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Experimental Research

The effect of NPWT in wound healing and bacterial count on deep dermal burn injury model: An experimental study

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ABSTRACT

Background: Sepsis is one of the main causes in burn victim's mortality. The use of negative pressure wound therapy (NPWT) provides an ideal environment to accelerate wound healing. We compare the use of normal saline (NS), intermittent NPWT, continuous NPWT and silver sulfadiazine in wound healing process.
Method: This study involved 6 Yorkshire pigs; each pig was induced with 20 burns on the flank area. Burns were divided into 4 treatment groups: NS gauze, intermittent NPWT, continuous NPWT, and silver sulfadiazine dressing. Burns were evaluated on day 1,3,7,14, and 21 for its morphology and bacterial colonization and on day 14 and 21 for the remaining burn surface area.
Result: Wound that received NPWT therapy appeared better in both granulation and crust formation. Remaining burn surface area (mm^2) on day 14 in NS group, intermittent NPWT, continuous NPWT, and silver sulfadiazine were 107.43 ± 83.43, 178.07 ± 74.83, 146.10 ± 69.1, 126.03 ± 83.22, respectively($p = 0.457$); on day 21 in NS group, intermittent NPWT, continuous NPWT, and silver sulfadiazine were 13.16 ± 16.86, 59.49 ± 20.72, 54.79 ± 46.59, 48.95 ± 39.84, respectively(p=0.169). There were no significant differences in each treatment group bacterial colonization(p>0.05). There were no significant correlation between bacterial colonization and remaining burn surface area (p>0.05).
Conclusion: While morphologically, the wound in NPWT treatment groups appeared better in granulation and crust formation, the remaining wound surface area and the number of bacterial colonization were not significantly difference compared to standard therapy (silver sulfadiazine and NS gauze). There were no significant correlation between the amount of bacterial colonization and remaining wound surface area on every treatment group.

1. Background

The leading cause of death in burn patients after initial resuscitation is multiple organ dysfunction syndrome (MODS), which can be caused by a direct response to sepsis [1]. Sepsis contributes to 75% of burn-related mortality, especially in developing countries [2]. Burn damages the first line of defence against microbes, namely the skin and suppresses the immune system. The wider the burn area with avascular necrotic tissue, the more it provides a good growth site for bacteria so that the risk of getting sepsis will increase [2,3].

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Some topical antimicrobials are cytotoxic to keratinocytes and fibroblasts; they can also impair the wound healing process [4]. Deciding the type, concentration, and duration of topical antimicrobials application should be based on the comparison of the risks and consequences of burn infection with the risk of delayed wound healing [5]. Accordingly, this condition results in the need for other wound therapies that can reduce the risk of infection but also have minimal side effects.

Negative pressure wound therapy (NPWT) has been used as a treatment for both acute and chronic wounds for 20 years around the world [5]. NPWT can provide a sterile and closed wound healing environment that can trigger re-epithelialization, increasing blood flow, and nutrients to the burn area [6]. By using a closed wound dressing and providing a sterile environment, it can reduce infectious complications and provide optimal moisture for wound healing [6,7].

This study aimed to compare the effect of normal saline gauze, intermittent NPWT, continuous NPWT, silver sulfadiazine dressing on wound healing and the number of bacterial colonization in deep dermal burn models.

2. Method

This experimental research was conducted at Prof. Soeparwi Veterinary Hospital, Faculty of Veterinary Medicine, Universitas Gadjah Mada. This research had been approved by the ethics committee of Faculty of Medicine, Public Health and Nursing - UGM KE/FK/0729/EC/2019. The population in this study was Yorkshire pigs (*Sus scrofa domesticus*). The inclusion criteria were: healthy male Yorskshire pig aged 2–3 months, well nourished, 10 kg in weight, and no skin continuities disturbance, while the exclusion criteria were: Yorkshire pig that was sick during the seven days of adaptation period, infected by other source of infection, or died during the experiment. In this research we used six Yorkshire pigs (*Sus scrofa domesticus*), then we gave 4 different treatment groups on every pig; the number of pigs was calculated using the degree of freedom sampling method. According to that formula, six pigs were the maximum sample size for our research that could significantly impact our final data analysis result.

