Clinical Application of Aseptic Biofilm to Improve Micro-Environment for Treatment of Post-Traumatic Refractory Wound

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Abstract

To investigate the effect of aseptic biofilm for improving the treatment of refractory post-traumatic wounds. From January 2020 to December 2021, 40 eligible patients with Refractory Post-traumatic wounds were admitted to the Department of Burn and Plastic Surgery of the First Affiliated Hospital of Soochow University in this retrospective analysis. 40 patients were assigned to the aseptic biofilm group. The aseptic biofilm group’s operation was performed including the debridement, aseptic biofilm covering, and Vacuum-Assisted Closure therapy. Mesh gauze group’s operation was performed including the debridement, mesh gauze covering, and Vacuum-Assisted Closure therapy. Skin grafting or skin flap transplantation was performed according to the condition of the wound. The time required for preparation of the substrate, VAS score during dressing changed, the time required for complete wound healing, the length of hospital stay, and cases with postoperative complications were recorded in this study. In this study, The time to wound healing and preoperative wound substrate preparation were significantly lower in the 20 patients in the aseptic biofilm group than in the petrolatum gauze group (t=-3.196, p<0.05) (t=-2.520, p<0.05). Pain scores during dressing changes were lower in the aseptic biofilm group compared to the mesh gauze group (p <0.05). Aseptic biofilm has the characteristics of temporarily protecting the wound, promoting wound healing, shortening the time of preparing for operations.

Keywords: Aseptic Biofilm; Porcine collagen-based dressing; Refractory post-traumatic wound; Trauma; Wound healing

Introduction

Skin flap transplantation and skin grafting have been used for the treatment of refractory post-traumatic wounds. However, the wound area is usually large, and simple skin flap transplantation cannot completely restore the wound, and there are risks such as microcirculatory disorder, infection, and necrosis of the skin flap. Free skin grafting puts forward high requirements on the substrate. Still, free skin grafting has risks of skin infection and necrosis. Wound healing theory holds that a ‘moist, sterile, hypoxia environment, no effusion’ can accelerate wound healing [1]. Aseptic Biofilm, as a new type of biological material to protect the stabilization of the micro-environment and to accelerate wound healing, is widely used in the treatment for deep burn wounds [2], but the treatment of post-traumatic refractory wounds is not reported domestically. In this paper, a retrospective analysis was performed on the comparison of wound repair in 20 patients with Aseptic Biofilm and 20 patients with mesh gauze in our hospital from January 2020 to December 2021. The clinical effect evaluation was conducted to improve the wound micro-environment and
promote post-traumatic refractory wound healing. The current reports are as follows:

**Materials and Methods**

**General Aspects**

A total of 40 patients with refractory post-traumatic wounds were hospitalized in our hospital from January 2019 to December 2020, including 26 males and 14 females. The mechanisms of the injury were traffic accident injury in 21 cases, bruise injury caused by heavy objects in 6 cases, high fall injury in 7 cases, and machine crush injury in 6 cases. According to the randomized clinical trial principle, there were 12 males and 8 females in the Aseptic bioprotective membrane group, with an average age of (49.73±11.91) years. In the mesh gauze group, there were 14 males and 6 females, with an average age of (47.14±10.74) years. The wound area of the patient was 4cm×5cm ~ 10cm×20cm. Inclusion criteria: 1) age from 16 to 75. 2) Full-thickness defect caused by trauma. If multiple wounds meet the test requirements at the same time, one of the wounds shall be designated to participate in the test; 3) Participants are required to voluntarily participate in the study and sign for operation. Exclusion criteria: 1) Patients with vasoergic diseases; 2) Allergy to porcine protein products; 3) Patients with tumor or uncontrolled severe diabetes; 4) Patients with severe systemic infection and septic shock; 5) Women who are pregnant or planning to become pregnant or breast-feeding; 6) Participants in other clinical trials within the last 6 months; 7) Other conditions that the researcher considers inappropriate to participate in this study. Exclusion criteria: 1) Cases that were mistakenly included; 2) Cases that change other dressings and biomaterials for other reasons during the test, affecting the judgment of the test; 3) No records have been made.

