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Antimicrobial Efficacy of Piperacillin- Tazobactam Loaded Alginate Scaffold in an Incisional Wound Model in Wistar Rats for Management of Surgical Site Infection: An in Vivo Study

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KEYWORDS:

Piperacillin–tazobactam, Sodium alginate, Surgical site infection, Wound healing, REEDA score, Colony-forming units.

ABSTRACT:

Introduction: Surgical site infections remain a significant postoperative complication, particularly in oral and maxillofacial procedures due to the inherent exposure of surgical wounds to the microbial flora of the oral cavity. Localised drug delivery systems using biocompatible scaffolds offer a promising approach for targeted antimicrobial therapy and improved wound healing. Sodium alginate, a widely used biopolymer, provides an effective platform for controlled drug delivery. This study aimed to evaluate the antimicrobial efficacy of a piperacillin-tazobactam loaded sodium alginate scaffold in an incisional wound model in Wistar rats.

Materials and Methods: This randomized controlled in vivo study was conducted on healthy adult Wistar rats divided into three groups: Group A (control), Group B (sodium alginate scaffold), and Group C (piperacillin- tazobactam loaded alginate scaffold). Standardised incisional wounds were created, and respective dressings were applied. Wound swab samples were collected on Days 1, 3, 7, 10, 14, and 21 for microbiological analysis, including isolation and identification of organisms and quantitative colony-forming unit (CFU) estimation. Clinical wound healing was assessed using the REEDA scoring system at corresponding time intervals.

Results: Pseudomonas aeruginosa and Staphylococcus aureus were identified as the predominant organisms. No significant difference in microbial growth was observed among groups at early time points; however, Group C demonstrated a statistically significant reduction in microbial load from Day 7 onwards. Quantitative CFU analysis validated these findings, showing progressive bacterial load reduction in the treated group. Clinically, Group C exhibited improved wound healing compared to Groups A and B.

Conclusion: The piperacillin-loaded sodium alginate scaffold demonstrated effective antimicrobial activity and enhanced wound healing in the incisional wound model. The findings support its potential as a promising localised drug delivery system for the management of surgical site infections.

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1. Introduction

Surgical site infections (SSIs) are among the most common postoperative complications, significantly contributing to patient morbidity, delayed wound healing, prolonged hospitalization, need for extensive surgical and antibiotic interventions, and increased healthcare costs. Despite advances in surgical techniques and infection control measures, SSIs continue to pose a major clinical challenge worldwide. Deep incisional SSIs extend beyond the superficial layers to involve the underlying muscles and fascia, often requiring more aggressive management. The development of SSIs is multifactorial, influenced by a combination of patient-related, procedure-related, and external factors. Among these, patient-related factors such as advanced age, immunocompromised status, diabetes, obesity, smoking, malnutrition, steroid use, and prolonged preoperative hospital stay play a crucial role in increasing susceptibility to infection [1]. Effective management of SSIs requires not only appropriate antimicrobial therapy but also the development of advanced wound care strategies that can control infection while promoting tissue regeneration. Conventional systemic antibiotic administration often fails to achieve adequate drug concentration at the wound site and may be associated with systemic side effects and the development of antimicrobial resistance [2].

This has led to growing interest in localized drug delivery systems, which provide targeted antimicrobial action, reduce systemic toxicity, and improve therapeutic outcomes. Biomaterial-based wound dressings, particularly those derived from natural polymers, have gained considerable attention for their ability to serve as both structural scaffolds and drug delivery platforms [3]. Among these, sodium alginate has emerged as a promising biopolymer due to its excellent biocompatibility, biodegradability, and capacity to maintain a moist wound environment conducive to healing. Alginate-based dressings are known to facilitate hemostasis, absorb exudate, and support cellular activities essential for tissue repair, including fibroblast proliferation and extracellular matrix formation. Furthermore, the incorporation of antimicrobial agents into alginate matrices enhances their functional performance by providing sustained and localized drug release, thereby improving infection control at the wound site [4,5].

In vivo studies have consistently demonstrated that antimicrobial-loaded biomaterial scaffolds can significantly reduce bacterial burden and accelerate wound healing by modulating inflammation and promoting tissue regeneration. Animal models, particularly Wistar rat incisional wound models, are widely used for evaluating wound healing and antimicrobial efficacy due to their reproducibility, ease of handling, and physiological relevance. These models enable the assessment of both clinical and microbiological parameters, providing a comprehensive understanding of the healing process [6].

