The Effect of Human Amniotic Membranes on the Bacterial Population of Infected Rat Burns

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The sine qua non of healing for the burn wound is the reestablishment of continuity of the skin. This may occur spontaneously in partial-thickness burns, or by the successful application of autografts in full-thickness burns. The non-uniformity of the thermal injury usually requires that both of these modalities be utilized in patients with major burns. Although the recent introduction of topical antibacterials has allowed a greater percentage of burns to proceed to healing without grafting, the need for wound closure by autografts remains. In a 50 per cent full thickness burn in an adult, the amount of skin required to obtain wound closure has been estimated at 6,000 square centimeters. Because this large amount of donor area is not always immediately available on the patient, other substances have been sought to obtain a temporary closure of the wound, while awaiting the time when donor areas may be used again.

Over 100 years ago, Pollock applied the first homograft onto a burned patient. Ten years later Lee attempted the first heterografting of burn wounds in the United States. Later, Ivanova suggested that fetal skin might have an advantage over adult skin when used as a homograft on burned patients because the infantile tissue possessed more "energetic vitality." Following the suggestion of W. L. Thornton, a senior medical student at The Johns Hopkins University, John Staige Davis in 1910 reported attempts at grafting pieces of the lining of the amniotic sac onto granulating wounds. Sabella treated a burned patient with amniotic membranes for the first time on June 16, 1912.

Early attempts at using these substitutes for the patient's own skin were designed to obtain epithelial continuity of the burn wound and to provide permanent coverage. Although Davis stated, in 1919, that these substitutes, if left in place would eventually be rejected; attempts at permanent replacement continued. By 1938, Bettman had reviewed all of these attempts and reported that substitute coverage could be gained while they were in place. The concept of homograft split-thickness skin being used as a temporary emergency biological dressing was popularized by Brown and associates. Douglas reported using amniotic membrane tissue as such a temporary covering for burns, later, however, to be replaced by split-thickness skin autografts.

Temporary biological dressings have a demonstrated usefulness in rendering the burn wound less painful, in decreasing the fluid and protein loss from the burn surface, and in gaining valuable time for allowing donor areas to re-epithelialize prior to the next harvesting of autografts. All of the biological dressings mentioned can serve these functions. In addition, Eade has shown that homografts, used as temporary dressings, were useful in decreasing the bacterial population in the granulating tissue of the burn wound. Switzer et al., showed a similar effect with heterografts.

The effect of amniotic membranes on the bacterial growth in the wound has not been previously reported. The purpose of this study was to re-evaluate the use of amniotic membranes as a temporary biological dressing both in the laboratory situation and in patients with thermal injuries.

Materials and Methods

Fresh amniotic membranes were obtained at the time of delivery from sero-negative mothers, who had no

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history of either premature rupture of the membranes or endometritis. The membranes were removed from the placenta aseptically, making no attempt to separate them into amnion or chorion. Using aseptic technic, the membranes were passed through five rinses of sterile isotonic saline. In addition, those not being used immediately in the laboratory studies, were passed through one rinse of 0.025 per cent sodium hypochlorite and an additional five rinses of saline prior to being stored at 4°C for clinical use. All membranes were cultured at 24 hours, 3 days, 1 week, 2 weeks, 4 weeks and 6 weeks.

Part “A”

Fifty white, Sprague-Dawley rats weighing 200–250 Gm. underwent a standard 20% full-thickness scald burn of the back using the Brooke burn model. They were immediately topically inoculated with 10⁸ Pseudomonas aeruginosa organisms obtained from fresh 18-hour broth culture. After 5 days, those rats surviving the burn wound sepsis, underwent burn escharectomy.

The area beneath the removed eschar was divided into three equal areas and tissue biopsies were obtained from each area for quantitative and qualitative bacteriologic analysis. After cleansing the surface of the wound with isopropyl alcohol, a piece of tissue was removed. It was aseptically weighed, flamed, and homogenized after being diluted 1:10 with thioglycollate. Serial tube dilutions and backplating were then performed to arrive at a bacterial count. Following biopsy, the three areas of the burn were treated as follows: one had an amniotic membrane sutured into the defect; another had a piece of human skin approximately 0.014 inches in thickness sutured into the defect; and the third area was left untreated as a control (Fig. 1). The positions chosen for each type of treatment were randomized on the various rats. The amniotic membranes and skin grafts were replaced by fresh material every 48 hours. At the time of each change, tissue biopsies were obtained for quantitative and qualitative bacteriology.

