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Biocompatibility and Antimicrobial Efficacy of a Piperacillin–Tazobactam Loaded Sodium Alginate scaffold for local drug delivery

[1] Tanya Takkar, [2] Vishnu Priya Veeraraghavan, [3] Gidean Arularasan S*, [4] Murugesan Krishnan, [5] M. P. Santhosh Kumar

[1] Post graduate, Department of Oral and Maxillofacial Surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Velappanchavadi, Chennai, Tamil Nadu, India.

[2] Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Velappanchavadi, Chennai, Tamil Nadu, India

[3] Assistant Professor, Department of Oral and Maxillofacial Surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Velappanchavadi, Chennai, Tamil Nadu, India.

[4] Head of the Department, Department of Oral and Maxillofacial Surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Velappanchavadi, Chennai, Tamil Nadu, India.

[5] Professor, Department of Oral and Maxillofacial Surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Velappanchavadi, Chennai, Tamil Nadu, India.

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KEYWORDS:

Antimicrobial activity, Cytocompatibility, Hemocompatibility, Local drug delivery, Sodium alginate.

ABSTRACT:

Introduction: Surgical site infections represent a major postoperative concern in oral and maxillofacial surgeries, contributing to increased morbidity and delayed healing. This emphasizes the imperative for the development of biocompatible wound dressing systems with effective antimicrobial properties. This study aims to evaluate the biocompatibility and antimicrobial efficacy of a piperacillin–tazobactam loaded sodium alginate scaffold.

Materials and Methods: Sodium alginate scaffolds loaded with piperacillin–tazobactam were evaluated for cytocompatibility using fibroblast NIH 3T3 and HDFa cell lines and hemocompatibility using an in vitro hemolysis assay. Antimicrobial efficacy was assessed through zone of inhibition studies against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Results: The drug-loaded scaffold demonstrated acceptable cytocompatibility and hemocompatibility, with cell viability remaining within non-cytotoxic to mildly cytotoxic ranges based on concentration and hemolysis below 5%. Significant antimicrobial activity was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Conclusion: The piperacillin–tazobactam loaded sodium alginate scaffold demonstrates effective antimicrobial activity along with acceptable biocompatibility, indicating its potential as a promising local drug delivery system for the management of surgical site infections.

1. Introduction

Surgical site infections remain one of the most significant postoperative complications in oral and maxillofacial surgery, contributing substantially to patient morbidity, prolonged hospitalisation, and increased healthcare burden worldwide. Compared to surgeries in other anatomical sites, the frequency of this complication is higher in patients with surgical wounds of the oral and maxillofacial region due to exposure of the surgical site to oral microflora, especially in cases with communication into the neck [1]. Necessitating the

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development of advanced wound dressings with both antimicrobial and biocompatible properties. Biopolymer-based membranes offer a promising platform for localized drug delivery and infection control [2].

Local antibiotic delivery systems have gained increasing attention as a strategy to provide sustained antimicrobial protection while supporting tissue repair in these high-risk wounds. Advances in biodegradable polymer technology have enhanced the clinical applicability of local drug delivery platforms by allowing gradual degradation without the need for secondary removal procedures. Such systems represent a shift toward targeted infection-control strategies that combine antimicrobial efficacy with improved wound-healing outcomes, supporting their potential role as an alternative or adjunct to conventional dressing materials in surgical-site infection management [3].

The effectiveness of a wound dressing is largely determined by its biocompatibility, as the material remains in direct contact with living tissues during the healing process. An ideal wound dressing should be inherently biocompatible, ensuring that it does not induce cytotoxic, inflammatory, or immunological responses. It should support cellular adhesion, proliferation, and migration, thereby promoting tissue regeneration and repair. While other functional properties such as moisture retention, exudate absorption, and protection from external contaminants are important, biocompatibility remains the most critical requirement, as it directly influences the safety and success of the wound dressing [4].