Based on a preliminary study, burns in pig were made under general anaesthesia; first, we did IM injection of atropine 0.06/kg, Zoletil® 4.4 mg/kg and xylazine 2.2 mg/kg then followed by endotracheal intubation; pigs were maintained anesthetized by inhalation of isoflurane (0.5–2.5%). Furthermore, flank areas were shaved and disinfected 3 times using povidone iodine, then deep dermal burn injuries were made using a stainless-steel round plate with 20 mm diameter which had been heated to a temperature of 92°C and affixed for 20 seconds with 1 kg pressure on the pig's back [8]. In total we created 120 burns on six pigs. To prevent bias, we made 20 burns with 4 different treatments in one pig (Fig. 1) to make sure that every treatment was performed under the same environment. Those burns were divided into four treatment groups, namely using normal saline gauze, intermittent NPWT, continuous NPWT, and silver sulfadiazine dressing. Normal saline gauze treatment group was considered as a negative control and silver sulfadiazine dressing was considered as a positive control. Intermittent and continuous NPWT treatment groups were considered as variable treatment groups. There were 6 samples in every treatment group that were assessed 5 times during this study (day 1,3,7,14 and 21). We would swabbed and sutured the wound each time we finished assessing the wounds on the specific day, thereby we made 120 burn wounds.

The maximum pressure used on the NPWT was −125 mmHg [9]. For the intermittent NPWT, the machine was set to stay on for 3 minutes and off for 9 minutes (V.A.C Original Veraflow® by KCI-USA). The wounds were evaluated on day 1, 3, 7, 14 and 21 following the burn injury. During the evaluation of the wounds, the picture of wound morphology was taken, measured, and transformed from pixels to mm^2 using *ImageJ* application, then samples were obtained by swabbing the wound using a sterile swab stick and diluted in 5 ml of normal saline. Samples were inoculated in blood agar and MacConkey agar, then incubated for 18–24h at 37°C. The data obtained were analysed using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY). Shappiro-Wilk test were used as a normal distribution test; if the data were normally distributed, we used ANOVA test to analyse the mean differences in each group, but if the data were not normally distributed, Kruskal Wallis test were used. For correlation testing of numeric and nominal data, we used the Pearson or Spearman test.

3. Result

On day 1 and 3, there were no burn wound closure, which was indicated by the absence of epithelialization and quite thick eschar. On the 1st day after burn injury, the eschar started to shrink (as seen from the shrinking burn mark) and did not exfoliate immediately. On the 3rd day, in the group that received NPWT treatment, the eschar appeared softer and partly exfoliated, as well as in the group that received silver

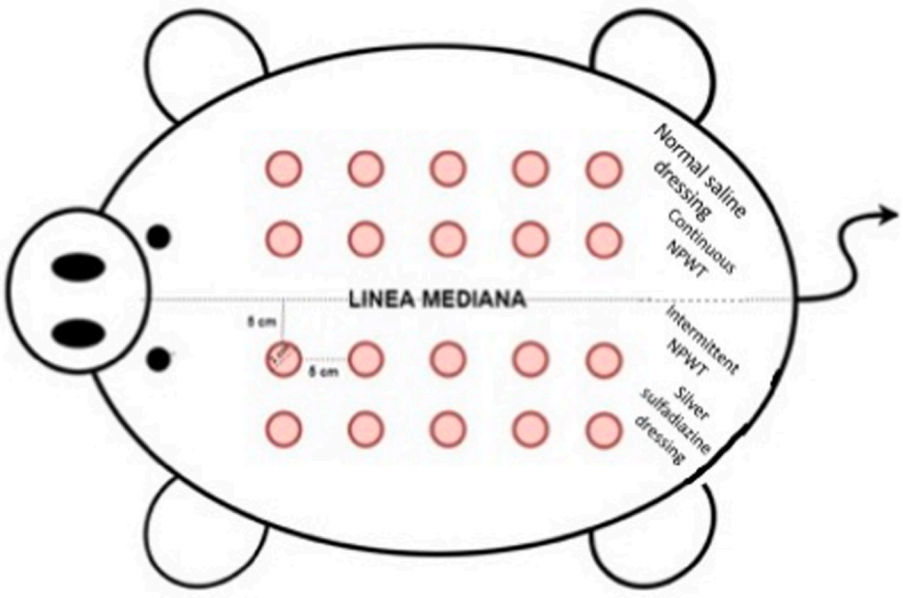

Fig. 1. Research burn model in Yorkshire pig.

sulfadiazine dressing; while in the normal saline gauze treatment group, the eschar appeared softer but there was no sign of significant exfoliation (Fig. 2).

