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EFFECTS OF POLYHEXAMETHYLENE BIGUANIDE ON INFECTED WOUND BED: HISTOLOGICAL

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ABSTRACT

Objective: *Evaluation of the effect of PBH (PBH) on histopathological and biofilm change at the infected wound bed.*

Subjects and methods: *Fifteen infected wounds of 15 inpatients at the Wound Healing Center - National Burn Hospital, from July to August 2024. The wounds were washed by PBH. Evaluation of histological progression on H&E-stained slides and Biofilm on the wound bed using scanning electron microscopy (SEM), at the time before (T0), after 7 days (T1) and after 14 days (T2) using PBH.*

Results: *The extracellular matrix structure at the wound site showed reduced inflammation, with a statistically significant decrease in the number of inflammatory cells at times T1 and T2. The proportion of wounds with biofilm decreased from 50% at T0 to 20.8% at time T1 and 8.3% at time T2. The biofilm structure was disrupted and eliminated at times T1 and T2 when observed under SEM.*

Conclusion: *PBH effectively eliminates bacteria, disrupts biofilm, and promotes wound healing.*

Keywords: *Infected Wound, Polyhexamethylene Biguanide, Bacteria, Biofilm*

1. INTRODUCTION

Microorganisms can exist harmlessly in wounds, but their growth may lead to infection, localized tissue damage, and impede wound healing. Wound infections can become more severe due to the development of biofilms. The prevalence of chronic wounds associated with biofilms is estimated to be approximately 78% [1]. Biofilms also contribute to the increasing problem of antibiotic resistance. Preventive measures, such as the rational use of antibiotics, infection prevention, and control, are crucial to addressing this threat. Removing biofilms from wound surfaces has been proven to significantly promote wound healing [2].

To effectively remove necrotic tissue and address biofilms at wound sites, a combination of debridement techniques and wound cleaning/irrigation is required [3]. For infected wounds or wounds at high

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risk of infection, wound irrigation solutions containing antimicrobial agents are particularly beneficial. Commonly used agents include hypochlorite, hypochlorous acid (HOCl), povidone-iodine, and Polyhexamethylene biguanide.

Notably, Polyhexamethylene biguanide is a polymer widely used as a disinfectant and antiseptic in various industries, including wound care. It exhibits low toxicity and is effective against a wide range of microorganisms, including bacteria, viruses, and fungi [4]. However, to date, there have been no systematic studies in Vietnam on the effects of Polyhexamethylene biguanide on infected wounds.

Based on this rationale, we conducted this study with the aim of evaluating the histological and biofilm changes at infected wound sites after the application of Polyhexamethylene biguanide.

2. SUBJECTS AND METHODS

2.1. Subject

Fifteen infection wounds from 15 patients aged over 18 years were hospitalized at the Wound Healing Center of the National Burn Hospital from July 2024 to August 2024.

2.1.1. Inclusion criteria

Patients over 18 years old with infection wounds.

2.1.2. Exclusion Criteria

Patients with any of the following conditions were excluded from the study: Patients in critical condition require emergency interventions; Patients with coagulation or bleeding disorders; Patients with infectious diseases such as HCV or HIV.

2.2. Materials and equipment

Including PBH, produced by Biopro Biopharmaceutical Joint Stock Company, Vietnam. JEM 1400 electron microscope by JEOL, manufactured in Japan.

2.3. Methods

2.3.1. Study design

A case-control study, with longitudinal clinical trials comparing pre- and post-treatment outcomes.

2.3.2. Research method using PBH

Patients enrolled in the study underwent wound culture testing, and those with positive culture results were included in the study. During dressing changes, the wounds were treated according to the following protocol: Clean the wound surface with a Natriclorid 0.9% solution to remove all medication and debris. Rinse the wound again with PBH solution. For wounds with biofilm, after rinsing with PBH solution, apply gauze soaked with PBH solution for 15 - 20 minutes until the biofilm softens and dissolves. Rinse the wound once more with PBH solution. Finally, apply iodine-containing dressings and cover the wound. This process was repeated at each subsequent dressing change until the wound healed naturally or surgical intervention was indicated to close the wound.

2.3.3. Study time points

Time T0: Before using PBH; T1: After using PBH for 7 days; T2: After using PBH for 14 days.

2.3.4. Clinical study

All patients were assessed for age; gender; co-mobilities; cause, location, and

duration of the wound. Characteristics of the Biofilm membrane were identified: Observation and description of Biofilm membrane characteristics including color and size (covering the entire or a part of the wound surface).