For such systems to be clinically effective, they must demonstrate significant antimicrobial efficacy while maintaining biocompatibility. Evaluation of antimicrobial activity in vitro is a critical initial step, as it provides direct evidence of the ability of the developed system to inhibit or reduce microbial growth against common pathogenic organisms. Standard assays, such as zone of inhibition and microbial growth studies, are widely employed to assess this property. In vitro antimicrobial evaluation is essential to establish the efficacy of biopolymer-based local drug delivery systems in controlling wound infections [5].

In view of the increasing need for effective antimicrobial wound dressings that are both safe and functionally efficient, the present study focuses on the development of a biopolymer-based scaffold for local drug delivery. Sodium alginate, a widely recognized and versatile biopolymer, offers excellent biocompatibility and favorable physicochemical properties for wound healing applications [6]. By incorporating the broad-spectrum antibiotic combination of piperacillin–tazobactam, the scaffold is expected to provide enhanced antimicrobial activity against gram positive and gram-negative pathogenic organisms. Therefore, the present study aims to evaluate the biocompatibility and in vitro antimicrobial efficacy of a sodium alginate-based scaffold loaded with piperacillin–tazobactam, thereby assessing its potential as an effective wound dressing material for infection control.

2. Material and Methods

Cytocompatibility- (MTT assay)

The cytocompatibility of the fabricated scaffolds was evaluated using an MTT cell viability assay in NIH 3T3 and HDFa cell lines. Cells were procured from NCCS Pune and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% ant0ibiotic solution under standard incubation conditions (37°C, 5% CO_2). About 5 $\times 10^3$ cells were seeded into 96-well plates and incubated for 24 h. Scaffold extracts were prepared by incubating sterilised samples in culture medium under aseptic conditions. The cells were treated with different concentrations and incubated for 24 h. Following treatment, MTT reagent was added to each well and incubated for 3–4 h to allow formazan crystals to form a metabolically active cell. The supernatant was carefully removed, and dimethyl sulfoxide (DMSO) was added to dissolve the crystals. Absorbance was measured using a microplate reader at 570 nm. Cell viability was calculated as a percentage relative to untreated control cells.

Hemocompatibility- Hemolysis Assay

Hemocompatibility of the fabricated scaffold was evaluated using a hemolysis assay. Freshly collected anticoagulated blood was centrifuged to isolate red blood cells (RBCs), which were washed with phosphate-buffered saline (PBS). Scaffold samples were incubated with diluted RBC suspension at 37°C for a specified duration. Following incubation, the samples were

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centrifuged, and the absorbance of the supernatant was measured using a UV–visible spectrophotometer at 540 nm to determine haemoglobin release. Distilled water and PBS served as positive and negative controls, respectively. Percentage hemolysis was calculated to assess blood compatibility.

Wound Healing Assay

About 5×10^5 HDFa cells/well were seeded in 12-well plates and incubated at 37 °C until they reached full confluence. A scratch was made in the centre of each well. The detached cells were removed by PBS washing. The cells were allowed to migrate in the presence and absence of drug-loaded scaffold for different time durations and imaged under a phase-contrast microscope (Lynx) Magnification x100.

Antimicrobial Properties- Zone of Inhibition

The antimicrobial activity of the fabricated scaffold was evaluated using the agar diffusion (zone of inhibition) method against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Bacterial strains were obtained from a standard microbiological culture collection and subcultured in nutrient broth at 37°C for 18–24 h to achieve active growth. The bacterial suspension was adjusted to match 0.5 McFarland turbidity standard to obtain a uniform inoculum concentration.

Sterile Mueller–Hinton agar plates were prepared and evenly inoculated by spreading the bacterial suspension across the agar surface using a sterile cotton swab to obtain a uniform bacterial lawn. Scaffold samples were cut into uniform circular discs under aseptic conditions and sterilised before testing. The prepared scaffold discs were carefully placed onto the inoculated agar plates using sterile forceps. Blank alginate scaffolds and standard antibiotic discs served as control groups. The plates were incubated at 37°C for 24 h under aerobic conditions. Following incubation, antimicrobial activity was assessed by measuring the diameter of the clear inhibition zone surrounding each scaffold sample using a digital caliper. Measurements were recorded in millimetres (mm), and the mean value was calculated from replicate experiments.