On the 7th day, in the normal saline gauze treatment group, the eschar started to soften and partly exfoliated. In the intermittent NPWT group, the eschar began to show increased exfoliation with minimal crust, while in the continuous NPWT group, there was increased exfoliation on the healthy area around the developed eschar. In the silver sulfadiazine dressing treatment group, the eschar was also easily exfoliated, but there was a pseudo eschar formed on the wound tissue. Although mechanical eschar removal was still needed when changing the dressing on the 7th day, the majority of eschar in each treatment group was easier to remove, compared to the 1st and 3rd day after burns (Fig. 2).

On the 14th day, we did mechanical eschar removal on each treatment group. In the normal saline group, there was a prominent wound contraction, although the growth of granulation tissue on the burn wound was not even with the surrounding tissue; wound closure was marked by the epithelialization that started to grow from peripheral to central wound. In the intermittent NPWT and continuous NPWT, there was abit wound contraction, but indirectly it showed a better granulation compared with normal saline groups, especially in the intermittent NPWT group; as a result, the height of the wound surface was almost the same as the surrounding skin tissue. In the silver sulfadiazine dressing group, it showed the same progression with the normal saline group where there was a wound contraction, but the granulation tissue on the silver sulfadiazine dressing group was not even with the surrounding tissue. There was increased crusting in the silver sulfadiazine dressing group compared to the other group treatment. (Figs. 2 and 4)

Epithelialization on day 21 in the normal saline gauze treatment group had almost completely covered the burn area, but visible wound contractions were also formed in the wound. When compared with the NPWT group, the epithelialization and granulation formed in the NPWT group was not completely covered, and the wound contraction was not as clear as in the normal saline gauze treatment group. In the silver sulfadiazine dressing group, the epithelialization almost completely covered the wound and showed hypergranulation activity, where the granulation tissue that grew in height exceeded the surrounding skin tissue (Figs. 2 and 4).

Wound contractions were seen on the 14th day, and the average remaining surface area in the normal saline gauze treatment group was the largest (initial area 314 mm^2) measuring 107.43 mm^2, while in the intermittent NPWT, continuous NPWT, and silver sulfadiazine dressing group the average remaining surface area was 178.07 mm^2, 146.1 mm^2, and 126.03 mm^2, respectively. However, the differences in each group's average surface area reduction were not significantly different ($p = 0.457$). On the 21st day, the normal saline gauze treatment group had almost completely epithelialized and the average surface area of the burn wound that had not healed was 13.16 mm^2, while in the intermittent NPWT, continuous NPWT, and silver sulfadiazine dressing treatment group the average remaining surface area was 59.49 mm^2, 54.79 mm^2, and 48.95 mm^2, respectively. These differences were not significant ($p = 0.169$) (Table 1).

The number of bacteria was obtained by taking a culture of the wound bed in each treatment group every day. The swab was soaked in normal saline and 1 ml aliquots were planted on blood agar and MacConkey agar. On day 3, there was increased contamination on the agar, so the total did not reach the minimum number of samples (4 samples) to be analysed so the samples on day 3 were excluded from the calculation.

As shown in Table 3, on the first day, the normal saline gauze treatment group grew more bacteria than the other group; the number of bacteria from normal saline gauze treatment group in blood agar culture

