Surface-modified and antibiotic-loaded polylactic acid implants: Experimental and clinical evaluation

Supplementary File

1. Histology

1.1. Detailed histology of each group

Histological analysis of polylactic acid (PLA) implant samples without antibiotic addition and infected with *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii* OXA-23 revealed specific characteristics for each group, as detailed in **Table S1**.

In the *E. coli*-infected group, a moderate presence of collagen fibers was observed, with significant disorganization. Angiogenesis was also moderate, suggesting an inflammatory response involving neovascularization. Fibroblasts were abundant, indicating intense tissue repair activity. Lymphocytes were present in low numbers, suggesting a weak immune response. Macrophages and neutrophils were scarce, indicating limited phagocytic and acute inflammatory responses. Eosinophils were also sparse, with no significant allergic response.

In the *S. aureus*-infected group, collagen fibers were moderately abundant but severely disorganized, indicating considerable disruption of normal tissue architecture. Angiogenesis was mild, suggesting a limited vascular response. Fibroblasts were abundant, indicating active repair and remodeling. Lymphocytes were moderately present, suggesting a persistent inflammatory response. Macrophages and neutrophils were scarce, indicating low phagocytic and acute inflammatory activity. Eosinophils were present in small numbers, with no evidence of allergic response.

In the *A. baumannii* OXA-23 group, collagen fibers were moderately abundant and notably disorganized, similar to the *E. coli* group. Angiogenesis was mild, indicating limited neovascular formation. Fibroblasts were abundant, suggesting an active tissue repair response. Lymphocytes were moderately present, indicating a continuous immune response. Macrophages were moderately abundant, suggesting increased phagocytic activity compared to other groups. Neutrophils were scarce, and eosinophils were mildly present, with no indication of a significant allergic response.

These results reveal distinct patterns of inflammatory response and tissue repair depending on the infectious agent, with *S. aureus* showing the most severe collagen disorganization and *A. baumannii* demonstrating the highest macrophage presence.

1.2. Histological analysis of antibiotic-loaded PLA implants

Histological analysis of PLA implants impregnated with antibiotics and intentionally infected revealed variations in tissue and inflammatory responses, as shown in **Table S2**.

In the C (I-PLA) + *E. coli* group, a moderate presence of collagen fibers with moderate disorganization was observed, indicating some structural alteration. Angiogenesis was also moderate, suggesting neovascular formation due to inflammation. Fibroblasts were moderately present, indicating tissue repair activity. Lymphocytes and macrophages were moderately present, reflecting active immune responses. Neutrophils were mildly present, indicating mild acute inflammation, while eosinophils were absent, indicating no allergic response.

In the vancomycin (V-PLA) + *S. aureus* group, collagen fibers were mildly present with slight disorganization, suggesting minimal structural changes. Angiogenesis was mild, with limited new vessel formation. Fibroblasts were moderately

Table S1. Semi-quantitative histological analysis of polylactic acid implants (without antibiotics) infected with different bacteria

Groups	*E. coli*	*S. aureus*	*A. baumannii*
Collagen fibers	++	++	++
Collagen fiber disorganization	++	+++	++
Angiogenesis	++	+	+
Fibroblasts	+++	+++	+++
Lymphocytes	+	++	++
Macrophages	+	+	++
Neutrophils	+	+	+
Eosinophils	+	+	+

Note: Classification: Absent/Rare (–), mild (+), moderate (++), severe (+++). *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, A. baumannii: Acinetobacter baumannii*

present, reflecting tissue repair activity. Lymphocytes and macrophages were present in low numbers, suggesting a less intense immune response. Neutrophils and eosinophils were absent, indicating no acute inflammation or allergic reaction.

In the polymyxin (P-PLA) + *A. baumannii* group, collagen fibers were moderately present with mild disorganization, indicating some preservation of tissue architecture. Angiogenesis was moderate, suggesting an active inflammatory response. Fibroblasts were abundant, indicating intense tissue remodeling. Lymphocytes and macrophages were present in only mild numbers, indicating a limited immune response. Neutrophils were mildly present, and eosinophils were absent, excluding a significant allergic response.

