Experimental Acceleration of Wound Healing

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PURPOSE
To show the effect of pulsed, high-frequency radio waves on wound healing in dogs.

MATERIAL AND METHOD

Twenty, unselected dogs were used. Each was anesthetized with i. v. Nembutal. The lateral aspect of each thigh was shaved and prepped; and a six-inch, longitudinal incision made. This extended in length from the tip of the greater trochanter to just above the knee and in depth through the subcutaneous tissue to the vastus lateralis. Immediately, the wound was closed using a continuous 00 black silk suture. The sutures were spaced one-half inch apart and included the full thickness of the wound. An effort was made to approximate all with as much uniformity as possible, and all 20 operations were performed within one and one-half hours. In only one dog (No. 59) was subcutaneous suture used. Each then received 800,000 units of Abbocllin intra-muscularly in the opposite hip.

Ten animals were designated controls and returned to their cages. The remaining ten were treated, under anesthesia, for 20 minutes twice a day: 10 minutes directly over the wound and 10 minutes over the liver. At 9:00 A.M. each 24-hour period for the next 10 days, two dogs (one control and one treated) were anesthetized with Nembutal. The wounds were prepped and draped and totally excised through two parallel incisions placed one-half inch away from the original wound. The resulting defect was closed with 00 silk. The gross specimens were photographed, sutured to a cardboard to prevent wrinkling, and fixed in formalin. They were then sent to the Methodist Hospital Pathology Department, Houston, and processed in the routine manner for sectioning.

Three areas were examined: at either end and in the center. This was done because it was found that the dogs scratched the distal portions and bumped the trochanter areas; thus it was felt that the central area was most representative. These sections were then stained with hematoxylin and eosin and mounted.

The specimens were studied microscopically and photographed using a Zeiss Photomicroscope. An attempt was made to show comparable areas, from superficial to deep, under different powers.

MICROSCOPIC STUDIES

24 HOURS

Untreated: Here and in subsequent sections the wound resembles an inverted mushroom with the hematoma in the deeper portion. The wound is inactive; there are few WBC's, no phagocytes, little or no fat activity, no histiocytes, and the edges are sealed with fibrin which is aligned longitudinally (Figs. 1 and 2).

Treated: The section is active: there is marked WBC infiltration and phagocytosis (Fig. 3).

48 HOURS

Untreated: The wound is still fairly inactive. It is soft and the edge extends through a soft clot. There is increased WBC infiltration as compared to the 24-hour untreated dog, but still no phagocytosis is present (Figs. 4 and 5).

Treated: This shows signs of marked stimulation: the hematoma has been absorbed and replaced by fat which seems to be extremely active. Strands of fat are migrating toward the edges and inflammatory cells stream out between the lobules. Masses of WBC's are noted, and there is infiltration of the entire wound. Histiocytes are seen within the wound edges and in the adjacent areas. Marked phagocytic activity is noted (Figs. 6 and 7).

72 HOURS

Untreated: The epithelium is sealed (this happens very rapidly in all wounds), there is beginning WBC infiltration, little fat activity, no phagocytosis, no histiocytic activity. Longitudinal alignment of fibrin is seen, and the hematoma is still evident without canalization (Figs. 8, 9, and 10).

Treated: The epithelium is sealed, there is marked fat activity which seems to have replaced the hematoma. The wound is tightly sealed and is a thin line in the mid-portion crowded between the infiltrating fat. The WBC infiltration has subsided as well as the phagocytosis, and histiocytic activity is much less. The hematoma has been canalized; and the alignment of fibers in the wound, while for the most part longitudinal, have turned in the transverse plane superficially and deep (Figs. 11 and 12).

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FIG. 1. Untreated, 24 Hours, 31X—The inactive wound. The inverted mushroom with the hematoma at the bottom.

FIG. 2. Untreated, 24 Hours, 125X—Showing little or no WBC infiltration. No phagocytosis.

FIG. 3. Treated, 24 Hours, 125X—Marked WBC infiltration. Phagocytosis present (not shown).

FIG. 4. Untreated, 48 Hours, 31X—The wound extends into a soft clot.

FIG. 5. Untreated, 48 Hours, 125X—Slight increase in WBC infiltration as compared to Fig. 1. No phagocytosis (not shown).

FIG. 6. Treated, 48 Hours, 31X—The hematoma has been absorbed. Replacement by fat which is active. Inflammatory cells stream out between fat cells.

FIG. 7. Treated, 48 Hours, 125X—Marked histiocytic activity as shown by presence of histiocytes.


FIG. 9. Untreated, 72 Hours, 31X—Hematoma still present without apparent canalization.

FIG. 10. Untreated, 72 Hours, 125X—WBC infiltration definite.

