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Zinc oxide, povidone-iodine, and polyurethane film: a comparative study on rabbit wound healing

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Abstract

Rabbits are popular pets and they are susceptible to injuries. Though various types of dressing materials have been available, the comparative analysis of the effectiveness of various dressing materials on external wound healing in rabbits is limited. So, the objective of this investigation is to assess the efficacy of Zinc oxide tape, Povidone Iodine, and Polyurethane film as wound dressing materials. Twenty rabbits were divided into four groups, each consisting of five rabbits: group A was the control group, group B was provided with zinc oxide tape, group C received 10% povidone iodine dressing, and polyurethane film was administered in group D. Each animal underwent three linear incisions, each 2 cm in length, in the flank area. The control group did not receive any dressing for external wound healing, whereas Groups B and D were administered zinc oxide tape and polyurethane film on day 0, and Group C got daily povidone iodine dressing. Morphological and histopathological changes of the wound sites were observed during the experiment. Full thickness skin tissue samples were obtained on days 3, 7, and 14 post-application of wound dressing materials. The wound length and width (1.28 ± 0.06 cm and 0.059 ± 0.0126 cm) were significantly decreased (ANOVA, $p < 0.001$, $\eta^2 = 0.755$ for length; $\eta^2 = 0.737$ for width) in 10% povidone iodine treated group compared to the zinc oxide tape group (1.46 ± 0.05 cm and 0.12 ± 0.06 cm), the polyurethane film (1.63 ± 0.03 cm and 0.193 ± 0.01 cm) and the control group (2.44 ± 0.05 cm and 0.251 ± 0.007 cm). The histological study revealed that 10% povidone iodine group exhibited the fastest reepithelialization, followed by zinc oxide tape, polyurethane film and control group. The study demonstrated that regular dressing with 10% povidone iodine promoted enhanced wound healing compared to zinc oxide and polyurethane film, making it appropriate for wound dressings in rabbits.

Keywords: Zinc oxide tape, povidone iodine, polyurethane film, wound healing, antimicrobial agents

Introduction

Rabbits are among the most recent domesticated animals in the world, it wasn't until the eighteenth and nineteenth centuries that the practice of keeping rabbits as pets became popular in North America and Europe (DeMello 2016). The popularity of domestic rabbits as household pets is increasing rapidly as more people discover what makes them suitable companions (Crowell-Davis 2007). In addition to injuries, rabbits may develop skin sores due to a variety of diseases, including obesity, sore hocks, arthritis, poor nutrition, dental disease, urinary disease, and neurologic disease. Rabbits frequently suffer from skin fold pyoderma and abscesses, which need to be treated with frequent and efficient dressings to heal (Vennen and Mitchell 2009). Wound dressings are used externally to maintain a healthy surrounding environment. The kind, depth, and severity of the wound, as well as the desired therapeutic outcome, all affect the choice of dressing. Wounds compromise the natural architecture of bodily tissues, and the healing process is intricate, encompassing steps such as hemostasis, inflammation, granulation tissue creation, extracellular matrix synthesis, connective tissue remodeling, and the fortification of the wound by collagen fiber deposition (Hashemi *et al.* 2015). Conventional dressings are often recommended for clean, dry wounds with low exudate. Gauze, lint, plasters, bandages, and cotton wool have all been employed as dressings to offer mechanical protection for wounds. Wounds frequently necessitate an optimal moisture level for healing, resulting in the predominance of contemporary dressings with sophisticated formulas over conventional ones (Boateng *et al.* 2008 Dhivya *et al.* 2015). Contemporary materials utilized for wound dressings encompass hydrocolloids, hydrogels, foam dressings, bioactive dressings, synthetic

polymers, etc. (Teixeira *et al*. 2020). Among the different dressing materials available on market, zinc oxide tape, povidone-iodine solution, polyurethane films are the most frequently used dressing materials for the treatment of any kinds of wounds under field conditions.

