

## RESEARCH

# NaCl vs distilled water in wound healing – is traditional wound care still effective?

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## Abstract

**Aims** To compare the effectiveness of distilled water (aquadest) and 0.9% sodium chloride (NaCl) in promoting wound healing, based on wound diameter, re-epithelialisation, granulation formation, neovascularisation and inflammatory response.

**Methods** An in vivo experimental study was conducted using 27 male Wistar rats (*Rattus norvegicus*), randomly assigned into three groups (control, aquadest, and NaCl). Full-thickness wounds were created on the dorsum of each rat and treated daily for 14 days. On days 2, 7 and 14, the wounds were assessed macroscopically and histologically, quantitatively and semi-qualitatively. Statistical analysis included Friedman and Wilcoxon tests, along with effect size calculations (Cohen's d and 95% confidence intervals).

**Results** Aquadest significantly improved reepithelialisation ( $p=0.018$ ), granulation tissue formation ( $p=0.022$ ), and reduced inflammation ( $p=0.039$ ) compared to baseline. NaCl did not demonstrate statistically significant improvements across any parameter. Although wound diameter reduction in the aquadest group was not statistically significant ( $p=0.117$ ), it showed a moderate-to-large effect size ( $d=0.78$ ), with qualitative analysis showing the reduction of inflammatory cells and increase of neovascular count and epithelial thickness ( $p=0.040$ ).

**Conclusions** Distilled water showed superior performance to 0.9% NaCl in supporting multiple aspects of wound healing in a rat model.

**Keywords** aquadest, compress therapy, distilled water, normal saline, wound healing.

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## Introduction

Wound healing is a complex biological process consisting of four main, interconnected phases: hemostasis, inflammation, proliferation, and tissue remodeling. Each phase involves a dynamic interaction between immune cells, inflammatory mediators, growth factors, and the extracellular matrix to restore the integrity of damaged tissue. The success of the wound healing process depends heavily on infection control, tissue perfusion, nutritional balance, and, most importantly, proper wound management, including irrigation techniques used in wound care. Wound irrigation is a fundamental step in modern wound management, aiming to remove necrotic debris, exudate, and contaminants, and reduce the number of microorganisms on the wound surface without damaging new granulation tissue.<sup>1,2</sup> The ideal irrigation fluid should be non-toxic to healthy tissue, not induce additional inflammatory reactions, have adequate cleansing properties,

and not disrupt the physiological balance of the wound. In clinical practice, normal saline (0.9% NaCl) has long been accepted as the standard of care due to its isotonicity to body fluids, widespread availability, and minimal risk to wound tissue.<sup>3,4</sup>

However, the use of 0.9% NaCl is not without controversy. Evidence from experimental and clinical studies indicates that although the solution is safe, it has no intrinsic antibacterial activity and does not significantly enhance epithelialisation. Some studies even show comparable results between irrigation with NaCl and sterile tap water or distilled water in terms of infection rates, healing times, and granulation tissue quality. This raises important questions about whether 0.9% NaCl truly offers clinical advantages over simpler and more economical alternatives. Distilled water (aquadest) is a hypotonic fluid traditionally used in various medical procedures, including minor wound cleansing, particularly

in healthcare facilities with limited resources. The primary advantages of aquadest lie in its low cost, widespread availability, and ease of storage. However, concerns have arisen regarding the potential for osmotic damage to healthy tissue due to its hypotonic nature, potentially compromising the integrity of epithelial cells and fibroblasts. Nevertheless, several experimental reports have shown that irrigation with sterile distilled water does not significantly affect inflammation or the rate of epithelialisation compared with 0.9% NaCl, especially when used for a limited duration and under sterile conditions.<sup>5-7</sup>

In the context of modern clinical practice, the effectiveness of irrigation with distilled water compared to normal saline remains a topic requiring scientific clarification. In some developing countries, resource constraints often make the routine use of 0.9% NaCl impractical due to cost, availability, and storage logistics. Therefore, a better understanding of the efficacy of distilled water as an irrigation fluid has the potential to significantly impact wound care policies at various levels of healthcare. If distilled water is proven to be equally effective without increasing the risk of complications, it could provide a more economical and accessible alternative without compromising wound healing.<sup>8-10</sup> Furthermore, recent developments in the biomedical field are prompting a reassessment of interventions considered standard of care to ensure their relevance to the latest scientific evidence. Normal saline, although safe and used for decades, has not necessarily been proven biologically superior to other alternatives. Therefore, comparative studies between 0.9% NaCl and distilled water in animal models are an important step in re-evaluating the scientific basis of conventional practices and determining whether traditional approaches remain relevant in the era of evidence-based medicine.<sup>11</sup>

This study aimed to objectively assess the effects of irrigation using 0.9% NaCl compared with distilled water on the wound healing process in a rat model. Assessment was conducted using macroscopic parameters, such as a decrease in wound diameter and histological parameters, including re-epithelialisation, granulation tissue formation, and inflammatory infiltration. The results of this study are expected to provide experimental evidence that supports data-based clinical decisions and help formulate new recommendations regarding the selection of effective, safe, and economical irrigation fluids in wound care.