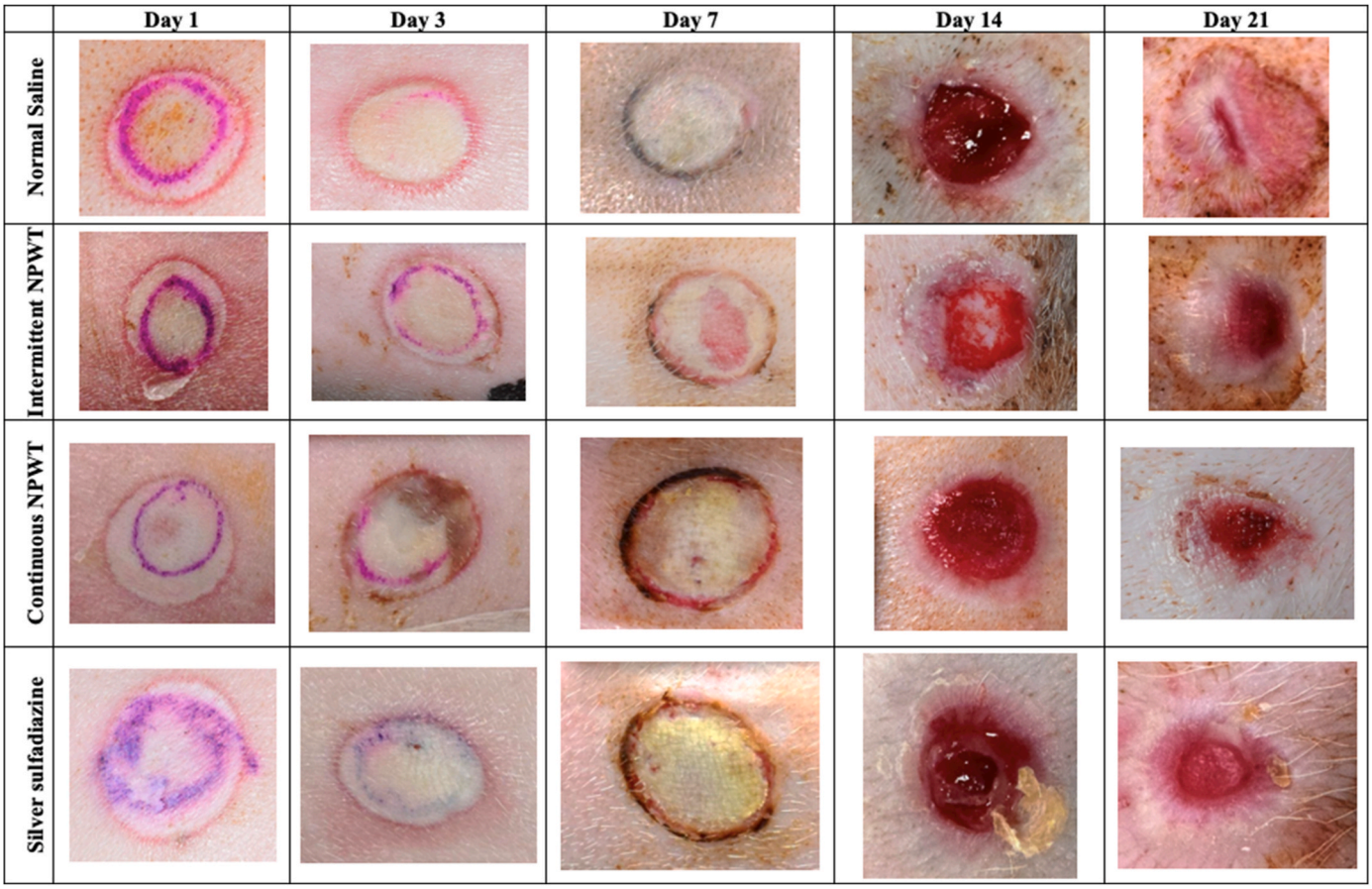

Fig. 2. Wound morphology.

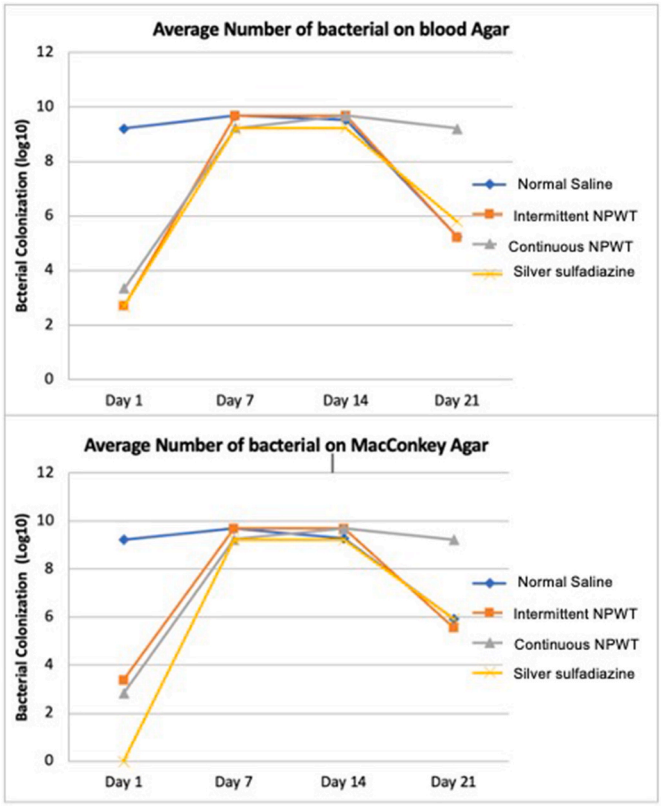

Fig. 3. Average number of bacteria on blood agar and MacConkey agar.

