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Macroscopic and microbiological changes in stage 4 pressure injuries after the use of polyhexanide

Alterações macroscópicas e microbiológicas em lesões por pressão estágio 4 após o uso da polihexanida

Alteraciones macroscópicas y microbiológicas en lesiones por presión nivel 4 después del uso de la polihexanida

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ABSTRACT

Objective: To describe improvement of healing, microbiological changes and presence of biofilm in stage 4 pressure injuries (PI) in patients with spinal cord injury, after the use of polyhexanide. **Methods:** A "before and after" study realized with five PI patients. Photographic records were collected, microbiological samples of the wound were collected before and after the use of polyhexanide with swab and irrigation-aspiration technique, and biopsy tissue was collected for light microscopy and scanning electron microscopy (SEM) analysis. **Results:** Wound healing improved. Regarding the microbiological analyses, it was observed a reduction of the number of colonies of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and a high number of *Acinetobacter baumannii complex* after the use of polyhexanide. SEM showed presence of biofilm in the wound in all samples, even after the use of the product. **Conclusion:** Further studies are necessary to confirm the benefit of the polyhexanide use.

DESCRIPTORS: Pressure injury; Polyhexanide; Biofilm; Stomatherapy.

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RESUMO

Objetivo: Descrever a melhora da cicatrização, as alterações microbiológicas e a presença do biofilme em lesões por pressão (LP) estágio 4, em pacientes com lesão medular, após o uso da polihexanida. **Métodos:** Estudo "antes e depois" realizado com cinco pacientes com LP. Foram realizados registros fotográficos, coletadas amostras microbiológicas da ferida, antes e após o uso da polihexanida com técnica de *swab* e irrigação-aspiração, e colhidos tecidos para biópsia para análise por microscopia de luz e microscopia eletrônica de varredura (MEV). **Resultados:** As feridas apresentaram melhora da cicatrização. Quanto às análises microbiológicas, observou-se a redução do número de colônias de *Pseudomonas aeruginosa* e *Staphylococcus aureus* e um maior número de casos de *Acinetobacter baumannii complex* após o uso da polihexanida. A MEV demonstrou a presença de biofilme na ferida em todas as amostras, mesmo após o uso do produto. **Conclusão:** Novos estudos são necessários para confirmar o benefício do uso da polihexanida.

DESCRITORES: Lesão por pressão; Polihexanida; Biofilme; Estomaterapia.

RESUMEN

Objetivo: Describir la mejora de la cicatrización, las alteraciones microbiológicas y la presencia del biofilm en lesiones por presión (LP) nivel 4, en pacientes con lesión medular, después el uso de la polihexanida. **Métodos:** Estudio "antes y después" realizado con cinco pacientes con LP. Fueron realizados registros fotográficos, se recolectaron muestras microbiológicas de la herida, antes y después el uso de la polihexanida con técnica de *swab* y de irrigación-aspiración, y se recolectaron tejidos para biopsia para análisis por microscopía de luz y microscopía electrónica de barrido (MEB). **Resultados:** Las heridas presentaron mejora en la cicatrización. Sobre los análisis microbiológicos, se observó la reducción del número de colonias de *Seudomonas aeruginosa* y *Staphylococcus aureus* y un mayor número de casos de *Acinetobacter baumannii complex* después del uso de la polihexanida. La MEB demostró la presencia de biofilm en la herida en todas las muestras, incluso después del uso del producto. **Conclusión:** Son necesarios nuevos estudios para confirmar el beneficio del uso de la polihexanida.

DESCRIPTORES: Lesión por presión; Polihexanida; Biofilm; Estomaterapia.

INTRODUCTION

The traumatic injury of the spinal cord causes important neurological changes that put the individual at risk of development of pressure injury (PI) throughout life. Several factors are involved in the appearance and chronicity of the wound, including physiological, microbiological, social, economic, educational and behavioral. PIs are chronic wounds that can remain open for long periods, favoring the adhesion and multiplication of bacterial communities[1]. The bacteria that proliferate in the wound are able to develop biofilm and represent a therapeutic challenge for the healing and to promote mechanisms of resistance against the antimicrobial action[1,2]. The presence of biofilm, associated to the high amount and diversity of microorganisms, increases the risk of infection and the delay in healing due to secretion of proteases by the organism [3,4].

The appropriate treatment of infected and severely colonized wounds is to maintain a controlled microbial load and create favorable conditions for healing. The care with PIs begins with cleaning, evaluating the necessity to remove devitalized and necrotic tissues and the presence of bacteria, in order to reduce the risk of infection [5].

