig. 4. Comparison of wound healing time in control and stimulated groups. Differences between control and experimental groups are statistically significant.

## RESULTS

In this study we used different frequencies of 2, 20 and 80 Hz. The results obtained in these experiments showed that the percentage of healing on the 4th, 8th and 10th days after surgery for control rabbits was 17.42%, 33.5% and 54.08% respectively, while wound healing on the same days was 17.6%, 39.1% and 71.7% following 20 Hz stimulation and 36.43%, 75.1% and 94% following 80 Hz stimulation in the study groups. Wound closure on day 10 in the 80 Hz group was significantly increased relative to the control and other experimental animals ( $P < 0.01$ ) (Fig. 1). Comparison of wound size changes at different frequencies is shown in Fig. 2.

Tensile strength was  $2013 \pm 192.56$  grams for the control group and  $2589 \pm 235.1$  grams in the 80 Hz stimulation group, but differences between means were not statistically

Table I. Comparison of wound surface area ( $\text{mm}^2$ ) of different groups during the healing period.

Day group	0	4	8	10
Control	195±25	137±11	97±5	41±8
Sham	185±7	143±5	103±22	58±19
2Hz	203±15	130±13	113±17	64±15
20Hz	197±9	111±20	54±2	15±2
80Hz	196±4	115±9	43±2	3±1

significant (Fig. 3). Duration of healing for controls was  $14.9 \pm 0.58$  days and  $12.66 \pm 0.55$ ,  $11.28 \pm 0.52$  and  $10.37 \pm 0.7$  days for 2, 20 and 80 Hz groups respectively (Fig. 4). The differences between control and experimental groups were significant ( $P < 0.01$ ), but although the duration of healing for the 80 Hz group was less than other experimental groups, it was not statistically significant. There was no significant difference between control and sham groups.

Wound surface area measurements during the healing period are shown in Table I.

## DISCUSSION

As the results have shown, low-voltage stimulation can affect and promote wound healing. LVS decreased the time of healing similar to the results of experiments using HVS.<sup>12</sup> Our results are also similar to those reported by Assimacopoulos and Alvare who used low-voltage direct current, in spite of the fact that pulsed current was used in our experiments.<sup>1,2</sup> Researchers using high-voltage positive polarity did not observe any effects on wound healing. On the other hand, those who used HVS and negative polarity on days 1-4 and positive polarity on days 4-7 reported a positive effect on healing.<sup>3</sup> These studies may prove the significance of the polarity of electrical current on different days. Although polarity seems to be an important factor in healing by ES, our study has shown that the frequency of current also plays a role in wound healing. By measuring the skin surface voltage, the existence of skin batteries have been shown. The role of these skin batteries is in the process of wound healing<sup>6</sup> and differences in response at positive and negative polarities are also related to the existence of these batteries.

One of the important mechanisms in the process of healing is re-epithelization, meaning that epithelial cells migrate from the intact border of the wound into the wound area. The existence of epidermal batteries and voltage gradient help this migration.

Furthermore, as shown in our previous study<sup>8</sup> and also reported by other studies,<sup>9,14,15</sup> ES can increase blood flow locally. Since any increase in blood flow would increase oxygen and food supply and facilitate removal of debris and

waste materials, it could promote healing. Holstein showed that low skin perfusion pressure can be used to predict ischemic wound complications, leading to reamputation at a higher level.<sup>10</sup> The results of this study indicate that LVS has the ability to accelerate wound healing, but it is not the same at different frequencies. Although the amount of tensile strength was greater in experimental animals compared to controls, it was not statistically significant. Our results are in agreement with those of Brown et al.<sup>3</sup> and Gentzkow.<sup>7</sup>

The therapeutic use of electrical stimulation must be investigated more extensively so that different characteristics of ES such as frequency, polarity and duration of pulse can be evaluated. Further investigation is needed in order to obviate the physiological mechanisms involved in the process of healing by different frequencies.

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