## Methods

This was an experimental *in vivo* study conducted at the Animal Laboratory of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Twenty-seven healthy male Wistar rats (*Rattus norvegicus*), aged 2–3 months and weighing 150–200 grams, were randomly divided into three groups (n=9 per group): (1) control (no treatment), (2) distilled water (aquadest) compress, and (3) 0.9% NaCl water (sodium chloride) compress. The animals were acclimatised for seven days before the intervention and housed under standard laboratory conditions with *ad libitum* access to food and water.

Each rat was anaesthetised using a ketamine (80mg/kg) and xylazine (10mg/kg) intraperitoneal injection. The dorsal surface was shaved and disinfected, and a full-thickness circular wound (1cm in diameter) was created on the back of each animal using a sterile biopsy punch. Immediately after wound induction, treatment was initiated and continued twice daily according to group allocation for 14 consecutive days. The NaCl group received sterile 0.9% sodium chloride, while the aquadest group received sterile distilled water. Wounds were treated twice daily using sterile gauze compresses soaked in either 0.9% sodium chloride (NaCl) or distilled water (aquadest), applied for 10 minutes per session. Following each compress, the wound was covered with sterile gauze. The control group received no fluid irrigation. The irrigation procedure was performed by flushing the wound with a solution according to the treatment group (0.9% NaCl or sterile distilled water) with a volume of 5mL per cm-squared of wound area using a sterile 10mL syringe without a needle to produce gentle pressure that did not damage the granulation tissue. After irrigation, the wounds were dried aseptically using sterile gauze by gently blotting for 10 minutes to absorb excess fluid without applying pressure that could interfere with tissue perfusion or induce additional mechanical trauma. This procedure was performed under close supervision to prevent tissue damage due to excessive pressure.

Macroscopic and histopathological evaluations were performed on days 2, 7, and 14. On each of these days, three rats from the NaCl group, three from the aquadest group, and three from the control group were euthanised using an overdose of ether. Wound healing was assessed macroscopically by measuring wound diameter in millimeters using a caliper. Tissue samples were then harvested and fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic analysis. Histological evaluations included assessment of re-epithelialisation, granulation tissue formation, and inflammatory cell infiltration using a semi-quantitative scoring system. Quantitative assessment was also performed using ImageJ software on images captured with Optilab 2.0. This assessment was used to measure epithelial thickness, inflammatory cell count, and neovascularisation.

All data were analysed using RStudio Version: 2025.05.1+ and SPSS version 25.0. Normality was assessed using the Shapiro–Wilk test. Non-parametric data were analysed using the Friedman test followed by the Wilcoxon signed-rank test for post hoc pairwise comparisons. Effect sizes were calculated using Cohen's *d* along with 95% confidence intervals to assess the magnitude of change. A *p*-value of <0.05 was considered statistically significant. Power calculation was not conducted due to the exploratory nature of this preclinical study.

All procedures involving animals were conducted in accordance with the ethical standards of the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University. Ethical approval for this study was granted under No. 450/UN4.6.4.5.31/PP36/2024, issued on 14 June 2024.

## Results

A total of 27 Wistar rats were evenly assigned to three groups: control, 0.9% NaCl, and distilled water (aquadest), with nine rats in each group. Wound healing outcomes were assessed macroscopically by measuring wound diameter and histologically using a semi-quantitative scoring system for three key parameters: re-epithelialisation, granulation tissue, and inflammatory response. The inflammatory response was evaluated by quantifying polymorphonuclear (PMN) cell infiltration in the wound bed, with scores of 1,2,3 indicating mild, moderate, and dense/heavy infiltration, respectively. Additionally, granulation tissue formation was graded based on the extent of capillary and fibroblast proliferation, where a score of 1 represented mild formation covering 0–25% of the wound area, a score of 2 indicated moderate formation covering 50–75% and a score of 3 denoted complete formation involving more than 75% of the wound area. Lastly, re-epithelialisation was assessed according to the proportion of the wound surface covered by new epithelial tissue, with a score of 1 indicating no re-epithelialisation, a score of 2 indicating less than 50% coverage, a score of 3 indicating 50–90% coverage, and a score of 4 indicating complete (100%) coverage. This study also evaluated the results of the analysis of epithelial thickness ( $\mu\text{m}$ ), the number of inflammatory cells evaluated on the side of the field most dense with inflammatory cells in each sample, and the number of neovascular. The results are provided in Table 1.