Currently, topical antimicrobial and antiseptic substances have been used in the treatment of wounds. It is believed that the use of antiseptics has a low potential for induction of bacterial resistance; its excessive use, however, can bring risks, reducing its effectiveness[6]. Incorrect and indiscriminate use of antimicrobial is a risk factor for the development of microbial resistance, and its adequate indication is important, with laboratory tests to determine the microbiological profile and detection of infection[7].

The solution of polyhexanide has been used for the cleaning of wounds and the reduction of bacterial biofilm, with action against gram-positive, gram-negative bacteria and fungi. Polyhexanide hinders the development of resistant bacteria[8], besides showing good tolerability and therapeutic efficiency [3,9], with better surface pH, but without penetration into deeper layers of the wound[3].

The visual inspection of the wound is often not sufficient to ensure that it is free of infection, and diagnostic tests are required for microbiological examination. Currently, there is no standard technique for microbiological collection and evaluation. Quantitative, semiquantitative and non-quantitative analyses have been realized as attempts to obtain better evaluation[10].

The PI treatment is complex and the therapeutic strategy depends on knowledge and personal experience[11], diverging between the different professionals and, thus, lacking the necessary scientific evidence.

OBJECTIVE

In this context, the present study is inserted, whose objective is to describe the care with the PI in the preoperative period of the surgical treatment, the macroscopic and microbiological changes and the presence of bacterial biofilm, before and after the use of the polyhexanide and topical therapy, according to institutional protocol.

METHODS

The study was realized in a descriptive exploratory way, in a "before and after" investigation, including patients aged between 18 and 65 years, diagnosed with traumatic spinal cord injury (paraplegics and quadriplegics), hospitalized in a rehabilitation hospital with proposal of surgical treatment for PI closure in stage 4, after approval of the Ethics Committee Institution Research (22329113.0.0000.0022). Those who were using systemic antimicrobials to treat PI infection were excluded from the sample. The sample consisted by five patients who were submitted to a surgical intervention of stage 4 PI, from November 2013 to June 2014, and who accepted to participate in the study and signed the Informed Consent Form and authorization for photographic registration. Data were collected by inspection and measurement of the wounds, by culture of lesion material obtained by swab, by irrigation-aspiration in the first 48 hours of admission and on the day of the surgical procedure and by biopsy on the day of surgery. During admission to the clinic, materials were collected for microbiological analysis of the wound bed after cleaning with 0.9% saline solution (SS) and the wounds were kept protected in the bath to avoid contamination. In the ward, the lesions were irrigated daily with a solution composed of 0.1% undecylaminopropyl betaine, 0.1% polyhexanide and 99.8% purified water; indicated coverage was applied according to the evaluation of the wound. Polyhexanide solution was used as it was indicated for cleaning and decontamination of the bed of acute or chronic colonized, critically colonized and infected wounds for removal of coatings and biofilms and for preparation of the wound bed for dressing application. The wounds were cleaned once daily with polyhexanide solution using the spray of the product vial or 20 mL syringe (in the fistulas) until the day of surgery. The time of use of the solution varied due to the individual evaluation of each patient and the appropriate time for surgical intervention, which involved evaluation of the aspect of the wound, adaptation to ventral positioning and control of spasticity. According to the evaluation of the wound, primary coverages were indicated as: oil with essential fatty acids (EFA), hydrogel or calcium alginate and secondary coverage with gauze and transparent film. On the day of surgery, after cleaning the wound with saline solution and collecting microbiological material, the wound was irrigated with polyhexanide solution between 2 and 3 hours before the patient was referred to the surgical block.

For swab collection, cotton-tipped rods were used in the middle of Stuart transport, using the technique of Levine et al.[12], which consists of pressing and rotating the swab on its own axis, on 1 cm^2 of the granulation tissue for 5 seconds in order to express the tissue fluid probably harbour the microorganisms. In order to collect the material by irrigation-aspiration, the wound was cleaned with physiological solution, applying 5 mL of saline solution 0.9% sterile to the injury bed, and 1 mL of the solution was aspirated that remained in wound bed, exercising slight suction on the granulation tissue, with adaptation of the technique described by Ferreira et al[13]. For culture technique and tissue antibiogram, approximately 0.5 cm of tissue diameter was used for culture; for scanning electron microscopy (SEM), samples of approximately 0.5 cm of wound tissue diameter were collected. The electron microscopy technique described by Haddad et al[14] was used and the tissue fragments used for analysis were removed by the plastic surgeon in the trans-operative period; the sites for withdrawal of the sample were indicated by the researchers in the trans-operative period. Samples for microbiological evaluation and SEM were removed from the wound bed at sites of viable granulation tissue with a punch after cleaning the wound with physiological solution and before to surgical degermation and antimicrobial prophylaxis.

