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ORIGINAL ARTICLE

Modeling chronic periodontitis in rats: Persistent alveolar bone loss mediated by periodontal pathogens

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Abstract

Background: Although widely used in periodontal research, rodents are naturally resistant to periodontitis. Conventional models, such as ligature-induced periodontitis, often fail to sustain defects due to spontaneous tissue regeneration after ligature removal. To address this, we refined a rat ligature-induced model of experimental periodontitis to better mimic the chronic, progressive nature of human periodontitis.

Methods: As a first step, following a split-mouth design, we compared the effectiveness of 3/0 silk thread and 0.008-inch orthodontic wire as ligature materials. Ligatures were applied around the left mandibular first molar for 6, 10, and 14 days. Periodontal pocket irrigation was performed every second day using a suspension of *P. gingivalis*, *P. intermedia*, and *S. gordonii*. As a second step, we evaluated whether silk-ligature alone, without human periopathogens, would be sufficient to induce a stable and progressive periodontal lesion. For that purpose, a silk ligature was removed on day 14, and the bone defect dynamics were monitored at 14-, 21-, and 28-days post-removal using micro-CT.

Results: Both wire and silk ligatures, in combination with bacterial irrigation, effectively induced rapid interproximal alveolar bone loss. However, silk ligature only, without periodontopathogen colonization, resulted in significantly lower bone loss (1.076 ± 0.22 mm vs. 2.012 ± 0.374 mm; $p = 0.003$) and the induced alveolar bone defects gradually resolved again over time.

Conclusions: The proposed rat model of periodontitis is well characterized and replicates human disease by sustaining colonization with viable periopathogens, leading to progressive disease with alveolar bone loss. The suggested model is straightforward, easy to establish and can be used reliably in preclinical studies.

KEYWORDS
alveolar bone loss, animal model, ligature-induced periodontitis, micro-CT, *Porphyromonas gingivalis*, *Prevotella intermedia*, rodent model of periodontitis

1 | INTRODUCTION

The prevalence of periodontal disease remains consistently high across all age groups and continues to rise worldwide. It is estimated to affect approximately 62% of all adults.[1–3] The progression of periodontitis is driven by genetic and systemic factors, dentition, lifestyle, and the interaction between microbial biofilm, particularly *Porphyromonas gingivalis*, and the host immune response.[4] A significant challenge in studying systemic conditions affecting the development and progression of periodontitis is the absence of a representative animal model of periodontitis.[5,6] Laboratory rodent models of periodontitis are the most widely used due to financial constraints, husbandry issues, and ethical considerations associated with the use of larger mammals such as dogs and non-human primates, which are more representative models.[5] Common laboratory rodents, such as mice and rats, are typically resistant to spontaneous periodontitis and less likely to develop the disease without external stimuli.[5,7] The various rodent models that have been introduced for experimental periodontitis include bacterial irrigation (infection/gavage), periodontal inoculation and chemically- and ligature-induced periodontitis.[8] The latter has been shown to induce rapid alveolar bone loss (ABL)—a key clinical indicator of periodontitis progression—through a combination of mechanical trauma and biofilm accumulation, thereby serving as a burst model of periodontal breakdown.[9] However, ligature placement alone does not replicate the pathogenesis of chronic periodontitis observed in humans and is therefore unsuitable for studying the effects of systemic diseases on immune response and bone metabolism in the periodontium, as laboratory rodents lack key human periopathogens. Based on the current understanding of periodontitis etiology, which follows the polymicrobial synergy and dysbiosis model, keystone pathogens such as *P. gingivalis* are essential and can enhance the virulence of the entire microbial community by interacting with supplementary pathogens, ultimately driving periodontitits progression.[10] Many studies performed in rodents have reported rapid bone regeneration, which occurs within 4–9 days after ligature removal.[11,12] In mice, complete healing occurred within 7 days without any treatment.[13] The administration of a bacterial suspension through oral gavage alone is not highly effective, as it requires more than 8 weeks to induce only minor ABL and necessitates the use of periodontitis-susceptible genetically modified animals.[8,14–16]

The present study aimed to optimize a well-established ligature-induced periodontitis model in rats by developing a reproducible, practical, and straightforward approach for inducing advanced periodontitis through the incorporation of viable human periopathogens. To achieve this, we employed the ligature-induced model to initiate ABL and gingival injury, followed by targeted irrigation of the periodontal pockets with viable periopathogens to ensure effective microbial colonization, thereby promoting sustained bone loss even after ligature removal.