**Main Sources of Materials**

Aseptic Biofilm is an acellular porcine peritoneum (trade name: Dermalgen) produced by Guangdong Guanhao Biotech Co. Ltd. Mesh gauze was purchased from Shaoxing Zhende Medical Dressing Co. LTD.

**Treatment methods**

**Preoperative preparation:** Preoperative preparation including blood routine, blood biochemical testing, blood infectious indicators, bacterial culture of the substrate, and antimicrobial susceptibility test was performed. The transparent square paper was used to draw the shape of the wound, and Adobe Photoshop CC2017 was used to estimate the wound area. The general condition of the patients was comprehensively evaluated, antibiotics were applied according to the results of bacterial culture, and symptomatic treatment such as anti-infection and nutritional support was given according to the results of laboratory tests. Preoperative dressing change was performed according to wound condition. As the aspect of nursing, the propaganda for patients includes quitting smoking and drinking, controlling Postprandial Blood Glucose within the normal range.

**Surgical treatment:** After the preoperative examination was normal, the patient underwent debridement and Aseptic Biofilm transplantation. After anesthesia and draping is complete, necrotic tissue was removed and the active tissue was retained as far as possible. Routine hemostasis was performed. The wound was repeatedly washed with 3% hydrogen peroxide solution, 0.5% iodophor solution, and normal saline 3 times each, followed by electrocoagulation and compression with warm saline gauze. The Aseptic Biofilm group cleaned the Biofilm with normal saline three times, then covered the wound surface and stitched it with the surrounding skin. The mesh gauze group was covered with mesh gauze, and the wound surface of the two groups was covered with vacuum-assisted closure material (V.A.C, KCI Company). Continuous negative pressure sealing drainage was performed, and the pressure was adjusted from -100mmHg to -125mmHg. One week after surgery, the vacuum-assisted closure therapy material was removed, and skin grafting or flap transplantation was performed according to the condition of the substrate. If the granulation tissue of the substrate was not fresh, the patients in the Aseptic biological protective membrane group were covered with the Aseptic biological protective membrane for another week. After removing the vacuum-assisted closure material, the mesh gauze group was treated with a conventional dressing change. If the basal granulation tissue of the wound surface was not fresh, the conventional dressing change was continued. After the basal granulation tissue was fresh, free skin transplantation or skin flap transplantation was performed. One week after free skin transplantation or flap transplantation, the vacuum-assisted closure material was removed and the skin grafting area or flap healing was observed immediately after the removal.

**Evaluation Indicators**

The wound healing time, the proportion of wound healing, the preparation time of the wound base, the pain scores during dressing change (Visual Analog Scoring), the length of hospital stay, and postoperative complications were observed.

**Main evaluation indexes:** Wound healing time: defined as the time it takes for the epithelialization area of the wound to reach 95%, and the degree of wound epithelialization needs to be observed by observational methods [3][3]. The preparation time of wound base refers to the time from the patient’s first debridement or the transplantation of Aseptic Biofilm to skin grafting and flap transplantation.

**Secondary evaluation indexes:** The proportion of wound healing: cases that wound healing after free skin transplantation or skin flap transplantation in 2 groups; the proportion of patients without
secondary debridement, free skin transplantation, or skin flap transplantation in the total cases in each group. Wound pain scores: After dressing was changed, the patients were evaluated by the Visual Analog Scale (VAS).

**Adverse events:** Postoperative complications include wound infection after free skin grafting or necrosis after skin flap transplantation. Wound infection manifests as severe redness, swelling, heat, and pain accompanied by a peculiar smell and purulent exudation during the week of the wound. Positive bacterial culture results on the wound are the standard.