was 167 × 10^7 CFU/ml and in MacConkey culture was 167 × 10^7 CFU/ml (see Table 3). In the intermittent NPWT group, the number of bacteria in blood agar was 500 CFU/ml and MacConkey agar was 2000 CFU/ml. In the continuous NPWT treatment group, the growth of bacteria in blood agar was 2166 CFU/ml and in MacConkey agar was 666 CFU/ml. The group that received silver sulfadiazine dressing treatment on the 1st day had the least bacterial growth which was 500 CFU/ml in the blood agar and there was no growth in the MacConkey agar (Fig. 3). There were no significant differences in each group's bacterial colonization rate in blood agar (p=0.128) and MacConkey agar (p = 0.052) (Table 3).

On the 7th day, bacterial colonization on blood agar culture in the normal saline gauze group, intermittent NPWT group, continuous NPWT group, and silvers sulfadiazine dressing group was 500 × 10^7 CFU/ml, 500 × 10^7 CFU/ml, 170 × 10^7 CFU/ml, and 166 × 10^7 CFU/ml, respectively (p = 0.16); while on the MacConkey agar the bacterial colonization was 500 × 10^7 CFU/ml, 501 × 10^7 CFU/ml, 170 × 10^7 CFU/ml, and 166 × 10^7 CFU/ml, respectively (p = 0.115) (Table 3, Fig. 3). On the 14th day of treatment, the number of bacterial colonization in each group was relatively equal, except in the group that received treatment with silver sulfadiazine dressing, where the growth of germs tended to be less than the other groups (Table 2); but there were no significant differences in each group's number of bacteria on blood agar (p = 0.244) and MacConkey agar (p = 0.473) (Table 3).

On day 21, the number of bacteria obtained in the normal saline gauze treatment group was 41 × 10^4 CFU/ml on blood agar and 84 × 10^4 CFU/ml on MacConkey agar. In the intermittent NPWT group, the bacterial colonization number was 16 × 10^4 CFU/ml on blood agar and 34 × 10^4 on MacConkey agar. In the continuous NPWT group, the bacterial colonization number was 166 × 10^7 CFU/ml and 166 × 10^7 CFU/ml on MacConkey agar, and in the group that received silver sulfadiazine dressing, the bacterial colonization number on blood agar was 63 × 10^4 CFU/ml and on MacConkey agar was 83 × 10^4 CFU/ml (Tables 2 and 3, Fig. 3). Statistical analysis of the bacterial colonization on day 21 showed that there were no significant differences in the number of bacterial colonization on blood agar (p = 0.594) and MacConkey agar medium (p = 0.752) between each treatment (Table 3).

In this study, we also calculated the correlation between the remaining wound surface area and the number of germs from the wound bed swab on a certain day. In all bacterial colonization treatment groups, Spearman correlation test were used. The results of the

Table 1
Remaining wound surface area on day 14 and 21.

Group	Remaining surface area on the 14th day (mm^2)	*p-value*	Remaining surface area on the 21st day (mm^2)	*p-value*
Normal Saline	107,43 ± 83,43	0,457	13,16 ± 16,86	0,169
Intermittent NPWT	178,07 ± 74,83		59,49 ± 20,72	
Continuous NPWT	146,10 ± 69,1		54,79 ± 46,59	
Silver sulfadiazine	126,03 ± 83,22		48,95 ± 39,84	