2 | METHODS

2.1 | Sample size calculation

The sample size was determined using a sample size calculator,[17] based on the ABL data reported by Bueno et al[18] A sample size of three per group was determined to be sufficient for detecting a significant difference in ABL with 80% power and a Type I error rate (α) of 5%. To account for potential animal loss following recovery from ketamine/xylazine anesthesia, an attrition rate of 10% was applied.[19]

2.2 | Animals

Animal experiments were approved by the Animal Ethics Committee and Animal Research Authority of the University of Sydney (No. 2024/2444) and conducted in compliance with the NSW animal welfare legislation and the Australian Code for the Care and Use of Animals for Scientific Purposes and were treated according to the ARRIVE guidelines.[20] Forty-two 20-week-old female Sprague–Dawley rats were used for this study (Ozgene, Perth, WA, Australia). A detailed distribution of animals across groups and the experiments performed is presented in Table 1, while the sequence of events is described later in this report. The rats were housed 3–4/cage with a 12-h light/dark cycle with chow and water available ad libitum. Room temperature was maintained at 21(±1)°C.

2.3 | Bacteria

All the bacteria were cultured in a liquid medium containing brain heart infusion (BHI) broth (Cat. 110493, Merck Life Science, Bayswater, VIC, Australia), supplemented with 5 g/L yeast extract (Cat. Y1625, Sigma-Aldrich, Scoresby, VIC, Australia) and 5% defibrinated sheep blood (Cat. SB100, Serum Australis Pty Ltd., Australia) or Columbia blood agar (Cat. CM0331B, Oxoid Ltd., Hants, UK) supplemented with 5 g/L yeast extract and 5% defibrinated sheep blood.

Anaerobic conditions for *P. gingivalis* W83 (Cat. BAA-308, ATCC, USA) and *P. intermedia* VPI 4197 (Cat. 25611, ATCC, USA) were established using Anaerocult system (Cat. 1.32369; Cat. 1.14226; Cat. 1.32371, Millipore, Darmstadt, Germany), with incubation at 37°C for 48 h in broth or 7–9 days on agar until visible growth was observed. *S. gordonii* (Cat. 35405, ATCC, USA) was cultured under aerobic conditions at 37°C for 24 h in 10% CO_2.

2.4 | Testing of bacterial viability in broth under long-term storage

Colonies of *P. gingivalis* and *P. intermedia* were cultured on blood agar under anaerobic conditions for 7–9 days, using the Anaerocult

TABLE 1 The experimental groups of animals.

Group	Timeline and number of animals per time point (*n*)		
Determination of the optimal material and time for the periodontal bone lesion formation			
Wire ligature	6 days, $n=3$	10 days, $n=3$	14 days, $n=4$
Silk ligature	6 days, $n=3$	10 days, $n=3$	14 days, $n=4$
Investigation of the stability of alveolar bone defects following ligature removal and the persistence of periopathogenic bacterial microflora in induced periodontal lesions			
Control	14 days, $n=3$	21 days, $n=3$	28 days, $n=5$
Bacterial suspension	14 days, $n=3$	21 days, $n=3$	28 days, $n=5$

system. After incubation, the bags containing the agar plates were opened inside an anaerobic chamber (Bugbox, Baker, Sanford, MA, United States) maintained with 10% CO_2, 5% H_2, and 85% N_2. Bacterial colonies inside the anaerobic chamber were picked and resuspended in BHI broth supplemented with 5 g/L yeast extract, with or without the addition of 10 g/L sodium carboxymethyl cellulose (CMC, Cat. 9004-32-4, Sigma-Aldrich, Bayswater, VIC, Australia). The suspension was then filtered through a 70 μm cell strainer (Cat. 833945070, Sarstedt, Germany). *S. gordonii* suspension was prepared using the same method but under aerobic conditions.