Despite advancements in biomaterial-based wound dressings, there remains a need for developing systems that combine effective antimicrobial activity with high biocompatibility to ensure safety and efficacy in clinical applications. The use of broad-spectrum antibiotics such as piperacillin–tazobactam in localized delivery systems presents a promising approach to combat both Gram-positive and Gram-negative pathogens commonly associated with surgical site infections. Incorporating such antibiotics into a biopolymer matrix may provide sustained antimicrobial activity, minimize infection risk, and enhance wound healing outcomes [3]. Therefore, the present study was designed to evaluate the in vivo antimicrobial efficacy and wound healing potential of a sodium alginate-based membrane loaded with piperacillin–tazobactam using an incisional wound model in Wistar rats.

2. Material and Methods

Study Design

This experimental study was designed as a controlled, prospective, randomised animal trial conducted on healthy adult Wistar rats aged 10-12 weeks and weighing approximately 250-300. The study aimed to evaluate the in vivo antimicrobial efficacy and wound- healing properties of a biopolymer-based piperacillin-loaded scaffold used as a dressing material in an incisional wound model.

The animals were randomly divided into three distinct groups:

Group A - control group receiving conventional dressing

Group B - experimental group receiving sodium alginate biopolymer scaffold dressing and

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Group C - experimental group receiving piperacillin–tazobactam-loaded scaffold dressing.

Each group comprised six animals, ensuring statistical reliability while minimising animal usage in accordance with ethical guidelines. The duration of the study consisted of a 21-day observation period, during which antimicrobial efficacy and wound healing were assessed. The surgical procedure and scaffold application were performed by the principal investigator and assistant, who were aware of the group allocation because of the nature of the intervention; hence, blinding during scaffold application was not feasible. However, the procedure was standardised and performed uniformly in all groups by the same operator to reduce procedural variability.

Ethical Approval

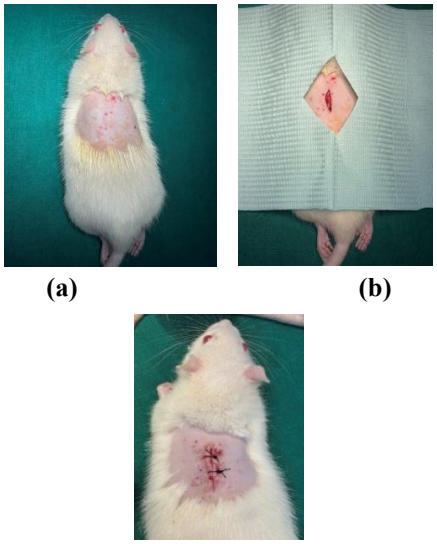
Prior ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) our of our institution. Approval number BRULAC/SDCH/SIMATS/IEAC/03-2026/03. The study adhered strictly to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All procedures involving animals were performed by trained personnel under veterinary supervision, ensuring strict compliance with ethical standards.

Experimental Procedure

After 7 days of quarantine, the animals will be subjected to induction of general anesthesia using ketamine (60 mg/kg) and xylazine (10 mg/kg) to ensure adequate analgesia, sedation, and immobility throughout the surgical procedure. Adequate depth of anesthesia will be confirmed prior to intervention by the absence of the pedal withdrawal reflex.

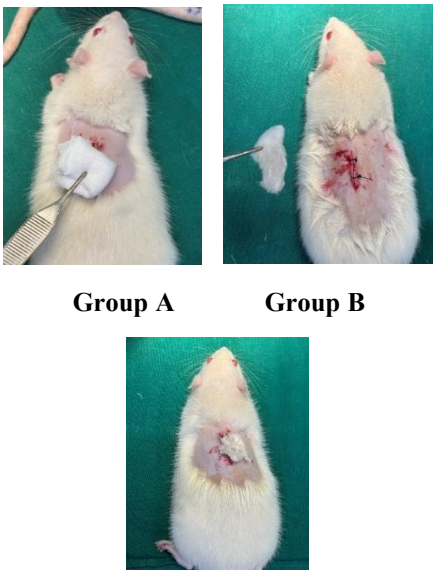
Under strict aseptic precautions, the dorsum of rat will be shaved and disinfected (Figure-1a). A standardised 2 cm linear full-thickness incision will be made on the dorsal surface of the rat; blunt dissection will be carried out to simulate a postoperative surgical wound (Figure-1b). The incision will then be closed using interrupted sutures with non- resorbable suture material to establish a reproducible incisional wound model (Figure-1c).

