Lanolin and Epidermal Growth Factor in Healing of Partial-Thickness Pig Wounds

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A total of 80 partial-thickness wounds (4.4 cm^2 size, 400 μm deep) was inflicted by electrokeratome in the dermal skin layer of four piglets, 15 kg body weight. The wounds were treated with gauze (control), lanolin cream (Lanolor or Lanolin with emulsifiers, Squibb) or with human epidermal growth factor (EGF) delivered in lanolin cream (10 μg EGF/mL cream). The treatment was applied every 12 hours for 12 to 120 hours after wounding. The reepithelization rate of the wound was determined by standardized morphometric method. In addition, we measured the thickness of the dermis and cell counts in the dermis.

We found that most of the statistically significant enhancement of the epithelization rate, thickness of the dermis, and higher cell count in the dermis were attributed to the effect of lanolin cream alone. The additional significant enhancement of healing by EGF over that of lanolin alone was documented in one of our experiments, but was only marginal. In another experiment using another commercial formulation of lanolin, we found no difference between the effect of EGF and lanolin. Several hypotheses were suggested to explain the effect of the two tested lanolin cream formulations, which induced strong inflammatory reaction in the wound.

Since no abnormalities stemming from excessive epithelization are known, it is a commonly accepted view in wound management that any enhancement of the healing of partial-thickness wounds is desirable. However, promoting the repair of deep skin wounds by either physical or chemical means could result in excessive scarring, wound contractures, etc.

Epidermal growth factor (EGF) is one logical candidate for enhancing epithelial coverage of shallow wounds. Indeed, this was documented by Franklin and Lynch[1] in rabbits by using a topical administration of mouse EGF. Also, a recent study by Brown et al[2] showed that EGF administered topically in a lanolin base on electrokeratome-inflicted skin wounds in adult swine significantly enhanced epidermal regeneration. Under similar experimental conditions, wounds epithelized at a slower rate when covered with either gauze alone or with a lanolin base. The effect of EGF as a potent mitogen of epithelial cells[3] was expected. In fact, it agrees with the results of others[4] who show wound healing in granuloma tissue stimulated by subcutaneously implanting a cellulose sponge.

Recently, we standardized a method for quantitation of the reepithelization rate of partial-thickness wounds in a pig model.[5] By statistical methods, we determined the number of histologic sections from each wound for morphometric analysis of epithelial cell coverage to obtain good sensitivity, ie, good discrimination of the magnitude of epithelization as a function of time posttreatment. The goal of the present study was to test EGF with the same treatment protocol used by Brown et al[2] to verify their data by using our alternative method of quantitating the rate of epithelization of partial wounds. However, this study will document that most of the enhancement may be attributed to lanolin alone, while the effect of adding EGF to lanolin cream was only marginal.

Materials and Methods

Principles for *Use of Animals* and *The Guide for Care and Use of Laboratory Animals* (National Institute of Health) were followed.

The dorsal aspects of four female Yorkshire piglets, 15 kg body weight, were prepared and shaved. In each animal, 20 partial-thickness wounds were inflicted by electrokeratome (Storz Instrument Co, St Louis). The

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wounds were 2.2 × 2.2 cm in size and 400 μm deep. Sets of four adjacent wounds were treated and dressed together, with treatments randomly assigned to the sets.

Three types of wound dressing regimens were tested in three of the piglets:

1. Gauze cover only (G) (Steri-pad, Type VII gauze, 3 × 3 in, Johnson & Johnson, New Brunswick, NJ) left in place until harvesting
2. Lanolin cream (L) (Lanolor, Squibb Inc, New Brunswick, NJ), redressed with 2.5 mL every 12 hours and covered with fresh gauze
3. Lanolin cream containing 10 μg human EGF (99% pure)/mL of lanolin cream (LE)

Each treatment was applied to five sets of four wounds totaling 20 wounds per treatment. One set of wounds from each treatment was harvested 12, 38, 62, 84, and 120 hours after infliction (Study 1).

To prevent the wound from drying and to keep the treatment on the wounds, the whole dorsal aspect of the skin was covered with semiocclusive polyurethane membrane with an adhesive layer (Tegaderm, 3M Company, Minneapolis).

The same three dressing modalities were tested in a fourth piglet, with two exceptions: (1) another Squibb lanolin cream (Lanolin with emulsifiers) was used as the base for the EGF instead of the Lanolor cream, and (2) the epithelization rate was studied at only two time intervals ÷ 62 and 84 hours (Study 2).