Part “B”

A comparison of the in vitro effect of human split-thickness skin and amniotic membrane on bacterial growth was performed. Varying inoculums of 18-hour broth cultures of Pseudomonas aeruginosa and Escherichia coli were placed into plain thioglycollate and into thioglycollate containing a homogenate of either amniotic membrane or split-thickness human skin. The inoculums ranged from 10³ to 10⁸ organisms. The amount of skin or membrane used in the homogenate ranged from 50 mg. to 400 mg. (Fig. 2). The cultures were incubated at 37°C for 18–24 hours. Following incubation, serial tube dilutions and backplating were performed to obtain the number of bacteria present in each culture.

The agents used were silver sulfadiazine (1%), sulfamylon acetate (10%), and gentamycin sulfate (0.1%). At that point, the topical ther-

![Fig. 1. Three areas of escharctomized infected burn treated with amniotic membrane, human split-thickness, or left untreated as a control.](image)

![Fig. 2. Design of In Vitro experiment to test the effect of biological dressing homogenate on bacterial growth.](image)
apy was discontinued and the biological dressings begun. In the deep partial-thickness burns, topical antibacterials were used during the acute stage of burn edema. Prior to the initial application of amniotic membrane of homograft split-thickness skin, tissue biopsies were obtained for bacteriologic analyses. The biological dressings were left in place 48 hours and then replaced. At each change of the biological dressings, tissue biopsies were taken from the underlying bed. Biological dressings were used in full-thickness burns until replaced by autografts and in partial-thickness burns until re-epithelialization occurred.

Results

Culture of amniotic membranes prepared in sterile isotonic saline alone occasionally yielded positive cultures after storing. A single rinse of 0.025 per cent sodium hypochlorite was found to sterilize the membranes sufficiently, so that all cultures have remained negative throughout the 6-week period tested.

![Graph](image)

**Table 1. Effect of Amniotic Membranes and Human Skin on Bacterial Growth in Burned Rats.**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>48 hours after treatment</th>
<th>96 hours after treatment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>animals decreased count (%)</td>
<td>animals increased count (%)</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Human Skin</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>Amniotic Membrane</td>
<td>58</td>
<td>13</td>
</tr>
</tbody>
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Fig. 3. Geometric means of bacterial counts in subeschar burn tissue versus time after initiation of treatment with biological dressings.

Part "A"

Fifty rats were initially burned and inoculated with $10^8$ Pseudomonas organisms. Thirty-eight rats survived burned wound sepsis for 5 days. All three methods of treating the infected burn were effective in decreasing the bacterial count in the sub-eschar tissue. (Table 1). At 48 hours, escharectomy alone had decreased the bacterial count in 18 per cent of the animals. However, in 50 per cent of the animals the bacterial count in the control area had increased. The application of human skin decreased the count in 29 per cent of the rats and brought increased quantitative growth in 50 per cent. This was not statistically significant ($p > 0.05$). Amniotic membranes decreased the count in 58 per cent of the rats and only resulted in increased bacteria in 13 per cent of the rats. This proved to be statistically significant ($p < 0.01$).

By 96 hours, or following two changes of biological dressings, both human skin and amniotic membranes had decreased the bacterial counts in virtually all of the animals. Reduced counts were seen in only 40 per cent of the control group. Both of these differences were statistically significant ($p < 0.01$). Although the percentage of rats with decreased counts at 96 hours were almost equal with human skin and amniotic membrane, the degree of decrease was significantly greater with amniotic membranes. The geometric mean of the bacterial counts after two changes of human skin was $10^6$ organisms per gram of tissue compared to $10^8$ organisms per gram for the amniotic membrane treated group (Fig. 3).

Part "B"

The in vitro studies showed no antibacterial effect of either skin or amniotic membrane on the growth of *Pseudomonas aeruginosa* or *Escherichia coli*. Within 24 hours all cultures contained $10^8$ organisms per milliliter. This was true irregardless of the initial inoculum or the type of tissue homogenate present (Table 2).

Part "C"

In the burned patients, the bacterial population was well controlled with the topical antibacterials. Bacterial counts were $10^5$ or fewer organisms per gram of tissue.
at the time the treatment was begun with the biological dressings. Both amniotic membranes and split-thickness homograft skin resulted in further decrease of the bacterial counts. In no instance did the bacterial count increase while biological dressings were being used. They appeared to be as effective as homografts in preparing the beds for grafting and in serving as a dressing to bide time while awaiting new donor areas for autografts.

