Influence of Microcrystalline Collagen on Wound Healing I. Wound Closure of Normal Excised and Burn Excised Wounds of Rats, Rabbits, and Pigs[1] (36575)

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During the past two decades, understanding of the general nature and control of the closely integrated process of wound healing has been expanded (1–4). The role of native collagen synthesized *de novo* in the process of healing cutaneous wounds has been referenced by many workers in the past decade (5–8). These investigators have implicated a relationship between collagen formation and increase in healing rate of wounds as measured by wound closure and tensile strength in animals and man. Recently, collagen has been utilized in many forms for its wound healing influence in promoting the repair process of surface defect wounds (9–13).

Microcrystalline natural collagen derived from edible bovine corium has been prepared as a water-insoluble partial acid salt of natural collagen (Avitene). As a partial acid salt of natural collagen, Avitene (14, 15) may be produced in various physical forms, one of which is an absorbent mat as a dressing for cutaneous wounds (16). This lyophilized collagen wound dressing has been investigated in several animal models to demonstrate improved wound closure. This paper describes some results of microcrystalline collagen lyophilized wound dressings as evaluated for wound closure rate in rats, rabbits, and pigs under various experimental conditions. A preliminary presentation has been made of this investigation (17).

Methods and Materials. Animals. The following animals were used in the experiments to be described: Male and female rats[2] weighing 180–220 g approximately 4 to 6 months of age, male and female rabbits[3] weighing 1.8 to 2.2 kg approximately 3 to 5 months of age, and male and female pigs[4] weighing 10 to 20 kg ranging from 4 to 6 months of age.

Normal excised wounds. Experimental cutaneous wounds were inflicted on shaved, depilated lateral sides of the anesthetized animal with a guarded scalpel blade in a surgically clean environment. The area excised was kept constant by keeping the animal resting on its side during the surgery, and outlining the area to be excised with a 3.0 cm square template. The wound size was uniform during the course of excision to conform to standard measurement of 2.8 to 3.2 cm^2.

The surgical excised areas were located paravertebrally toward the lateral surfaces in the thoracolumbar region. The excised would in the rabbit and rat was a full thickness excision to the panniculus carnosis. Complete hemostasis was secured by pressure with sterile gauze pads before covering the wound with the dressing. Collagen wound dressing[5] or gauze dressing[6] was placed onto the wound, allowing for overlap on all edges of approximately 1 cm and then covered with an overlapping gauze square held in place on the edges with Blenderm tape.[7] This dressing was followed by an overwrap of stretch gauze.[8] Following surgical excision and dressing of the wound on one side, the animal was laid on the contralateral side and the surgical procedure and dressing was repeated as described.

[1] Presented in part at the Amer. Burn Ass. Meet., San Antonio, TX, Apr., 1971.

[2] Holtzman Corporation; Madison, WI.

[3] L. D. Palmer Farm; Fort Worth, TX.

[4] Murrell Farms; Fort Worth, TX.

[5] Microcrystalline collagen wound pad; Avicon, Inc., Fort Worth, TX.

[6] Curity gauze sponges; Kendall Hospital Products Div.; Chicago, IL.

[7] 3M Co., Minneapolis, MN.

[8] Sta-tite, Cheeseborough-Pond; New York, New York.

In the pig, the excised wound was full thickness down to the muscle layer as described by Winter (18). Dressing of the wound was undertaken in the same manner described above for rats and rabbits with the exception of substituting a cohesive bandage[9] in place of the stretch gauze bandage.

Excised burn wounds. A burn was inflicted from a branding iron with a 3 cm^2 surface to produce a full thickness third degree burn. Twenty-four hours after burning, the animals were reanesthetized and the burn area was excised and the wound was subsequently dressed with either gauze or collagen in the manner described above for excised wounds. Verification of the third degree burn was undertaken by observing the absence of Evans blue dye in excised burns after dye injection and by histological examination of the tissue.