In the aztreonam (Z-PLA) + *E. coli* group, moderate collagen fiber presence and mild disorganization were observed. Angiogenesis was mild, and fibroblasts were abundant, indicating robust tissue repair. Lymphocytes were moderately present, suggesting an active immune response. Macrophages were present in low numbers, indicating low phagocytic activity. Neutrophils were present in small numbers, suggesting mild acute inflammation. Eosinophils were mildly present, indicating a slight allergic response.

These results demonstrate that incorporating antibiotics into PLA implants, when combined with infection by different bacterial strains, influences inflammatory and tissue repair responses, with notable differences among groups in connective tissue organization and cellular infiltration (**Figure S1**).

2. Antimicrobial activity and antibiotic elution

In each group, five samples were prepared for microbiological evaluation: PLA-C (control), PLA-V, PLA-P, PLA-I, and PLA-Z.

Table S2. Semi-quantitative histological analysis of antibiotic-loaded PLA implants with induced infection

Groups	I-PLA+*E. coli*	V-PLA+*S. aureus*	P-PLA+*A. baumannii*	Z-PLA+*E. coli*
Collagen fibers	++	+	++	++
Collagen fiber disorganization	++	+	+	+
Angiogenesis	++	+	++	+
Fibroblasts	++	++	+++	+++
Lymphocytes	++	+	+	++
Macrophages	++	+	+	+
Neutrophils	+	–	+	+
Eosinophils	–	–	–	+

Note: Classification: Absent/Rare (–), mild (+), moderate (++), severe (+++).
Abbreviations: I: Imipenem; P: Polymyxin; PLA: Polylactic acid; V: Vancomycin; Z: Aztreonam.