**Statistical Methods**

SPSS 20.0 statistical software was used for analysis, and the measured data were represented as $\bar{x}\pm s$. The mean value comparison of measurement data between the two groups was performed by analysis of variance T-test, and counting data was performed by Chi-square test. $p<0.05$ was statistically significant.

**Results**

**Comparison of General Conditions of Patients**

There was no significant difference in general data between the 2 groups ($p>0.05$), See Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Gender (case)</th>
<th>Age $(\text{year, } \bar{x}\pm s)$</th>
<th>Estimated wound area $(\text{cm}^2, \bar{x}\pm s)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Aseptic Biofilm</td>
<td>20</td>
<td>14</td>
<td>6</td>
<td>51.80±16.16</td>
</tr>
<tr>
<td>Mesh, gauze group,</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>49.95±15.83</td>
</tr>
<tr>
<td>Statistics</td>
<td></td>
<td></td>
<td></td>
<td>$c^2=0.440$</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td>0.507</td>
</tr>
</tbody>
</table>

Table 1: General data of refractory post-traumatic wounds between 2 groups ($\bar{x}\pm s$).

**Condition of Wound Healing**

The preparation time of the wound base of the 20 patients in the sterile biologic preservation film group was 7-14 days, with an average of $(9.00\pm2.83)$ days, which was significantly lower than that of the 20 patients in the mesh gauze group (7-18 days, with an average of $(11.70\pm3.87)$ days) ($t=2.520$, $p<0.05$). The wound healing time of patients in the Aseptic Biofilm group was 14-28 days, with an average of $(18.45\pm5.42)$ days. The wound healing time in the mesh gauze group was 15-34 days, with an average of $(23.75\pm5.06)$ days. There was a significant difference in the wound healing time between the Aseptic Biofilm group and the mesh gauze group ($t=-3.196$, $p<0.05$). The hospital stay in the Aseptic Biofilm group is 13-29 days, with an average of $(21.00\pm5.69)$ days. The length of hospital stay in the mesh gauze group ranged from 17 to 37 days, with an average of $(26.95\pm6.11)$ days, and there was a significant difference ($t=-3.186$, $p<0.05$). The proportion of wound healing was 85% in the Aseptic Biofilm group and 80% in the mesh gauze group. As is shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Preparation time of substrate (day)</th>
<th>Time of Wound healing (day)</th>
<th>Length of hospital stay (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptic Biofilm</td>
<td>9.00±2.83</td>
<td>18.45±5.42</td>
<td>21.00±5.69</td>
</tr>
<tr>
<td>Mesh, gauze group,</td>
<td>11.70±3.87</td>
<td>23.75±5.06</td>
<td>26.95±6.11</td>
</tr>
<tr>
<td>$t$ value</td>
<td>$-2.520$</td>
<td>$-3.196$</td>
<td>$-3.186$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
</tr>
</tbody>
</table>

Table 2: Post-traumatic refractory wound healing ($\bar{x}\pm s$).
Pain Score of Dressing Change

The pain score of 20 patients in the experimental group was (3.80±1.20) points during dressing change. Most patients had mild pain during dressing change and could tolerate the pain, which was significantly lower than that of 20 patients in the control group (5.05±1.15) points during dressing change (p <0.05).

Adverse Events

Among the 20 cases in the experimental group, 17 patients had wound healing after one-time skin grafting and skin flap transplantation after wound preparation, 2 patients had wound infection and 1 patient had partial necrosis of the wound flap, and the wounds in the above cases were healed after dressing changes. Among the 20 cases in the control group, 16 patients had wound healing after one-time skin grafting and skin flap transplantation, 2 patients had wound infection and 2 patients had partial necrosis in the skin graft area. The wounds in these cases healed after multiple dressing changes, and there was no statistical significance in adverse events between the two groups. (p > 0.05)