### Wound diameter

Across all groups, the wound area showed a general trend of reduction after 14 days of observation. However, no statistically significant change in wound diameter was observed in any group. The control group had a p-value of 0.231 with a moderate effect size (Cohen's  $d=0.55$ , 95% CI: 0.00–1.00). The NaCl group showed a p-value of 0.448 with a small effect size ( $d=0.33$ , 95% CI: 0.00–1.00), while the aquadest group had a p-value of 0.117 with a moderate-to-large effect size ( $d=0.78$ , 95% CI: 0.01–1.00) as seen in Figure 2 and Figure 4.

### Inflammatory response

Only the aquadest group exhibited a statistically significant reduction in inflammation ( $p=0.039$ ;  $d=0.778$ , 95% CI: 0.365–1.00), indicating a large effect. The control group showed no statistical significance ( $p=0.150$ ), though it had a moderate effect size ( $d=0.633$ , 95% CI: 0.251–1.00). Similarly, the NaCl group had a p-value of 0.097, not reaching significance, but with a large effect size ( $d=0.778$ , 95% CI: 0.365–1.00) as seen in Figure 3 and Figure 5. In addition, quantitative assessment results using ImageJ with inflammatory cell counting in the preparations showed no significant differences between days

2, 7, and 14 in all groups. In the control and NaCl groups, a relative increase in inflammatory cells was found over time ( $p=0.250$  and  $p=0.082$ ). However, in the distilled water group, there was a decrease in the number of inflammatory cells on day 14 compared to days 2 and 7 ( $p=0.347$ ), as seen in Table 1 and Figure 1.

### Granulation tissue formation

The aquadest and control groups both exhibited statistically significant increases in granulation tissue. Both had a p-value of 0.022 and large effect sizes ( $d=0.939$ , 95% CI: 0.582–1.00). The NaCl group showed a p-value of 0.059, not reaching statistical significance, with a small effect size ( $d=0.233$ , 95% CI: 0.00–0.616) as seen in Figure 3 and Figure 5.

### Re-epithelialisation

Significant improvement in re-epithelialisation was observed in both the control and aquadest groups. The control group demonstrated a p-value of 0.037 and a large effect size ( $d=0.833$ , 95% CI: 0.451–1.00), while the aquadest group had a p-value of 0.018 and a very large effect size ( $d=0.933$ , 95% CI: 0.551–1.00). The NaCl group did not show significant improvement ( $p=0.156$ ), though it demonstrated a moderate effect size ( $d=0.619$ , 95% CI: 0.206–1.00) as seen in Figure 3 and Figure 5. The results of the evaluation of epithelial thickness showed that in the control and NaCl groups, there was no significant change in epithelial thickness between the evaluations on days 2, 7, and 14 ( $p=0.717$  and  $p=0.414$ ), but showed an increasing trend. The group with distilled water showed a significant increase between the evaluations on day 14 ( $194.43\pm 34.06 \mu\text{m}$ ) compared to day 2 ( $69.52\pm 18.72$ ) and day 7 ( $53.57\pm 9.87$ ) ( $p=0.040$ ), as seen in Table 1 and Figure 1.

### Neovascular count

Neovascular counts showed no significant changes on days 2, 7, and 14 in all groups ( $p=0.153$ ,  $p=0.471$ , and  $p=0.264$ , respectively). However, there was a trend towards an increase in neovascularization from baseline across all groups, as seen in Table 1 and Figure 6.

## Discussion

This study compared the effects of 0.9% NaCl and distilled water (aquadest) on histopathological parameters of wound healing, as seen in Table 1 and Figures 1–6. Although 0.9% NaCl is widely used for wound irrigation due to its affordability and isotonic nature, it did not significantly enhance re-epithelialisation, granulation tissue formation, or reduction of inflammatory cell infiltration in our model. Its primary benefit lies in maintaining a moist environment, but over time, as water evaporates, it may become hypertonic, potentially disrupting osmotic balance and failing to actively stimulate tissue repair. Several studies have echoed these limitations, noting that isotonic saline primarily plays a passive role in wound healing.<sup>7,12–15</sup>