2.3.6. Morphological study

- Histopathological identification of the wound tissue and inflammatory cell count on H&E-stained slides: Tissue biopsy at the VT site, preparation of H&E-stained slides, and identification of histological images using a Carl Zeiss optical microscope manufactured in Germany, with objective lenses magnified at 100x, 200x, and 400x.

- To determine the number of inflammatory cells per 1 area unit: When observing H&E-stained slides, count the total number of inflammatory cells in a field of view at 400x magnification. Inflammatory cells are counted on six fields of view in six different locations. The total number of inflammatory cells from all six fields was added and divided by six. This provides the average number of inflammatory cells per field of view. This task is carried out at the Department of Anapathology, Military Hospital 103.

- Determination of Biofilm Ultrastructure on the Wound Surface Using Scanning Electron Microscopy (SEM): Samples were prepared and analyzed using the JEM 1400 electron microscope (manufactured by JEOL, Japan). This task was carried out at the Morphology Department, Institute 69, President Ho Chi Minh Mausoleum Protection Command.

2.3.7. Data analysis

Data Analysis: The collected data were compared before and after treatment. At a 95% confidence level, the comparison was considered statistically significant when $p < 0.05$. Data were analyzed by STATA 12.0 software.

3. RESULTS

3.1. Characteristics of study patients

Table 3.1. Characteristics of patients and wounds (n = 15)

Feature	X ± SD	Min - Max
Age (year)	56.4 ± 13.6	28 - 76
Wound size (cm^2)	75.6 ± 49.5	10 - 110
Duration of wound existence (month)	2.37 ± 1.45	0.5 - 4
	N	%
Gender		
Male	9	60
Female	6	40
Wound position (n = 15 wounds)		
Upper extremity	2	13.3
Lower extremity	13	86.7

The study patients had a male-to-female ratio of 1.5, with an average age of 56.4 ± 13.6 years. The wound area was relatively large, averaging 75.6 ± 49.5 cm^2.

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The wounds had an average duration of 2.37 ± 1.45 months. The main causes of the wounds included cellulitis (54%), diabetes (33%), and other causes such as gout and Cushing's syndrome, accounting for 13% (Chart 3.1). Wounds on the lower extremity were the most common, comprising 86.7%, while 13.3% were located on the upper extremity.

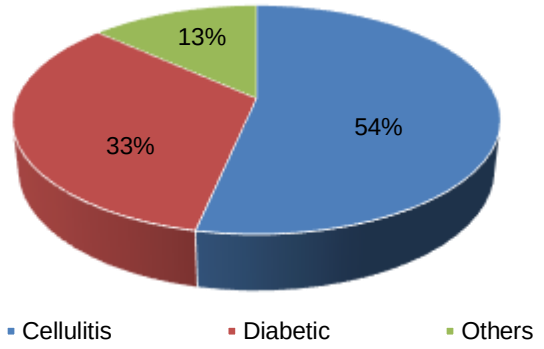

Chart 3.1. Distribution of patients by cause

3.2. Histological changes after using PBH

Table 3.2. Histological Changes after using Latex HB® (n=15)

Time	Histological feature	
T0	Feature	The entire skin layer was damaged. The subcutaneous connective tissue showed inflammatory edema. Intercellular spaces had numerous inflammatory cells (primarily neutrophils and lymphocytes - Figure 3.1A).
	Number of inflammatory cells	16.3 ± 2.7/ area unit
T1	Feature	The wound bed was connective tissue of the dermis layer, with many collagen fibers and fibroblasts. Scattered infiltration of neutrophils and lymphocytes was observed. The spinous cells proliferated from the wound edges to the wound bed center (Figure 3.1B).
	Number of inflammatory cells	8.2 ± 1.6/ area unit
T2	Feature	The proliferation of the extracellular matrix (ECM) was evident as the proliferation of numerous fibroblasts and myofibroblasts. Only a few neutrophils, lymphocytes, and macrophages remained (Figure 3.1C)
	Number of inflammatory cells	4.9 ± 1.5/ area unit
P		$P_{0-1} < 0.05$; $P_{0-2} < 0.001$; $P_{1-2} < 0.05$

After using Latex HB, the wound histology showed significant improvement, and the number of inflammatory cells was statistically reduced compared to before using Latex HB.