Zinc compounds have been used for centuries in the treatment of skin disorders and it remains in place for extended periods. Zinc oxide tape is a robust tape widely used for strapping in the medical industry, and it is strong enough to protect the injury (Hallböök and Lanner 1972). It has been demonstrated that topical zinc oxide therapy enhances the healing of leg ulcers. After the tape is used, zinc is absorbed systemically (Strömberg *et al*. 1984, Wetter *et al*. 1986). Besides, it carries some disadvantages like not being suitable for heavily exudative wounds, adhesive may cause irritation and can be painful to remove (Morales-González *et al.* 2022)

Povidone-iodine is a widely used commercial antimicrobial agent for skin disinfection, surgeical procedure, and local anti-infective treatment (Ruder and Springer 2017). The advantages of povidone iodine are that it is suitable for various wound types, including infected wounds (Kanno *et al*. 2016). Numerous studies have assessed the efficacy of povidone-iodine as a therapeutic agent for promoting wound healing. According to some, povidone-iodine possesses a greater variety of antibacterial activities, significant antibiofilm efficacy, no acquired bacterial resistance or cross-resistance, low cytotoxicity, advantageous tolerability, and the ability to hasten wound healing. Povidone-iodine has virucidal, fungicidal, and bactericidal effects on encapsulated viruses within 1–5 minutes (Sauerbrei 2020, Bigliardi *et al*. 2017). Povidone iodine kills bacteria by releasing free iodine, which binds to proteins (Panchmatia *et al*. 2019). Once bound, the iodine is carried across the cell membrane into the cell cytoplasm via polyvinylpyrrolidone. The free iodine has a microbicidal effect within in time (Edmiston *et al*. 2018). Although it carries some disadvantages, such as damaging healthy cells, it may cause irritation or allergic reactions.

Polyurethane film is considered an excellent candidate for preparing modern dressings (Wang and Wang 2012a). These environmentally-friendly Polyurethane film has widely favorable chemical and physical properties such as good oxygen and water vapor permeability and barrier properties, variable mechanical properties, and nontoxicity (Mohammadi *et al*. 2013). Despite many drawbacks, including potential harm to adjacent skin with extended usage on moist wounds, susceptibility to tearing during application or removal due to its thin composition, and restricted antibacterial efficacy (Gultekin *et al*. 2009).

Despite the widespread application of zinc oxide tape, povidone-iodine solution, and polyurethane film for wound management, currently there is no existing research about the efficacy of these materials in promoting the healing of cutaneous wounds in rabbits. This investigation will be beneficial in identifying an appropriate dressing material for the successful management of skin wounds in rabbits.

Materials and methods

The study was conducted in the Department of Surgery and Radiology, Gazipur Agricultural University (GAU). The ethical approval for this study was obtained as the ethical code number FVMAS/AREC/2023/49 from the Animal Research Ethics Committee of Gazipur Agricultural University (GAU), Gazipur, Bangladesh.

A total of 20 male rabbits, aged between 5 and 6 months, were selected for this investigation. Their body weight ranged from 1 kg to 1.5 kg. The animals were obtained from several pet stores and a rabbit farm in Gazipur. All the animals demonstrated robust health and had no visible indicators of health problems. The animals were reared in a specialized housing system, particularly designed for rabbits, in the Department of Surgery and Radiology at GAU. Before the study began, the animals underwent a 14-day quarantine period. The rabbits had enough access to food and water.

Experimental design

Twenty rabbits which appeared to be in good condition, were categorized into 4 groups. They participated in this experiment with the authorities' approval, following their

rules and recommendations. The rabbits were categorized into four groups (Group A, B, C and D) using a simple randomization method. Each rabbit was assigned a group number by drawing lots to ensure unbiased distribution.

Wound creation: Following a 12-hour period of withholding feed from the rabbits, they were anesthetized using a combination of ketamine HCl (Ketalar®, Popular Pharmaceuticals Ltd., Bangladesh) at 35 mg/kg body weight and Xylazine HCl (Xyla®, Interchemie, Netherlands) at 5 mg/kg body weight intramuscularly (IM) before making the incisions. As a premedication atropine sulphate (Inj. Tropin vet®, ACME Laboratories Ltd., Bangladesh) at 0.065 mg per kg body weight IM was used. For maintenance, half of the initial dose of ketamine HCl was used. Following induction, the surgical site was trimmed, shaved, and disinfected. Three surgical incisions, each measuring 2 cm in length, were made in the left flank of each rabbit. The skin was detached from the underlying tissues by blunt incision. Simple interrupted sutures were used using nylon thread to seal the surgical incisions. Each rabbit had three incision sites for the application of zinc oxide tape (Zinc Oxide Adhesive Bandage®, Dearon, China), 10% povidone iodine (Viodin®, Popular Pharmaceuticals Ltd., Bangladesh), and polyurethane film (Transparent Film Dressing®, Kastormed Med. Inc., USA) to its external lesion. Povidone-iodine was provided daily, whereas zinc oxide tape and polyurethane film were not removed until the day of sample collection. Post-surgery, the animals were relocated to their shelter.