Table 1. Comparison of wound healing parameters across treatment groups

Treatment	Times	Mean±SD	Δ (Delta mean)			p-value and test
			D2-D7	D2-D14	D7-D14	
<b>Diameter</b>						
Control	D2	8.52±2.37	-0.21	-2.09	-1.88	0.231 repeated measures
	D7	8.31±2.33				
	D14	6.43±4.17				
NaCl	D2	7.79±0.75	-0.79	-1.59	-0.8	0.448 repeated measures
	D7	7±1.46				
	D14	6.2±1.91				
Aquadest	D2	7.99±1.76	-1.69	-3.73	-2.04	0.117 repeated measures
	D7	6.3±0.27				
	D14	4.26±0.66				
<b>Inflammatory response</b>						
Control	D2	3±0	-0.67	-1	-0.33	0.150 Friedman test
	D7	2.33±0.58				
	D14	2±1				
NaCl	D2	3±0	-0.33	-0.67	-0.34	0.097 Friedman test
	D7	2.67±0.58				
	D14	2.33±0.58				
Aquadest	D2	3±0	-0.67	-1	-0.33	<b>0.039*</b> Friedman test*
	D7	2.33±0.58				
	D14	2±0				
<b>Granulation response</b>						
Control	D2	1±0	1	1.67	0.67	<b>0.022*</b> Friedman test
	D7	2±0				
	D14	2.67±0.58				
NaCl	D2	1±0	0.33	1.33	1	0.059 Friedman test
	D7	1.33±0.58				
	D14	2.33±1.15				
Aquadest	D2	1±0	0.67	2	1.33	<b>0.022*</b> Friedman test
	D7	1.67±0.58				
	D14	3±0				
<b>Re-epithelialisation</b>						
Control	D2	1±0	0.67	2	1.33	<b>0.037*</b> Friedman test
	D7	1.67±0.58				
	D14	3±1				
NaCl	D2	1±0	0.33	1	0.67	0.156 Friedman test
	D7	1.33±0.58				
	D14	2±1				
Aquadest	D2	1±0	0	2.33	2.33	<b>0.018*</b> Friedman test
	D7	1±0				
	D14	3.33±0.58				
<b>Epithelial thickness</b>						
Control	D2	88.49±24.93	-0.001	36.27	36.27	0.717 Friedman test
	D7	88.49±44.53				
	D14	124.77±80.42				

Treatment	Times	Mean±SD	Δ (Delta mean)			p-value and test
			D2–D7	D2–D14	D7–D14	
NaCl	D2	73.09±30.26	-13.56	68.83	82.39	0.414 repeated measures
	D7	59.52±18.16				
	D14	141.92±89.59				
Aquadest	D2	69.52±18.72	-15.95	124.90	140.86	<b>0.040*</b> repeated measures*
	D7	53.57±9.87				
	D14	194.43±34.06				
<b>Inflammatory cell count</b>						
Control	D2	243.33±38.72	432.67	462.67	30.00	0.250 repeated measures
	D7	676.00±556.39				
	D14	706.00±530.12				
NaCl	D2	854.67±550.12	-250.00	1,201.33	1,451.33	0.082 repeated measures
	D7	604.66±417.91				
	D14	2056±554.52				
Aquadest	D2	1060.66±664.95	24.33	-594.00	-618.00	0.347 repeated measures
	D7	1085.00±515.18				
	D14	466.66±267.71				
<b>Neovascular count</b>						
Control	D2	8.33±5.85	8.00	6.67	-1.33	0.153 repeated measures
	D7	16.33±5.51				
	D14	15.00±10.54				
NaCl	D2	7.33±2.08	9.33	4.33	-5.00	0.471 repeated measures
	D7	16.67±7.64				
	D14	11.667±6.66				
Aquadest	D2	11.33±11.84	-3.33	9.67	13.00	0.264 Friedman test
	D7	8.00±8.66				
	D14	21.00±4.00				

In the test column \* means <0.05

In the Delta mean columns no change is represented by yellow, decrease by red and increase by blue.

In contrast, distilled water (aquadest) demonstrated significant histological improvements, particularly in re-epithelialisation, granulation tissue formation, and reduction of inflammatory cell infiltration. These results are consistent with the findings of Fernandez et al<sup>7</sup>, who reported a shorter mean wound healing time in the distilled water group (2.7 weeks) compared to the isotonic saline group (3.1 weeks), although this difference did not reach statistical significance (p=0.389). The similarity in outcomes may be due to the transient hypotonic effect of distilled water, which, while capable of causing osmotic lysis in vitro, is rapidly neutralised in vivo. This transient effect may promote early inflammatory clearance and facilitate the transition to the proliferative phase without prolonged cytotoxicity.<sup>7</sup> More recent clinical data reinforce the potential of distilled water. Similarly, Jeo et al<sup>18</sup> demonstrated that distilled water irrigation in post-laparotomy patients reduced superficial surgical site infection rates without adverse microbiological outcomes and was more cost-effective than standard care.<sup>18</sup> These findings align with the cytokine

modulation theory, which postulates that hypotonic fluids aid in removing pro-inflammatory mediators while promoting tissue repair.<sup>17–19</sup>

Furthermore, a randomised clinical trial by Riza et al<sup>15</sup> found that wound cleansing using distilled water was as safe and effective as sterile saline, particularly in low-resource settings. This study adds practical validation to the use of aquadest, especially considering its economic feasibility and broad accessibility<sup>15</sup>. The lack of solutes in distilled water may also reduce antigenic load and microbial colonization, indirectly enhancing keratinocyte and fibroblast proliferation.<sup>8–9</sup> Granulation tissue formation, a hallmark of the proliferative phase, depends on angiogenesis, fibroblast activity, and extracellular matrix deposition.<sup>19</sup> Aquadest's ionic neutrality may help stabilise wound pH and reduce leukocyte chemotaxis, supporting tissue remodeling and collagen synthesis.<sup>2,20</sup> Additionally, the method of application via compress (rather than direct irrigation) may allow a more