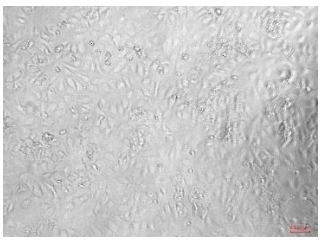
3. Results

Cytocompatibility- (MTT assay)

The cytocompatibility of the fabricated scaffolds was evaluated using a cell viability assay in NIH 3T3 and HDFa cell lines at varying concentrations.

The cytocompatibility of the sodium alginate scaffold and the piperacillin-tazobactam loaded alginate scaffold was evaluated in NIH 3T3 fibroblast cell lines across concentrations ranging from 0.16 to 5 mg/mL (Figure-1). The sodium alginate scaffold demonstrated high cell viability at all tested concentrations. At 0.16 mg/mL, cell viability was 102%, indicating excellent cellular proliferation. At 0.31 mg/mL, viability was 96%, followed by 89% at 0.63 mg/mL. Even at higher concentrations of 1.25 mg/mL and 2.5 mg/mL, viability remained 86% and 83%, respectively. At the highest tested concentration of 5 mg/mL, cell viability was maintain at 73%, confirming the intrinsic biocompatibility of the alginate scaffold.

The piperacillin–tazobactam-loaded alginate scaffold exhibited a concentration-dependent reduction in cell viability compared to the control scaffold. At 0.16 mg/mL, viability was 86%, decreasing to 81% at 0.31 mg/mL and 75% at 0.63 mg/mL. At 1.25 mg/mL, viability further declined to 67%, followed by 59% at 2.5 mg/mL. At the highest concentration of 5 mg/mL, cell viability was recorded at 55%. Although a gradual decline in cell viability was observed with increasing concentration of the drug-loaded scaffold, values remained above 50% even at the highest concentration tested. According to ISO 10993-5 standards, cell viability greater than 70% is considered non-cytotoxic, while 50–70% indicates mild cytotoxicity. Most drug-loaded scaffold concentrations remained within the non-cytotoxic to mildly cytotoxic range (Graph-1).



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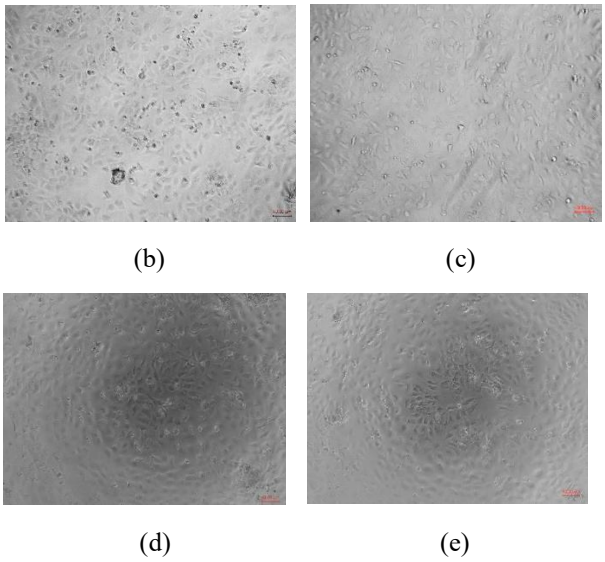
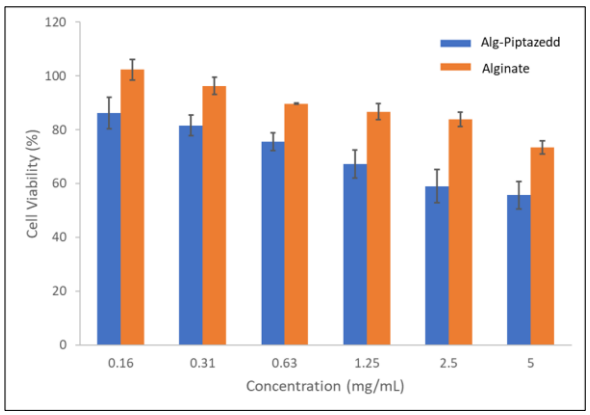


