WOUND HEALING AND PERFUSION OF PRESSURE ULCERS IN DIRECT CURRENT STIMULATED DENERVATED TISSUES

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ABSTRACT

A recently developed animal model for stage 3 pressure ulcers was used to study the influence of DC electrical stimulation on wound healing. Two groups of animals were used. One group was treated daily using DC stimulation while the second nonstimulated group served as control. Changes in wound size (area and volume) and tissue perfusion were measured and recorded as dependent variables. The exponential wound healing model of Vodovnik and Stefanovska was used to calculate healing time constants. Our results indicated reduced healing time constants as measured by area and volume for the DC stimulated animals versus controls.

During wound healing, denervated tissue perfusion at the edge of the pressure ulcer was estimated by the measurement of the transcutaneous oxygen partial pressure. Perfusion of the denervated healing tissues with or wihout stimulation was greater than that in normal tissues at the same anatomical sites. Initially, stimulation reduced perfusion but subsequently increased and equalized at the healing unstrimulated control sites.

INTRODUCTION

Research on electrically enhanced pressure sore healing using controlled experimental methods has been sparsely reported. One of the earliest reports was by Carey and Lepley (1) who indicated changes in histology and the ensile strength of the wounds as a result of direct current stimulation. Moe recent work by Stefanovska and Vodovnik (2) presented a quantitative analysis of the effects of surface stimulation on pressure sore healing in 18 spinal cord injured patients (3). Using a group of subjects for control, they have shown an exponential relationship between photographed wound area and the time of healing. The use of healing time constants offers a powerful technique for comparing wound closure rate with and without stimulation.

The objective of this report is to show the effect of direct current (DC) stimulation on the healing time constants and the perfusion of denervated tissues as measured by wound area, volume and the transcutaneous partial pessure of oxygen (TcPo_2). The measurement of TcPo_2 is a recognized noninvasive method to determine the local perfusion state of deeper skin tissues (4,5). Wyss *et al.* (6) have shown the dependence of TcPo_2 on the local arteriovenous pressure gradient in normal subjects and demonstrated variations relating to perfusion of the capillaries. more recently, they have shown anatomic variations of TcPo_2 in the legs of patietns before amputation (7). Others have shown a near linear relationship between externally applied pressure and the measured TcPo_2 (8,9). Reed *et al.* (9) pointed out additional systemic variables influencing the measurement of TcPo_2. They found that arterial oxygen tension, change in cardiac output and change in body position had a significant effect and caused fluctuations in TcPo_2. In this study, the use of TcPo_2 was introduced to analyze changes in tissue perfusion in the stimulated trochanteric area at the wound edge of high grade pressure ulcers. The paper will present a simultaneous recording technique from two locations on the skin to overcome the confounding effects of systemic variations on the measurement of TcPo_2.

METHODS

The denervated trochanteric pressure sore model reported earlier (11) was used to study the healing and perfusion of the stimulated and control wounds. This technique allowed the development of deep, grade 3 or 4 tissue ulcer in the flaccid monoplegic hind limb of the mini-pig. The applied DC amplitude was less than 1 mA with a current density between surface electrodes vaiable from 30 to 80 $\mu A/cm^2$. Healing pressure sores were examined, treated daily and photographed at three- to five-day intervals. The measured areas from the photographs were recorded as a function of time after removal of tissue compression. In this study of the effect of DC electrical stimulation of the healing process, five animals were used in the control group of unstimulated specimens and five other animals for the stimulated group of specimens. The photographed wound area was measured by tracing the wound at the border of the epidermis on a digitizing tablet with Sigma Scan attachment on an IBM PC/AT. A disk of known area, included in each photograph, was also traced for calibration of the measuring system. Wound area from the transparent overlay was measured also by "counting squares" on a millimeter grid within the marked wound perimeter. These methods of wound area measurements correlated well and yielded highly reproducible results among different observers without statistically significant differences.

To evaluate the bulk changes in the wound cavity, the volume of wounds was also measured by filling the tissue defect with sterile isotonic saline to the level of intact skin surface while the wound surface was maintained horizontally. The volume of saline filling the wound was reported as the wound volume. Four pairs of control and stimulated wounds were used in these measurements. The initial wound volumes, after debridement on the day of removal of the indentor, were averaged and found to be 12.2 ± 3.3 ml for the control wounds and 12.0 ± 5.1 ml for the experimental wounds, representing a statistically nonsignificant difference at $p<0.05$.