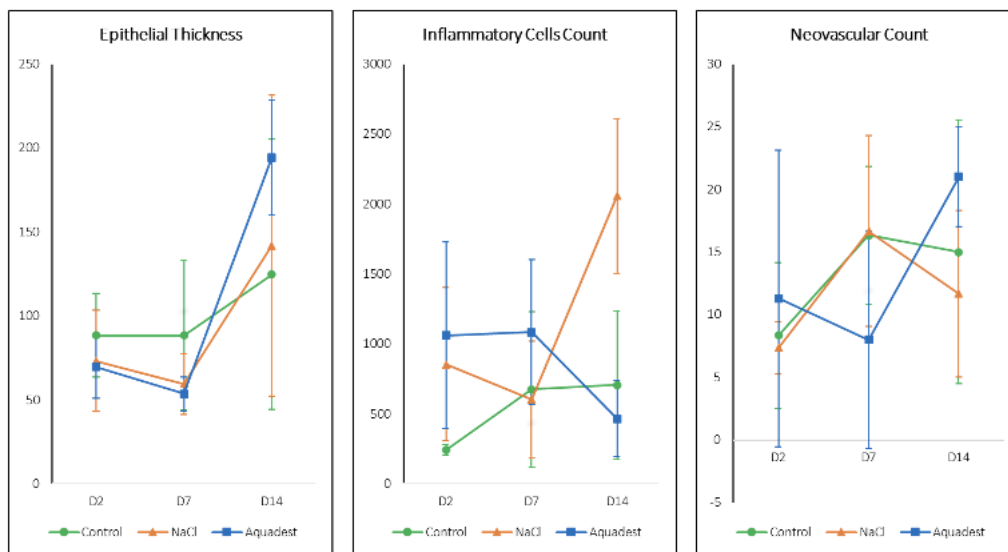
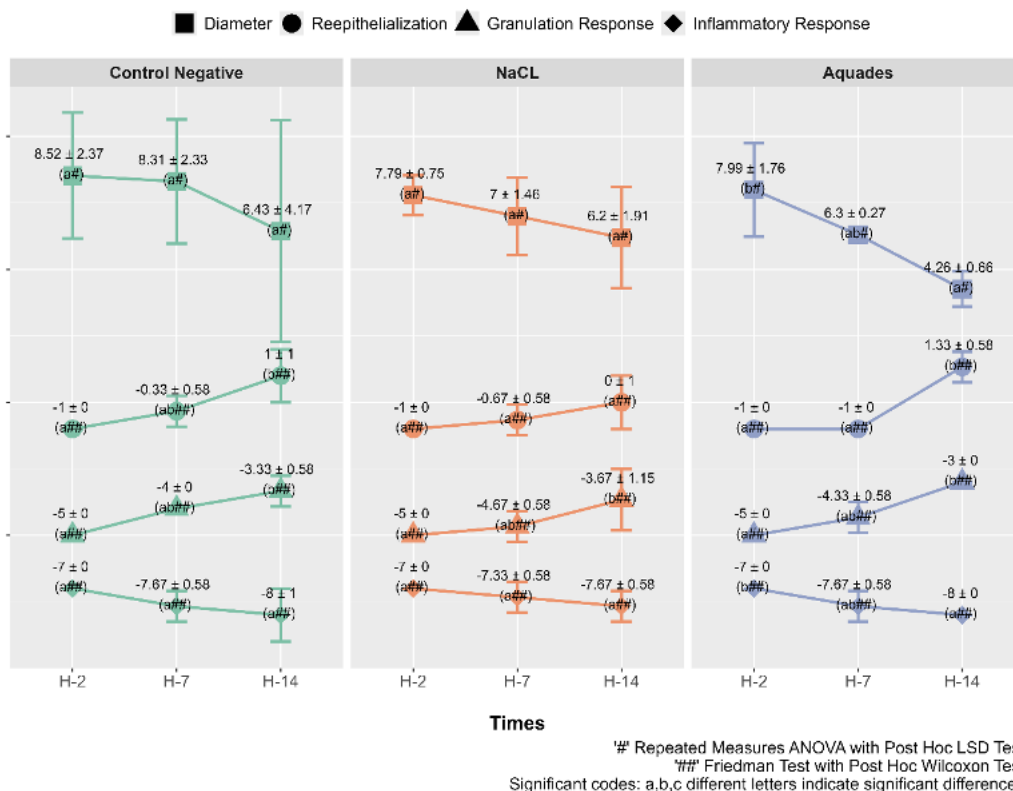