Dressing material was carefully applied over the sutured wound site, ensuring adequate contact with the incision area according to the group allocation and a bandage was given (Figure-2). Following the procedure, the animals were isolated and housed individually in separate cages according to their respective study groups. Animals will be monitored regularly, and serial wound swab cultures will be collected to assess microbial colonisation (Figure-3). Clinical signs of wound healing, and discharge; will be recorded throughout the study period.



(a) **(b)** **(c)**

Figure 1. Experimental procedure: (a) Preparation of surgical sit, (b) Linear incision and blunt dissection, (c) Sutured incisional wound



Group A **Group B** **Group C**

Figure 2. Different dressing material Group A- Conventional gauge dressing, Group B- Sodium

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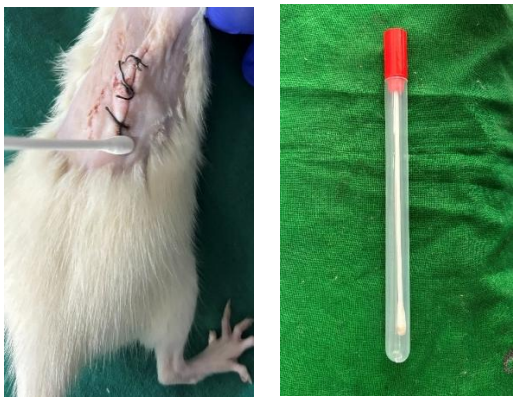
alginate scaffold dressing, Group C- piperacillin–tazobactam-loaded scaffold dressing.



(a) **(b)**

Figure 3. Specimen collection- (a) Swab specimen collection, (b) Collected specimen

Outcomes Measured

The study outcomes were assessed using microbiological and clinical parameters to evaluate antimicrobial efficacy and wound healing.

Isolation and Identification

Antimicrobial efficacy was evaluated by serial wound swab analysis. Swabs were collected at predetermined intervals Day 1, Day 3, Day 7, Day 10, Day 14 and Day 21 and subjected to isolation and identification of microorganisms. The presence or absence of pathogenic organisms was recorded and compared among the study groups.

Quantitative Microbiological Assessment – Colony-Forming Unit (CFU) Analysis

Antimicrobial efficacy was primarily evaluated by quantifying of bacterial load using colony-forming unit (CFU) analysis. Wound swab samples were collected at predetermined intervals on Day 1, Day 3, Day 7, Day 10, Day 14 and Day 21 cultured on appropriate media, and the number of colonies was counted and expressed as CFU/mL. Reduction in CFU count over time was used as an indicator of antimicrobial effectiveness of the scaffold.

Clinical Wound Assessment (REEDA Score)

Wound healing was evaluated using the REEDA scoring system, assessing redness, edema, ecchymosis, discharge, and approximation. Each parameter was graded on a scale of 0 to 3, and the total score was calculated on Day 1, Day 3, Day 7, Day 10, Day 14 and Day 21, with lower scores indicating better wound healing (Table-1). The total REEDA score ranges from a minimum of 0 (optimal healing) to a maximum of 15 (severe wound impairment).

Table 1: Clinical wound assessment (REEDA Score)

Score	Redness	Oedema	Ecchymosis	Discharge	Approximation
0	None	None	None	None	Close
1	Within 0.25 cm of the incision bilaterally	<1 cm from incision	Within 0.25 cm bilaterally or 0.5 cm unilaterally	Serous	Skin separation ≤ 3 mm
2	Within 0.5 cm of the incision bilaterally	1–2 cm from incision	0.25–1 cm bilaterally or 0.5–2 cm unilaterally	Serosanguinous	Skin and subcutaneous fat separation
3	Beyond 0.5 cm of the incision bilaterally	>2 cm from incision	>1 cm bilaterally or >2 cm unilaterally	Bloody, purulent	Separation involving skin, subcutaneous fat, and fascia

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3. Results

Isolation and Identification

Microbiological analysis of wound swab samples revealed the presence of two predominant bacterial strains. Strain 1 was identified as a Gram-negative *Pseudomonas aeruginosa*. Strain 2 was identified as Gram-positive *Staphylococcus aureus* (Figure 4).