Table 1. Groups and sample size of the study

Group	No. of rabbits	Materials for Experiment
Group A (Control group)	5	No dressing materials was applied to the wound area
Group B (Zinc oxide tape)	5	Zinc oxide tape was applied to wound area
Group C (Povidone iodine)	5	10% Povidone iodine solution was applied to wound
Group D (Polyurethane film)	5	Polyurethane film was applied

Follow-up data were collected from the first day of surgery to the fourteenth day post-surgery. To evaluate the efficacy of wound healing, morphological parameters, including swelling surrounding the wound, the elevation of the sutured line above the skin's surface, and the dimensions of the sutured area were recorded. To determine the length of wound contraction, the width of the sutured region was measured on Days 3, 7 and 14. Tissue samples for histopathological analysis were collected from all treatment groups on Day 3, 7 and 14.

Morphological data evaluation: After collecting the skin sample, the size was observed and measured. The wound size was measured using a digital slide caliper. The length and the width were measured. In order to minimize observer bias, morphological measurements conducted under blinded conditions. The assessors of wound contraction did not know the treatment groups. All samples were coded prior to analysis, and the code was only revealed after data collection was completed.

Histopathological study: The study was done using the standard hematoxylin and eosin staining procedure with slight adjustments. Initially, the tissues were preserved by immersing them in a 10 % formalin for 1 week. Subsequently, the tissues were thoroughly washed in flowing water for overnight to eliminate the fixative. To dehydrate the tissues, they were sequentially immersed in increasing concentrations of alcohol, starting with 70 %, 80 %, 95 %, and finally 100 % for 2 hours each. The alcohol solution was changed every 2 hours. Then the tissues were kept in xylene for 2 hours before impregnation. At a temperature of 60°C, the tissues were immersed in molten paraffin wax. This process involved two changes, with each change lasting one hour. Paraffin blocks were created by using rings and placing tissue pieces in the middle. The paraffin blocks that had been made were left at room temperature for a few hours. Subsequently, the tissues were sliced into sections of 5 µm thickness using a rotary microtome machine. The sections were immersed in the heated water (45°C) within the paraffin bath. Next, the required parts were placed on glass slides, free from grease and oil. The prepared slides were airdried

and stored in a refrigerator at 4°C until further use. The histopathological analysis of slides were also done under blinded condition to minimize observer bias.

Statistical analysis: Statistical analyses were performed using R Studio version 4.4.1. An alpha level of 0.05 was considered statistically significant. When assumptions were met, a one-way ANOVA was conducted to compare treatment groups, followed by Tukey's HSD post-hoc test for pairwise comparisons. For each analysis, effect sizes (η^2 and ω^2) were calculated to assess the magnitude of differences between groups. Descriptive statistics (mean ± SD) were also calculated.

Results

The effects of Povidone iodine, Zinc oxide tape, and Polyurethane film on wound healing were assessed using morphological and histological parameters in rabbits. Morphological parameters including swelling of wound area and contraction rate of wound bed. Histological features include observation of inflammatory cells, hemorrhage, and re-epithelialization of skin wounds.

Morphological changes in skin wound: In morphological observation, superior wound healing was noted in the Povidone iodine treated groups (Figure 3), followed by the Zinc oxide tape groups (Figure 2), then the Polyurethane film groups (Figure 4), and control (Figure 1), respectively.

In morphological observation, superior wound healing was noted in the Povidone iodine treated groups (Figure 3), followed by the Zinc oxide tape groups (Figure 2), then the Polyurethane film groups (Figure 4) respectively.

Swelling and exudation were diminished within 4 days in all experimental groups, with the exception of the control group (Group A) and polyurethane film treated group. Fluid exudation was seen in the Polyurethane film treatment group and the control group until day 6 and day 8 respectively. Complete wound healing in patients treated with Povidone iodine was noted within 14 days.

The most significant wound contraction was observed in the povidone iodine treated group followed by zinc oxide, polyurethane film, and control group. Table 2 shows the changes in wound length and width in all groups.