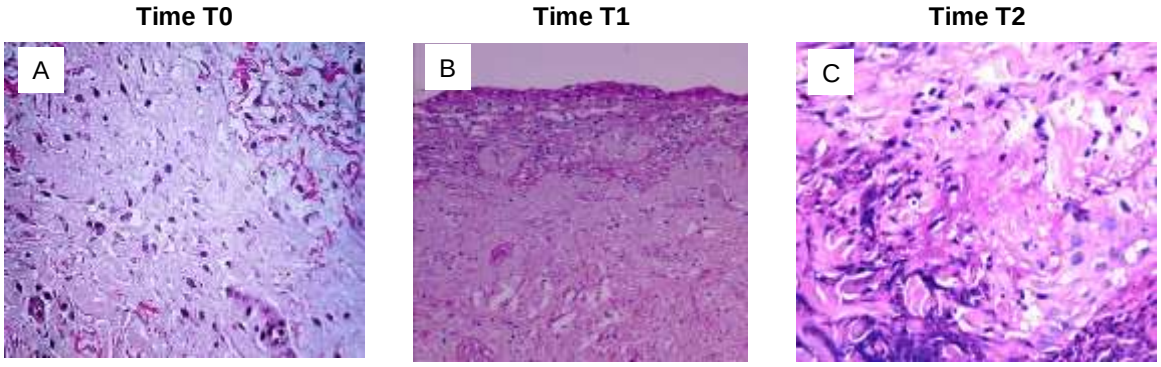

Figure 3.1. Histological characteristics on H&E-stained slides of the wound at different study time points observed under 100x, 200x and 400x magnifications

3.3. Changes in the Biofilm Membrane

Table 3.3. Clinical characteristics of the Biofilm membrane after using PBH (n = 12)

Time / Feature	T0 (n = 12) n (%)	T1 (n = 5) n (%)	T2 (n = 2) n (%)
Color			
- Green	4 (33.3)	1 (20)	1 (50)
- Yellow	7(58.3)	3 (60)	1 (50)
- Yellow + Green	1 (8.3)	1 (20)	
Size			
- Part of the wound surface	4 (33.3)	3 (60)	2 (100)
- Entire wound surface	8 (66.6)	2 (40)	-

At time T0, there were 12 wounds with Biofilm membranes, predominantly yellow, accounting for 58.3%. The second most common color was green (33.3%). 8.3% of the wounds had biofilm membranes with a combination of yellow and green.

Regarding the macroscopic morphology of the Biofilm membranes, at time T0, 66.6% of the wounds had biofilm membranes covering the entire wound surface. At time T1, 33.3% of wounds had biofilm membranes covering only part of the wound surface. At time T2, Biofilm membranes persisted and covered only a portion of the surface in two wounds.

Observing the biofilm membrane under a scanning electron microscope, the biofilm on the wound surface exhibited various morphologies. At time T0, bacteria were densely colonized, covering the wound surface entirely and protected by a polysaccharide membrane. At time T1, the Biofilm structure was destroyed, and the bacterial density on the wound surface significantly decreased compared to T0, with bacteria forming colonies. At time T2, many tissue samples showed no bacteria, or bacteria appeared only sparsely on the wound surface (Figures 3.2 and 3.3).

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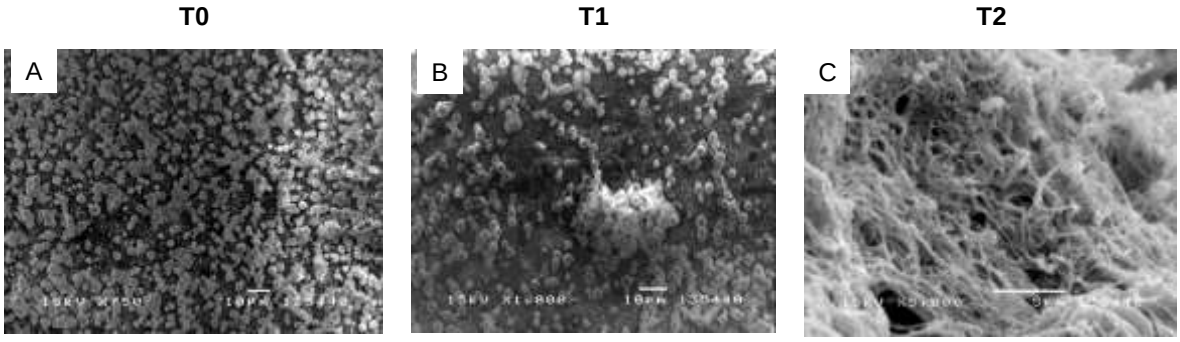

Figure 3.2. Images of the Biofilm Membrane Observed Using Scanning Electron Microscopy (SEM): At time T0, Cocci bacterias were densely distributed, invading the entire wound surface. At time T1, the density of Cocci bacterias decreased compared to time T0, with cocci colonizing and tending to cluster, forming bacterial colonies. By T2, no Biofilm membrane was detected..