In both studies, wounds were harvested in sets of four by a wide excision with a scalpel and fixed in 10% formalin. Only three wounds from each set were embedded in paraffin and evaluated by the established procedure[5]. Eight randomly selected sections, 7 μm thick, were cut from each wound. The percent of wound surface covered by epithelization, at least one cell thick, was recorded for each section by reading the slide with an ocular micrometer. Thus, the results on each treatment at each time period are based on 24 section readings.

Besides measurements on the rate of epithelization, dermal thickness and cell density in the dermis were studied in Study 1. Dermal thickness was measured as the layer between the assumed line connecting the lower aspects of epithelial cells and the borderline between the dermis and the subdermal fat. A Zeiss Photomic III microscope optical picture was projected through a TV camera adaptor on a television screen monitor and connected with a video micrometer (Colorado Video, Inc), which showed a digital display of thickness in microns.

Results

Gross Observations

In most wounds, the gauze was easy to remove; if partially adherent, wetting with sterile saline released it without causing any visible damage to the wound surface. This was confirmed by microscopic evaluation. In later stages (62 hours and later), scab formation was evident. In wounds treated with L or LE, the cream remained on the surface and between dressings was minimally resorbed (Figure 1).

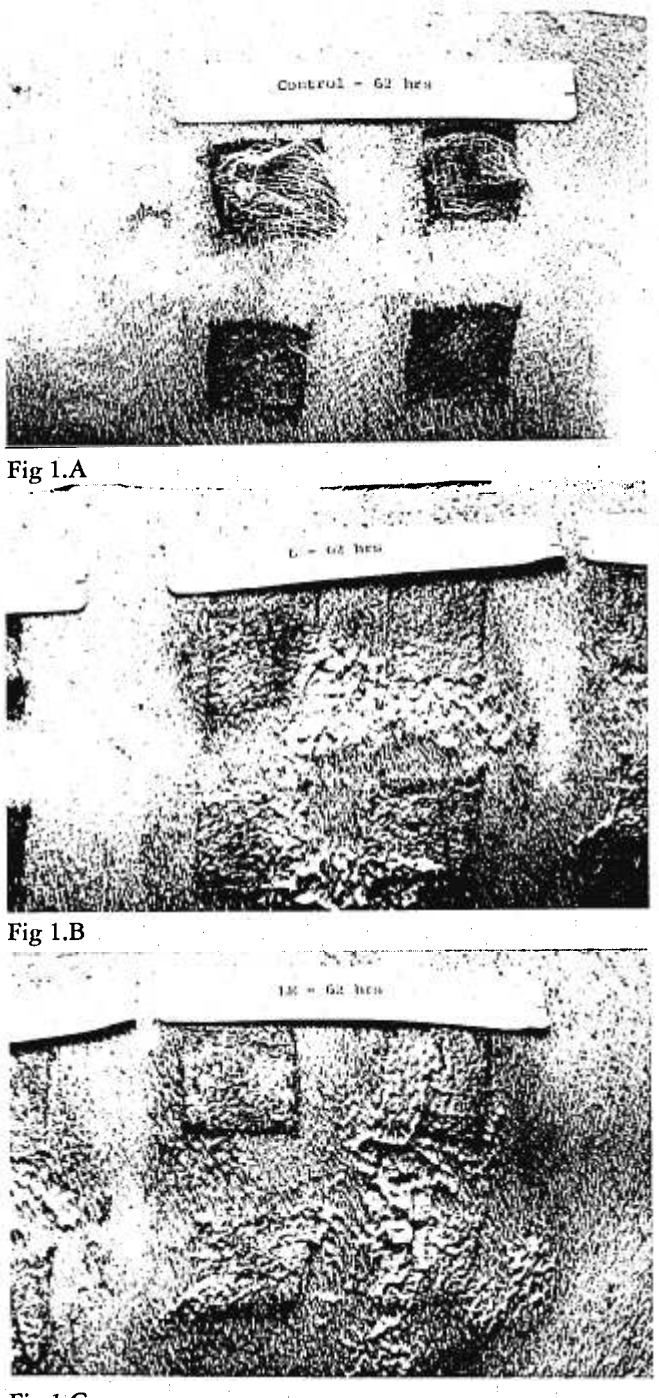

Fig 1.A

Fig 1.B

Fig 1.C

Fig 1. Gross appearance of partial-thickness wounds at 62 hours of A, control; B, wound treated with lanolin cream (L); and C, wound treated with lanolin cream and EGF (LE). Before redressing, gauze was exceptionally adherent to wound. Most lanolin cream remained on wound and was not removed between dressings.