Figure 1. Apposition of wound edges in (A) day 3, (B) day 7, (C) day 14 in group A (Control).



Figure 2. Apposition of wound edges in (A) day 3, (B) day 7, (C) day 14 in group B (Zinc oxide tape).

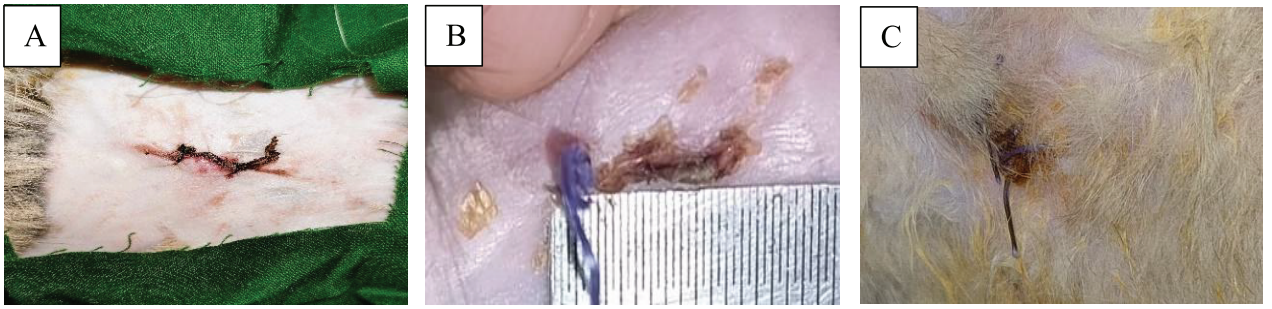


Figure 3. Apposition of wound edges in (A) day 3, (B) day 7, (C) day 14 in group C (Povidone iodine.



Figure 4. Apposition of wound edges in (A) day 3, (B) day 7, (C) day 14 in group D (Polyurethane film).

Swelling and exudation were diminished within 4 days in all experimental groups, with the exception of the control group (Group A) and polyurethane film treated group. Fluid exudation was seen in the Polyurethane film treatment group and the control group until day 6 and day 8 respectively. Complete wound healing in patients treated with Povidone iodine was noted within 14 days.

The most significant wound contraction was observed in the povidone iodine treated group followed by zinc oxide, polyurethane film, and control group. Table 2 shows the changes in wound length and width in all groups.

The post-hoc Tukey HSD testing revealed that the control group demonstrated a higher length (2.44 ± 0.0583 cm) than the other treatment groups. The polyurethane film (1.63 ± 0.0361 cm) exhibited a higher length compared to povidone iodine (1.28 ± 0.0673 cm) and zinc oxide tape (1.46 ± 0.0504 cm).

Povidone iodine exhibited the shortest length and statistically showed significant differences from all other groups.

The control group (0.251 ± 0.0076 cm) exhibited substantially higher width than zinc oxide tape (0.12 ± 0.0643 cm) Polyurethane film (0.193 ± 0.0140 cm) and povidone iodine (0.059 ± 0.0126 cm). Povidone iodine showed the smallest width and had a significant difference from all other groups.

The interaction plot reveals a significant interaction effect between treatment and time on length ($F(6, 24) = 5.23$, $p < 0.001$). This shows that the impact of the different treatments on length changed over time. The control group demonstrated an increasing trend in length at all time intervals, but the other treatment groups indicated a reduction in length with time. The degree of the reduction in length was most significant for the povidone iodine group. The interaction plot demonstrates a significant interaction effect between treatment and time on width ($F(6, 24) = 4.87$, $p < 0.01$). This indicates that the effect of the various treatments on width fluctuated over time. The povidone iodine group exhibited the most significant reduction in width also in that case.

Table 2. Effects of povidone iodine, zinc oxide tape, polyurethane film and control group for wound contraction

Treatment	Length (cm) (Mean ± SD)	Width (cm) (Mean ± SD)	Overall ANOVA (p)	Effect size (η^2; ω^2)
Control	2.44 ± 0.0583[a]	0.251 ± 0.0076[a]		
Zinc oxide tape	1.46 ± 0.0504[c]	0.120 ± 0.0643[c]	0.00056 (Length)	Length: 0.755; 0.724
Povidone iodine	1.28 ± 0.0673[d]	0.059 ± 0.0126[d]	0.00085 (Width)	Width: 0.737; 0.702
Polyurethane film	1.63 ± 0.0361[b]	0.193 ± 0.0140[b]		

Values are presented as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences between groups (Tukey HSD, $p < 0.05$). Overall ANOVA results are reported with exact p-values and effect sizes (η^2 = eta squared; ω^2 = omega squared). Higher effect size values indicate a stronger influence of treatment on wound contraction.