Typical Cases

Case 1: A 37-year-old female was admitted to the hospital for 3 days due to a car accident that caused a full-thickness skin defect of the left foot. Admission diagnosis: left foot skin avulsion with skin defect. Irregular skin defect of 11cm×7cm on dorsalis was estimated to be 70cm². Necrotic skin was observed during the trauma and at the heel. The wound cavity was poorly healed, and dark red bloody exudation could be accumulated in the cavity (Figure 1A). On the third day after admission, ‘left foot debridement + Aseptic Biofilm covering + vacuum-assisted closure therapy’ was performed (Figure 1B-1C). On the 7th day after surgery, the vacuum-assisted closure material and the Aseptic Biofilm were removed. After the granulation tissue of the substrate grew well (Figure 1D), ‘left foot debridement + free skin transplantation of the left foot + negative pressure closed wound drainage’ was performed (Figure 1E), vacuum-assisted closure material was removed on the fifth day after the second surgery, and the skin graft healed well. The patient was discharged 14 days after skin grafting (Figure 1F). As is shown in Figure 1.

Figure 1: Case 1 1A. Wound condition before debridement; 1B. The substrate after debridement; 1C. The wound was covered with Aseptic Biofilm; 1D. On the 7th day after the first surgery, the vacuum-assisted closure therapy material and Aseptic Biofilm were removed, and the granulation tissue on the substrate was fresh; 1E. Wound surface of the left foot after skin grafting; 1F. The wound healed 14 days after skin grafting.

Case 2: A 65-year-old male patient was admitted to the hospital with skin necrosis of the anterior medial left lower leg due to injuries sustained in a car accident. Admission diagnosis: skin necrosis of the left lower limb. A 15 cm x 7 cm irregular skin defect could be found on the dorsal surface of the foot, with marked peri-invasive redness and swelling, and a small accumulation of purulent exudate at the substrate. The estimated area of the wound is 97cm² (Figure 2A). On the third day after admission, ‘left foot debridement + Aseptic Biofilm covering + vacuum-assisted closure therapy’ was performed (Figures 2B-2C). On the 7th day after the debridement, the vacuum-assisted closure therapy material and the Aseptic Biofilm were removed, and good granulation tissue growth was seen on the surface of the left calf (Figure 2D). The vacuum-assisted closure therapy material was removed on the 6th day after the second operation and the skin graft healed well. As is shown in Figure 2.
Discussion

Severe injuries, such as traffic injuries, smashing injuries, crush injuries, and shock and explosion injuries, often cause damage to deep tissues, such as muscles, tendons, nerves, blood vessels, bones, joints, and even organs, in addition to skin injury and necrosis [4]. After acute trauma, related factors of the wound microenvironment affect the regulation of wound inflammation, angiogenesis, and fibrous tissue proliferation, and then affect wound healing. Wound microenvironment refers to the composite of physical, biochemical, cellular, and other factors affecting wound healing in the wound area and adjacent area, which can be divided into external microenvironment and internal microenvironment [5]. The external microenvironment comprises temperature, humidity, tension, pH, and other factors, while the internal microenvironment includes ECM and cellular factors. The effect of different reasons on wound healing is finally reflected in the change of internal microenvironment. The essence of regulating the wound microenvironment is to directly or indirectly improve the internal microenvironment through materials, drugs, physical therapy, and other different methods, and regulate the behavior of immune and non-immune cells in biochemistry, mechanical force, spatial structure, and other aspects, thus affecting the wound healing process [6]. Microenvironmental regulation is the only way to promote wound regeneration and repair [7]. Due to the poor condition of the wound base, the treatment of deep tissue injury is very difficult. These wounds can become chronic under the influence of infection and other factors, thus increasing the difficulty of treatment. Conventional skin grafting and skin flap transplantation after large area trauma are highly demanding, and postoperative complications such as microcirculatory disorder, infection, and necrosis may occur. And then cause an increase in the occurrence of post-traumatic wounds.