A series of measurements were also taken to establish the normal and pressure-exposed tissue response with and without stimulation to changes in perfusion as indicated by the chane in transcutaneous partial pressure of oxygen. These measure-

ments were carried out using a two-channel Clark electrode type instrumentation (Radiometer TCMI, Radiometer America) with built-in heating system for closed loop controlled skin temperature at 42° C to cause vasodilation of the small vessels of the skin for reduced oxygen diffusion resistance.

The electrode outputs were calibrated in room air for 152 mmHg oxygen partial pressure, and the outputs were monitored from the digital display of the instrument. Oxygen partial pressure was measured simultaneously over the shoulder musculature for control data recording and at the edge of the wound and near the trochanter of the hind leg for experimental observations. The recording sites were prepared by shaving the skin and cleansing with alcohol. Support wells for the electrodes were applied to the skin with adhesive, and a drop of glycerine diffusion medium was plced in the well for airtight coupling of the electrodes to the skin. After electrode application, a 25-minute time delay was needed for the development of the steady state oxygen diffusion before recording the characteristic $TcPo_2$.

There are several factors which influence the magnitude of the $TcPo_2$ at any one site of recording. Such factors as cardiac output, arterial oxygen tension and body position have to be kept constant or otherwise accounted for. The controllable variables such as body position were kept constant, but the contribution of the other systemic variables was difficult to separate from the local effect of denervation and stimulation. Thus a two channel recording process was employed to obtain differential $TcPo_2$ information from the skin. $TcPo_2$ was recorded from the right side of the animal (to maintain constant position) at the shoulder and the trochanter. At any time, circulatory changes from systemic variables are expressed similarly at these sites and their ratio can indicate local trochanteric effects normalized to the shoulder site. The ratio of simultaneously recorded values (trochanter/shoulder $TcPo_2$) free of systemic effects can be then used to analyze the local changes of $TcPo_2$ and tissue perfusion.

RESULTS

Healing sores were followed for up to four weeks or until complete wound closure. The average length of observation for the control animals was 26±4 days and for the stimulated animals 23±3 days. All wounds showed an initially rapid reduction in surface area followed by aprogressively reduced rate of closure. Overall, the reduction in wound area appeared to follow an exponential function, as suggested by Vodovnik and Stefanovska (2). Thus the measured surface areas were fitted to this exponential function and the time constant calculated for each wound. Figures 1 and 2 show the observed data points and the curves calculated from the exponential model for the control and stimulated animals, respectively. Healing time constants and the corresponding initial current density applied by DC stimulation are shown in Table 1. The current density was calculated from the initial wound area and the average current applied daily for 2 hours across the wound between the stimulating electrodes.

The average values of the wound area time constants were found to be longer for the control group of animals (n=5) than the stimulated group (n=5). DC stimulation yielded an average wound-healing area time constant of 9.7±3.1 days compared to 13.4±8.5 days for the nonstimulated control time constant, resulting in a mean difference of 28% in healing rate between the groups of animals. i is interesting to note that the standard deviation in the control group is also larger than that in the stimulated