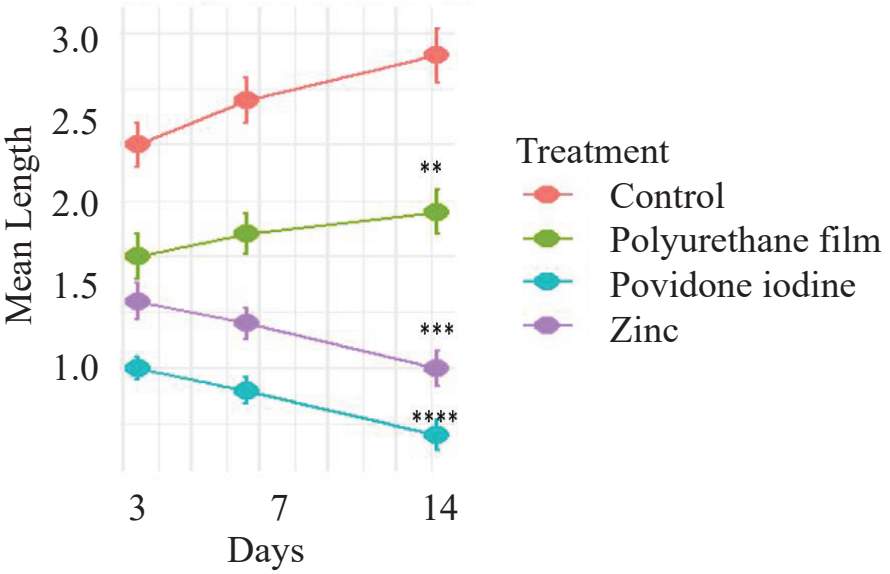


Figure 5. Mean interaction plot for length.

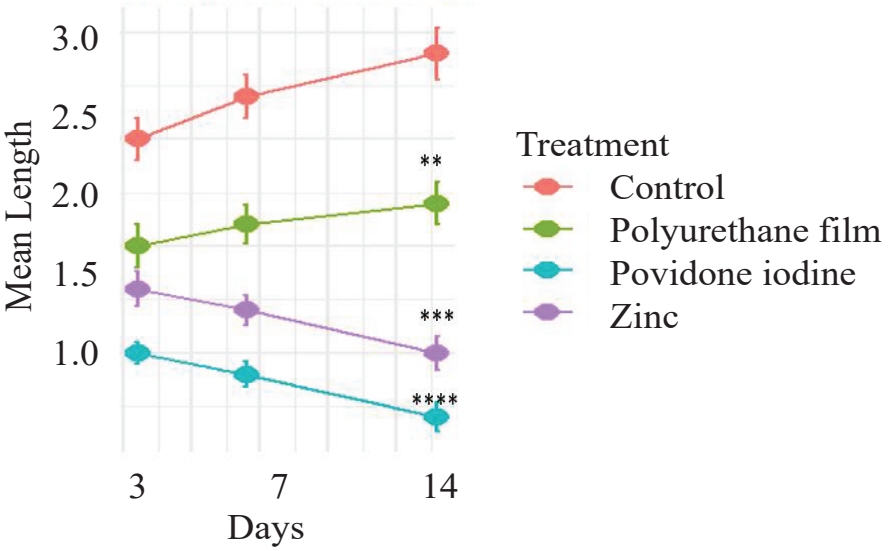


Figure 6. Mean interaction plot for width.

On day 3, all the samples had gaps between the wound edges. Group A and D showed less epithelialization (Figure 7). Group B and C showed signs of healing with the presence of reactive cells on the wound bed.

On day 7, Group B and C had shortest gaps between the wound edges. Group A and C showed less epithelialization. Group A still had exudates and swelling (Figure 8). Vascularization and presence of reactive cells were present in all the groups.