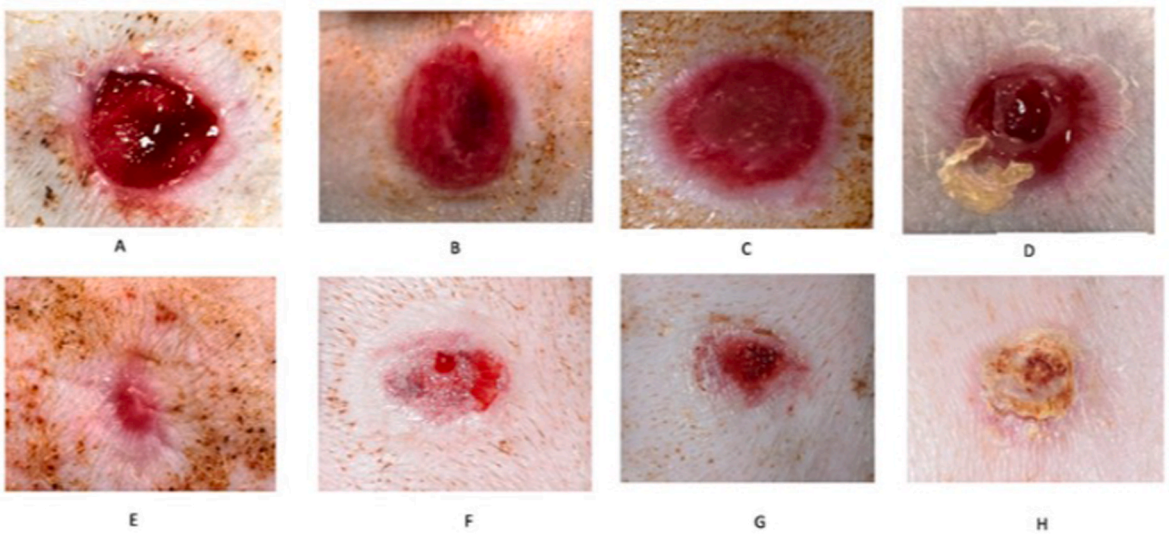

Fig. 4. Wound morphology on day 14 and 21 after burn.

Table 2
Average burn wound area on 14th and 21st day.

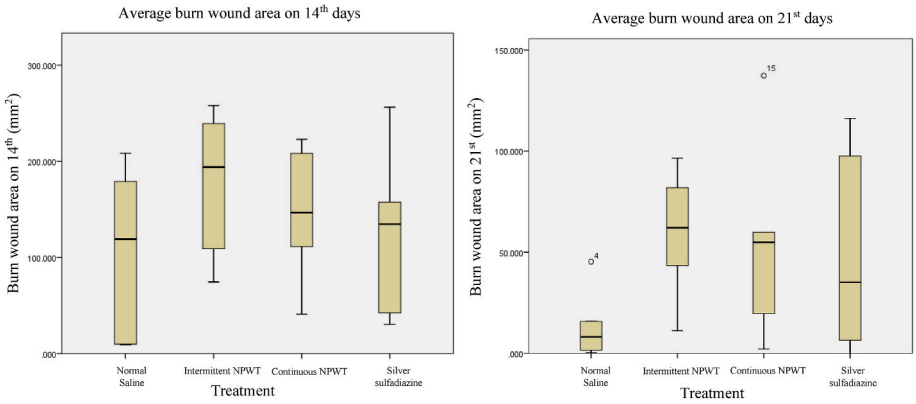

Table 3
Bacterial colonization rate on deep dermal burn base swabs based on days (CFU/ml).

	Treatment Group				*p*
	Normal Saline	Intermittent NPWT	Continuous NPWT	Silver sulfadiazine	
Day 1					
Blood Agar	$167 \times 10^7 \pm 408 \times 10^7$	500 ± 1224	2166 ± 4355	500 ± 836	0,128
MacConkey Agar	$167 \times 10^7 \pm 407 \times 10^7$	2000 ± 4427	666 ± 1221	0	0,052
Day 7					
Blood Agar	$500 \times 10^7 \pm 547 \times 10^7$	$500 \times 10^7 \pm 547 \times 10^7$	$170 \times 10^7 \pm 406 \times 10^7$	$166 \times 10^7 \pm 408 \times 10^7$	0,16
MacConkey Agar	$500 \times 10^7 \pm 546 \times 10^7$	$501 \times 10^7 \pm 546 \times 10^7$	$170 \times 10^7 \pm 408 \times 10^7$	$166 \times 10^7 \pm 408 \times 10^7$	0,115
Day 14					
Blood Agar	$333 \times 10^7 \pm 516 \times 10^7$	$500 \times 10^7 \pm 547 \times 10^7$	$501 \times 10^7 \pm 545 \times 10^7$	$169 \times 10^7 \pm 406 \times 10^7$	0,244
MacConkey Agar	$333 \times 10^7 \pm 516 \times 10^7$	$500 \times 10^7 \pm 547 \times 10^7$	$501 \times 10^7 \pm 545 \times 10^7$	$167 \times 10^7 \pm 494 \times 10^7$	0,473
Day 21					
Blood Agar	$41 \times 10^4 \pm 45 \times 10^4$	$16 \times 10^4 \pm 26 \times 10^4$	$166 \times 10^7 \pm 408 \times 10^7$	$63 \times 10^4 \pm 96 \times 10^4$	0.594
MacConkey Agar	$84 \times 10^4 \pm 120 \times 10^4$	$34 \times 10^4 \pm 58 \times 10^4$	$166 \times 10^7 \pm 408 \times 10^7$	$83 \times 10^4 \pm 109 \times 10^4$	0,752

correlation tests between bacterial colonization with the remaining surface area in the normal saline gauze, intermittent NPWT, continuous NPWT, and silver sulfadiazine dressing treatment group on day 14 and 21 in blood agar and MacConkey agar were not significantly different ($p > 0.05$) (Table 4)