FIG. 11. Treated, 72 Hours, 31X—Hematoma canalizing. Wound tightly sealed. WBC infiltration subsiding.

FIG. 12. Treated, 72 Hours, 312X—Beginning transverse alignment of fibers in superficial portion of wound.

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4TH DAY

Untreated: The epithelium is not sealed. There is WBC infiltration, phagocytosis, and minimal histi cytotic activity. Some minimal fat activity is noted in the depths of the wound. The alignment of the wound is longitudinal and fibroblasts are seen "flowing into" the wound margins. The hematoma is still present (Figs. 13 and 14).

Treated: A violent, old dog who continually fought with the others and constantly reinjured his wound. The epithelium has healed; the original hematoma has been removed, but another is present from secondary injury. The old hematoma now has fibers transversing. The stage of WBC, phagocytosis, histi cytotic activity is diminishing. Considerable fat activity is present. Fibrocytes and fibroblasts are noted (Figs. 15 and 16).

5TH DAY

Untreated: This wound has become infected and broken open which is a definite stimulation to the wound healing process as noted by the marked increase of fat activity throughout the tissue. There is marked WBC infiltration, phagocytosis, fibrocytes, fibroblasts, and canalization. This latter finding is unique in that fat is seen within the lumen. As yet, the alignment is not specific, but is certainly not organized or transverse (Figs. 17 and 18).

Treated: The epithelium is healed; fibers are seen as they align themselves transversely across the wound in the line of stress; fat activity is in evidence; the hematoma is canalized; fresh RBC’s are noted within the interstices of the wound. Fibers, fibrocytes and fibroblasts are present (Figs. 19 and 20).

6TH DAY

Untreated: The wound is healing well, but there is considerable edema and softness. The epithelium is healed, and the fibroblasts are aligned longitudinally in the superficial areas; deeper there is no organization. Fibrocytes are not seen to any appreciable extent throughout the wound. The area of the hematoma inflammatory cells and fibroblasts are noted (Figs. 21, 22, and 23).

Treated: The wound is quite dense, and there is a lack of edema. The epithelium is well healed, there is increasing transverse alignment of all fibers in the line of stress; WBC infiltration, phagocytosis, histi cytotic activity, and fat stimulation seem to be diminishing. Collagen is noted throughout, mainly in the superficial areas. The hematoma has been completely canalized, and a new vessel is present (Figs. 24, 25 and 26).


FIG. 14. Untreated, 4th Day, 312X—Fibroblasts "flowing into" the edge of the wound.


FIG. 17. Untreated, 5th Day, 31X—Wound is infected. Edges show marked WBC infiltration. Increased fatty activity.
FIG. 18. Untreated, 5th Day, 31X-The hematoma area showing the fat activity within the hematoma itself. No alignment of fibers.


FIG. 20. Treated, 5th Day, 125X—Transverse alignment of fibers. WBCs in interstices. Fibroblasts, fibrocytes and fat activity.

FIG. 21. Untreated, 6th Day, 31X—Wound is healing well, but note edema and softness. Epithelium healed.

FIG. 22. Untreated, 6th Day, 125X—Longitudinal alignment of superficial areas of fibroblasts and fibrocytes.

FIG. 23. Untreated, 6th Day, 312X—Deeper there is no organization of fibroblasts.

FIG. 24. Treated, 6th Day, 31X—The wound is dense. Lack of edema. Epithelium well healed with increasing transverse alignment of fibers. WBC infiltration, phagocytosis, histocytic activity, and fat stimulation diminishing. Collagen noted superficially.

FIG. 25. Treated, 6th Day, 125X—Collagen traversing wound.

FIG. 26. Treated, 6th Day, 31X—Complete canalization of hematoma.


FIG. 29. Untreated, 7th Day, 312X—Fibrocytes “flowing in” transverse manner to wound edge. Transverse alignment and fresh RBCs present in interstices.

FIG. 30. Treated, 7th Day, 31X—The wound is tight and healing. Fat has crossed deep. Hematoma canalized.
7TH DAY

Untreated: The wound is healing well, however there is still considerable edema present. Transverse alignment of the fibers is beginning, and the hematoma is becoming canalized. In the deeper areas the wound is still in the early inflammatory stages. Fibrocytes can be seen “flowing in” a transverse manner into the wound edges; transverse alignment of the fibers is seen as are fresh RBC’s (Figs. 27, 28, and 29).

Treated: The wound is healing and is tight. Fat has crossed the wound deep; the hematoma is incompletely canalized here and may heal with scar. All the cells are aligned transversely, and the wound has healed with fibrocytes and little with fibers or collagen. Capillaries are noted within the wound itself (Figs. 30, 31, and 32).

