ORIENTATION AND BIOMECHANICAL PROPERTIES OF DC ELECTRICALLY STIMULATED AND HEALED PIGSKIN PRESSURE SORES

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ABSTRACT

Previous work has demonstrated that stimulation of pressure ulcers in denervated limbs accelerates wound healing. This preliminary study evaluated the viscoelastic properties of direct current (DC) stimulated and heald skin obtained from male hanford mini-pigs. Using the denervated hind limb trochanteric pressure sore model, the hind limbs of 12 pigs were divided into three groups: A) 1 mA stimulus amplitude maintained with a current density of 30-80 μA/cm^2 hours daily for 28 days; B) unstimulated denervated healed skin; and C) normal skin. After thawing, the mean thickness and cross-sectional area of dumbbell shaped specimens from selected areas around the wound site were measured. All specimens were uniaxially loaded at an extension rate of 150 mm/min on a Chatillon ET1100 test system and the load, extension, deformation stiffness and slope were determined.

The mean stress (MPa) from Groups A and B parallel to the current flow was 2.5±1.4 and 2.9±2.1 compared to 7.3±1.9 for controls; samples perpendicular to current flow showed increases (12.4±3.4 and 9.1±3.2) in the mean stress values when compared to controls (9.44±3.6). There were no statistical differences in the slope (N/mm), and Youngs modulus (MPa) were lower than identical samples located perpendicular to current flow. Perpendicular specimens located at areas 3 cm from the wound sites may be at the boundary of the effective electrical field.

Although stimulated tissue healed faster, the scar did not achieve the overall tensile strength of the normal skin at 30 days after pressure sore formation. The regional mechanics of the extensible skin and collagen deposition for hind limb joint motion require further investigation.

KEY WORDS: Pressure sores, Pig skin, DC stimulation, Mechanical properties.

INTRODUCTION

Electrical stimulation is one technique to facilitate and accelerate skin and hypodermic wound healing. The course of wound healing in different tissues is based on the analyses of the strength of the wound in relation to that of the intact tissue (1). With knowledge of the tensile strength properties one can predict the tensile stresses experienced by the skin until healing is complete. The extensibility of the skin may show directional variations. To evaluate the mechanical properties of skin with healing pressure sores, a controlled pilot study was performed using six 15 kg mini-pigs with denervated limbs. Swine were chosen because their skin is in many ways similar to the human skin in terms of thickness, blood supply and subcutaneous attachments. This paper presents the results of mechanical tests on three types of isolated pig skin: denervated DC stimulated, denervated control and normal controls. The effect of strain rate, stress and sample orientation on the mechanical behavior (slope and Youngs modulus) of the healing pig skin were investigated. This report describes the preparation and evaluation of these mechanical factors of excised skin. The contrinutions of increased vascularity, tissue perfusion, wound size and collagenous skin components affecting the mechanical peformance are reported separately.

MATERIALS AND METHODS

Pressure Sore Model and Stimulation

The denervated limb trochanteric pressure sore model in our previous studies (2) permitted the use of a 2.5 cm percutaneous cancellous bone screw for wound formation and a 3 cm diameter spring compression indentor to maintain a constant pressure of 800 mmHg for 3 days. Using this technique, a reproducible and uniformly controlled Grade 3 or higher grade tissue ulcer was developed in the trochanteric area of the flaccid monoplegic hind limb of the mini-pig. Constant direct current electrical stimulation was applied on healthy skin by electrodes placed 3 cm distal and proximal from the edge of the wound. The applied current amplitude was less than 1 mA and the maximum current density between the electrodes of 30-80$\mu A/cm^2$ (Fig. 1). Wounds were stimulated two hours per day for five days per week until termination approximately 30 days later. After elective sacrifice, all limbs were frozen intact (-20° C).

Figure 1. Appearance of the wound just prior to autopsy of the mini-pig. The 3 cm plastic indentor used for compression of the skin is shown at the left.

Mechanical Testing

A dumbbell shaped template, used to outline the tissue area of constant dimensions was centered through the wound and surrounding tissue. After marking the template, the entire skin sample was shaved, cut and stored in saline. Sections were oriented both parallel and perpendicular to the current flow as shown in Fig. 2. Stimulated and nonstimulated control animals underwent similar sampling procedures. Evaluation of specimens included quantitation of tissue thickness at the various regions using a Mitutoyo thickness gauge to within an accuracy of 0.01 mm. Mechanical testing was perrformed using a Chatillon ET1100 testing machine with a 500 kg load cell. The load force and deformation elongation were recorded. The gauge length of all samples was determined by setting the distance between the cross head grips at 15 mm. All specimens were gripped by two clamps fitted with rough metal jaw surfaces across the shoulders of the dumbbell specimens. Specimens were uniaxially loaded in tension until they failed at an extension rate of 150 mm/min. The tests were conducted in air at room temperature (21° C), but samploes were kept in saline until tested and moist throughout the procedure. From the load deformation curve, the following mechanical characteristics were calculated: stress (MPa), strain (%), slope (N/mm) and Youngs moudlus (MPa). All data are presented as mean ± standard deviation. A paired t test was used for evaluating samples cut from parallel or perpendicular sites.