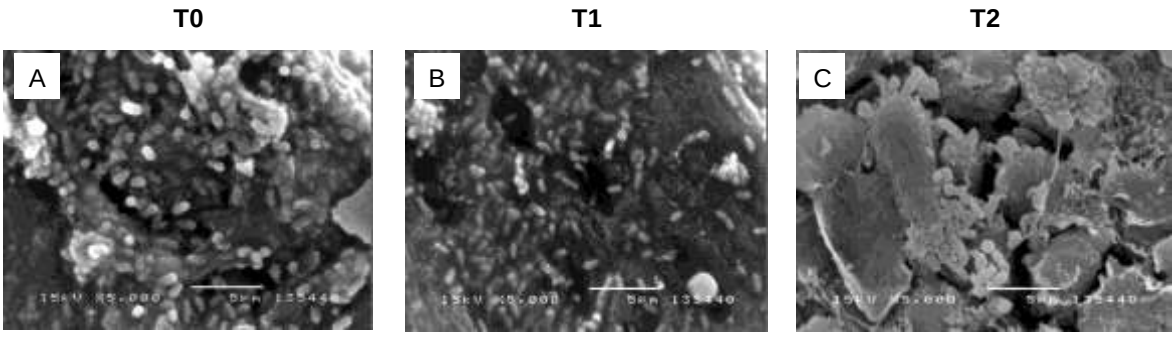

Figure 3.3. Images of the Biofilm Membrane Observed Using Scanning Electron Microscopy (SEM): At time T0, Bacilli were densely distributed and protected by a polysaccharide membrane covering the entire wound surface. At time T1, the Biofilm membrane was disrupted, and the bacilli were aggregated (colony) and still enclosed by the polysaccharide protective membrane, with single bacilli scattered on the outside. At time T2, the wound surface only showed scattered bacilli, with a tendency to cluster together.

4. DISCUSION

In clinical practice, the use of solutions to irrigate wounds aims to reduce infection and remove foreign bodies, cellular debris, or exudates from the wound surface. Sodium chloride 0.9% is recommended for wound irrigation as it is readily available, non-toxic to wound tissue, does not interfere with the wound healing process, and is cost-effective [5].

Additionally, the market currently offers various solutions containing antimicrobial agents that are highly effective in wound care, such as sodium hypochlorite, hypochlorous acid, povidone-iodine, and PBH. PBH is recommended for wound care due to its affordability and availability. Experimental evidence has demonstrated the antimicrobial effects of PBH against a wide range of microorganisms, including

Gram-negative and Gram-positive bacteria, pathogenic fungi, viruses, and protozoa [6].

Several clinical studies have reported that PBH solutions effectively reduce bacterial load in various types of chronic wounds (venous ulcers, diabetic foot ulcers, pressure ulcers) [7] as well as acute wounds (burns, surgical wounds, trauma wounds, skin graft wounds) [8]. Furthermore, when PBH is used in combination with negative pressure wound therapy, it has shown synergistic effects in treating infected wounds [9].

In addition to its antimicrobial properties and ability to eliminate surface bacteria from wounds, some studies suggest that applying dressings impregnated with PBH can improve wound healing [10]. Our research findings align with these observations. When wounds were irrigated and soaked with PBH solution, histological analysis of H&E-stained specimens showed progressive improvements over time, including reduced connective tissue inflammation, increased fibroblast proliferation, and decreased inflammatory cell counts (Table 3.2).

Biofilms are quite common and are one of the primary causes of delayed wound healing. The effectiveness of PBH against biofilms has been demonstrated in several experimental studies. By impregnating dressings with PBH and applying them to wound surfaces, Bazire A et al showed that biofilms were disrupted by betaine which was a surfactant component in PBH [11]. Mueller SW et al reported that PBH effectively eliminates methicillin-resistant Staphylococcus aureus (MRSA) [12].

Chai et al studied the effects of PBH on diabetic foot ulcers infected with drug-resistant Pseudomonas. They observed a significant reduction in bacterial load in the wounds after using PBH [10]. PBH's ability to disrupt biofilms formed by drug-resistant bacteria is achieved by promoting the transfer of antibiotic resistance genes among bacterial species [13].

In this study, we found that PBH was effective well both cocci and bacilli. At T0, bacteria were present in high density and were protected by a polysaccharide membrane covering the entire wound surface. After 7 days of using PBH (T1), the biofilm was disrupted; cocci and bacilli aggregated into colonies and were surrounded by a protective polysaccharide membrane. At time T2, many tissue samples showed no bacteria, or bacteria appeared only sparsely on the wound surface (Figures 3.2 and 3.3).

5. CONCLUSION

PBH effectively eliminates bacteria, disrupts biofilm, and promotes wound healing.

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