The case analyses were performed as a strategy to monitor wound microbiology before and after the use of polyhexanide, in search of evidence to justify the benefits of its use in wound microbiology and in the preparation of the wound bed in the preoperative closure of PI.

The tissue fragments were collected trans-operative and sent for histopathological, microbiological and SEM analysis, in order to evaluate the wound tissue and the bacterial profile change in the wounds, as well as the biofilm presence. The sequence for data collection was performed according to Fig. 1.

RESULTS

All patients were men, and the majority were young (mean age 34 years), single (80%), black colour, paraplegic by firearm projectile (80%), low schooling (80% with elementary school), low income and without formal employment. Of the total sample, 80% presented PI stage 4 in the ischial region, followed by the sacral region (20%), all with indication of surgical closure. Most patients had PI open for more than 3 years (60%) and 60% had PI scars in other regions (ischial, sacral, trochanteric, leg and calcaneum). In the hospital environment, the wounds were cleaned daily with polyhexanide solution, evaluated and treated with EFA, calcium alginate and hydrogel. Analysis of the measurement (Table 1), inspection (Table 2) and photographic record (Fig. 2A and Fig. 2B) showed improvement of wound bed conditions after preoperative care.

Microbiological analyses of the collected material showed that, after topical treatment, there were a reduction in the number of colonies of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and an increase in the cases of *Acinetobacter baumannii complex* (Table 3).

The culture of the tissue fragment withdrawn in the trans-operative was only positive for two samples with growth of *A. baumannii complex, P. mirabilis, S. agalactiae* and *P. aeruginosa* (Table 4).

Histopathological analysis revealed ulceration containing granulation tissue and borders with hyperkeratosis

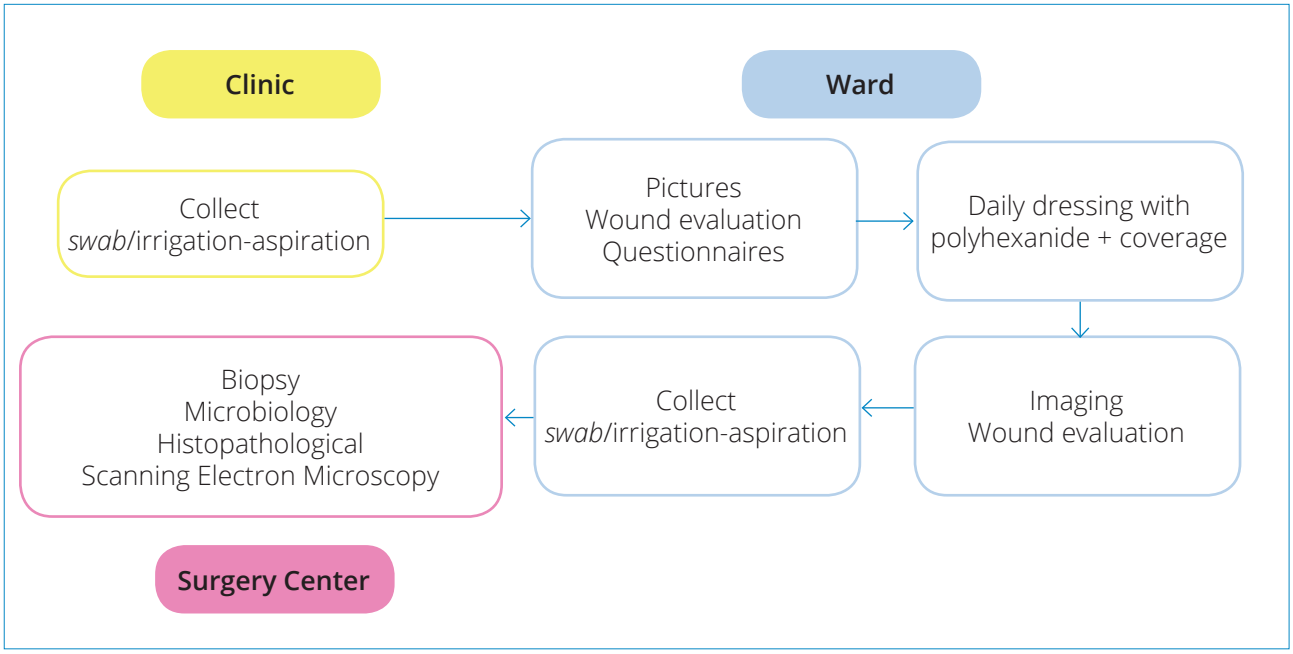

Figure 1. Sequence for data collection.

