Inert Wound Dressing Is Not Desirable

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Four Yorkshire piglets were inflicted with a total of 92 split-thickness wounds 4.8 cm² in area and 400 μm deep. The wounds were treated with eight dressing regimens under the same experimental design. The rate of reepithelialization of the wound was quantitated by a morphometric method. The magnitude of inflammatory reaction of the wound to the dressing was scored from histological slides. The results indicate a relationship between the rate of reepithelialization of split-thickness wounds and the inflammatory response of the wound to the dressing. Dressings, such as collagen sponge, polyethylene glycol, Duoderm, and lanolin ointment, induce moderate to severe inflammatory changes when placed on the wounds. These wounds reepithelialize significantly faster than control, gauze-covered wounds. This contrasts with inert dressings, such as hydrated hydrogel membrane, Carbopol 934P, or Silvadene cream, which did not affect the rate of reepithelialization when compared with the healing of control wounds. Simultaneously, these dressings induced no or minimal inflammatory reaction in the wound tissue. Only when the inflammatory reaction to the wound dressing was excessive (methylcellulose) was the rate of reepithelialization of the wounds significantly inhibited in comparison with control wounds. We hypothesize that wound dressings, by inducing inflammatory reaction, enhance healing by activating cells, such as macrophages or fibroblasts, that produce growth factors and other mediators of the repair process.

INTRODUCTION

Many studies that evaluate the acceptability of various wound dressing materials stress the need for a bioinert, nontoxic, biocompatible dressing [1-6]. This opinion has been incorporated into review articles on optimal wound dressing characteristics [2, 4] that consider the induction of a moderate to intense inflammatory response in the wound from the dressing as an adverse reaction [7]. The healing process can be viewed as a form of inflammatory reaction starting with a production of factors needed for promoting the repair of the defect [8]. In 1969, Lykke and Cummings [9] suggested that the inflammatory response, accompanying the early phase of wound healing, "might exert a beneficial effect on healing..." However, is the magnitude of this inflammatory process adequate to achieve optimal healing? It is an accepted theorem that wounds heal at a maximum rate in healthy, nutritionally balanced subjects. Nevertheless, in clinical practice, there are several conditions that delay healing with suboptimal cell reaction within the wound. Examples are patients on corticoid treatment or immunosuppressive therapy, after radiation therapy, and in diabetics [10]. Topical treatments with vitamin A, vitamin E, oxygen, etc., have been used in an effort to correct delayed healing [10].

In principle, wound healing activators stimulate several aspects of cell life, such as mitosis, synthesis of structural macromolecules (collagen and glycosaminoglycans), and synthesis and discharge of activators and mediators of the intercellular communications. These cellular activities are suppressed under conditions that result in delayed healing.

Over several years, we tested various factors to promote healing of skin wounds by drugs incorporated into various "vehicles," ranging from creams and gels to preformed membranes. We noticed highly variable wound tissue response to these vehicles. A study by Eaglstein and Mertz [11, 12] indicated that some so-called "inert" vehicles do affect wound healing, yet the mechanisms are not understood. After comparing the morphology of the wound tissue reaction with the quantitative determination of the actual rate of healing, we noticed that the higher inflammatory response of the wound tissue invariably paralleled faster wound healing. This study aims to document this statement.

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 method used to inflict shallow wounds in piglets and to quantitate the rate of reepithelialization was published earlier [13]. In principle,
Rate of Reepithelialization of Split-Thickness Wounds Treated with Carbopol and Lanolin in a Swine Model

<table>
<thead>
<tr>
<th>Duration of the treatment (hr)</th>
<th>Rate of reepithelialization (%)</th>
<th>Control</th>
<th>Carbopol 934P</th>
<th>Lanolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>17.1 ± 5.4</td>
<td>18.4 ± 9.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>40</td>
<td>38.8 ± 7.8*</td>
<td>38.7 ± 8.9*</td>
<td>54.3 ± 13.2*</td>
<td>—</td>
</tr>
<tr>
<td>54</td>
<td>50.5 ± 8.9*</td>
<td>48.2 ± 9.1*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>62</td>
<td>60.8 ± 12.4*</td>
<td>97.3 ± 22.1*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>85</td>
<td>99.7 ± 1.0</td>
<td>96.6 ± 4.7</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note. Data based on 22 to 24 analysis in each group/time period are presented as X ± SD. Control wounds were covered with Tegaderm and gauze. The tested medication in experimental wounds was administered in gauze. The wounds were covered with Tegaderm. The significance calculated for a certain time period (horizontally) is shown by letters. Different letters indicate statistically significant difference at \( P < 0.05 \) (\(*\) vs \( \delta \)) or at \( P < 0.01 \) (\(*\) vs \( C \)).