8TH DAY

Untreated: The wound is healing well, but there is still edema and looseness of cells. Fat is noted within the wound, and a strand of fat cells can be seen approaching the wound on one side. Transverse alignment is seen superficially and is progressing to the deeper areas. Deep there is very little organization. Fibrocytes but no collagen are seen (Figs. 33 and 34).

Treated: The wound is healing well. Transverse alignment of the fibrocytes, fibers, and collagen are noted. Considerable fat activity is present (Figs. 35 and 36).

9TH DAY

Untreated: The wound is becoming tighter, and there is less evidence of edema. No fat activity is present; higher power shows that the cells are immature fibroblasts aligned transversely. Some fresh RBC’s are noted in the interstices. No collagen is present. Deeper, the alignment is not organized (Figs. 37 and 38).

Treated: The wound is well healed but continues to mature. Little edema is present, but there is fat activity on both sides. Transverse alignment of fibrocytes is noted with RBC’s in the interstices. Spots of collagen are noted throughout; and deep, the wound has matured with transverse alignment of cells and fibers and complete canalization of the hematoma (Figs. 39, 40, and 41).
FIG. 37. Untreated, 9th Day, 31X—Wound is tighter. Less edema. No fat activity.

FIG. 38. Untreated, 9th Day, 125X—Immature fibroblasts. Some RBCs in interstices. Fibroblasts with no definite alignment. No collagen.


FIG. 40. Treated, 9th Day, 125X—Transverse alignment of fibrocytes. RBCs in interstices. Spots of collagen.

FIG. 41. Treated, 9th Day, 31X—Hematoma canalized, blood clot in the vessels.

FIG. 42. Untreated, 10th Day, 31X—Wound is healing. Edema abating. Superficial transverse alignment of fibroblasts and fibrocytes.

FIG. 43. Untreated, 10th Day, 125X—Transverse alignment of fibroblasts and fibrocytes.

FIG. 44. Treated, 10th Day, 31X—Wound is healing tight. No edema. Collagen apparent. Fibrocytes adjacent to collagen not aligned transversely. Are they in new stress lines?

FIG. 45. Treated, 10th Day, 125X—Collagen with disorganization of fibrocytes adjacent. Fat activity has ceased.

FIG. 46 through 56. See Text (Conclusions).
Untreated: The wound is healing and the edema abating. Superficially there is transverse alignment of fibroblasts and fibrocytes, but deeper there is less organization without collagen or appreciable fat activation (Figs. 42 and 43).

Treated: The wound is healing and tight without edema. Collagen is apparent and seems to relieve the stress of the cells which then seem to align themselves along new lines of stress. Fat activity has now virtually ceased, and deeper the cells are not as well organized as the more superficial ones (Figs. 44 and 45).

SUMMARY

A wound of the skin and subcutaneous tissues in dogs presents itself as an inverted mushroom with the hematoma deep and a narrow neck extending to the superficial structures. Healing occurs first in the epithelium, then in the superficial areas, and last in the deeper structures. Initially there is acute inflammation with invasion of the fibrin-seal by WBC's and phagocytes. Histiocytes appear not only in the adjacent areas but also within the wound edges itself. Fat is stimulated and becomes active and seems to grow from the juxtaposing areas to the wound edge and seems to help evacuate the hematoma, which either becomes solid with scar or, in most cases, canalized.

WBC infiltration, phagocytosis, and histiocytic activity are seen in the early phases and do not persist throughout healing. Fat stimulation appears early, within hours and persists until the later stages but fades late.

Fibroblasts seem to "swim in" from the adjacent areas; and, at first, align themselves longitudinally within the fibrin-seal. However, later they turn in a transverse plane, the superficial ones first and the deep ones later. After these appear, fibrocytes are noted and the wound is much less edematous. Collagen is then deposited in the transverse alignment (line of stress) along the lines of the fibrocytes. After collagen appears, the cells seem to lose their original alignment and fall into new lines of stress so that they appear less organized.

As healing progresses, fresh RBC's are noted in the interstices along with capillaries.

CONCLUSIONS (FIGS. 46-56).

The use of pulsed high-frequency waves accelerated wound healing in 10 dogs by stimulating the following:

1. Transverse alignment: treated, three days (Fig. 46); untreated, eight days (Fig. 47).
2. Collagen formation: treated, six days (Fig. 48); untreated, never appeared within ten days.
3. WBC infiltration: treated, appeared in 24 hrs. (Fig. 49); untreated, three days (Fig. 50).
4. Phagocytosis: treated, appeared in 24 hrs.; untreated, four days.
5. Histiocytic activity: treated, appeared in 48 hrs. (Fig. 51); untreated, four days (Fig. 52).
6. Fat activity: treated, appeared in 48 hrs. (Fig. 53); untreated, five days (Fig. 54).
7. Hematoma canalization: treated, three days (Fig. 55); untreated, seven days (Fig. 56).