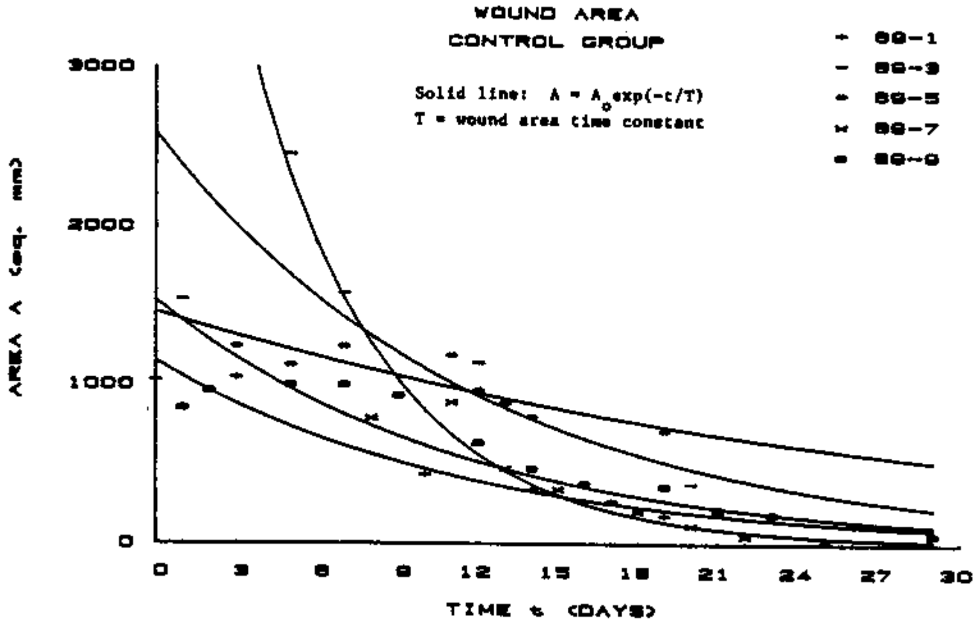

Figure 1. Wound area control group

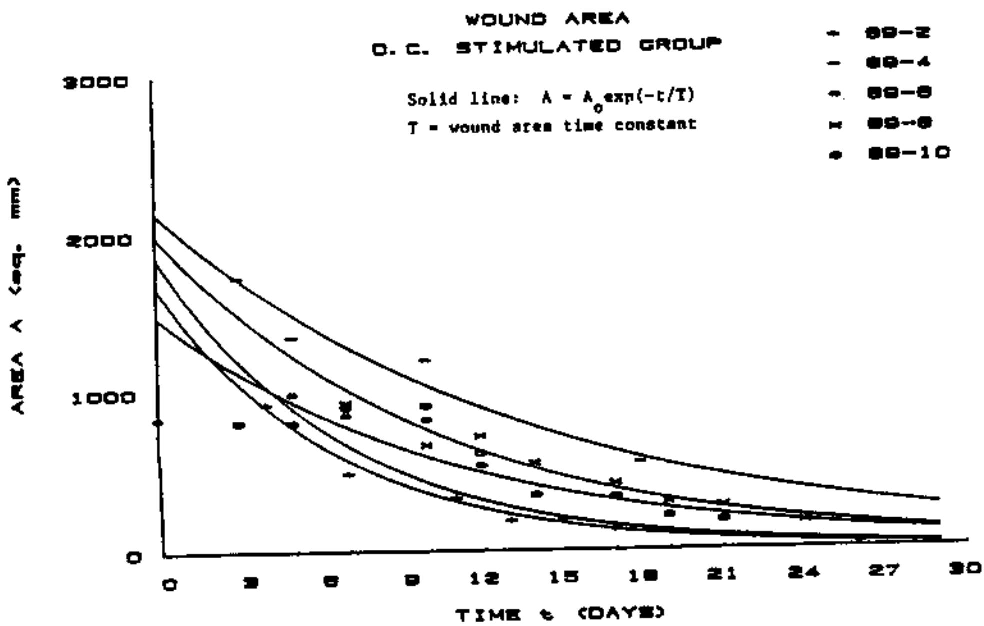

Figure 2. Wound area D.C. stimulated group

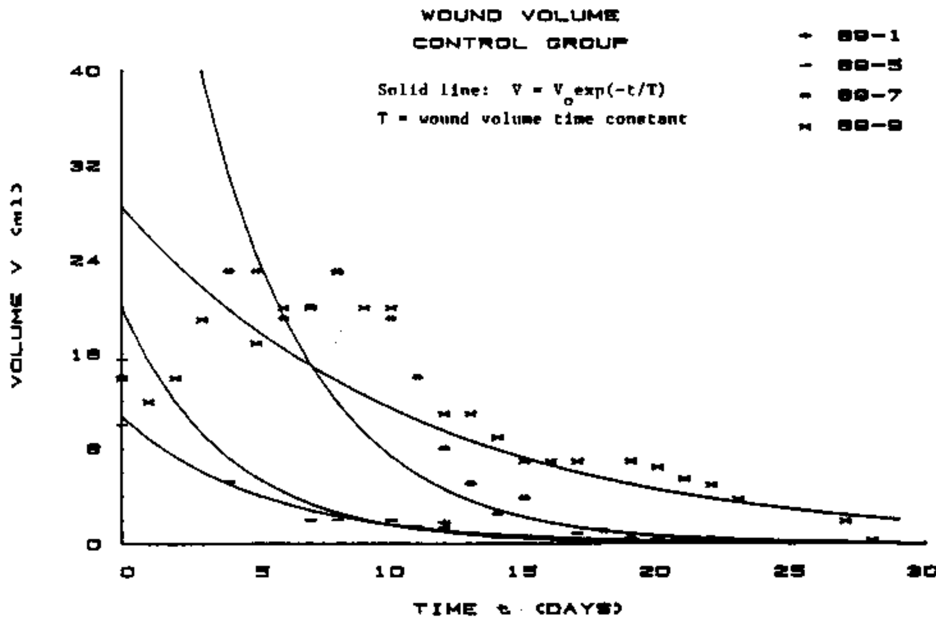

Figure 3. Wound volume control group

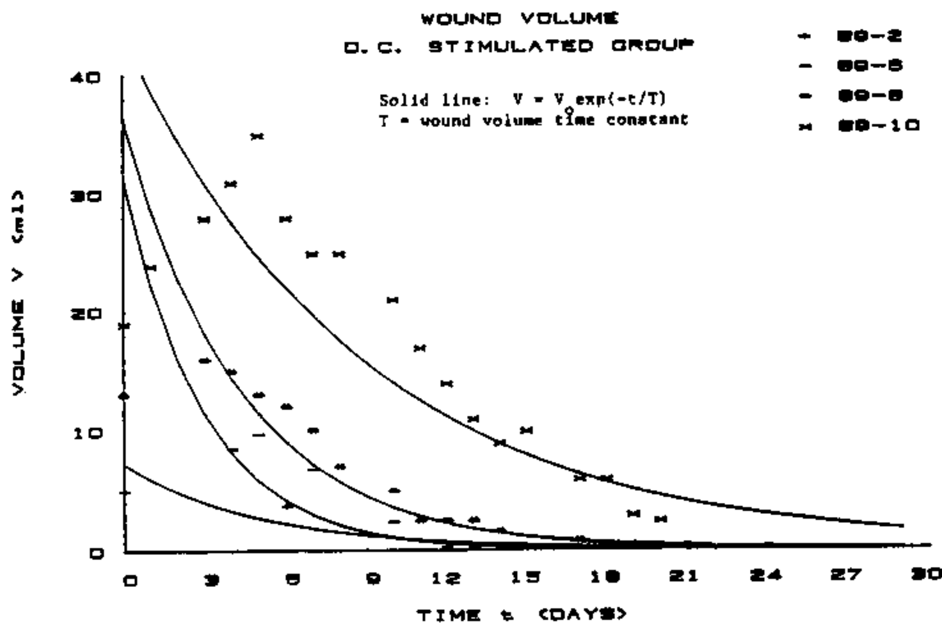

Figure 4. Wound volume D.C. stimulated group

group, inidcating that the usual standard tratment of the wounds in the absence of stimulation results in greater variance of the healing rate of the pressure sores. This higher variance, however, also resulted in no significant difference at p=0.05 between the means of the two groups.

The rate of wound closure was also evaluated from reduction of wound volume measured by the saline filling technique described earlier. Thus wound volumes were measured as a function of time after removal of tissue compression in four experimental and four control animals. The volume change of the tissue defect was again observed to follow an exponential function shown in Figures 3 and 4, and the Vodovnik and Stefanovska model (2) was again applied. From the model, the volume-based healing time constants were also calculated and are also shown in Table 1.

	Area Time Constant (Days)	Volume Time Constant (Days)	Average DC Current Density ($\mu a/cm^2$)
Control			
89-1	11.2	3.9	0
89-3	11.8	NM	0
89-5	27.8	5.2	0
89-7	5.0	4.1	0
89-9	11.0	10.9	0
Mean ± S.D.	13.4 ± 8.5	6.0 ± 3.3	0
Stimulated			
89-2	6.6	5.1	70.0
89-4	13.9	NM	38.5
89-6	6.8	3.0	80.1
89-8	10.4	4.3	66.5
89-10	11.0	8.8	76.4
Mean ± S.D.	9.7 ± 3.1	5.3 ± 2.5	66.3 ± 16.4

NM = Not Measured

Table 1. Wound area, volume time constants and applied direct current densities

DC stimulation produced an average wound volume time constant of 5.3±2.5 days compared to 6.0±3.3 days for the nonstimulated time constant. This difference in the means, however, was only 12%, and the variance in the data was again higher in the nonstimulated group.