Figure 1. Error plot analysis. Qualitative (semi-quantitative) analysis (above). Quantitative analysis (below)

uniform and less abrasive exposure of the wound bed to the fluid, minimising trauma and enhancing therapeutic absorption. Despite these histologic improvements, macroscopic wound diameter reduction was not statistically significant in the aquadest group. This highlights a known discrepancy in wound healing dynamics: cellular and biochemical repair may precede visible wound contraction, particularly in rodent models where loose skin can mask underlying changes. Our sample size was modest, and inter-animal variability may have diluted statistical signals despite moderate-to-large effect sizes.<sup>5,7</sup>

The quantitative analysis results in this study indicate that the application of distilled water to wounds increases epithelial thickness, reduces the number of inflammatory cells, and increases neovascularisation compared to the baseline, as seen in Table 1 and Figure 1. These three findings are biologically related to the acceleration of the wound healing process. Faster re-epithelialisation and epithelial thickening indicate more efficient keratinocyte proliferation and migration to close the epithelial defect. Reduced inflammation indicates a faster transition from the inflammatory to the proliferative phase. Increased neovascularisation supports the supply of oxygen and nutrients necessary for extracellular

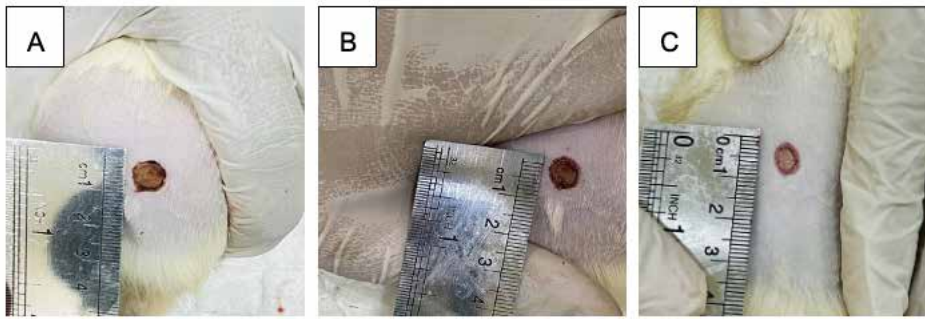


Figure 2. Wound healing in rats: (A) Day 2, (B) Day 7, and (C) Day 14 in the Aquadest group

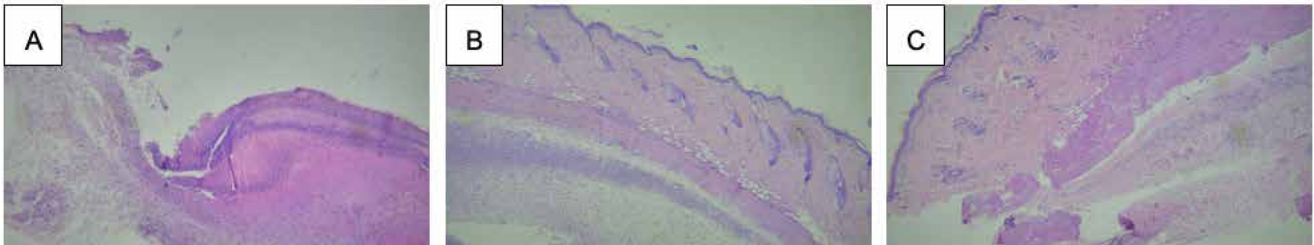


Figure 3. Histopathology images of the Aquadest group at different time points. (A) Day 2: Light granulation tissue formation (0–25%), no re-epithelialisation observed, dense/heavy inflammatory cell infiltration. (B) Day 7: Moderate granulation tissue formation (50–70%), no re-epithelialisation observed, moderate inflammatory cell infiltration. (C) Day 14: Complete granulation tissue formation (>75%), re-epithelialisation covering 50–90% of the wound area, moderate inflammatory cell infiltration.

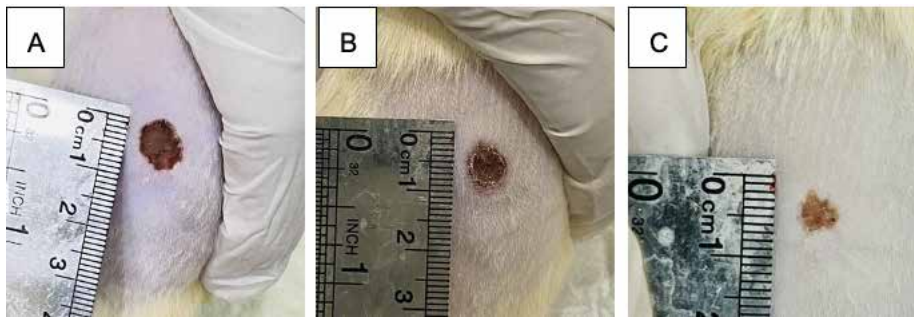


Figure 4. Wound healing in rats: (A) Day 2, (B) Day 7, and (C) Day 14 in the 0.9% NaCl group