Measurements. Measurement of the size of the wound was facilitated by placing Blenderm tape over the wound and tracing the advancing edges of new epidermal ingrowth with a marking crayon. The tape was transferred to a paper where the area was traced with a planimeter to determine the area of the wound. This procedure was undertaken initially with the excision of the wound (Day 0) and subsequently with each change of the wound dressing without disturbing the wound bed.

Wounds were examined every 2 to 4 days. The collagen wound pad or gauze dressing was changed with removal of surface debris and minimal disturbance of the new granulated surfaces. If the wound edges were separated from the dry, thin film of collagen serum exudate, the area was cleaned of loose nonadherent material and the wound was redressed with another collagen wound pad. No attempt was made to remove a well-adhering collagen dressing which in turn would inhibit or destroy the integrity of the wound edges where epithelization was occurring. Removal of the dressing was facilitated by irrigation with sterile saline. Measurement of the wound area was undertaken after this debridgement of loose and nonadherent material.

[9] Peg; Becton-Dickinson Company; Rutherford, New Jersey.

Observations were carried out under blind conditions by removing the wrap outside the surgical suite and allowing the observer making the quantitative observations to evaluate the wound only after the dressing had been removed to eliminate any bias which might exist toward the different wound dressings. After evaluation by the observer, the animal was removed from the surgical suite and redressed in a different locale. At the end of the experiment, the code was broken for evaluation of the data.

To evaluate the progress of wound healing and presence of infection, tissue biopsies of the wound sites were taken from the animals for histological examination and microbiological culture. A description of the histologic and microbiologic findings of these wounds are the subject of a subsequent paper (19).

Statistics. Analyses of variances were done among the different test treatments, utilizing the percent closure of the wounds from the initial area (Day 0). The analysis of variance was undertaken to determine the intertest and intratest differences, as well as differences between dressings on different days as evaluated by various observers. Individual analysis on any one day was undertaken by standard t test procedures (20).

Materials. The gauze was of USP grade (Type I, Class B, made of Type 7 gauze) applied as a 12-ply mesh measuring 5 cm^2 as a primary dressing. Gauze sponges applied as a secondary dressing measured 7.25 cm^2 and were of the same quality. Microcrystalline collagen wound dressings consisted of a 0.5% aqueous gel of microcrystalline collagen prepared by lyophilization and measuring 7.5 $\times$ 10 $\times$ 0.5 cm thick as a primary dressing. All microcrystalline collagen wound dressings were sterilized by use of ethylene oxide or heat (125° for 28 hr). No detectable ethylene oxide residuals were observed following ethylene oxide determinations. Gauze dressings were sterilized by heat at 125° for 30 min.

Results. Normal excised wounds were studied in rats and rabbits for comparison of collagen dressing versus gauze dressing. In pigs, multiple wounds (3 to 4) were studied on each side of the animal with an equal

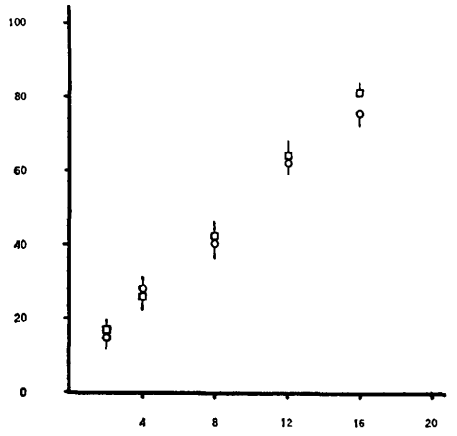

FIG. 1. Mean percentage change of wound closure in paravertebral burn wounds covered with gauze dressings in rats. (□) Right side; (○) left side; (ordinate) time (days); (abscissa) mean percentage change ± standard error.

number of collagen-dressed and gauze-dressed wound sites. The influence of multiple wounds in animals on healing rate has been demonstrated to be equivalent in these type of tests (21).

A validation of the experimental procedures was undertaken initially to compare healing of two paravertebral excised burn wounds in the same animal, both covered with gauze dressing. As demonstrated in Fig. 1, no significant difference occurred in wound closure rate of two wounds in the same rat.