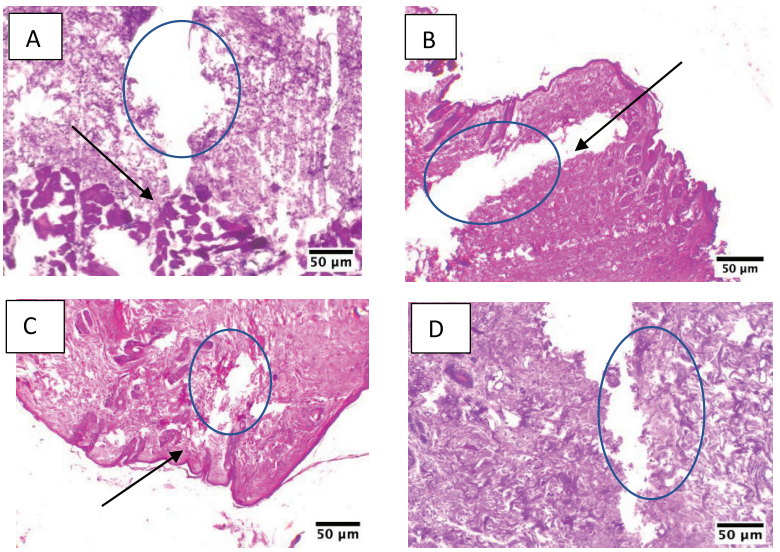


Figure 7. Histopathology of the skin of the rabbits after 3 days of incision. (A) Presence of reactive cells on wound bed (Black arrow) with distinctly separated wound edges (Black circle) on day 3 of the control group. (B) Epithelialization is present with inflammatory cells with shortening of gaps between wound edges on the zinc oxide group (C) Epithelialization with reactive cells on wound bed with little gaps between wound edges on the povidone iodine treated group. (D) The wound edges are distant (Black circle) and it indicates little wound healing on the Polyurethane film group.

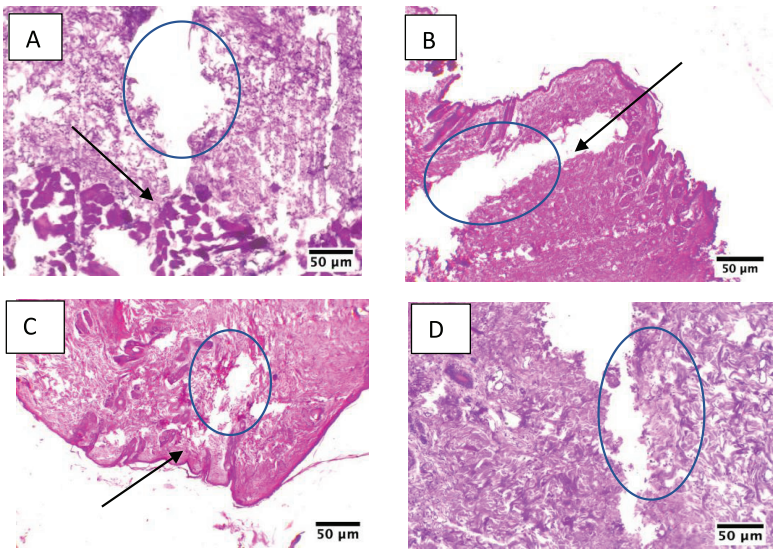


Figure 8. Histopathology of the skin of the rabbits after 7 days of incision. (A) Distinctly separated wound edges (Black circle) on day 7 of the control group. (B) Epithelialization (Black arrow) is present with shortening of wound edges (Black circle) on the zinc oxide group (C) Epithelialization (Black arrow) with reactive cells (Yellow arrow) on wound bed with little gaps between wound edges on the povidone iodine treated group. (D) The wound edges are little closer (Black arrow) indicating wound healing on the Polyurethane film group.

Discussion

Significant differences in wound contraction were observed among the groups in terms of both length and width, as indicated by the statistical analysis. Povidone iodine exhibited the minimum wound length and width, while the control group showed the maximum wound length without any treatment. The most significant reepithelialization and vascularization was facilitated by Povidone iodine, followed by Zinc oxide tape, Polyurethane film, and the control group, as indicated by the histopathological analysis. These findings indicate that Povidone iodine is a more effective wound dressing material than zinc oxide and polyurethane film, which corroborates its suitability for rabbit wound-care.