Table 4
Correlation of bacterial colonization with the remaining wound surface area on day 14 and 21 in various treatment group.

Correlation of bacterial colonization with the remaining wound surface area on day 14 in various treatment group			
		Blood Agar	MacConkey Agar
Normal Saline	p-value	0,084	0,125
	ρ	0,754	0,696
Intermittent NPWT	p-value	0,686	0,864
	ρ	0,213	−0,091
Continuous NPWT	p-value	0,439	0,439
	ρ	0,395	0,395
Silver sulfadiazine	p-value	0,208	0,05
	ρ	0,6	0,812
Correlation of bacterial colonization with the remaining wound surface area on day 21 in various treatment group			
Normal Saline	p-value	0,468	0,787
	ρ	−0,371	−0,143
Intermittent NPWT	p-value	0,208	0,072
	ρ	0,6	0,771
Continuous NPWT	p-value	0,544	0,266
	ρ	−0,314	−0,543
Silver sulfadiazine	p-value	0,468	0,208
	ρ	0,371	0,6

4. Discussion

In this study, we used Yorkshire pigs because of its similarity in anatomy and histology with human skin. Each pig received twenty burns in the flank area, then the burns were divided into four different treatments. Wound closure was calculated based on the remaining raw surface that had not been epithelialized. Observable epithelialization occurred on day 14 and day 21, while on the 1st, 3rd, and 7th day, epithelialization was not observed because the wound healing phase of deep dermal burn injury started in more than 7 days following the burn treatment. In some samples, the eschar still not exfoliated completely on the 7th day after the burn injury.

Data analysed with the SPSS program showed that there were no significant differences in the remaining wound surface area between those four treatment groups on day 14 ($p = 0.457$) and day 21 ($p = 0.169$) following burn injury (Table 1). Several studies on NPWT

revealed that NPWT improved microcirculation and accelerated epithelialization therefore it could close the raw surface faster [6,10]. Previous study by Saxena et al., showed that the use of NPWT could improve the stretch of the cells and accelerate the cell growth, apart from the presence of growth factors secreted by these cells [11]. Research conducted by Daniel and Wilson made an observation on 20 patients in NPWT therapy, it showed that NPWT could stimulate the growth of infection free scar granulation in a relatively short time, the application of NPWT also considered as an easy and convenient therapy therefore it could be an alternative infected wound therapy. In our study, there were no distinct differences in the epithelialization of each treatment groups; it was possible that the wound size in this study was too small (the burn area was made 314 mm^2 in size) and other factors such as wound contraction could also made the epithelialization look almost similar in each group [12]. But, in the group that received either continuous or intermittent NPWT treatment, the wound appeared better in both granulation and crust formation (crustation).

The use of silver sulfadiazine dressing which is still the standard therapy in deep dermal burns [13] also did not have significant differences in the remaining burn surface area. Although several previous studies have stated that silver has a toxic effect on wound healing [14,15], in this study, there were no significant different effects on the residual surface area, whether in the silver sulfadiazine dressing group nor in the other groups of treatment. However, crustation appeared more prominent in the silver sulfadiazine dressing treatment group, and it could interfere with the epithelialization process and disturbed the wound closure process. Repeated usage of silver sulfadiazine could produce pseudo eschar formation and interfere with the granulation process on the wound bed, thus it could relatively interfere with the wound healing process [16].