**Discussion**

The concept of temporary biological dressings, introduced in the 1930's, has been developed to the point where their use has been extended from burns to all types of granulating wounds. Homograft and heterograft split-thickness skin have both proved to serve the functions required of a biological dressing. However, each has its drawback. Originally, homografts required that another individual donate his skin as a life-saving measure. This often meant up to 20 to 30 donors to deal with for a single patient with major burns. In 1953, Brown et al. reported that it was practical to use postmortem homografts as biological dressings. Since that time, cadavers have provided the usual source for homografts. In the general hospital, however, the supply of cadavers suitable to provide sufficient homografts is limited. Those cadavers with a history of malignancy, hepatitis, or syphilis are usually excluded. Personnel must be kept available at all times to procure the skin in a sterile manner, often in the operating room.

Following the recommendations of Silvetti et al., heterografts were introduced to eliminate the problem of a lack of available homografts. Porcine heterografts are now commercially available. They are not, however, always immediately available when needed, and when purchased, add to the already overwhelming cost incurred by the burned patient. Additionally, heterografts have not proved as effective as homografts in decreasing bacterial contamination of the wounds in our hands. Both homografts and heterografts are limited by the size in which they are available. The size and shape being determined by the existing dermatomes being used to obtain them.

Amniotic membranes were chosen for evaluation in the present study because of their ready availability, large size, lack of cost, and their structural similarity to skin. Histologically the membranes are made up of two loosely connected tissues, the amnion and the chorion. The amnion or inner layer is derived from the epiblast and is continuous with the ectoderm or the embryo. Its inner surface is composed of cuboidal or flattened epithelial cells and its outer surface covered with mesenchymal connective tissue. The chorion has mesenchymal connective tissue in contact with the amnion and an external ectoderm composed of a fairly thick layer of transitional epithelium. Pigeon has stated that since amniotic membrane is formed by the ectoderm of the fetus, it is like an extension of the baby’s skin.

Amnion, chorion, and the combined amniotic membranes have been used by various investigators as a substitute for skin in the past. Most investigators have had a preference for one or the other of the membranes. Jenner studied amnion and chorion side by side in the same wound and found no demonstrable difference between the two. Similarly, Dino et al. experimented with various layers of fetal membranes and found the end result practically the same whether using amnion alone, chorion alone, or combined amnion and chorion. Since Sabella’s first case describing the use of amniotic membrane on the burn wound 50 years ago, multiple reports have appeared in the world’s literature. Most of these were reporting the attempts to use amniotic membrane as a permanent substitute for skin autografts or as a dressing over partial-thickness burns. Pigeon has demonstrated their value in partial-thickness burns as a cover under which reepithelialization can occur. Their use in full-thickness skin loss has not been rewarding. Dahinterova and Dobrkovsky observed failure when amniotic membranes were applied on deep burns or on seriously infected areas. They found that the membranes became autolyzed in 48 hours and disintegrated. Furthermore,
they stated that the same was true on all granulating surfaces even if they were clean. Similar findings have been reported by others.\textsuperscript{9,20,29} In all of these studies, the amniotic membranes were left in place until disintegration occurred. Once the softening and dissolution process begins, it seems obvious that any beneficial effect of the amniotic membrane would be negated. Therefore, in the present study, the membranes were changed every 48 hours. As demonstrated by Shuck and Moncrief\textsuperscript{27} for homograft skin, in a less tidy wound more frequent changes prevent collections of purulent material from developing under the biological dressing. This allows for firm adherence of the membrane to the underlying granulations. In the present studies, no disintegration occurred within 48 hours when adherence of the membrane was maintained.

Because of the occasional positive cultures discovered in the amniotic membranes prepared for laboratory use in sterile saline, those prepared for clinical use underwent a slightly different preparation. Dino et al. have reported the testing of many agents to sterilize amniotic membranes. They found sodium hypochlorite effective. It is felt that a single rinse of 0.025 per cent sodium hypochlorite together with rinses of sterile saline, will not change the biologic effectiveness of the amniotic membrane to any significant degree.