Rate of Epithelization

A comparison of the epithelization rate (expressed as percent of wound area covered) in the three treatment

modalities was made; the findings are summarized in Table 1 and Figure 2. An analysis of variance by treatment and time indicates that:

- During the first 12 hours of treatment, all methods were equally effective.
- At 38- and 62-hour treatment periods, L alone induced significant enhancement of epithelization; LE was significantly more effective than L alone.
- At 84- and 120-hour treatment periods, both L and LE significantly stimulated epithelization without any difference between these two treatment modalities.

Table 1. Effect of EGF Administered in Lanolin Cream on Rate of Epithelization of Split-Thickness Wounds in a Standardized Pig Model: Statistical Evaluation of Results

Sampling Time (hrs)	Epithelization Rate (% wound area covered)* G	L	LE
12	5.7 ± 0.7[a]	8.0 ± 0.6[a]	7.1 ± 1.1[a]
38	9.0 ± 1.0[a]	16.2 ± 0.8[b]	26.5 ± 3.8[c]
62	28.8 ± 1.9[d]	46.1 ± 3.0[e]	58.8 ± 3.5[f]
84	34.4 ± 1.7[d]	69.2 ± 3.5[g]	69.7 ± 3.7[g]
120	68.7 ± 2.3[g]	91.3 ± 2.3[h]	98.0 ± 0.6[h]

*Data shown as $\bar{X} \pm$ SEM are based on 23 measurements in each treatment sampling period.

[a-h]If the superior letters differ, they indicate a statistically significant difference at least at $P > 0.05$.

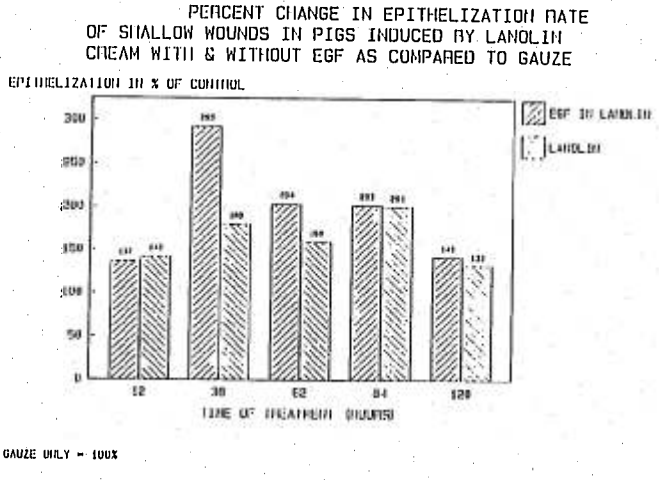

Fig 2. Increase in epithelization rate of partial-thickness wounds treated by lanolin cream or lanolin cream with EGF. Only a 38- and 62-hour treatment periods did EGF significantly increased rate of epithelization above lanolin effect.

Logistic regression curves of the form: $E(t) = 1/[1+\exp[-A-(t-t_{50}]]$ were fitted to these reepithelization data as an alternative analysis (Figure 3). The paramater t_{50} describes the time at which the expected degree of epithelization, E(t), reaches 50%. The larger the value of the shape parameter, A, the more rapidly E(t) is increasing at t_{50}. The logistic curve was used simply as a good empirical description of the data although such curves are not uncommonly used as theoretical models for bounded growth processes. Estimates of A and t_{50} for each treatment were derived by means of nonlinear regression and are presented, along with their estimated standard errors, in Table 3. Two major conclusions can be shown.

1. Times when half of the wound areas treated with the three methods are covered with epithelial cells (t_{50}) are statistically distinguishable, although the time difference between L and LE is small (six hours).
2. The healing rate of wounds (slope of the areas, A) treated with L or LE is not significantly different. Both curves differ from the slope A for gauze.