According to Ahmad *et al.* (2014), on skin wounds in rabbits, the wound area started to diminish from day 2, and this tendency persisted until day 14. On day 14, there was a complete contraction of the wound area. Zinc applied to wounds stimulates skin regeneration by attracting new skin cells and promoting blood flow to the site, a process known as neoangiogenesis. Zinc oxide medical tape is especially beneficial for skin and soft tissue injuries. However, it may be used to treat nearly any external lesion (Hughes and McLean 1988, Lin *et al.* 2017, Thompson *et al.* 2017). Xie *et al.* 2011, demonstrated the antibacterial properties of zinc oxide tape, indicating improved conditions at the infected location for wound healing (Xie *et al.* 2011). A study revealed that zinc oxide enhances epithelialization of full-thickness skin wounds by activating zinc-dependent MMPs, which increase keratinocyte migration. The research indicated that zinc oxide enhanced the endogenous expression of insulin-like growth factor (IGF-1), which is essential for granulation tissue formation (Moore 2003). But on the contrary, prolonged moisture collection beneath the adhesive tape can cause skin injury and impede recovery (Fumarola *et al.* 2020). However, in this study, zinc oxide tape performed better than polyurethane film, showing that it can be used in any cutaneous surgical wounds in rabbits.

Bigliardi *et al.* (2017), suggested that povidone iodine, owing to its swift, powerful, broad-spectrum antibacterial characteristics and advantageous risk/benefit ratio, is anticipated to continue being an efficacious therapy for both acute and chronic wounds. Sindelar and Mason (1979) executed a prospective randomized trial involving patients undergoing various general surgical procedures, wherein wounds were irrigated with either 10% povidone iodine or normal saline before closure; across all wound types, the incidence of wound infections was significantly lower in the povidone iodine irrigated group. Some investigations have demonstrated that povidone-iodine can accelerate fibroblast proliferation, angiogenesis, epithelialization, and epithelialization, similar to specific herbal formulations (Ravishankar *et al.* 2019). But Sakhavar and Khadem 2008 conducted a study to evaluate the efficacy of 10% povidone-iodine in facilitating the healing of surgical wounds in rabbits. The findings indicated that povidone-iodine is an effective antibacterial agent; yet, it may not consistently be the optimal choice for accelerating recovery or seeming superior to other therapy (Sakhavar and Khadem 2008) In this study, povidone iodine was found to be the most effective among the 3 treatment groups. Although it can be mildly cytotoxic, the results suggest that the antimicrobial properties of povidone iodine improved better environment for epithelialization and wound healing.

Research using animals, especially mice, has demonstrated that occlusive dressings, such as transparent polyurethane films, can accelerate wound healing relative to non-occlusive methods (Guillen *et al.* 2023). Incorporating active chemicals such as silver, asiaticoside, or natural polyols into polyurethane foam dressings enhances their antimicrobial properties and promotes wound healing. This demonstrates the versatility of polyurethane as a foundational material for effective wound dressings (Rybka *et al.* 2022). Transparent films effectively retain moisture; nevertheless, their absorption capacity is limited. They are most effective on wounds with minimal or no exudate. Excessive exudate accumulation underneath the covering may lead to maceration (The Wound Pros 2022). The relatively poorer performance of polyurethane film

could be due to the low antimicrobial properties of the film and bacterial proliferation on the surgical site resulting in delayed healing.

However, this study has some limitations. First, the sample size was relatively small (n = 5 per group), which may limit the statistical power and overall e-findings. Second, although randomization and blinding were applied, complete elimination of potential bias cannot be guaranteed. Third, only male rabbits within a narrow age and weight range were included to keep the population uniform. The result may vary based on different age and sex groups. Fourth, although surgical technique, suture material, and post-operative care were standardized, these factors may still have influenced healing outcomes. Finally, gross wound photographs were available at days 3, 7, and 14; more frequent documentation could provide finer resolution of the healing process.

Conclusion

Wound healing is essential since it enables the body to independently repair damaged tissues and restore the integrity and functionality of the affected area following an injury. This study's findings demonstrate that 10% povidone-iodine is more efficient than zinc oxide tape and polyurethane film in facilitating wound healing in rabbits with surgically induced skin wounds. Future research should involve a larger sample size, diverse wound types, and various dressing materials to identify the optimal dressing for rabbit skin wound treatment before generalizing these findings.

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