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Geometric Determinants of Full-Thickness Wound Healing in Adult Male Yucatan Miniature Swine

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ABSTRACT

Our objective was to obtain quantitative data regarding the relationships between wound healing rates vs. initial size and shape of full-thickness wounds.

Nine full-thickness paraspinous wounds were created on each of three adult male Yucatan miniature swine. The wounds for each animal were comprised of all combinations of three sizes (10, 20, and 30 cm^2) in three shapes (square, circle, and equilateral triangle—apex pointing to spine). Dressing changes were performed three times per week for seven weeks, and then weekly until termination. During dressing changes, all wounds were photographed and planimetric measurements of perimeters, total wound areas, and areas of epithelialization were obtained.

Time to complete healing was determined by clinical observation and photographic documentation. Although larger wounds took longer to heal than smaller ones, there was no appreciable association with wound shape, e.g., average healing times for 10 cm^2 wounds were 33, 35, and 33 days for circles, squares, and triangles respectively.

Absolute wound healing rates were calculated using planimetric data. There was a strong correlation between healing rate and initial wound area. Mean healing rates were 2.1, 2.6, and 3.3 cm^2 per week for 10, 20, and 30 cm^2 wounds, respectively. These differences did not appear to be affected by wound shape.

Results for the outcome of linear healing rates were determined from changes in planimetric measurements of area and perimeter using the following calculations:

Linear Healing Distance (cm):

$$\frac{\text{Change in area from Day X to Day Y (cm}^2\text{)}}{\text{Mean of Day X and Day Y perimeters (cm)}}$$

Linear healing rate = Linear healing distance per unit of time (day, week, etc.)

Conclusions: Linear healing rates in adult Yucatan MS were largely unaffected by initial wound size or wound shape. This confirms the appropriateness of this test system for further preclinical wound healing studies.

INTRODUCTION

The exploration of interventions that may favorably affect wound healing represents an important area of research for the clinical sciences. The development of a satisfactory dermal model for preclinical research should include considerations of similarities between animal and human skin based on dermal anatomy and physiology. The use of miniature swine for the purpose of wound healing assessments has been well-established. It is also important to identify similarities and differences between human and animal model wound healing, in both normal physiologic healing and responses that may be attributable to potential interventions.

Wound healing rates can be described in multiple ways. Time to complete healing, and rates based on changes in wound area, perimeter, length, and/or width are commonly reported. However, these parameters can all be influenced by initial wound area or shape. Previous human research on 49 wounds from 39 patients has found that a linear healing rate parameter can measure healing rates that are largely unaffected by initial wound area or shape (Gorin et al., 1996).

Miniature swine skin has multiple characteristics that, due to similarity with human dermal anatomy and physiology, make it an attractive model for studies of wound healing. In order to optimize study design for such studies, some knowledge of the geometric determinants of wound healing is necessary.

RATIONALE AND SCOPE

This experiment was an attempt to obtain useful quantitative information regarding the relationships between wound healing rates and initial size and shape of full-thickness wounds in adult Yucatan miniature swine. The primary outcomes of interest (objectives) were:

1. Time to complete healing (defined as full epithelialization)
2. Healing rate
   a. Absolute change in area from baseline over time
   b. Linear healing rate (change in wound radius over time)

Box 1. What are the geometric determinants of healing rates?

Three adult male Yucatans (4.0 to 4.5 years old)
Nine wounds per animal
Three shapes with three sizes per shape
10, 20, and 30 cm^2 circles, squares, and triangles
Different wound arrangements (layout) on each pig

EXPERIMENTAL METHODS

Under general anesthesia, each of three adult male (4 to 4.5 years old) Yucatan miniature swine had nine full thickness surgical wounds created along the paraspinous dorsal area between the base of the scapula and the iliac crest (**Table 1**). The wounds for each animal were comprised of all combinations of three sizes (10, 20, and 30 cm^2) in three shapes (square, circle, and equilateral triangle—apex pointing to spine).

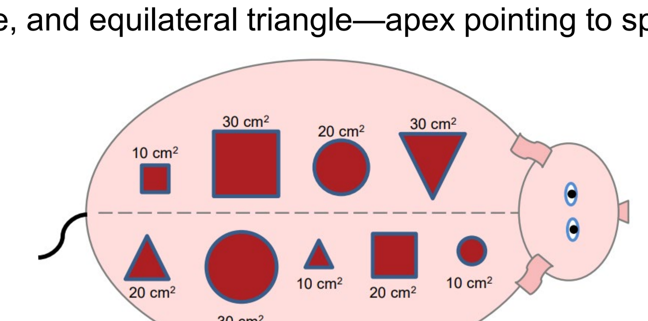

Figure 1. Combinations of different shapes and sizes

Following surgery, all wounds were photographed (**Figure 2**), and then covered by Optifoam, followed by Tegaderm™ and Reston Foam™, all of which were held in place by Stockinette. Simple dressing changes were performed three times a week for seven weeks, and then weekly until all wound were fully epithelialized (Day 70). During each dressing change, all wounds were photographed and scored using a modified version of the Bates-Jensen Wound Assessment Tool.