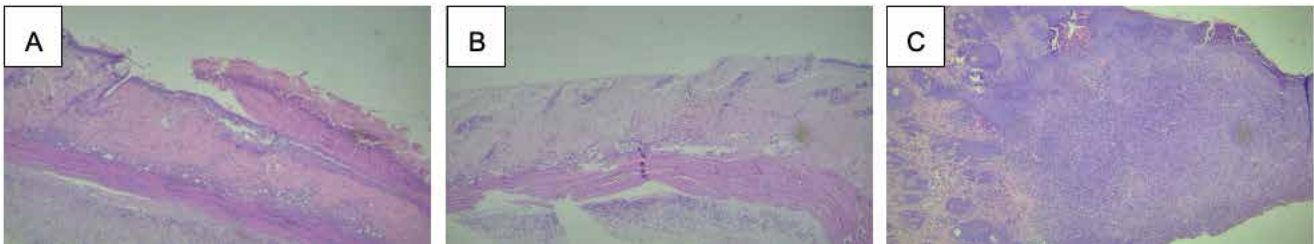


Figure 5. Histopathology images of the 0.9% NaCl group at different time points. (A) Day 2: Light granulation tissue formation (0–25%), no re-epithelialisation observed, dense/heavy inflammatory cell infiltration. (B) Day 7: Moderate granulation tissue formation (50–70%), re-epithelialisation covering 1–50% of the wound area, moderate inflammatory cell infiltration. (C) Day 14: Complete granulation tissue formation (>75%), reepithelialization covering 50–90% of the wound area, moderate inflammatory cell infiltration.

matrix synthesis and new tissue formation. All of these processes accelerate wound closure and restoration of tissue function. The basic concept that a moist wound environment accelerates epithelialisation has been described in a comprehensive analysis by Pastar et al (2014).<sup>20</sup>

Mechanistically, the benefits of distilled water appear to be related to the creation of a less irritating and relatively moist

wound microenvironment. A moist environment reduces scab formation, which inhibits keratinocyte migration, and simultaneously retains growth factors and proteolytic enzymes that support tissue remodeling. This facilitates epithelial cell movement and proliferation, thereby increasing the thickness of the new epithelium. In addition, reduced osmotic stimulus or foreign ion content in sterile distilled water can reduce excessive phagocyte activation and pro-

inflammatory cytokine secretion, so that the number of inflammatory cells in the wound decreases more quickly and minimises secondary tissue damage. These micro conditions are also conducive to angiogenesis because pro-angiogenic factors produced by resolving macrophages and fibroblasts can survive longer in a non-destructive environment, thus explaining the increase in neovascularisation measured in the distilled water group. The statement about the influence of a moist environment on reducing inflammation and promoting angiogenesis is supported by studies that link moist wound healing with increased re-epithelialisation, reduction of necrotic tissue, and angiogenic stimulation.<sup>21-23</sup>

Several limitations warrant discussion. First, the study did not evaluate molecular biomarkers (such as IL-6, VEGF, TNF- $\alpha$ ) that could clarify the biological mechanisms underlying histological findings.<sup>4,19,21</sup> Second, wound healing was assessed through linear diameter measurements, which may underestimate subtle volume or perfusion changes. Data ranges that are too wide cause a wide data distribution and the use of nonparametric analysis. More advanced imaging methods, such as 3D wound profiling or perfusion studies, may offer better assessment of healing dynamics. This study also did not assess wound infection status, although infection can affect the inflammatory response and slow healing, making it an important limitation that needs to be examined in further research. Finally, this study was conducted in a preclinical

rat model and cannot yet be generalised to humans. Future studies should include standardised application protocols, blinded histological analysis, and molecular profiling. Testing in diabetic or immunocompromised models and extended observation into the remodeling phase would better reflect clinical complexity and validate the translational value of aquadest.<sup>24,25</sup>

## Conclusions

This preclinical study demonstrated that distilled water (aquadest) significantly improved key histological markers of wound healing, namely re-epithelialisation, granulation tissue formation, improvement of the epithelial thickness, neovascularisation, and inflammation reduction, when compared to the baseline. These results suggest that aquadest may offer a safe, accessible, and cost-effective alternative for wound irrigation. However, procedural differences and the lack of molecular biomarker analysis limit the generalisability of these findings. Further studies are warranted to confirm these results in human populations and under standardised clinical protocols.