Frequently changed amniotic membranes were more successful in decreasing the bacterial count in contaminated rat burns than was human skin (Fig. 3, Table 1). This raised the question as to whether there was a substance in amniotic membrane which was specifically antibacterial. One such possibility is allantoin, which is known to exist in amniotic membrane. Needham\textsuperscript{10} reported studies by A. W. A. Brown showing the beneficial effect of maggots on contaminated wounds was due in part to the allantoin and urea excreted by them. Another possibility is lysozyme, a bacteriolytic protein of low molecular weight which is present in amniotic tissues.\textsuperscript{13} To test for the presence of such a subcellular substance being responsible for the increased antibacterial effect seen in rats, amniotic membrane homogenate was compared to human skin homogenate \textit{in vitro}. The complete lack of any demonstrable effect on bacterial growth by the varying dosages of tissue homogenate was not unexpected (Table 2). Rubin and Bongiovi\textsuperscript{24} recently stated that skin itself possesses bactericidal substances in its biological makeup such as lysozymes and certain fatty acids. Neither, however, could they demonstrate bacterial inhibitory activity of split-thickness human skin \textit{in vitro} when measured by a disc sensitivity technic.

Another hypothesis for the observed decrease in the bacterial count under the amniotic membrane lies in the intimate biologic closure of the open wound by the membrane.\textsuperscript{18} Restoration of the functional circulation through the covered granulations allows a more rapid turn-over of phagocytes, serum bacteriolytic factors, and may accelerate the removal of the necrotic debris. Therefore, repeated applications of the membranes allow the host resistance factors in the granulating bed to function at peak efficiency. This same mechanism of obtaining a biologically closed wound would function, not only for amniotic membranes, but also for other temporary biological dressings. The same hypothesis could thus be used to explain the effect on the bacterial count seen in rats treated with human skin. The increased antibacterial effect seen with the amniotic membrane may be due to the fact that it is less well-differentiated than skin. Although the amniotic membranes used on the rat were from human sources and therefore heterologous as was the skin; Douglas \textit{et al.}\textsuperscript{11} have shown that there is very little difference between the heterologous and homologous chorion grafts in the mouse. This is compared to a threefold difference that they demonstrated between heterologous and homologous skin. In the present experiment, the amniotic membrane may have had an effect in the rat comparable to rat homograft skin and therefore greater than human heterograft skin. Rappaport \textit{et al.}\textsuperscript{21} have shown that heterograft left in place longer than 24 hours, as we might normally use a homograft, often will not control the underlying infection. This correlated with data of our own showing that homografts are more effective than heterografts in decreasing bacterial growth in both laboratory animals and in the clinical situation.\textsuperscript{23}

The nonuniformity of a clinical burn make comparative studies in the clinical situation difficult to interpret. Although the number of patients and comparative observations were too small for statistical analysis, amniotic membranes appeared to decrease the bacterial count in the burn wound at least as well as homografts. In patients, significant gross infection is not often present in the burn wound managed with topical antibacterials. Therefore, any changes due to biological dressing will be of small magnitude and will require a great many observations to obtain statistically significant data.

The amniotic membranes fulfilled all of the functions of an ideal biological dressing. In terms of their large size and ready availability at no cost to the patient, they are actually superior to homograft and heterograft skin. In addition, the membranes appear to have another property. Subjectively the rapidity of ingrowth of epithelium form the borders of the wound in full-thickness defects and the rate of re-epithelialization of partial-thickness burns appear to be increased by their use. Chao \textit{et al.}\textsuperscript{2} and Troensgaard-Hansen\textsuperscript{81} also have noted that amniotic membrane seemed to possess some specific healing power. They have reported a stimulation of both fibrous-
tissue growth and more rapid epithelial repair. Needham has summarized the available information on the growth-promoting factor found in embryo extract. Although the available evidence seems to point to an acceleratory action of amniotic membranes on the healing of wounds, that phase of their action was not objectively studied in the present investigation.

Summary

Amniotic membranes have not previously been studied using the standard methods currently in vogue for biological dressings. The present study has evaluated amniotic membranes changed every 48 hours on a standard infected escharectomized scald burn in the rat. Compared to human skin the amniotic membrane was more effective at decreasing the bacterial counts in the burn wound. A specific antibacterial substance was sought using in vitro technics with amniotic membrane homogenate. No such substance was found. It is proposed that the in vivo antibacterial effect seen is due to the achievement of a biologically closed wound by the membrane, thus allowing the host's own defense mechanisms to deal with the bacterial population as did other biological dressings. However, their large size, ready availability, and lack of cost make them more desirable.

References