Extensive control measurements were carried out with the oxygen electrodes to help understand their behavior and the tissue characteristics on the control and

recording sites of the pig tissues. The results are shown in Table 2. Individual electrode responses were tested by placing both oxygen electrodes at the control site on the shoulder musculature of the pig, 3 cm apart. The electrodes responded nearly identically to oxygen tension trends and showed no more than a 3 mmHg difference between them during a 5-minute recording interval. The oxygen electrodes were also placed at the trochanter and shoulder of five different normal animals on two and three separate days each. These measurements, prior to denervation, were made to establish representative normal $TcPo_2$ values specific to these anatomic sites and body position of the pigs. The means, their ratios and standard deviations of these observations are also shown in Table 2. The mean $TcPo_2$ values at the trochanter and shoulder were found to be significantly different in the normal pigs at the $p<0.05$ level.

1. Recording from neighboring sites on the shoulder.

   Electrode 1: 55.6 ± 0.7 mmHg

   Electrode 2: 55.0 ± 2.2 mmHg

2. Recording from shoulder and trochanter from five normal pigs on 11 separate days as pairs of simultaneous observations in five 1-minute intervals, 25 minutes after the placement of electrodes.

   Normal Trochanter: 42.7* ± 19.2 mmHg

   Normal Shoulder: 60.9* ± 12.6 mmHg

   Normal Ratio (NR = trochanter/shoulder): 0.67 ± 0.22

* Difference is significant at $p < 0.05$.

Table 2. Control recordings of $TcPo_2$ from normal pigs without pressure sores

Data from the $TcPo_2$ electrodes was recorded after establishing consistent outputs from the instruments 25 minutes after electrode application. The mean of five values and the ratio of each shoulder-trochanteric point in 1-minute intervals were calculated and used as the skin oxygen tension at the electrode site for each week of healing during these experiments. The results are tabulated in Table 3 for the first, second and third weeks of healing, respectively. The $TcPo_2$ ratios (trochanter/shouler)

obtained from the dervated healing trochanter and normal shoulder of the unstimulated control animals were defined as Control Denervated Ratio (CDR), while the ratios from the denervated healing trochanter and shoulder of the DC stimulated animals were defined as Stimulated Denervated Ratio (SDR). Statistical analysis was done to test for equal means of these ratios.

Duration of Wound Healing (week)	Control Denervated Ratio (CDR)	DC Stimulated Denervated Ratio (SDR)	Normal Ratio (NR)
1	0.92 ± 0.16	0.78 ± 0.23	0.67 ± 0.22
2	0.78 ± 0.18	0.91 ± 0.19	
3	0.91 ± 0.20	0.79 ± 0.31	

Table of Significant Differences ($p < 0.05$)

Duration of Wound Healing (week)	NR vs CDR	NR vs SDR	CDR vs SDR
1	S.D.	N.S.	S.D.
2	S.D.	S.D.	S.D.
3	S.D.	N.S.	N.S.

S.D. = Significant difference in mean ratios; reject null hypothesis
N.S. = No significant difference of mean ratios; accept null hypothesis

Table 3. Variability of Trochanter/Shoulder TcP_{O_2} Ratios

Three hypotheses were tested:

1. The effect of denervation is zero.
2. The effect of stimulation is zero.
3. The interaction of the two effects is zero.

If the hypothesis of equal means is rejected for any effect, then it can be concluded that there are differences among the means of the ratios, and pairs of means may be examined by a t-test to determine if differences exist between any two effects. The results of the statistical analysis are also shown in Table 3, indicating the variability of the mean TcP_{O_2} ratios during the first, second and third weeks of healing of the

control and stimulated pressure sores compared to the normal ratio (NR vs. CDR and NR vs. SDR) and the interaction (CDR vs. SDR).

CONCLUSIONS

Direct current stimulation of denervated pressure sore wounds in monoplegic pigs accelerated the healing and altered the perfusion of the wounds. The wound area time constants indicated an average of 28% increase in the healing rate with stimulation. The averaged results of the wound volume time constants were similar to the results with the area time constants but not as dramatic. The wound volume time constants showed an average of only 12% increase in wound healing rate with stimulation. The lesser percent increase in the volume time constants may indicate that the volume filling effects of DC stimulation are less prominent than the surface area reduction effects. The possibility exists that DC stimulation is more èffective in the hypodermic tissues near he skin surface than in the tissues deep in the wound cavity.