The preformed membranes or layers (Duoderm, Geliperm, or collagen sponge) were cut in a way to cover all four wounds. To keep the treatment in place and to prevent dehydration of some dressings and the wound surface, a Tegaderm (3M) semiocclusive adhesive polyurethane film was placed over all the wounds. Control wounds were treated in the same manner. Two sets of experiments are presented; in the first study (Table 1) the control wounds were covered with sterile gauze and Tegaderm. Experimental groups treated with Carbopol or lanolin were also covered with gauze and Tegaderm. The data in Table 2 are based on wounds, when the treatments and control wounds were without gauze and only Tegaderm film was used.

Each treatment was harvested 54 hr after infliction except for Carbopol 934P and lanolin, which were left on the wounds for time periods shown in Table 1 with daily change of the treatment. Before harvesting the wound granulation tissue, most dressings were removed, if possible. However, dressings that adhered too strongly to the wound (collagen sponge, Duoderm) were left on the wound to prevent any damage to the epithelial layer. Even with the dressing removed it was still possible to ascertain some characteristics of the inflammatory exudate especially because several wounds were evaluated for each dressing. Wounds were harvested in sets of four by a wide excision with a scalpel, then fixed in 10% formalin. Only three randomly selected wounds from each set were embedded in paraffin and evaluated by the established procedure [13]. In principle, eight randomly selected sections, 7 μm thick, were cut from each wound and stained with hematoxylin and eosin. The percentage

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Rate of reepithelialization (± SD)</th>
<th>Inflammatory reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(in percentage of original wound area)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68.6 ± 13.7*</td>
<td>1</td>
</tr>
<tr>
<td>Silvadene</td>
<td>58.8 ± 6.2*</td>
<td>1.5</td>
</tr>
<tr>
<td>Carbopol</td>
<td>65.4 ± 11.3*</td>
<td>2</td>
</tr>
<tr>
<td>Hydrogel</td>
<td>68.8 ± 15.1*</td>
<td>2.5</td>
</tr>
<tr>
<td>Polymethylene glycol</td>
<td>87.4 ± 14.8*</td>
<td>3.5</td>
</tr>
<tr>
<td>Collagen sponge</td>
<td>cross-linked</td>
<td>86.7 ± 19.1*</td>
</tr>
<tr>
<td>Duoderm</td>
<td>92.2 ± 18.4*</td>
<td>4</td>
</tr>
<tr>
<td>Methylocellulose</td>
<td>53.1 ± 12.3*</td>
<td>5</td>
</tr>
</tbody>
</table>

Note. Each value is based on 22 to 24 measurements. Each dressing, as well as control wounds, were covered with adhesive polyurethane film (Tegaderm, 3M). Statistical significance tested by Duncan’s multiple variability range test is shown by letters; different letters indicate statistical difference at \( P < 0.05 \) (\(*\) vs \( \delta \)) or at \( P < 0.01 \) (\(*\) vs \( C \)). Inflammatory reaction was scored on a scale from 1 to 4, where 1 refers to normal; 2, minimal; 3, moderate; 4, severe; 5, excessive.
of wound length covered by epithelial cells, at least one-cell thick, was recorded for each section by reading the slide with an ocular micrometer. Thus, the results on each treatment and each time period are based on 24 section readings, which were subjected to statistical analysis by Duncan’s multiple range variability test.