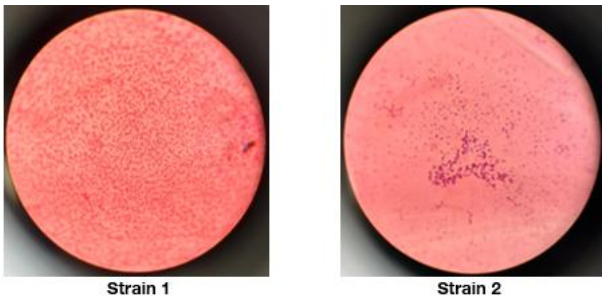

Figure 4. Isolation and Identification: Strain 1 - Gram-negative, non-spore-forming, aerobic rod-shaped bacteria, *Pseudomonas aeruginosa;* Strain 2 – Gram-positive spherical cocci, typically arranged in irregular, grape-like clusters, *Staphylococcus aureus*

The comparison of microbial growth across the three groups demonstrated no statistically significant difference on Day 1 (p = 0.074) and Day 3 (p = 0.377). However, significant differences were observed from Day 7 onwards (Day 7: p = 0.007; Day 10: p = 0.004; Day 14: p = 0.001; Day 21: p = 0.012). At later time points, the Piperacillin + Tazobactam group showed a higher proportion of no growth, indicating effective microbial elimination, whereas the control and sodium alginate groups showed persistent bacterial presence. On Day 7 (p = 0.007), pairwise comparison revealed a significant difference between Group A and Group C (p = 0.006) and between Group B and Group C (p = 0.015), while no significant difference was found between Group A and Group B (p = 1.000). On Day 10 (p = 0.004), a significant difference was observed between Group A and Group C (p = 0.006), whereas comparisons between Group A and Group B (p = 0.061) and Group B and Group C (p = 0.121) were not statistically significant. On Day 14 (p = 0.001), significant differences were found between Group A and Group B (p = 0.015) and between Group A and Group C (p = 0.002), while no difference was noted between Group B and Group C (p = 1.000). On Day 21 (p = 0.012), a significant difference persisted between Group A and Group C (p = 0.015) (Table 2). This indicates that Group C achieved significantly better microbial clearance compared to Group A and B, particularly from Day 7 onwards (Graph 1).

Table 2: Comparison of microbial growth between three groups at different time intervals

Day	Category	Control (Group A)	Sodium Alginate (Group B)	Piperacillin + Tazobactam (Group C)	p-value
Day 1	No Growth	0 (0.0%)	0 (0.0%)	3 (100.0%)	0.074
	GP	6 (40.0%)	6 (40.0%)	3 (20.0%)	
Day 3	No Growth	0 (0.0%)	0 (0.0%)	2 (100.0%)	0.377
	GP	2 (40.0%)	1 (20.0%)	2 (40.0%)	
	PA	0 (0.0%)	2 (100.0%)	0 (0.0%)	
	SA	2 (40.0%)	1 (20.0%)	2 (40.0%)	
	PA + SA	2 (50.0%)	2 (50.0%)	0 (0.0%)	
Day 7	No Growth	0 (0.0%)	0 (0.0%)	5 (100.0%)	0.007*
	GP	0 (0.0%)	1 (100.0%)	0 (0.0%)	
	SA	2 (40.0%)	2 (40.0%)	1 (20.0%)	
	PA + SA	4 (57.1%)	3 (42.9%)	0 (0.0%)	
Day 10	No Growth	0 (0.0%)	2 (28.6%)	5 (71.4%)	0.004*