With the development of material science, the emergence of temporary covering plays an important role in refractory wound repair. Porcine-derived biological dressings showed good histocompatibility among many heterogeneous biological dressings. Jardelino, C. (2010), et al [8] manifested that porcine peritoneum after a series of sterile treatments could provide a good scaffold for the growth of wound granulation. Porcine peritoneum collagen membrane was biocompatible and bioabsorbable within 3 weeks after the implantation into mouse subcutaneous tissue. Porcine peritoneum biological dressing could promote the reconstruction of wound blood vessels and has a wide field of application with good prospects [9]. Since it was first reported in 2001, porcine-derived biological dressings have been widely used to promote wound healing and repair damaged human tissues [10,11], with a wide range of applications and mature technology.

Porcine collagen-based dressing causes platelet adhesion and then activate platelets to release various proteolytic enzymes the secretion of various cytokines, stimulates white blood cell chemotaxis, promotes such as transforming growth factor (TGF-beta), platelet-derived growth factor (PDGF), and other cytokines, thus inducing the migration of vascular endothelium and promoting the vascularization [12,13]. Chen X et al. (2020) proved that the porcine collagen biological dressing membrane regulates the wound microenvironment by promoting capillary hyperplasia, affecting oxygen concentration in the wound microenvironment, regulating the regulatory function of miRNAs in ESCs, and other
ways to provide appropriate conditions for wound healing [14].

As the collagen-based dressing is prepared from pig visceral membrane, the Aseptic Biofilm can effectively fit the wound surface, seal the wound surface and relieve pain. In terms of anatomy, the porcine peritoneum is composed of mesenchymal and elastic fiber, thin and smooth surface, parietal peritoneum is translucent, developed from mesoderm of the body wall. Immunogenicity is lower than that of acellular dermal matrix materials. Depending on the semi-permeability of the porcine visceral membrane, it can prevent the loss of protein and water on the wound. When there is a large amount of exudate and secretions on the wound, porcine peritoneal material becomes moist to regulate the gas-liquid exchange inside and outside the wound and reduce exudation [15], and the exudate on the wound is discharged through osmosis or evaporation. In addition, dressings made of porcine peritoneal have sufficient sources, a simple preparation process, low requirements for equipment conditions, and are convenient for utilization, disinfection, and storage. The Aseptic biofilm can promote wound healing by maintaining wound pH value, oxygen concentration, temperature, and material exchange conditions as new biological material. In addition, it can also improve wound blood transport and relieve hypertrophic scar.

The aseptic biological protective membrane is usually used for wound repair after burns. Guo Yun, et al. (2013) proved that Aseptic biofilm can control infection, reduce exudation and promote wound healing through clinical observation of 24 pediatric deep Ⅱ° burn wounds covered with Aseptic biofilm [2]. Cao Shengjun, et al. (2012) pointed out that covering deep Ⅱ° burn wounds with Aseptic biofilm can reduce wound exudation and relieve pain [16]. However, the application of an aseptic biological protective membrane on refractory wounds after trauma has not been reported. Among the 20 patients with refractory post-injury wounds in this group, the patients using an Aseptic biofilm had shorter wound healing times, higher one-time wound healing rates, and less pain during dressing change than those in the mesh gauze group. To a certain extent, the use of Aseptic biofilm can improve the wound microenvironment, reduce the pain of dressing change, shorten the time of wound preparation, improve the success rate of one-time skin grafting and skin flap transplantation, and relatively promote wound healing, shorten the course of the disease and reduce the length of hospital stay.

Conclusions

In this study, the aseptic biological trauma membrane has the characteristics of temporarily protecting the wound, promoting wound healing, shortening the preparation time of the wound base, alleviating the wound pain, and improving the wound microenvironment. It is an effective new biomaterial for the treatment of refractory post-trauma wounds and is worthy of clinical application.

References