Figure 1.
Photomicrographs showing (a) Control cells- NIH3T3 (b) Cells treated with 0.625 mg/mL of sodium alginate scaffold (c) Cells treated with 2.5 mg/mL of sodium alginate scaffold (d) Cells treated with 0.625 mg/mL of Piperacillin- loaded alginate scaffold (e) Cells treated with 2.5 mg/mL of Piperacillin- loaded alginate scaffold.



Graph 1: In vitro cell viability (%) in NIH 3T3 cells of sodium alginate scaffold and piperacillin–tazobactam loaded alginate scaffolds

The cytocompatibility of the scaffolds was evaluated in HDFa (Human Dermal Fibroblast adult) cell lines across concentrations ranging from 0.13 to 2 mg/mL (Figure 2).

The control sodium alginate scaffold demonstrated the highest percentage of cell viability at all tested concentrations. At 0.13 mg/mL, viability was approximately 115%, indicating enhanced cellular proliferation. At 0.25 mg/mL and 0.5 mg/mL, viability remained high at approximately 95% and 90%, respectively. Even at higher concentrations of 1 mg/mL and 2 mg/mL, viability was maintained at approximately 75% and 65%, confirming the excellent intrinsic biocompatibility of alginate.

The 10% drug-loaded sodium alginate scaffold exhibited slightly reduced but acceptable cell viability compared to the control scaffold. At 0.13 mg/mL, viability was approximately 100%, decreasing to 92% at 0.25 mg/mL and 80% at 0.5 mg/mL. At 1 mg/mL and 2 mg/mL, viability was approximately 70% and 60%, respectively.

The 20% drug-loaded sodium alginate scaffold demonstrated comparatively lower viability across all concentrations. At 0.13 mg/mL, viability was approximately 88%, decreasing to 75% at 0.25 mg/mL and 67% at 0.5 mg/mL. At 1 mg/mL and 2 mg/mL, viability further declined to approximately 58% and 50%, respectively. A clear concentration-dependent reduction in cell viability was observed in all groups, particularly in drug-loaded scaffolds (Graph-2).

The 10% formulation remained largely within the non-cytotoxic range at therapeutically relevant concentrations, whereas the 20% formulation approached mild cytotoxic levels at higher concentrations.

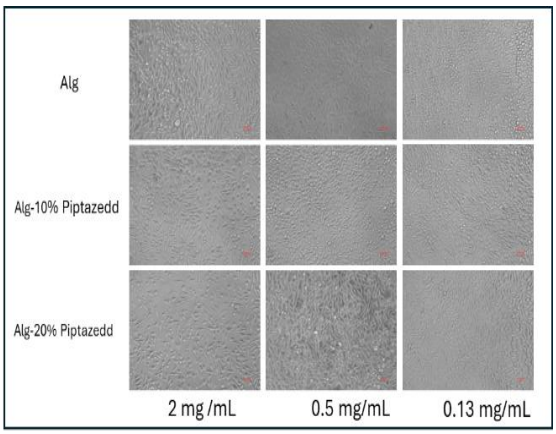


Figure 2.
Photomicrographs of HDFa cells treated with sodium alginate scaffold and 10% and 20% of piperacillin-tazobactam loaded alginate scaffolds at varying concentrations at 2 mg/mL, 0.5 mg/mL, and 0.13 mg/mL.