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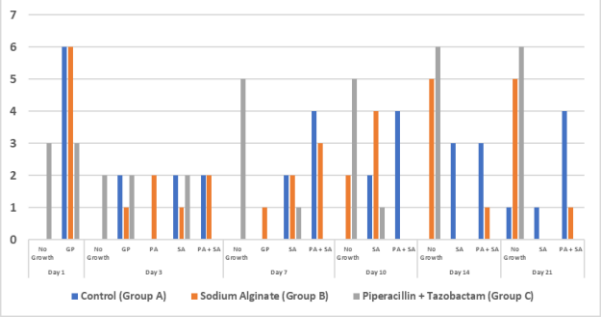
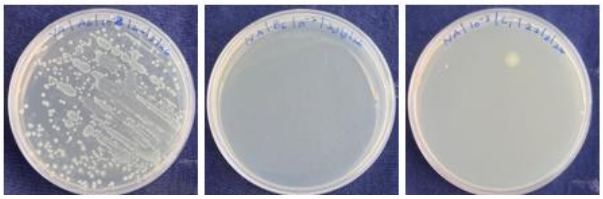
Day	Category	Control (Group A)	Sodium Alginate (Group B)	Piperacillin + Tazobactam (Group C)	p-value
	SA	2 (28.6%)	4 (57.1%)	1 (14.3%)	
	PA + SA	4 (100.0%)	0 (0.0%)	0 (0.0%)	
Day 14	No Growth	0 (0.0%)	5 (45.5%)	6 (54.5%)	0.001*
	SA	3 (100.0%)	0 (0.0%)	0 (0.0%)	
	PA + SA	3 (75.0%)	1 (25.0%)	0 (0.0%)	
Day 21	No Growth	1 (8.3%)	5 (41.7%)	6 (50.0%)	0.012*
	SA	1 (100.0%)	0 (0.0%)	0 (0.0%)	
	PA + SA	4 (100.0%)	1 (100.0%)	0 (0.0%)	



Graph 1: Comparison of microbial growth between three groups

Quantitative Microbiological Assessment - Colony Forming Unit (CFU) Analysis

Swab samples were collected on serial days and cultured to assess colony-forming units; Group A demonstrated a higher colony count compared to Group C (Figure 5).



Group A **Group B** **Group C**

Figure 5. Representative agar plates obtained on Day 10 illustrating bacterial colony growth used for quantitative estimation of colony forming units (CFU/mL): (a) Group A (control), (b) Group B (sodium alginate scaffold) and (c) Group C (piperacillin–tazobactam loaded scaffold).

The comparison of colony-forming unit (CFU) counts among the three groups on day 1 and day 10 was performed using the Kruskal-Wallis test, no statistically significant difference in CFU counts was observed among the three groups ($p = 0.341$). Although Group C showed a markedly higher mean CFU count (25022.11 ± 6126.42) compared to Group A (5.19 ± 7.61) and Group B (20.29 ± 39.83), the difference was not statistically significant due to high variability and skewed distribution, as reflected in the median and interquartile range values.

On day 10, there was also no statistically significant difference in CFU counts among the groups ($p = 0.757$). Group A demonstrated a higher mean CFU count (1356 ± 3255.04) compared to Group B (271.50 ± 602.17) and Group C (858.36 ± 2029.38); however, the wide standard deviations and overlapping interquartile ranges indicate substantial variability within groups (Table 3).

These findings suggest that absolute CFU values alone did not show statistically significant intergroup differences at either time point, likely due to baseline heterogeneity and non-normal distribution (Graph 2).

Table 3: Comparison of CFU count (10^3) among three groups at day 1 and day 10

Interval	Group	Mean ± SD	Median (IQR)	p-value
Day 1	Group A	5.19 ± 7.61	0.56 (14.99)	0.341
	Group B	20.29 ± 39.83	0.80 (39.88)	
	Group C	25022.11 ± 6126.42	26 (37559.54)	

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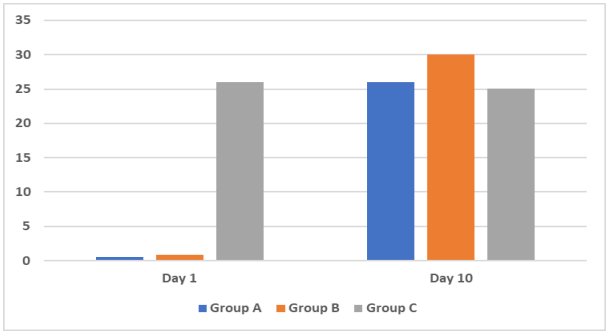
Day 10	Group A	1356 ± 3255.04	26 (2058)	0.757
	Group B	271.50 ± 602.17	30 (411.75)	
	Group C	858.36 ± 2029.38	25.08 (1324.99)	



Graph 2: Comparison of median CFU count among three groups

The percentage change in CFU count from baseline to 10 days post-treatment was analysed using the Kruskal-Wallis test. A statistically significant difference was observed among the three groups (p = 0.019), indicating that the magnitude and direction of microbial change varied significantly between interventions.