The number of bacterial colonization (bacterial count) was one of the factors that affect the rate of wound healing. Unlike the research conducted by Ahmed et al. that stated NPWT had the ability to reduce bacterial colonization [17] and similar research conducted by Daniel and Wilson that showed the ability of NPWT to triggers the growth of infection-free tissue [12], in this study, NPWT treatment did not show significant differences in reducing bacterial colonization, compared to normal saline gauze and silver sulfadiazine dressing treatment group neither on the 1^{st}, 7^{th}, 14^{th}, nor 21^{st} day ($p > 0.05$) (Table 3). There was controversy on this matter concerning some of the results on the previous research. Glass et al. conducted a systematic review on the effect of NPWT on bacterial count and found that eight studies reported no effect of NPWT on germ numbers, seven studies reported that NPWT had a bacteriostatic effect, and five other studies stated that NPWT had suppressive effect on bacterial growth. For certain bacterial species, similar to this study, NPWT did not have statistically significant differences in the numbers of bacterial colonization against other treatments (normal saline and silver sulfadiazine) [18]. Lalezari et al. also stated that many studies revealed that there were no significant number of germs differences in the use of NPWT compared to the standard dressings (wet to dry dressing). Although the effect of NPWT on wound healing was good, especially intermittent NPWT, its relationship with germ numbers was still difficult to explain, because there were many factors that could affect the wound healing process [9,19].

In this study, the use of silver sulfadiazine dressing can only prevent the bacterial growth on the 1^{st} day as shown on Table 3 that there were no bacteria in MacConkey agar medium. However, the number of bacterial colonizations in the silver sulfadiazine dressing group did not have significant differences compared to the other treatment groups. Silver class drugs such as silver sulfadiazine had bacteriostatic effect, but repeated application would create pseudo eschar; if the eschar was still present in the burn, the penetration rate of silver sulfadiazine would be poor [4]. This aspect was considered to be a strong reason why the application of silver sulfadiazine also produces germ numbers that are not much different from other groups (Table 3). Several recent studies had shown that silver-based dressings can dramatically reduce the growth of methicillin resistant *Staphylococcus aureus* (MRSA) at all stages of burn healing [4], but in this study, although the mean bacterial count showed high results in all treatment groups (including silver sulfadiazine), we did not analyse the type of bacteria, because some bacteria had high virulence that could interfere the wound healing process [4,20,21].

In this study, there were no statistically significant correlation between the number of bacteria and the remaining wound surface area in all treatment groups. In vitro studies showed that cells would grow faster when it stretched [11]. The administration of NPWT on wounds could trigger the cells to stretch so it could accelerate the epithelialization process, reduce oedema and absorb cellular debris (exudate control), therefore the risk of infection which could interfere with cell proliferation in the body would be reduced. In this study, there were no signs of wound and systemic infection even though the swab results were more than $>10^5$ CFU/ml. Previous study stated that high bacterial numbers were not always associated with the incidence of infection because there was an interaction between the host and the pathogen [11]. Daniel et al. conducted a research related to topical antimicrobials in burns and found that eschar that was not completely removed will cause microbes to proliferate more easily; however, the use of topical antimicrobials can prevent the growth of pathogenic bacteria and prevent systemic spread [4].

This study was purely an experimental study in which all confounding factors were controlled as much as possible, but the immunological factors, host-pathogen response and pig's behaviour could not be controlled. We presumed that the method of bacterial colonization number (bacterial count) measurement in each wound that were done by using the wound-base swab method (CFU/ml) study instead of using a punch biopsy (CFU/gram of tissue) could be one of the causes for the unsignificant differences in each group bacterial colonization number.

5. Conclusion

Although there were no statistically significant differences in the remaining wound surface area and the number of bacterial colonization in the intermittent and continuous NPWT groups compared to the standard therapy (silver sulfadiazine dressing and normal saline gauze) groups, morphologically, wound in the group that received either continuous or intermittent NPWT treatment appeared better in both granulation and crust formation (crustation). There were no significant correlation between the amount of bacterial colonization and the remaining wound surface area that received NPWT compared to the normal saline gauze and silver sulfadiazine dressing.

Availability of data and materials

The datasets used during the study are available from the corresponding author on reasonable request.

Sources of funding

This study was partly funded by community grant of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

Ethical approval

This study was approved by the Institutional Review Board of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta, Indonesia (KE/FK/0729/EC/2019).

Consent

Not applicable.

Authors' contributions

RR and MRS conceived the study. RR and GCG drafted the manuscript. MRS, EP, SA, ID, TA, YWW critically revised the manuscript for important intellectual content. RR, MRS, EP, SA, ID, TA, and YWW facilitated all project-related tasks.

Registration of research studies

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Register ID: 202202114.

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Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors declare that they have no competing interests.

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List of abbreviations

NPWT	Negative pressure wound therapy
MRSA	Methicillin resistant *Staphylococcus aureus*

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103367.

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