- Five parameters (edges, exudate type, exudate amount, granulation, epithelialization estimates)
- Five-point scale for each parameter

Planimetric measurements of total wound areas and areas of epithelialization were obtained using Photoshop. At the end of the study all animals were humanely euthanized.

Figure 2. Representative Yucatan wounds post surgery (Day 0) illustrating different shapes and sizes

RESULTS

Table 1. Adult Yucatan wound type distribution (N=3 animals; N= 9 wounds of each shape and size)

Animal	Wound Shape	Number (N)	Wound Size (cm^2)
1M1:0670	Circle	3	1 ea. 10, 20, 30
	Square	3	1 ea. 10, 20, 30
	Equilateral triangle	3	1 ea. 10, 20, 30
1M2:1382	Circle	3	1 ea. 10, 20, 30
	Square	3	1 ea. 10, 20, 30
	Equilateral triangle	3	1 ea. 10, 20, 30
1M3:1397	Circle	3	1 ea. 10, 20, 30
	Square	3	1 ea. 10, 20, 30
	Equilateral triangle	3	1 ea. 10, 20, 30

Table 2. Yucatan wound healing results: average healing time and rate of linear healing

Wound Shape	Wound Size (cm^2)	Average Healing Time (d)	Average Healing Rate (cm^2/wk)	Average Linear Healing Rate (cm/wk)
Circle	10	33	2.1	0.57
Square	10	35	2.1	0.46
Equilateral triangle	10	33	2.1	0.65
Circle	20	56	2.6	0.62
Square	20	56	2.6	0.53
Equilateral triangle	20	49	2.6	0.57
Circle	30	70	3.3	0.65
Square	30	54	3.3	0.70
Equilateral triangle	30	70	3.3	0.57

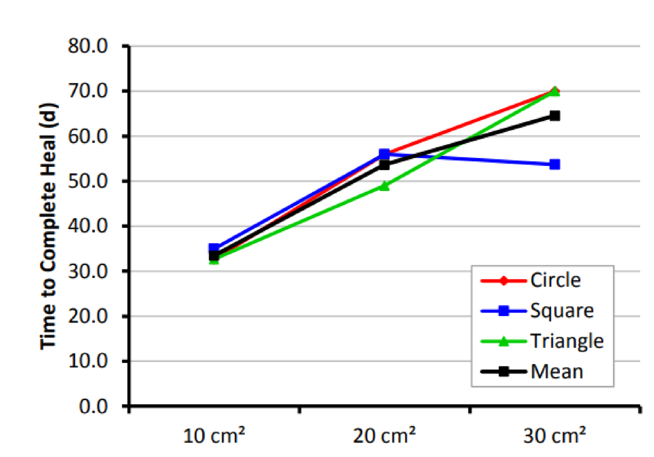

Figure 3. Average time to complete heal

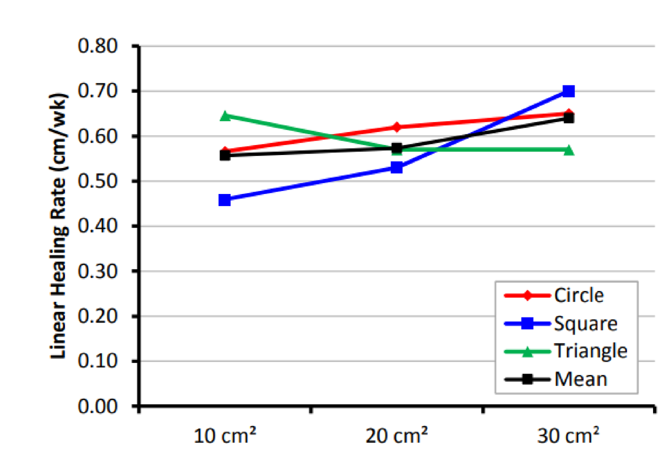

Figure 4. Average linear healing rate (cm/week)