Group A demonstrated a mean percentage change of -1.48 ± 67.60, with a median of -35.55 (IQR: 135.40). The negative mean and median values indicate an overall increase in CFU count, suggesting deterioration in microbial status. The large standard deviation and wide interquartile range reflect substantial variability, indicating that while some subjects may have shown improvement, the majority exhibited worsening or inconsistent response.

Group B showed a mean percentage change of −5.32 ± 55.35, with a median of −31.50 (IQR: 111.73). Similar to Group A, the negative values indicate an increase in CFU count, implying inadequate microbial control. Although the variability was slightly lower than Group A, the wide dispersion still indicates heterogeneous treatment response, with no consistent reduction in microbial load.

Group C exhibited a mean percentage change of 74.75 ± 54.24, with a median of 96.51 (IQR: 40.46). The positive values indicate a substantial reduction in CFU count, reflecting effective microbial elimination. Compared to Groups A and B, Group C showed a higher magnitude of improvement and a relatively narrower IQR, indicating more consistent response among subjects.

Based on percentage change in CFU count, Group C demonstrated a significant reduction in microbial load, whereas Groups A and B showed an increase in CFU counts. Pairwise comparison confirmed a statistically significant difference between Group A and Group C (p=0.036), indicating superior antimicrobial efficacy of the intervention in Group C. Comparison between Group B and Group C shows a trend toward better performance of Group C (p=0.056), but not statistically significant, possibly due to small sample size and variability (Table 4).

Table 4: Comparison of % change in CFU between three groups

Group	Mean ± SD	Median (IQR)	p-value
Group A	-1.48 ± 67.60	-35.55 (135.40)	0.019*
Group B	-5.32 ± 55.35	-31.50 (111.73)	
Group C	74.75 ± 54.24	96.51 (40.46)	

Clinical Wound Assessment (REEDA Score)

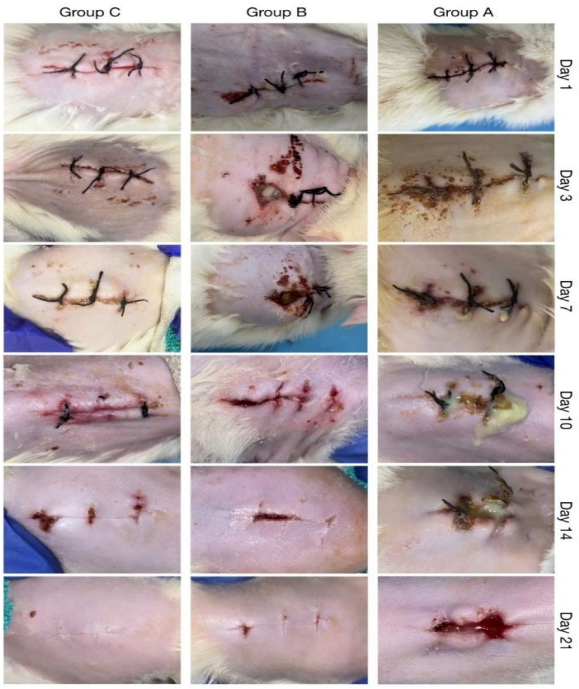


Figure 6. Sequential clinical wound assessment in Wistar rat models from Day1- Day 21 in different showing improved healing in Group C compared to Groups A and B

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On comparison of REEDA scores, Group C consistently demonstrated superior wound healing outcomes compared to Groups A and B. Group C showed faster resolution of redness, reduced edema, and minimal ecchymosis at later stages, indicating improved inflammatory control and vascular recovery. A significant reduction in discharge was observed in Group C from early time points, suggesting effective infection control. Additionally, Group C exhibited significantly better wound edge approximation across multiple time intervals, reflecting enhanced tissue repair and closure. Overall, these findings indicate that the drug-loaded scaffold (Group C) promoted improved wound healing parameters compared to the other groups (Figure-6).