Table 2. Slope and Epithelization Half Time of Healing of Partial-Thickness Wounds Treated with Gauze, Lanolin, or Lanolin with EGF*

	Treatment G	L	LE
Slope A°	0.322 ± 0.0022[a]	0.0469 ± 0.0034[b]	0.0454 ± 0.0035[b]
Epithelization Half time T_{50} (hrs)	99.010 ± 2.1300[a]	64.7100 ± 1.6000[b]	58.7500 ± 1.7200[c]

*Data derived from the ANOVA program treatment of the experimental data are presented as X ± SE.

[a-c]Superior letters refer to statistical difference. The same letter signifies no difference.

Table 3. Thickness of Dermis Underneath Partial-Thickness Wounds Treated with Gauze, Lanolin, or Lanolin with EGF*

Duration of Treatment (hrs)	Dermal thickness (μm) G	L	LE
12	1704 ± 480	1749 ± 540	1926 ± 690[b]
38	1872 ± 480[a]	2463 ± 600[a]	2787 ± 450
62	1308 ± 570[a]	1752 ± 510	1740 ± 690[b]
84	1251 ± 510[a]	1674 ± 630	1842 ± 570[b]
120	1221 ± 420	1389 ± 660[a]	1872 ± 480

*Data shown as $\bar{X} \pm$ SEM are based on 23 measurements in each treatment sampling period.

[a]Indicates a statistically significant difference at $P > 0.05$.

[b]Indicates significant difference between gauze (G) and lanolin cream with EGF (LE) treatments.

Thus, analysis by logistic regression supports the conclusions of the analysis of variance evaluation: Lanolin cream alone promotes the rate of epithelization by 35%; adding EGF to lanolin stimulates further healing, although its effect over lanolin alone is much less (10%).

Dermal Thickness

During evaluation of the epithelization dynamics of various wounds, we observed that the dermis appeared thicker in both L- and LE-treated wounds. We measured the dermal thickness by an objective morphometric method (Table 3), thus confirming our observation. A graphic plot of these data (Figure 4) clearly shows that the dermal layer was significantly thicker after 12, 36, and

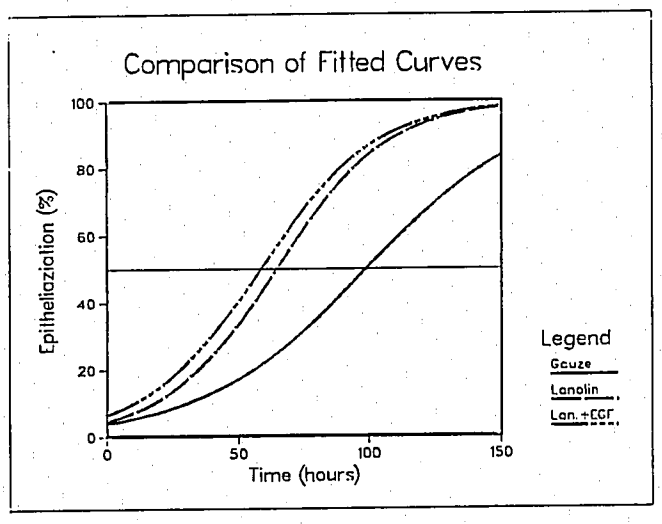

Fig 3. Logistic regression curves of epithelization rates of partial-thickness wounds under three treatments.

120 hours of the LE application. At the 62- and 84-hour periods, L and LE induced the same thickening of the dermis and was significantly different from the gauze treatment.

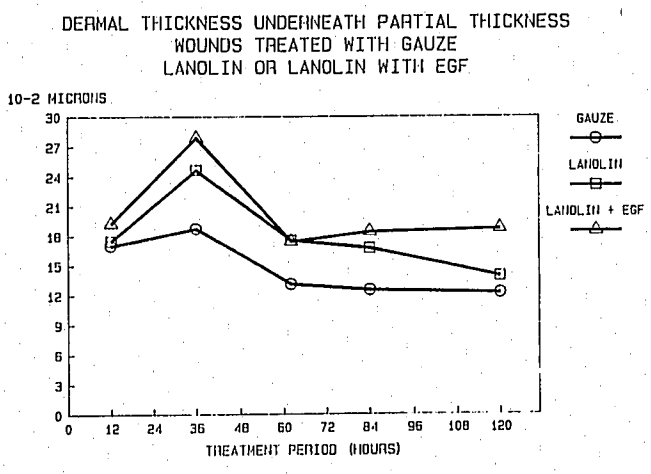

Fig 4. Thickness of dermis underneath partial-thickness wounds treated with gauze, lanolin cream, or lanolin with EGF. Thickness of dermis over intact skin was 1148 ± 95 μm. For details, see Table 3.