The findings of this study have practical implications for wound care, particularly in resource-limited settings. Given its efficacy and affordability, aquadest may be considered as an alternative irrigation solution where isotonic saline is unavailable or cost-restrictive. Its ability to enhance

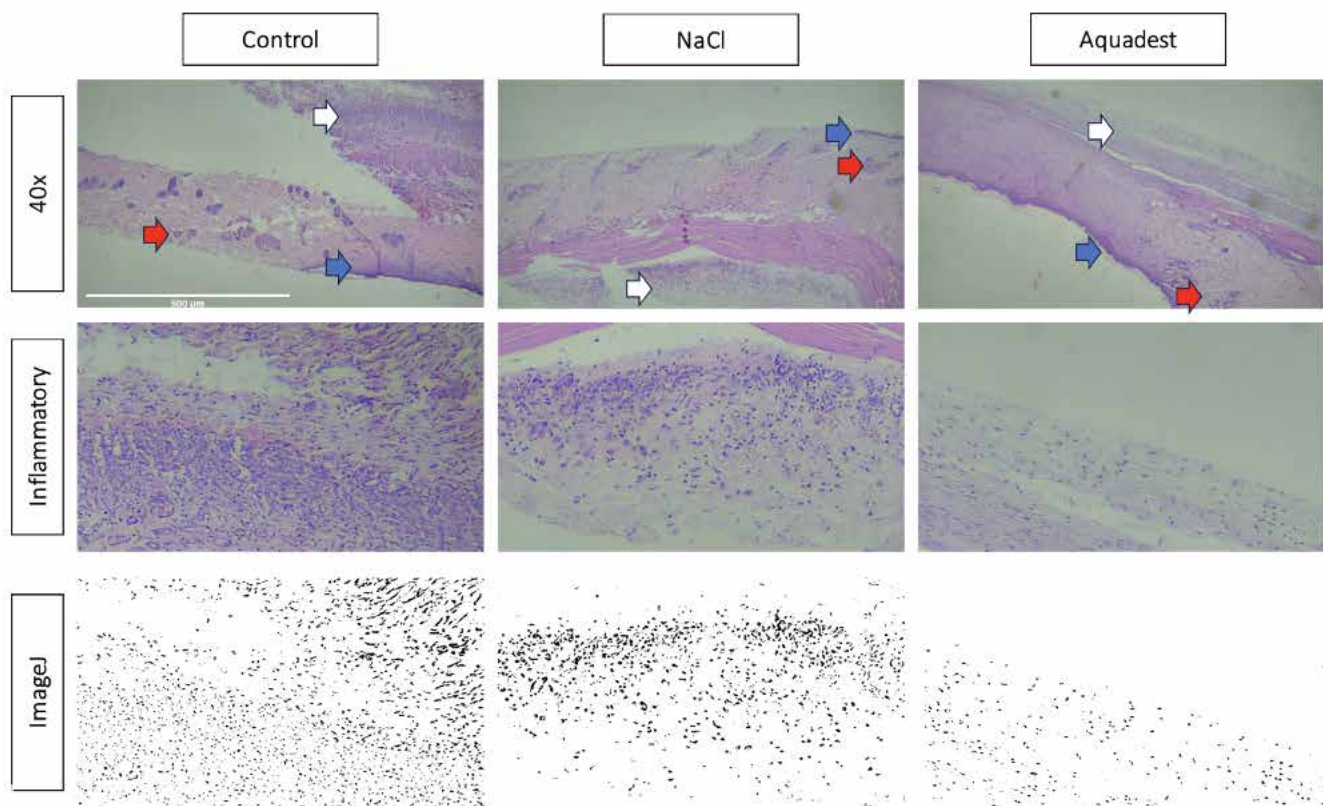


Figure 6. Histological evaluation and basic quantitative counting of the samples. The image shows 40x magnification, inflammatory cells in H&E staining, and inflammatory cells in ImageJ. Epithelial thickness (blue arrow); inflammatory cells (white arrow); neovascularisation (red arrow).

tissue healing through reduced inflammation and improved granulation formation may be especially beneficial for acute and chronic wound management. Implementation in clinical settings, however, should be preceded by clinical trials evaluating not only efficacy but also patient-reported outcomes, safety and long-term healing trajectories.

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## Conflict of interest

The authors declare no conflicts of interest related to this study.

## Ethics statement

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. The ethical clearance number is 450/UN4.6.4.5.31/PP36/2024, issued on 14 June 2024. All animal handling and experimental procedures were performed in accordance with institutional guidelines and international standards for the ethical treatment of laboratory animals.

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## Author contributions

Concept, method, validation and analysis: SW and KD.

Supervision and methodological development: UM.

Software, data visualisation and management: SC and AP.

Writing (original draft): SW, KD, SC and AP.

Writing (review and editing) SW, KD, SC and KD.

Funding acquisition: SW, KD, SC and AP.

All authors read and approved the final version of the manuscript.

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