The total REEDA score demonstrated statistically significant differences from Day 3 onwards ($p < 0.05$), with progressively stronger significance at later time points.

On Day 1, all subjects in all three groups had a score of 1 (100%), indicating that all wounds were in the moderately healed category, with no statistically significant difference between groups ($p = 1.000$).

On Day 3 ($p = 0.045$), most subjects in all groups remained in the moderately healed category (scores 1-5). Group C showed a relatively higher proportion of lower scores (closer to healing), but pairwise comparisons did not reveal any statistically significant differences after Bonferroni correction.

On Day 7 ($p = 0.007$), a shift in healing pattern was observed. The majority of subjects in Groups A and B remained in the moderately healed category, whereas Group C demonstrated improvement with a higher proportion approaching lower scores. Pairwise comparison showed a significant difference between Group B and Group C ($p = 0.009$), while comparisons between Group A vs B ($p = 1.000$) and Group A vs C ($p = 0.052$) were not statistically significant.

On Day 10 ($p = 0.003$), Group C showed marked improvement, with a substantial number of subjects falling into the moderately healed and approaching healed category, whereas Group A still had higher scores indicating delayed healing. Pairwise analysis revealed a significant difference between Group A and Group C ($p = 0.002$), while Group A vs B ($p = 0.277$) and Group B vs C ($p = 0.277$) were not significant.

On Day 14 ($p = 0.024$), further progression in healing was evident. Group C demonstrated a greater proportion of subjects nearing the healed category (score 0), while Groups A and B largely remained in the moderately healed category. Pairwise comparison showed a significant difference between Group A and Group C ($p = 0.030$), with no significant differences between Group A vs B ($p = 1.000$) and Group B vs C ($p = 0.109$).

On Day 21 ($p = 0.001$), the highest level of healing was observed. Group C showed the greatest proportion of subjects in the healed category (score 0), followed by Group B, while Group A continued to show delayed healing with higher scores. Pairwise analysis demonstrated significant differences between Group A and Group B ($p = 0.025$) and between Group A and Group C ($p = 0.001$), whereas Group B vs C ($p = 0.874$) was not statistically significant. Group C consistently showed lower total scores, indicating superior overall wound healing, followed by Group B, while Group A showed the highest scores, indicating delayed recovery (Table 5, Graph 3).

Table 5: Comparison of total REEDA score among three groups

Time	Score	Group A	Group B	Group C	p-value
Day 1	1	6 (100.0%)	6 (100.0%)	6 (100.0%)	1
Day 3	1	1 (16.7%)	0 (0.0%)	4 (66.7%)	0.045*
	2	4 (66.7%)	6 (100.0%)	2 (33.3%)	
	3	1 (16.7%)	0 (0.0%)	0 (0.0%)	
Day 7	2	1 (16.7%)	0 (0.0%)	5 (83.3%)	0.007*
	3	5 (83.3%)	6 (100.0%)	1 (16.7%)	
Day 10	1	0 (0.0%)	0 (0.0%)	4 (66.7%)	0.003*

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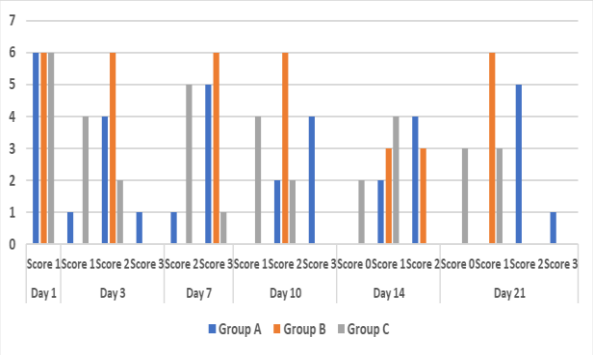
	2	2 (33.3%)	6 (100.0%)	2 (33.3%)	
	3	4 (66.7%)	0 (0.0%)	0 (0.0%)	
Day 14	0	0 (0.0%)	0 (0.0%)	2 (33.3%)	0.024*
	1	2 (33.3%)	3 (50.0%)	4 (66.7%)	
	2	4 (66.7%)	3 (50.0%)	0 (0.0%)	
Day 21	0	0 (0.0%)	0 (0.0%)	3 (50.0%)	0.001*
	1	0 (0.0%)	6 (100.0%)	3 (50.0%)	
	2	5 (83.3%)	0 (0.0%)	0 (0.0%)	
	3	1 (16.7%)	0 (0.0%)	0 (0.0%)	