The shape of the curves for individual treatments indicates maximum response of the dermis to the healing process-treatment method at the 36-hour period. As the healing progresses, the dermal layer becomes thinner but remains well above the dermal thickness of the intact pig skin (compare 0 time v 120 hours time readings).

Wound Morphology

Slides from each wound were also evaluated under high magnification. Wounds treated with gauze showed no or minimal inflammatory reaction, or the presence of an irregular scab infiltrated by few cells. Epithelial coverage occurred either directly on the clean wound surface or underneath the scab (Figure 5A). Lanolin-treated wounds showed a thick layer of cream infiltrated by an excess of

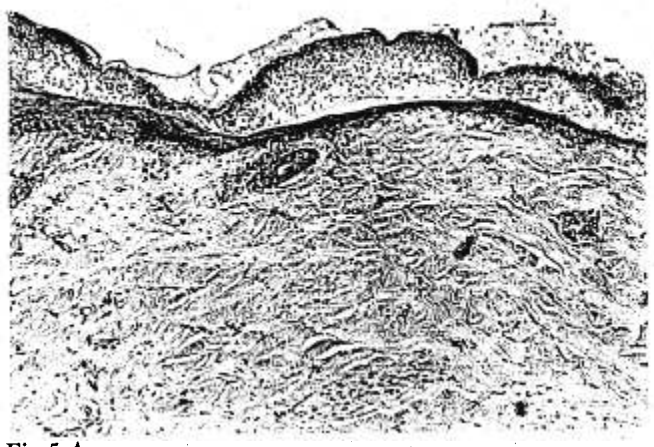

Fig 5.A

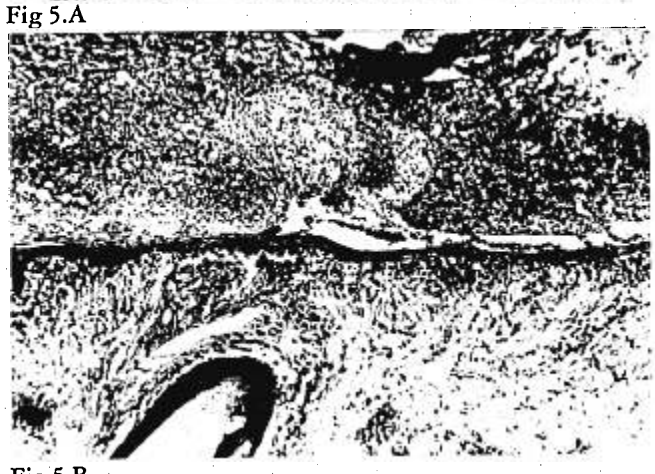

Fig 5.B

Fig 5.C

Fig 5. Reaction of wound to various treatments: A, wound covered for 62 hours with gauze. Note continuous epithelial layer underneath scab. Few inflammatory cells are present in scab. B, wound covered for 62 hours with lanolin cream. Layer of lanolin cream is permeated with many inflammatory cells, which are also present under newly formed epithelization layer. C, wound covered for 62 hours with lanolin cream and EGF. Lanolin layer infiltrated with inflammatory cells, which are also present in upper dermis. This section does not show any epithelization.

inflammatory cells (Figure 5B), more at the 36-hour period than at 12 hours. Inflammatory cells were also sporadically found underneath the newly formed epithelial layer, which was often thicker in gauze-treated wounds (Figure 5A). Lanolin cream with EGF induced the same strong inflammatory reaction and cell infiltration as lanolin alone (Figure 5C).

Density of Cells in Dermis

We tested the hypothesis that the above-documented inflammation induced by L or LE in the wounded skin will result in a higher number of cells in various fields of the dermis. The data shown in Table 4 show certain value trends that are lower in the G group and higher in the L and LE groups.

Table 4. Density of Cells in Dermis of Skin Wounds Treated With Gauze, Lanolin, and EGF*

Sampling Time (hrs)	Density (cell/6080 μm^2) G	L	LE
12	20.9 ± 1.9	28.9 ± 3.4	26.1 ± 2.9
38	21.4 ± 1.5	26.7 ± 2.3	22.6 ± 2.5
62	21.0 ± 1.4	28.5 ± 3.7	25.5 ± 2.5
84	19.6 ± 5.8	27.3 ± 1.8	25.6 ± 2.3
120	23.8 ± 1.9	26.8 ± 1.8	27.7 ± 2.5

*Data shown as $\bar{X}$ ± SEM are based on 23 readings in each treatment-time group.