Journal of Chemical Health Risks
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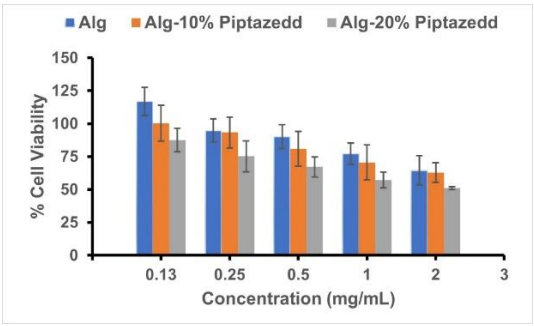
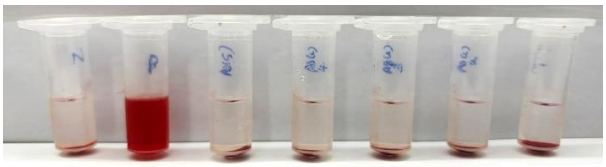


Graph 2: Cell viability (%) of HDFa cells treated with sodium alginate scaffold, Alg 10% piperacillin–tazobactam, and Alg-20% piperacillin–tazobactam scaffolds at concentrations ranging from 0.13 to 2 mg/mL.

Hemocompatibility- Hemolysis Assay

Hemocompatibility of the control sodium alginate scaffold and the piperacillin–tazobactam loaded alginate scaffold was evaluated using an in vitro hemolysis assay at concentrations ranging from 12.5 µg/mL to 200 µg/mL. Both scaffolds demonstrated a concentration-dependent increase in hemolysis percentage. At lower concentrations (12.5–50 µg/mL), minimal hemolysis was observed in both groups, indicating excellent erythrocytes compatibility. At higher concentrations (100–200 µg/mL), a moderate increase in hemolysis was noted; however, the percentage hemolysis for both scaffolds remained below 5%, which is generally considered the acceptable threshold for non-hemolytic biomaterials according to ISO 10993-4 standards (Figure-3). These findings indicate the hemocompatibility of the alginate scaffold (Graph 3).



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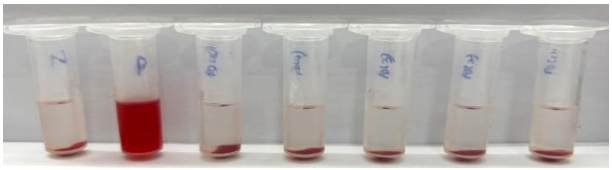
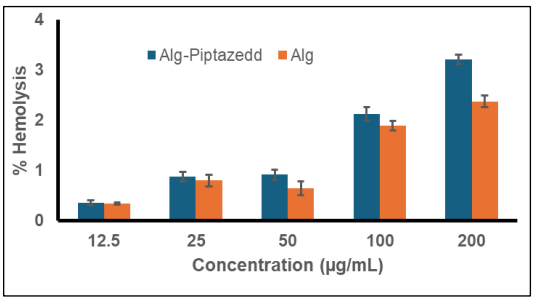


(b)

Figure 3.

Hemolysis assay demonstrating the hemocompatibility of (a) sodium alginate scaffold and (b) piperacillin–tazobactam loaded alginate scaffolds. The negative control (normal saline) shows the absence of hemolysis, while the positive control shows complete red blood cell lysis.



Graph 3: In vitro hemolysis (%) of sodium alginate scaffold and piperacillin-tazobactam loaded alginate scaffold at different concentrations

Wound Healing Assay- Scratch Test

The scratch assay demonstrated progressive wound closure in all groups over 48 hours. The untreated control demonstrated progressive wound closure over time. At 24 hours, partial cell migration into the wound area was observed, and by 48 hours, near-complete closure, indicating normal fibroblast migratory behaviour.

The sodium alginate and Alg-10% drug-loaded scaffolds showed enhanced fibroblast migration with near-complete closure at 48 hours. The 20% drug-loaded formulation exhibited slightly slower closure, possibly due to higher local antibiotic concentration. These findings suggest that the 10% piperacillin–tazobactam loaded scaffold effectively supports cellular migration while maintaining antimicrobial potential (Figure- 4).