Graph 3: Comparison of total REEDA score among three groups

4. Discussion

Animal models play a crucial role in evaluating wound healing and antimicrobial interventions under controlled experimental conditions. Rat skin wound models are widely accepted and reliable due to their ease of handling, cost-effectiveness, and reproducibility. These models allow systematic assessment of healing parameters, infection progression, and therapeutic response. In the present study, a standardized incisional wound model in Wistar rats was employed, which provided a consistent and reproducible platform for evaluating both antimicrobial efficacy and wound healing. The findings of this study further support the suitability of rat models as an effective experimental system for preclinical evaluation of wound dressing materials [6].

Alginate-based wound dressings have been widely investigated for their dual ability to support tissue regeneration and provide antimicrobial protection. Existing evidence suggests using alginate dressings in infected wound models have demonstrated significant reduction in bacterial load along with enhanced wound healing, attributed to the biocompatible and moisture-retentive nature of alginate. In a rat model of purulent wounds, alginate-based dressings were shown to exhibit both antibacterial and regenerative properties, leading to improved wound closure and reduced inflammation. Similarly, in the present study, the sodium alginate-based membrane loaded with piperacillin–tazobactam demonstrated effective antimicrobial activity and improved wound healing outcomes, as evidenced by reduced CFU counts and better REEDA scores. Moreover, the incorporation of an antibiotic in the present study enhances the antimicrobial efficacy of the alginate matrix, providing an added advantage in managing surgical site infections [7,8].

Quantitative microbiological parameters, particularly colony forming unit (CFU) analysis, are widely used to objectively evaluate bacterial load and antimicrobial efficacy [9] . In the present study, an incisional wound model in Wistar rats was utilized, and antimicrobial efficacy was assessed using CFU counts along with clinical wound parameters. The observed reduction in CFU values in the treated group indicates effective local antimicrobial activity of the developed scaffold.

REEDA scoring system serves as a reliable and reproducible clinical tool for assessing wound healing in experimental models. Previous studies utilizing incisional wound models in Wistar rats have employed the REEDA scoring system to assess healing parameters, including redness, edema, ecchymosis, discharge, and wound approximation. The use of REEDA scoring enables systematic monitoring of inflammatory response

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and tissue repair over time. In the present study, a similar approach was adopted to evaluate wound healing progression, where REEDA scores provided clear differentiation between the study groups [10].

However, a limitation of the present study is that a standardized infection model using specific bacterial inoculation could not be replicated, and the study relied on natural microbial colonization. Future studies incorporating histological analysis are warranted to strengthen the translational relevance of the findings. Despite this, the findings still demonstrate a clear trend of reduced bacterial load and improved wound healing, supporting the potential of the developed scaffold for managing surgical site infections.

5. Conclusion

The present in vivo study demonstrated that the piperacillin–tazobactam loaded sodium alginate scaffold exhibited superior antimicrobial efficacy and enhanced wound healing compared to control and plain alginate groups. Microbiological analysis revealed a significant reduction in bacterial load, with predominant organisms identified as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The drug-loaded membrane showed a higher proportion of no microbial growth from Day 7 onwards, indicating effective and sustained antimicrobial action. Quantitative CFU analysis further substantiated these findings, demonstrating progressive reduction in bacterial load in the treated group.

Clinically, wound healing assessment using the REEDA scoring system demonstrated improved outcomes in the drug-loaded group, with faster resolution of redness, edema, ecchymosis, and discharge, along with better wound edge approximation. These findings suggest that localized delivery of piperacillin–tazobactam through a biocompatible alginate scaffold not only enhances infection control but also promotes favorable wound healing conditions. However, further studies incorporating histopathological evaluation and clinical trials are recommended to validate its translational applicability.

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