When collagen wound pads were compared to gauze pads in excised wounds placed paravertebrally in the same rabbit, a significant ($p < .025$) difference in the wound closure was observed on Days 4–16. The mean percentage difference in wound closure, among the significant days of improvement, ranged from 15 to 22%, favoring collagen wound pads (Fig. 2), for days with significant differences.

In pig wounds there was observed a significant ($p < .025$) improvement in wound closure rate with the collagen wound pads on Days 6, 8, 12, and 16; the difference on Day 10 was not significant. The difference in wound closure on these days ranged from 13 to 20% (Fig. 3).

In paravertebral burn wounds in rats covered with a collagen wound dressing, there was observed an improved wound closure rate on Days 4–16 over the gauze dressings. This significant ($p < .025$) improvement in wound closure was evident with mean percentage differences which ranged from 12 to 23% (Fig. 4). In a previous experiment described above, if paravertebral wounds in rats were covered with gauze, no significant interanimal differences were observed in wound closure (Fig. 1).

The collagen dressings were permeated with serum exudate with resulting discoloration of the dressing at the site of the wound interface for the first 10–12 days. Collagen dressings overlapping the wounds maintained a white appearance. At the wound site, the collagen dressings form a thin film which becomes continuous with the wound edges forming (Days 1–4) a moist, smooth, protective covering over the wound. On occasions when the wound bed became infected, dissolution of the wound pad would become apparent at the site of contact of the pad with the infected site. As uncomplicated healing progresses (Days 5–10) this film drys with the formation of the scab and appears histologically to be incorporated into the scab. Animals which were obviously infected to the point of impairing wound closure were elimi-

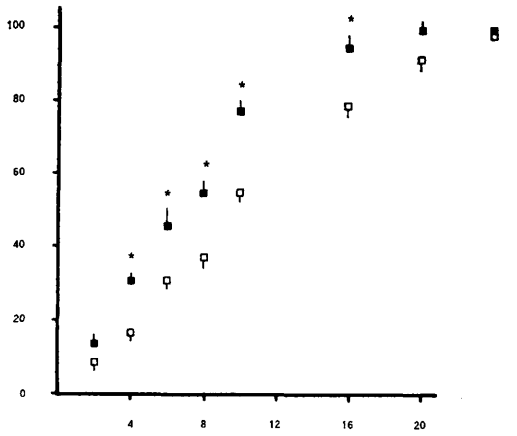

FIG. 2. Mean percentage change in wound closure of paravertebral excised wounds covered with microcrystalline collagen dressing in rabbits. (□) Gauze dressing; (■) microcrystalline collagen dressing ($n = 37$); * $p < .025$; (ordinate) time (days); (abscissa) mean percentage change ± standard error.

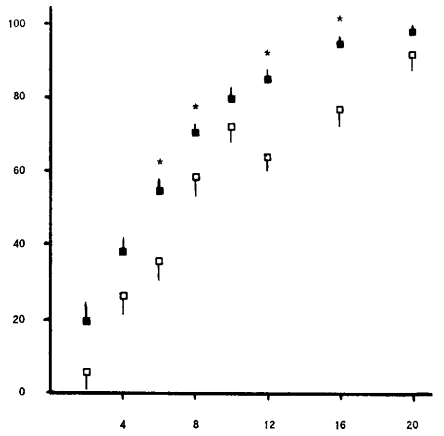

FIG. 3. Mean percentage change in wound closure of multiple excised wounds of pigs covered with microcrystalline collagen dressing or gauze dressings. (□) Gauze dressings ($n = 20$); (■) microcrystalline collagen dressing ($n = 27$); * $p < .025$; (ordinate) time (days); (abscissa) mean percentage closure ± standard error.

nated from the study and represented less than (7/111) 7% of the total animals evaluated.

Discussion. The use of paravertebral wounds allows for evaluation of a test dressing versus a reference dressing in the same animal, reducing the intraanimal variability. Earlier workers have described an inhibitory influence of wound healing in primary wounds in the presence of secondary wounds. The validation experiment in rats (Fig. 1) demonstrated uniform healing rates with no side to side variability, supporting some later work in excised wounds observed by Levenson *et al.* (21).