Table 1. Measurement of wound on admission and after topical treatment with polyhexanide.

Measurement of wound	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	B	A	B	A	B	A	B	A	B	A
Length (cm)	3.5	**3.2**	2.0	2.0	1.5	1.5	4.5	**4.0**	5.0	**4.0**
Width (cm)	2.5	**2.0**	1.5	1.5	1.0	1.0	3.5	**2.0**	4.0	**3.0**
Area (cm^2)	8.7	**6.4**	3.0	3.0	1.5	1.5	15.7	**8.0**	20	**12**
Depth	4.5	4.5	10	10	1.5	1.5	4.5	5.0	5.5	4.0

In bold, measures that have changed after treatment; B = Before; A = After.

Table 2. Evaluation of the wound and exudate at admission and after the use of polyhexanide in the preoperative pressure lesion associated with daily dressings.

Wound evaluation	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	B	A	B	A	B	A	B	A	B	A
Granulation tissue	×	×	×	×	×	×	×	×	×	×
Shattering	×	×	–	–	×	×	×	×	×	–
Coagulation necrosis	–	–	–	–	–	–	×	×	–	–
Ischemia tissue	–	–	–	–	–	–	×	–	–	–
Hyperkeratosis borders	×	×	×	×	×	×	–	–	–	–
Curling borders	×	×	×	×	×	×	–	–	–	–
Plain borders	–	–	–	–			×	×	×	×
Serous exudate	–	–	×	×	×	×	–	–	×	–
Serosanguineous exudate	–	×	–	–	–	–	–	×		×
Purulent exudate	×	–	–	–	–	–	×	–	–	–
Exudate amount	Medium	Small	Medium	Medium	Small	Small	Large	Small	Medium	Small

× = Presence; – = Absence; B = Before; A = After.

Table 3. Microorganisms found in the sample by swab (S) and irrigation-aspiration (A) on admission and after the use of polyhexanide.

Microrganisms	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
A. complex		S/A1+		S/A1+			A 1+	S 1+	A 1+	S/A1+
E. coli							S/A1+			
Pasteurella multocida	A 1+									
P. aeruginosa					S/A3+	S/A1+	S/A2+	S/A1+	S/A1+	S/A1+
P. mirabilis				S/A1+			S/A2+	S/A1+	S 1+	
S. aureus	A1+	S/A1+				S1+	S/A3+		S/A1+	A1+
S. haemolyticus	S1+									
S. agalactiae			S/A1+	S/A1+	S/A1+	A1+			S/A1+	
S. anginosus							A 3+			
S. dysgalactiae ssp	S/A1+									
S. capitis						A 1+				
S. lugdunensis						A1+				

1+, 2+ and 3+ = semi-quantification in plaque.

and epithelial hyperplasia (Fig. 2C). PI presented fibrosis, inflammatory cells, neovascularization, acanthosis, hemosiderin deposits, giant-cell reaction (Fig. 2D) and gram-positive bacteria (Table 5).

SEM analysis revealed tissue with keratinized regions and exposed fibrous connective tissue, presenting numerous recesses, with presence of coccoid and bacilliform cells, hyphae, structures suggestive of biofilm and inflammatory

response (Table 6).Structures suggestive of bacterial biofilms were observed containing numerous coccoid and bacilliform cells of smaller diameter and length, surrounded by a discrete polymer matrix associated with fibrous substrate (Fig. 2E). Also suggestive formations of bacterial biofilms were observed exhibiting coccoid cells of equal or smaller diameter surrounded by a well-defined polymeric matrix containing granular deposit areas (Fig. 2F).