Besides measurements on the rate of epithelialization, the morphology of the wounds with shown treatments and appropriate controls was evaluated and the magnitude of the inflammatory reaction including the assessment of both inflammatory infiltrate and exudate was scored independently by two pathologists on a scale from 1 to 5:

1. No or borderline cellular inflammatory reaction
2. Minimal inflammation
3. Moderate density of inflammatory cells with some exudate
4. Severe, high density of inflammatory cells in the wound tissue with thicker layer of exudate
5. Excessive, with signs of dense foci of cells infiltrating the wound tissue and forming a thick layer of inflammatory exudate.

FIG. 1. Morphology of the split-thickness wound covered for 54 hr with gauze and Tegaderm film. This wound served as control. Note minimal presence of inflammatory cells remaining on the wound after removing the gauze dressing (H & E, 100X magnification).

FIG. 2. Split-thickness wound covered with Carbopol 934P for 54 hr. A Tegaderm film was placed over the gel. Note minimal inflammatory cells exudate. An epithelial cover extends from skin edge (H & E, 100X magnification).
We determined that using this semiquantitative score of the inflammatory reaction was more reliable than our efforts to quantify the number of cells per wound area. A Zeiss Photometric III microscope was used.

RESULTS

Initially, we studied the healing rate of two treatments, including Carbopol 934P and lanolin ointment. The data on the rate of reepithelialization of split-thickness wounds for these two formulations compared to appropriate tissue controls are shown in Table 1. The administration of Carbopol 934P did not affect healing but lanolin significantly enhanced healing [14]. Morphological evaluation of the wounds showed the typical, previously reported dynamics of the reepithelialization of the control wounds covered with wet gauze and semipermeable polyurethane adhesive film (Tegaderm 3M) (Fig. 1). Minimal inflammation with few granulocytes infiltrating the upper layer of the wound was found in wounds covered with Carbopol 934P, despite the duration of the treatment (Fig. 2). There was, however, severe inflammation in wounds treated with lanolin with both inflammatory exudate and infiltrate present in the upper layer of the dermis (Fig. 3). Due to the removal of gauze from the harvested wounds, the picture of the inflammatory exudate was sometimes distorted, being partially removed with the dressing. These wounds were not used for semiquantitative scoring, as shown in Table 2.

This finding was the first indication of a possible relationship between the rate of healing by reepithelialization and the magnitude of the inflammatory response. In light of this finding, we decided to study this aspect in detail.

We treated the split-thickness wounds with seven selected dressing materials for 54 hr. Both the rate of reepithelialization and the magnitude of the cellular inflammatory reaction to the dressings were evaluated. The results are shown in Table 2.

The dressings can be divided into three categories for their effect on the rate of healing by epithelialization:

- No effect was obtained with Silvadene, Carbopol, and hydrogel membrane.
- Significant enhancement of the reepithelialization was achieved with polyethylene glycol (PEG), Duoderm, and collagen sponge dressing.
- Significant inhibition of the healing was caused by administration of methylcellulose.

Except for methylcellulose, the individual dressing materials fit the same classification categories for inflammatory response. The most inert dressing was gauze or Silvadene (Fig. 4), followed by Carbopol 934P (Fig. 2), and hydrogel membrane (Fig. 5). PEG (Fig. 6), collagen sponge (Fig. 7), and Duoderm (Fig. 8) induced strong exudate with inflammatory cells. This exudate was trapped either within the sponge matrix or inside the paste of Duoderm or PEG. The cellular reaction induced by the methylcellulose application onto the wound was excessive, infiltrating the upper third of the dermis and forming a thick exudate mixed with methylcellulose. Few areas showed fused cellular aggregates (Fig. 9).

The results of the semiquantitative scoring of the inflammatory reaction, averaging the values obtained by two pathologists, are shown in the second column of Table 2. If the data for methylcellulose are omitted, there is
a significant statistical relationship between the healing rate and the magnitude of the inflammatory reaction induced by the dressing ($r = 0.903, P < 0.01$). Epithelization always occurred underneath the dressings. The epithelial cover originated equally from hair follicle epithelial cells and the epithelium of the skin margins.