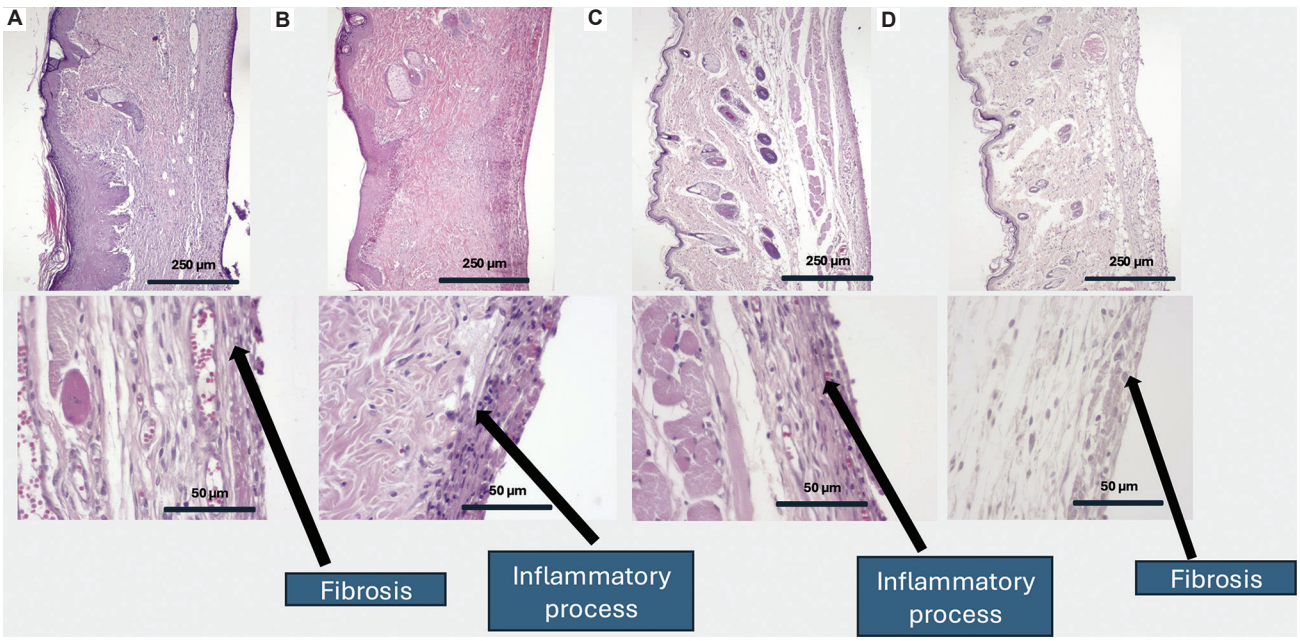

Figure S1. Histological evaluation of peri-implant tissues following implantation of polylactic acid (PLA) and vancomycin-loaded PLA constructs. Representative hematoxylin and eosin-stained sections are shown for the (A) PLA, (B) PLA-infected, (C) vancomycin (V)-PLA-infected, and (D) V-PLA groups. Upper panels show low-power views of the implant-tissue interface, while lower panels show higher-magnification images of the indicated areas. The green bars identify the region of tissue directly adjacent to the implant surface. Increased fibrosis was observed in the non-infected groups (PLA and V-PLA), whereas inflammatory cell infiltration was more prominent in the infected groups (PLA-infected and V-PLA-infected groups), consistent with the local tissue response to infection. Arrows indicate representative areas of fibrosis or inflammatory infiltrates. Scale bar: Upper: 500 µm, lower: 50 µm; magnification: Upper: 4×, lower: 40×.

The microorganisms used for testing included *S. aureus* ATCC® 25923 (to assess vancomycin activity), *E. coli* ATCC® 25922 (to assess imipenem and aztreonam), and carbapenem-resistant *A. baumannii* OXA-23 (to assess polymyxin). Subsequently, these samples were placed on Mueller–Hinton agar plates containing the respective bacterium, adjusted to a turbidity equivalent to 0.5 McFarland, corresponding to 3×10^8 CFU/mL. The diameter of the inhibition zone surrounding the samples was measured after 24 h of incubation at 37°C, serving as an indicator of their antibacterial activity. To evaluate the shelf-life antimicrobial activity of impregnated PLA implants, microbiological testing was performed at 30, 60, 90, 120, 150, and 180 days. The disk diffusion assay was used as a qualitative method to confirm the presence of bioactive antibiotics released from the PLA matrix.

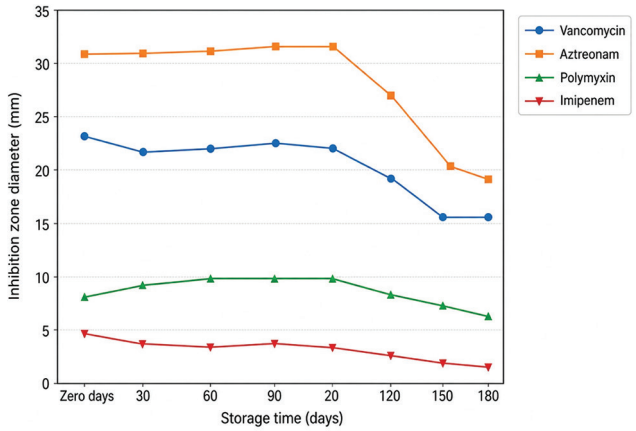

Figure S2. Inhibition zones of polylactic acid (PLA) discs impregnated with antibiotics over 180 days of storage. Line graph showing the inhibition zone diameters (mm) for PLA discs loaded with vancomycin, aztreonam, polymyxin, and imipenem at different storage times (0, 30, 60, 90, 120, 150, and 180 days). Vancomycin and aztreonam maintained relatively stable antimicrobial activity throughout the evaluation period. Polymyxin exhibited consistently small inhibition zones, reflecting its limited agar diffusion. Imipenem showed a marked decline in activity after 120 days, reaching a diameter of 5 mm at 150 and 180 days, corresponding to the disc size and indicating complete loss of antimicrobial activity.