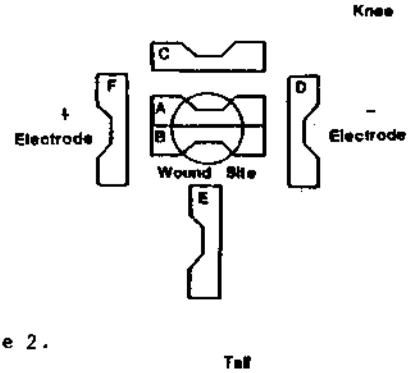

Figure 2.

Figure 2. Harvested skin and the orientation of cut specimens around the wound site. The positive and negative electrodes are placed on the skin parallel to the femur.

RESULTS

There was no significant difference between the skin thickness of the regions A-B or D-F of individual animals, therefore the data from each site was pooled for statistical analyses. Tissue samples in which ruptures occured at the grips were excluded from the study. Rupture in the dumbbell specimens usually occurred in the center of the test length of the samples. The measured variables determined from samples cut parallel and perpendicular to the current flow are listed in Tables I and II.

Stress is the force exerted on the skin while strain is the elongation of the skin prior to rupture. The maximum stiffness is derived from the linear slope of the curve between 20% and 80% of the stress at failure and the amount of skin elasticity is correlated to the stiffness; the larger the maximum stiffness, the less elastic is the skin.

Comparison of the Properties of Denervated and Controls Tissue
(Sites A-B)
Parallel to Current Flow*

	Denervated Control	Denervated Stimulated	Normal Controls†
Stress (MPa)	2.9±2.1	2.5±1.4	7.3±1.9
Strain (%)	34.0±7.2	42.7±14.6	62.2±16.7
Slope (N/nm)	10.6±5.5	13.2±8.4	23.8±10.5
Young Modulus (MPa)	4.3±2.9	5.7±2.2	12.4±3.8

* mean ± S.D., n= 6, no significant difference at $p \leq 0.05$ level
† n = 10

TABLE I

Comparison of the Properties of Denervated and Control Tissue
(Sites D-F)
Perpendicular to Current Flow*

	Denervated Control	Denervated Stimulated	Normal Controls†
Stress (MPa)	9.1±3.2	12.4±3.4	9.44±3.6
Strain (%)	67.0±12.6	72.0±22.5	96.3±51.1
Slope (N/nm)	22.8±17.2	19.0±6.1	23.8±12.2
Young Modulus (MPa)	14.4±6.7	18.3±6.4	11.5±5.7

* mean ± S.D., n= 6, no significant difference at $p \leq 0.05$ level
† n = 10

TABLE II

DISCUSSION AND CONCLUSIONS

The transfer of data from the wound healing process from animals to man is difficult due to the many differences in the anatomy of the skin and the histological appearance of the healing procss (3,4). This has led to the continual awareness of the need for improved methods for the measurement of the mechanical properties of skin and wound healing (5,6). The present study has shown that the mechanical properties of the pig skin on denervated limbs shows directional variation. Generally, stimulated skin samples perpendicular to the current flow and perpendicular to the wound had higher measured values for all parameters tested than those obtained at corresponding parallel sites. The relative strength and elasticity of the fibrous scar in stimulated wounds was evident. Although samples were evaluated after 30 days, the properties of the skin have not fully reached the tensile properties of normal skin. The rapid return of the strain (stretch) has been suggested to allow the wound to absorb energy by stretching and is an important mechanism in protecting early wounds from dehiscence (7). Madden and Peacock (8) report that the smaller average diameter of collagen fibrils in wound tissues compared to normal skin may contribute to low tensile strength.

The skin of the pig when compared to rodents, dogs and humans is very inelastic. There exists a significant difference in the total skin thickness between pigs and many other species due in part to the thickness of the fat layer. The sequential changes in the elasticity of the scar and natural maturation processes with interventional therapy such as electrical stimulation require further quantification. During the initial period of wound healing a direct relationship has been established between the accumulation of collagen within the wound and the development of mechanical strength. Mechanical differences in normal skin and repair tissue may be a result of structural differences in the collagen network in wound tissue. Our studies have shown a reduced healing time for stimulated wounds (2); electrical stimulation may orient newly formed collagen in a similar pattern to that of normal skin even in the absence of neural influences. With open wounds, Madden (8) reported that the mechanical properties increase from 15 to 90 days and the geometry of the wound edge does not influence the collagen deposition within the wound tissue.

The mechanical properties of wound tissues are related to factors other than only orientation of the new collagen. Electrical stimulation may be involved in modifying factors on cells and the crosslinking of collagenous molecules that alter the tensile properties of heald tissues. In the pig model, wound and healed skin is initially under tension and compression due to the indentor and later due to wound contraction. Arem (9) reported that in wounds under tension, newly deposited collagen is oriented along the direction of the stress. The role of electrical stimulation and its influence on collagen synthesis, maturation and function, as scars and wounds approach the strength of unwounded skin is the aim of our further studies.

Collagen fiber orientation in the wound and normal tissue is one possibility to explain the variations between the parallel and perpendicular specimens. In conclusion, for the determination of the elastic properties of wound and electrically stimulated skin, a controlled pilot study was conducted using an established pressure sore technique and experimental testing and analysis methods were developed and described.

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