DISCUSSION

We observed growth of different species of pathogens colonizing the wounds, with growth of microorganisms in the different collection techniques (swab and irrigation-aspiration) and with a reduction in the number of colonies in two cases. Knowing that the removal of the infeasible tissue from the wound reduces the microbiological load we cannot confirm that this reduction in the number of colonies occurred due to the use of the product, since one of the patients underwent debridement of necrotic tissues at the edge of the bed with technique of Slice preoperative and another made use of cefepime for the treatment of bacteriuria by *P. aeruginosa* - the same microorganism present in the wound.

As for the samples obtained by superficial fragment of the viable tissue of the wound, only two showed growth, in one of them the growth of *P. aeruginosa*, even with the use of the antibiotic (cefepime), which confirms the placements of other studies, when reporting that bacteria that grow in biofilm develop defense mechanisms that make them resistant to phagocytosis by cells of the immune system and to the penetration of antibiotics.

We believe that patients with spinal cord injury and PI have a significant risk of being colonized by resistant microorganisms due to their own wound, the use of frequent antimicrobials for urinary tract infection or prophylaxis for urological exams, urinary catheter use and the hospital environment, which provides cross-transmission of multiresistant bacteria.

Hyperkeratosis and keratin contact with connective tissue may cause giant-cell reaction and folliculitis, both were observed in some patients.

SEM showed signs of association between different bacterial genera sharing the same biofilm, which may give them greater resistance[15]. The granular deposits may refer to areas of degradation of the biofilm polymer matrix. This suggests that 0.1% polyhexanide works, but its efficacy is questionable when used in irrigation and wound cleaning for biofilm removal. In the same way, the microorganisms described as being associated with a discrete amount of matrix appear to be unaffected by the product.

In this study, it was possible to demonstrate an improvement in wound healing with topical care and the use of the polyhexanide solution. We cannot say that improvement of the wounds was due only to polyhexanide, but by multiple

Table 4. Culture of tissue fragment after use of polyhexanide.

Microrganisms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Acinetobacter complex	–	5 × 10(2) UFC/mL	-	-	-
Proteus mirabilis	–	6 × 10(2) UFC/mL	-	-	-
Streptococcus agalactiae	–	6 × 10(3) UFC/mL	-	-	-
Pseudomonas aeruginosa	–	-	5 × 10(2) UFC/mL	-	-

CFU = colony forming units.

Table 5. Histopathological and Gram analysis for tissue after the use of polyhexanide.

Data analyzed	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Inflammatory cells	×	×		×	×
Fibrosis	×	×	×	×	×
Neovascularization	×	×	×	×	×
Acanthose	×				×
Hemosiderin Deposits	×			×	×
Giant-cell reaction				×	×
Gram for tissue	Negative	Negative	Negative	Gram-positive coccus	Negative