DISCUSSION

The results of this study support the view that wound dressings that inflict irritation to the split-thickness wound, as manifested by enhanced inflammatory reaction, also promote wound healing by epithelialization. We assume that the same conclusion will apply to the healing of deep wounds: we expect more mediators or activators will be produced by activated inflammatory cells to additionally stimulate myofibroblasts and enhance the formation of epithelium from the epidermis of the intact skin. This remains a working hypothesis awaiting future experimental testing. If a specific dressing provides additional stimulus to healing of all wounds, this can be of clinical importance mainly in pa-

FIG. 4. Split-thickness wound covered with Silvadene ointment for 54 hr. Few inflammatory cells infiltrate the ointment, which causes minimal cellular reaction in the wound tissue (H & E, 100× magnification).

FIG. 5. Hydrogel membrane covering split-thickness wound for 54 hr. Note the minimal inflammatory reaction. The epithelial cells grow underneath the dressing (H & E, 100× magnification).
FIG. 6. Polyethylene glycol covering the wound for 54 hr. Moderate to severe inflammatory cell accumulation within the dressing and in the upper stratum of the repair tissue (H & E, 75X magnification).

tients with delayed healing due to radiation, chemotherapy, immunosuppression, corticoid treatment, diabetes, etc.

Experimental evidence is sufficient to consider these results predictable. To some the implications of this study may be controversial. The role of endogenous mediators and activators such as histamine, serotonin, prostaglandins, and growth factors in the wound healing process have been well established. Recently, we showed [14] that the effect of exogenously administered EGF was either minimal or absent when the factor was administered through the lanolin ointment vehicle. Lanolin ointment alone significantly promoted healing (possibly by increasing the endogenous production of growth factors).

The consensus of those who develop new dressing materials or who approve their use (FDA) is that only bioinert, nontoxic, nonirritating wound tissue materials should be used as a wound cover. However, our data suggest that inert wound dressings are not desirable. Efforts

FIG. 7. Interface of the wound covered with collagen sponge for 54 hr. Cellular infiltrate is concentrated within the sponge matrix. Sponge is fixed to the wound until the reepithelialization occurring underneath the sponge releases the bond between the wound and dressing (H & E, 100X magnification).
to enhance wound healing of certain wounds by drugs could be avoided by using active wound dressings. It is not always clear what makes a wound dressing active. In Duoderm, we believe that it could be the presence of a readily extractable hydroxyproline-containing protein (gelatin), which forms up to 15% of the weight of the dressing. Both gelatin and collagen proteins, as well as their degradation products, were chemotactic to some cells, including fibroblasts [15, 16]. Both proteins induced the production of a superoxide anion by activated granulocytes [17]. The availability (release) of those chemotactic proteins or peptides from a collagen sponge moiety may not be the only mechanism for the activity of collagen sponge dressings. The cross-linked sponge, containing minimal amounts or no extractable collagen, still induces cellular infiltration not only when in contact with skin wounds [8, 18, 19] but also when inserted into articular cartilage or bone defects [20]. In these situations, the unique surface morphology of collagen was considered the factor for promoting cell adhesion, migration, and mitosis. Alvarez and Biozes [21] showed that a collagen sponge matrix facilitated reepithelialization and reduced contraction of full-thickness wounds in the porcine model. Benfer and Struck [22] showed the

FIG. 8. Duoderm dressing induced severe inflammatory cell reaction (H & E, 45X magnification).

FIG. 9. Cellular inflammatory reaction to the methylcellulose dressing of a split-thickness wound. This reaction was scored as severe to excessive (H & E, 100X magnification).
advantage of using collagen "instead of an inert material" to stimulate wound healing, thus eliminating the need for a two-stage transplantation. Doillon and Silver [23] showed that a collagen sponge dressing induced the spatial deposition of wound tissue within its matrix.

The activity of lanolin in promoting the rate of healing was discussed in our previous paper [14] and referenced the presence of cholesterol ester in the ointment. This component is a known tissue irritant.

"Inert" dressings were studied and discussed by Eaglstein and Mertz [11, 12]. They found that treatment with some of these agents increased, while others decreased, the rate of reepithelialization of shallow wounds. They found it unlikely that this effect could be ascribed to the effect of these vehicles on the documented DNA synthesis, regulation of oxygen transport to the wound, or control of the moisture or crust quality on the wound. This study now indicates that, to a certain degree, the production of inflammatory reaction with its consequences is reflected in the activity of the epithelial cells.

REFERENCES