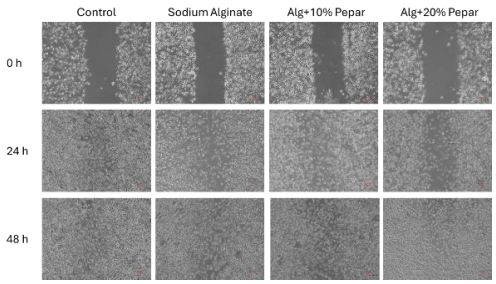


Figure 4.

Representative microscopic images of the in vitro wound healing (scratch) assay demonstrating cell migration in different treatment groups

Journal of Chemical Health Risks
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JCHR (2026) 16(3), 775-782 | ISSN:2251-6727

Antimicrobial Properties

Zone of Inhibition

The antimicrobial activity of the fabricated scaffolds was evaluated using the agar diffusion method against *Staphylococcus aureus and Pseudomonas aeruginosa*. The sodium alginate scaffold without drug (negative control) did not exhibit any zone of inhibition against either microorganism (0 mm), confirming the absence of inherent antibacterial activity.

Against *Staphylococcus aureus*, the standard antibiotic amikacin demonstrated an inhibition zone of 22 mm. The drug-loaded scaffolds showed superior antibacterial activity compared to the antibiotic control, with inhibition zones of 26 mm and 24 mm observed for the 10% and 20 % drug-loaded scaffolds, respectively (Figure-5). The 10% formulation demonstrated comparatively higher inhibition against *Staphylococcus aureus* (Table 1).

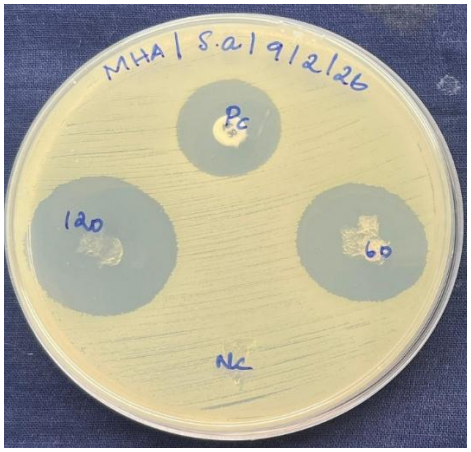
Table 1: Zone of inhibition (in mm) showing antimicrobial activity of drug-loaded scaffolds against *Staphylococcus aureus*

Antimicrobial activity against *Staphylococcus aureus*	**Zone of inhibition (in mm)**
Amikacin	22 mm
Sodium alginate scaffold without drug (negative control)	0 mm
10% drug loaded scaffold	26 mm
20% drug loaded scaffold	24 mm

Similarly, against *Pseudomonas aeruginosa*, the standard antibiotic ciprofloxacin demonstrated a zone of inhibition measuring 18 mm. The piperacillin-tazobactam loaded scaffolds exhibited enhanced antibacterial activity, with inhibition zones measuring 24 mm and 25 mm for the 10% and 20% drug-loaded scaffolds, respectively (Figure-5). The increase in inhibition zone indicated improved antimicrobial effectiveness with increasing drug concentration (Table-2).

Table 2: Zone of inhibition (in mm) showing antimicrobial activity of drug-loaded scaffolds against *Pseudomonas aeruginosa*

Antimicrobial activity against *Pseudomonas aeruginosa*	**Zone of inhibition (in mm)**
Ciprofloxacin	18 mm
Sodium alginate scaffold without drug (negative control)	0 mm
10% drug loaded scaffold	24 mm
20% drug loaded scaffold	25 mm