Antibiotic release and biological activity were evaluated using an eluate-based agar diffusion assay. PLA samples loaded with antibiotics (vancomycin, polymyxin, imipenem, and aztreonam) were individually immersed in sterile phosphate-buffered saline (PBS) and incubated under controlled conditions. At predetermined time points (1 h, 24 h, 72 h, 7 days, and 14 days), the entire volume of PBS was collected and replaced with fresh sterile PBS to ensure continuous elution. The collected eluates were immediately used to assess antimicrobial activity. Agar plates were previously inoculated with standardized bacterial suspensions, including *S. aureus* for vancomycin, *Acinetobacter* spp. for polymyxin, and *E. coli* for imipenem and aztreonam. Wells were aseptically created in the agar surface, and 30 µL of each eluate was deposited into the wells. Plates were incubated under appropriate conditions, and antimicrobial activity was qualitatively assessed by the presence of inhibition zones surrounding the wells. The persistence

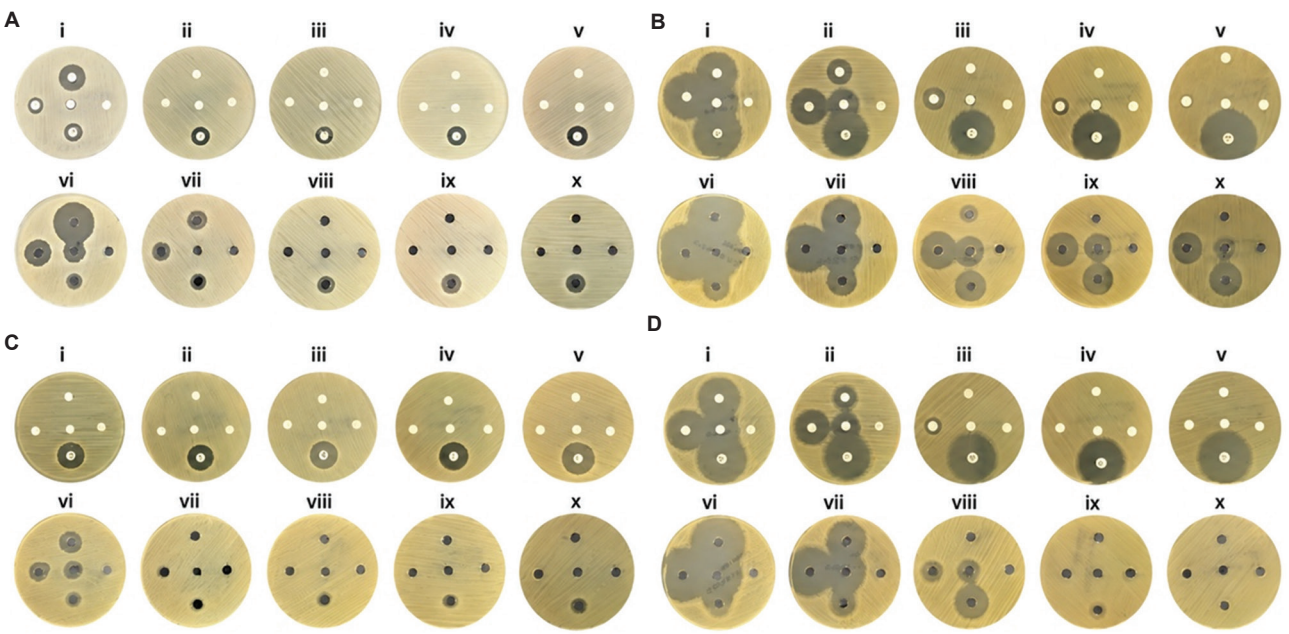

Figure S3. Representative agar diffusion assay demonstrating the antimicrobial activity of eluates obtained from polylactic acid (PLA) samples loaded with (A) vancomycin, (B) aztreonam, (C) polymyxin, and (D) imipenem. PLA discs were immersed in phosphate-buffered saline (PBS), and eluates were collected at (i) 1 h, (ii) 24 h, (iii) 72 h, (iv) 7 days, and (v) 14 days, with the medium replaced in full at each interval. (i–v) The eluate over a nitrocellulose disk; and (vi–x) the eluate in the medium hole. A 30 µL volume of each eluate was applied to wells on agar plates previously inoculated with the respective bacterial strains: *Staphylococcus aureus* (vancomycin), *Acinetobacter spp.* (polymyxin), and *Escherichia coli* (imipenem and aztreonam). Zones of inhibition were observed at all-time points, indicating sustained antimicrobial activity, even after repeated PBS replacements. These findings suggest continuous antibiotic release from the PLA matrix with preserved biological activity. In each plate, the inferior disc is a control nitrocellulose disk, and the right is a negative control (PLA without antibiotic).