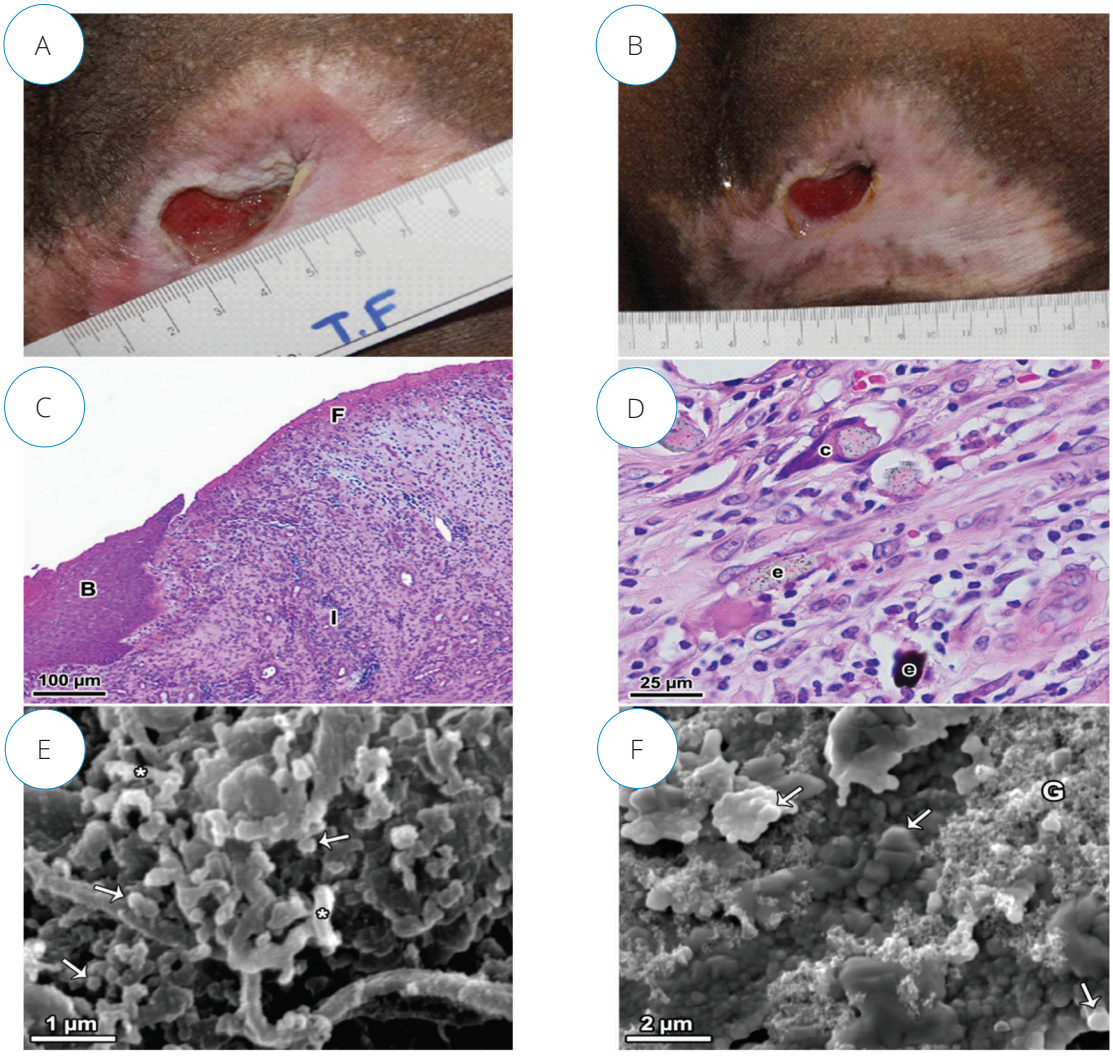

Figure 2. Pressure injury (PI) and the use of polyhexanide, macro and microbiological scales. (A) PI in the left ischial region, on admission, measuring 3.5 cm × 2.5 cm, with granulation tissue, shattering, curling borders and perilesional maceration area. (B) PI in the left ischial region in reduction, after six days of use of the polyhexanide, measuring 3.2 cm × 2 cm, with granulation tissue. (C) Light microscopy showing general histological appearance of the wound: B-border, F-fundus, I-inflamation (HE 100x). (D) Light microscopy showing foreign body reaction: c-giant cell, e-foreign body (HE 400x). (E) SEM showing association between different types of microorganisms. * - baciliform cells, arrows - cocoid cells. (F) SEM showing well-defined matrix biofilm with granular deposits (G) on its surface.

Table 6. Analysis by scanning electron microscopy (SEM) after the use of polyhexanide.

Data analyzed	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Coccoid cells (250 - 500 nm)	×	×	×	×	×
Bacilliform cells (0.5 - 1 µm)		×			×
Hyphae	×				×
Inflammatory cells	×	×			
Biofilm presence	×	×	×	×	×
Polymer Matrix	Discrete	Discrete	Well-defined	Discrete	Discrete
Granular deposit					×

factors, such as the withdrawal of the pressure factor with the adoption of the ventral position; daily cleaning and dressing of wounds; nutritional supplementation; and abandonment of risk habits.

Our discoveries suggest that the protocol established with the use of polyhexanide, the daily performance of dressings, the control of risk factors and pressure relief, promoted the improvement of macroscopic aspect of the wound, change of the microbiological profile and maintenance of the biofilm in the bed of the wound.

CONCLUSION

It is believed that this sample was small and that it is necessary to develop further investigations, looking for evidence on the efficiency of polyhexanide on PI microbiology and on the macroscopic improvement of the wound bed.

AUTHOR'S CONTRIBUTION

Conceptualization, Tabari L and Kamada I; Methodology, Tabari L and Kamada I; Research, Tabari L; Cordeiro BA; Mello MT; Gomes EAP and Brandão ICS; Writing - First version, Tabari L; Kamada I; Cordeiro BA; Mello MT; Gomes EAP and Brandão ICS; Writing - Review & Editing, Tabari L; Kamada I and Cordeiro BA; Acquisition of Financing, Tabari L; Resources, Tabari L; Cordeiro BA; Mello MT; Gomes EAP and Brandão ICS; Supervision, Tabari L; Kamada I and Cordeiro BA.

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