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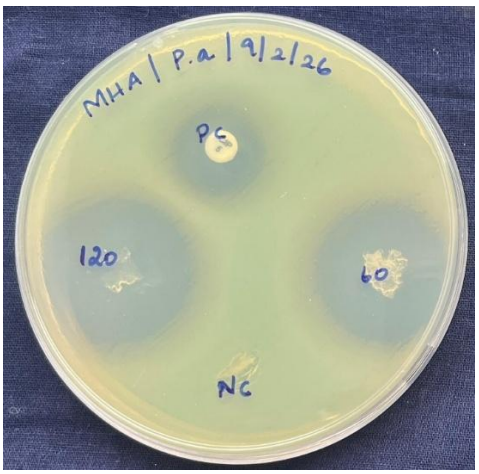

(b)

Figure 5.
Antimicrobial activity of drug-loaded scaffolds against (a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa* measured by zone of inhibition (mm).

Journal of Chemical Health Risks
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4. Discussion

Although systemic antimicrobials remain the standard treatment modality in management of surgical site infection, their effectiveness may be compromised by poor penetration into poorly vascularized tissues, biofilm formation. Local drug delivery enables the administration of high concentrations of antimicrobial agents directly at the surgical wound site while minimizing systemic toxicity and adverse effects. Such targeted therapy is especially beneficial in contaminated surgical fields where bacterial colonisation is common. Antibiotic-loaded biomaterials, including hydrogels, polymer membranes, collagen matrices, and alginate-based dressings, have demonstrated the ability to provide sustained and controlled drug release. Controlled-release systems improve antimicrobial exposure at the wound interface and reduce the need for repeated the need for systemic dosing [5,6].

Biocompatibility remains a critical parameter for evaluating the suitability of scaffold materials for clinical applications. Various studies have reported that composite scaffolds incorporating antimicrobial agents can maintain favorable cell viability and support cellular proliferation. Similarly, in the present study, cytocompatibility assessment-MTT assay using fibroblast cell lines demonstrated acceptable cell viability, indicating that the incorporation of piperacillin–tazobactam did not induce significant cytotoxic effects. The concurrent demonstration of antimicrobial activity through disk diffusion methods and biocompatibility through cell line studies reinforces the dual functional potential of the developed scaffold. Thus, the results of the present study, supports the use of antibiotic-loaded biopolymer scaffolds as effective and safe materials for antimicrobial applications [5,7,8,9].

Human dermal fibroblast adult (HDFa) cells are widely used as an in vitro model for evaluating the biocompatibility. Marwah et al have demonstrated the use of HDFa cells to assess cell viability, inflammatory response, and regenerative potential of biomaterial-based scaffolds. In the present study, cytocompatibility was assessed using HDFa cell lines, which demonstrated acceptable cell viability, indicating that the developed membrane is biocompatible [10].

The present study has certain limitations. The biological evaluation was limited to in vitro cytocompatibility and hemocompatibility assays, which, although essential, do not fully represent the complex in vivo environment. Long-term cellular responses, tissue integration, and immunological reactions were not assessed. The study was also restricted to selected microbial strains, and the effectiveness of the system against a broader spectrum of clinically relevant pathogens remains to be explored. Furthermore, the absence of in vivo validation limits clinical applications. Therefore, further studies involving in vivo models and extended biological assessments are necessary to confirm the safety and antimicrobial effectiveness of the developed membrane.

5. Conclusion

Biocompatibility of the fabricated scaffold was assessed during cell viability assays, which demonstrated acceptable cytocompatibility across the tested concentrations. Hemocompatibility studies using hemolysis assays further confirmed that the scaffolds induced minimal hemolysis, demonstrating good compatibility with blood components. The wound healing potential of the scaffold was further evaluated using an in vitro scratch assay, which demonstrated progressive cell migration and closure of the wound gap over time, indicating that the scaffold supports cellular proliferation and tissue regeneration. These findings highlight the potential of the developed scaffold not only in infection control but also in promoting wound healing. Significant antimicrobial activity was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Overall, the results of this study demonstrate that the piperacillin–tazobactam loaded alginate scaffold possesses effective antimicrobial activity, good biocompatibility, and promising wound healing properties.

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