A comparison of the magnitudes and the significance of the mean $TcPo_2$ ratios from Table 3 can establish the relative order of tissue perfusion between normal, denervated and stimulated denervated tissues. Thus, based on the $TcPo_2$ ratios, the unstimulated control denervated (CDR) tissues and the stimulated denervated (SDR) tissuees appear to be better perfused than the normal tissues through all three weeks of healing. The comparison between stimulated denervated and unstimulated control denervated tissues was variable and the order depended on the time or phase of healing. Initially the stimulated perfusion was less than the control, but it became greater in the second week and equalized with the control in the third week. The measurement of $TcPo_2$ in future studies will continue to further define the effect of stimulation on the underlying tissue perfusion.

In summary, the stimulation of pressure ulcers in denervated limbs seems to accelerate both the rate of wound closure and the rate of wound filing and seems to reduce the variance in the outcome of the healing process. Furthermore, the application of direct current stimulation affects perfusion and causes a reduction of wound area more than the reduction of the wound volume.

Acknowledgements:

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REFERENCES

1. Carey L.C., Lepley D., Effect of continuous direct electric current on healing wounds. Surgical Forum 13:33-35, 1962.

2. Stefanovska A., Vodovnik L., *et al.*, Enhancement of ulcerated tissue healing by electrical stimulation. RESNA 10th Ann. Conf., 585-587, 1987.

3. Savin R., Benko M., *et al.*, Clinical experiences in wound healing by electrical stimulation in SCI patients. RESNA 12th Ann.Conf., 171-172, 1989.

4. Tremper K.K., Shoemaker W.C., Transcutaneous oxygen monitoring of critically ill adults, with and without low flow shock. Crit Care med 9(10):706-709, 1981.

5. Smith D.J., Madison S.A., Bendick P.J., Transcutaneous Po_2 monitoring in the vascular laboratory. J. Clin Eng. 8(2), April-June 1983.

6. Wyss C.R., Matsen F.A., King R.V., Simmons C.W., Burgess E.M., Dependence of transcutaneous oxygen tension on local arteriovenous pressure gradient in normal subjects. Clin Sci 60:499-506, 1981.

7. Wyss C.R., Harrington R.M., Burgess E.M., Matsen F.A., Transcutaneous oxygen tension as a predictor of success after an amputation. JBJS 70A(2):203-207, 1988.

8. Seiler W.O., Stahelin H.B., Skin oxygen tension as a function of imposed skin pressure: Implication for decubitus ulcer formation. J. Am. Geriatr.Soc. 27(7):298-301, 1979.

9. Sangeorzan B.J., Harrington R.M., Wyss C.R., Czerniecki J.M., Matsen F.A., Circulatory and mechanical response of skin to loading. J. Orthop. Res. 7:425-431, 1989.

10. Reed R.L., Maier R.V., Landicho D., Kenny M.A., Carrico C.J., Correlation of hemodynamic variables with transcutaneous PO2 measurements in critically ill adult patients. J. Trauma 25(11):1045-1053, 1985.

4. Tremper K.K., Shoemaker W.C., Transcutaneous oxygen monitoring of critically ill adults, with and without low flow shock. Crit Care med 9(10):706-709, 1981.

5. Smith D.J., Madison S.A., Bendick P.J., Transcutaneous Po_2 monitoring in the vascular laboratory. J. Clin Eng. 8(2), April-June 1983.

6. Wyss C.R., Matsen F.A., King R.V., Simmons C.W., Burgess E.M., Dependence of transcutaneous oxygen tension on local arteriovenous pressure gradient in normal subjects. Clin Sci 60:499-506, 1981.

7. Wyss C.R., Harrington R.M., Burgess E.M., Matsen F.A., Transcutaneous oxygen tension as a predictor of success after an amputation. JBJS 70A(2):203-207, 1988.

8. Seiler W.O., Stahelin H.B., Skin oxygen tension as a function of imposed skin pressure: Implication for decubitus ulcer formation. J. Am. Geriatr.Soc. 27(7):298-301, 1979.

9. Sangeorzan B.J., Harrington R.M., Wyss C.R., Czerniecki J.M., Matsen F.A., Circulatory and mechanical response of skin to loading. J. Orthop. Res. 7:425-431, 1989.

10. Reed R.L., Maier R.V., Landicho D., Kenny M.A., Carrico C.J., Correlation of hemodynamic variables with transcutaneous PO2 measurements in critically ill adult patients. J. Trauma 25(11):1045-1053, 1985.