Due to the high variability of the data, no statistical significance was obtained among the various treatment-period groups. In pooling all the values for a given treatment across healing times, we found significantly ($P < .05$) more cells in the dermis under the L or LE treatment than for gauze only.

Therapy	cells/10,000 μm^2
G	35.7 ± 1.22
L	45.4 ± 1.96
LE	43.9 ± 1.91

Table 5. Rate of Epithelization of Partial-Thickness Wounds Treated with Gauze, Lanolin with Emulsifiers, or EGF

Group	Time (hrs)	N	% of Epithelization ($\bar{X}$-SE)
1 G	62	22	31.4 ± 3.0[a]
2 L	62	24	53.4 ± 3.7[b]
3 LE	62	23	55.8 ± 3.8[b]
4 G	84	20	58.6 ± 2.5[b]
5 L	84	24	84.3 ± 3.3[c]
6 LE	84	24	78.1 ± 3.8[c]

[a-c]Superior letters refer to statistical significance. Values with different letters are statistically significant at $P > 0.05$.

Rate of Epithelization

After completing the whole study, we realized that Brown and associates[2] used another lanolin formulation. To insure that this oversight was not the reason for the conflicting results, we repeated the same experiment using "Lanolin with emulsifiers" (Squibb) as a vehicle for EGF. The treatment and healing were observed at only two periods—62 and 84 hours—using the method described above and as in Brown's study.

The results (Table 5) show a significant enhancement in the rate of epithelization of partial-thickness wounds treated for 62 or 84 hours with lanolin cream alone. Adding EGF to the vehicle did not enhance the healing.

Discussion

This study presents several original observations. Most interesting is the finding that lanolin cream alone promotes epithelial healing. Although the actual mechanism of such an effect is not known, we speculate that retention of moisture at the wound surface is an important factor. Thus, the "healing"-promoting effect of lanolin will be comparable to the petrolatum used in Xeroform and other dressings. The importance of a moist environment for epithelization of partial thickness wounds has been sufficiently documented.[6-9] Another possibility is that some components of the lanolin cream—for example, cholesterol ester—have a direct effect on cell mitosis and possibly other cell functions as well. Further, another possibility is an indirect effect caused by stimulation of the inflammatory response. Striking inflammatory cell exudate was found within the layer of lanolin cream covering the wound. These inflammatory cells could be producing stimulatory substances. The overall activation of inflammatory skin response may also explain the significantly thicker dermis and cell content in the dermis underneath the lanolin cream.

In our first study, the effect of human EGF administered in lanolin cream was detectable, but marginal. In the second study, it was absent. These results are very different from the large positive effect reported by Brown et al[2]. We also saw a fairly large positive effect due to lanolin alone, whereas they saw no difference between lanolin-treated and untreated wounds. A discussion of the different aspects of the two experiments is therefore in order.

The first difference is in the method of evaluation. We used the morphological evaluation of the wound area covered by epithelial cells, which allows for detection of a single layer of epithelial coverage. Brown et al used the method developed by Eaglstein and Mertz in which the epidermis and dermis are separated and wounds assessed as either healed or not healed based on macroscopically visible defects. Histology of wounds assessed as healed indicate a five- to six-cell layer and is the endpoint of this method (P. Mertz, private communication).

The second difference was the depth of the wounds. We cut wounds at 0.4 mm, which allows for complete excision of the epidermis, leaving subepidermal hair follicles as the only source for reepithelization. Brown[2] cut wounds to a depth of only 0.125 mm. It seems likely that

this wound depth would have significantly more epidermis in the bed of the original wound; epidermal rete pegs would be expected to extend at least 0.2 mm in depth.

The third difference is that they used mature animals while we used young animals, essentially infants. There are reports that wound healing slows with advancing age, which could make a meaningful difference.

A fourth possible difference must be considered. Although we designed our study to deliver the same dose as Brown et al[2], we do not know how the EGF we used compares in potency with that used in their study. It is possible that the EGF dose we used was not optimum. A recently published study by Schultz et al[16] indicates that doses that are too high and doses that are too low will give negative results.

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