The use of the pig excised wounds utilizes a higher species with an integument more analogous to the human skin. Multiple wounds were undertaken in these animals because of the size of the species with no wounds closer than 10 cm to each other. This distance was sufficient to avoid the influence of fibrogenesis of one wound on an adjacent wound site as described by Peacock (23).

The role of endogenous and exogenous collagen in the wound healing process has been discussed in the literature as recently reviewed by Peacock and Van Winkle (3). Endogenous collagen is formed from fibroblasts which begin to infiltrate the wound area from subcutaneous tissue within 4 to 5 days. The role of exogenous collagen can only be surmised to be that of scaffolding for the proliferation of fibroblasts into the wound area. Thus, only in the initial few days of wound healing can any dressing accelerate the natural process of wound healing. Validation of this hypothesis awaits further well-defined experiments on the influence of exogenous collagen on synthesis of endogenous collagen by fibroblasts *in vitro* and *in vivo*.

The wounds covered with a gauze dressing heal in a linear uniform rate as denoted by the open symbols in Figs. 1–4. In wounds covered by microcrystalline collagen wound dressing, two phases appear to be identified; an initial accelerated healing phase, especially shown by wound closure rates on Days 2 to 4 and a late phase in which wound healing proceeds at a normal rate. Because of the initial accelerated phase, the microcrystalline collagen-dressed wounds demonstrate almost completed (≧ 95%) closure 2 to 4 days earlier than the gauze-covered wounds.

By comparison, the difference in the closure rate of wounds dressed with microcrystalline collagen wound pad compared to gauze-dressed wounds is less dramatic in pigs

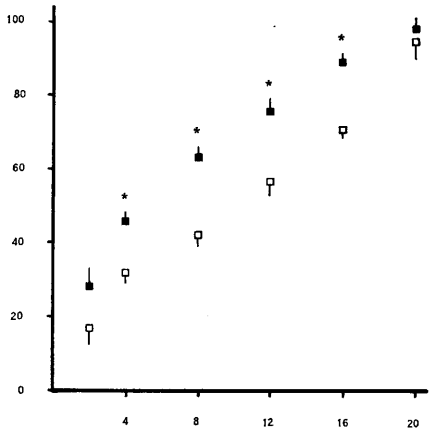

FIG. 4. Mean percentage change in wound closure in paravertebral burn wounds covered with microcrystalline collagen wound dressing versus gauze dressing in rat. (□) Gauze dressings ($n = 6$); (■) microcrystalline collagen dressings ($n = 6$); * $p < .025$; (ordinate) time (days); (abscissa) mean percentage closure ± standard error.

than in rodents. In rodents a statistically significant wound healing was observed by Day 4 (Figs. 2 and 4) compared to Day 6 (Fig. 3) for pigs. In addition, the closure rate (between sites on Day 8 or 12) for microcrystalline collagen wound pad on rodents was as much as 24% greater than gauze, whereas in pigs the difference was never greater than 15%. It should be kept in mind that wound healing influences which have been demonstrated at lower species usually becomes less discernible as one goes up the phylogenetic scale indicative of species variability (3).

In this investigation, the microcrystalline collagen wound dressing demonstrated improved wound closure in excised wounds of rodents and swine. The results indicate that further evaluation of collagen on the components of wound healing are merited using *in vitro* and *in vivo* models.

Summary. A collagen wound dressing derived from edible bovine corium has been investigated for application as a primary dressing for normal-excised and burn-excised wound in rodents and pigs.

Utilizing the rate of wound closure as an end point, the microcrystalline collagen wound pad was superior to a gauze dressing. A significant enhanced wound closure rate was demonstrated by the microcrystalline collagen wound dressing earlier in wound healing (Days 4–6) with a resultant earlier total closure of the wound as evaluated against control wounds dressed with gauze.

The microcrystalline collagen wound dressing demonstrated no untoward reaction indicative of inflammation or infection, but does appear to assist in the normal wound healing process. The above results merit further investigation of the material in other wound healing models.

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