of inhibition zones over time was interpreted as evidence of sustained antibiotic release with preserved biological activity.

The PLA discs impregnated with antibiotics were evaluated for their ability to maintain antimicrobial activity over a 180-day storage period by measuring inhibition zone diameters. PLA-V showed relatively stable activity, with inhibition zones ranging from 11 to 16 mm throughout the experiment, despite minor fluctuations. PLA-Z demonstrated the highest and most consistent activity among all antibiotics tested, maintaining large inhibition zones (26–35 mm) across all time points. PLA-P exhibited consistently small inhibition zones (6–8 mm), a finding attributed to its limited diffusion in agar rather than loss of activity. In contrast, PLA-I displayed marked instability: although inhibition zones increased up to 90 days (20 mm), a sharp decline was observed at 120 days (12 mm), followed by complete loss of activity at 150 and 180 days, when the measured 5-mm diameter corresponded solely to the size of the disc without any inhibition halo (**Figure S2**).

The antimicrobial activity of eluates obtained from antibiotic-loaded PLA samples was evaluated over time using an agar diffusion assay. Clear zones of inhibition were observed for all antibiotics at all evaluated time points, including 1 h, 24 h, 72 h, 7 days, and 14 days, despite complete PBS replacement at each interval. Vancomycin-loaded PLA demonstrated consistent inhibitory activity against *S. aureus*, while polymyxin, imipenem, and aztreonam maintained activity against *Acinetobacter* spp. and *Escherichia coli*, respectively. Although a slight reduction in inhibition zone intensity was visually noted over time, antimicrobial activity remained detectable even at 14 days. These findings indicate sustained release of active antibiotics from the PLA matrix throughout the experimental period (**Figure S3**).

3. Mechanical tests

3.1. Mechanical properties

Biomechanical analysis of the incorporated membranes was conducted using an EMIC DL 500 universal testing machine (INSTRON, Brazil). Four samples were tested from each group. The polymer sample was prepared in accordance with the American Society for Testing and Materials D1708-13 standard. Before each test, sample thickness, width, and initial length values were provided to the equipment. Thickness and width are necessary to calculate the cross-sectional area and, consequently, the stress (Equation S1). The initial length is necessary to calculate the strain and, consequently, the elongation percentage (Equation S2). Parameters measured included tensile strength and elongation. A preload of 0.1 N and a constant strain rate of 5 mm/min were applied.

$$\sigma=F/A \quad (1)$$

where σ is the stress in megapascal; F is the force in newton; and A is the cross-sectional area in mm^2.

$$\%El=(\Delta L/L_i)\times 100=\varepsilon\times 100 \quad (2)$$

where %El is the elongation percentage; ΔL is the variation of sample length in mm; L_i is the initial sample length in mm; and ε is the strain in mm/mm.

3.2. Results of the mechanical properties analysis

The stress–strain analysis demonstrated typical elastoplastic behavior in all PLA samples loaded with antibiotics, characterized by an initial linear elastic region, followed by progressive plastic deformation and post-peak softening. The ultimate tensile strength ranged from approximately 48.0 MPa to 50.5 MPa across the groups, while the estimated Young's modulus varied between 1.71 and 1.95 GPa. Although polymyxin-loaded PLA showed numerically higher values and imipenem slightly lower values, statistical analysis revealed no significant differences among the groups ($p>0.05$). Overall, the incorporation of antibiotics did not adversely affect the mechanical properties of PLA, as all formulations remained within the polymer's expected range, supporting its suitability as a structurally stable drug-delivery scaffold.