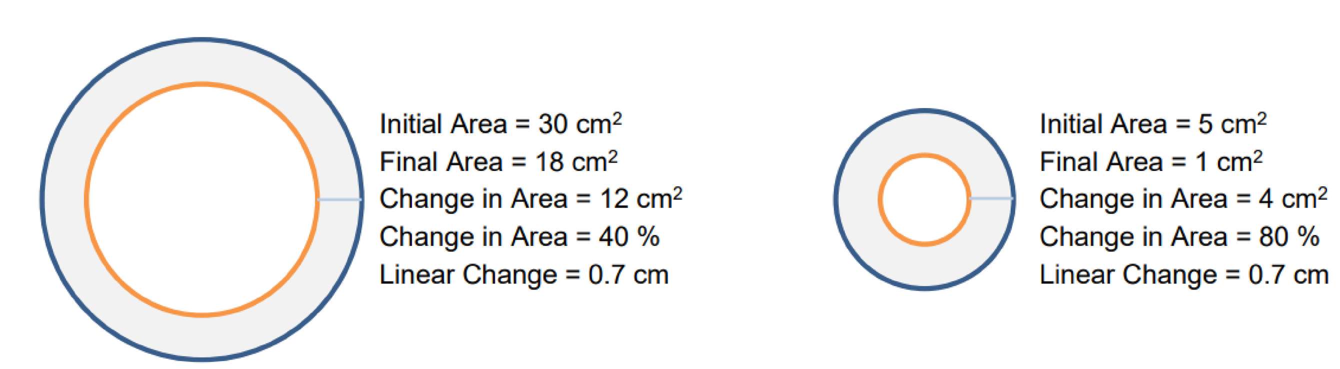

Figure 5. Linear healing rate calculation schematic

- Linear change = Change in area/mean perimeter
- Result is a distance measurement which can be expressed per unit of time (e.g., 0.7 cm/week)

Figure 6. Example wound contracture & healing on 20 cm^2 wound, Day 21

Wound areas and perimeters for each wound on each animal for every timepoint were entered into an electronic database. Results for the outcome of time to complete healing were determined by clinical observation and photographic documentation. Although larger wounds took longer to heal than smaller ones, there was no appreciable association with wound shape. For example, the average healing times for 20 cm wounds were 56, 56, and 49 days for circles, squares, and triangles, respectively (**Table 2**).

Results for the outcomes of absolute wound healing rates were calculated using planimetric data. There was a strong correlation between healing rate and initial wound area. Mean healing rates were 2.1, 2.6, and 3.3 cm^2 per week for 10, 20, and 30 cm^2 wounds, respectively (**Figure 3**). These differences did not appear to be affected by wound shape.

It was determined that linear healing rates were largely unaffected by either initial wound area or wound shape (**Figure 4**).

Results for the outcomes of linear healing rates were determined from changes in planimetric measurements of area and perimeter using the following calculations:

Linear Healing Distance (cm):

$$\frac{\text{Change in Area from Day X to Day Y (cm}^2\text{)}}{\text{Mean of Day X and Day Y Perimeters (cm)}}$$

Linear healing rate = Linear healing distance per unit of time (day, week, etc.)

For a circle, this formula is simply the linear decrease in radius between timepoints, which corresponds to the linear distance the wound margin migrates toward the center of a circle during a specified time interval (**Figures 5 and 6**).

DISCUSSION

The linear healing rate was not significantly affected by initial wound area or by wound shape. This aspect of wound healing was reported earlier by Gorin et al., 1996 for human chronic skin ulcers. Some pig-to-pig variability occurred in our study with respect to wound healing rates, but was consistent with normal biologic variation. Cardinal et al., 2009, reported that human wounds that show a consistent linear rate of healing are those most likely to completely heal. Our miniature swine findings correlate well to human wound healing aspects previously reported by Gorin et al., 1996, Cukjati et al., 2001, Cardinal et al., 2009, and Cardinal, Phillips et al., 2009.

CONCLUSION

Consistent linear wound closure rates, unaffected by initial wound size or wound shape, were recorded in adult male Yucatan miniature swine. These findings, which are similar to human linear wound closure, confirm the appropriateness of this system for further preclinical research in wound healing.

REFERENCES

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Footnote: Altasciences adheres to national guidelines that address the humane care, use, and treatment of laboratory animals. We have an Animal Welfare Program, and our procedures clearly explain that all research performed on live vertebrate animals will be performed humanely. We ensure the highest ethical standard of animal care and use. IACUC approvals are required for all live animal protocols. Our laboratory adheres to and exceeds the highest ethical and scientific standards when conducting *in vivo* studies.