The total viable bacterial concentration in the suspension was determined by measuring absorbance at OD 600 nm. The OD was set to 1.5 for *P. gingivalis* and *P. intermedia*, and 0.6 for *S. gordonii*, corresponding to 1×10^{10}–10^{11} viable cells/mL. After preparing the bacterial suspension in the BHI broth with or without CMC, the suspension was loaded into a 1 mL insulin syringe (Cat. SS10M2913KA, Terumo, USA), by removing the syringe plunger and carefully dispensing the liquid into the barrel. This method was necessary due to the high viscosity of the liquid, which made it impossible to draw the suspension directly into the syringe. Photos of the syringes loaded with bacterial suspension are shown in Figure S1L. Syringes with bacterial suspension were stored at +4°C in a light-proof box. At 0, 3, 24, 48 h, 8 and 14 days after suspension preparation, 20 μL of bacterial suspension in broth, with or without CMC, was cultured on blood agar.

2.5 | Ligature placement

Each animal was anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) (Ketamav 100, Mavlab, Australia) and xylazine (3 mg/kg) (Xylazil-20, Ilium, Australia). Eye lubricant was applied, and the rat was positioned on the table with a mouth gag (Cat. T2000, iM3, Australia) (Figure S1K). For the wire ligature, a stainless-steel orthodontic ligature wire ($d=0.008$ inches, ORMCO, Glendora, CA, USA) was used, and for the silk ligature, a 3/0 silk suture (Look Silk Sutures, Cat. X786B, Westwood, MA, USA) was used. Local infiltration anesthesia was administered on the facial aspect of the mandibular first left molar with 3% Mepivacaine (Scandonest 3%, Septodont, USA).

The silk suture was placed as follows. The gingival papilla was retracted on both the lingual and buccal sides using a Gracey curette (Figure S1A), a pre-bent ligature wire (Figure S1B) was passed through the interdental space from the lingual side on both sides to create a loop, and the silk thread was inserted (Figures S1C–F). The wire was threaded through the interdental space and positioned around the first molar, where a ligature was placed (Figures S1G–J). For the wire ligature placement after gingival retraction, a pre-banded wire was threaded through the interdental space between the first and second molars (Figures S1A–D) and twisted around the first molar using a needle holder.

After the successful placement of the ligature, ketamine/xylazine anesthesia was reversed with a subcutaneous injection of atipamezole 1 mg/kg (Atipamezole, Ilium, Australia). For the first 3 days after ligature placement, rats were provided with chow pellets broken into small pieces and napa nectar gel (Cat. NC9479557, Se Lab Group Inc., NC, USA) to reduce any pain associated with mastication following the ligature placement.

2.6 | Periodontal pocket irrigation

The suspension for periodontal pocket irrigation was prepared by mixing bacterial suspensions of *P. gingivalis* and *P. intermedia* at OD 1.5 and *S. gordonii* at OD 0.6 in the BHI broth with 10 g/L CMC in equal volumes under anaerobic conditions. The prepared suspension was then stored in syringes at +4°C in a light-proof box for up to 14 days until use. For periodontal pocket irrigation, rats were anesthetized with isoflurane. Once anesthetized, they were placed on their backs, and the mouth was opened using a rodent wire mouth gag (Cat. D1032, iM3, Australia). The cheek was retracted with a spatula, and access to the pharynx from the oral cavity was blocked with a cotton stick to prevent excess absorption of the suspension and prevent the risk of aspiration (Figure S1M). Periodontal pocket irrigation was performed by inserting the tip of the needle between the ligature and the first molar, irrigating the gingival sulcus from both the vestibular and lingual surfaces. For each periodontal pocket irrigation, 80–100 μL of the suspension was used. After completing the bacterial irrigation procedures, the remaining bacterial suspension from the syringe was seeded onto

blood agar and incubated under anaerobic and aerobic conditions to assess bacterial viability.

2.7 | Bacterial PCR

The isolated single colony was resuspended in 200 μL of nuclease-free water and heated on a heat block at 100°C for 10 min. The sample was then cooled and centrifuged at 12 000 *g* to remove cell debris. The reaction volume was set to 25 μL and was prepared according to the manufacturer's instruction for GoTaq® Colorless Master Mix (Cat.M7132, Promega, USA).

The following primer pair sequences were used in this study:

P. gingivalis (*fimA*) F: 5′-ATCTGAACGAACTGCGAC-3′,
R: 5′GTTCTGTCCTCGTTGTCTT-3′;
P. intermedia (*16sRNA*) F: 5′-GTCCACATATGGCATCTG-3′,
R: 5′-ATACGTTGCGTGCACTCA-3′;

The PCR reaction consisted of 30 amplification cycles and was performed according to the manufacturer's guidelines. PCR products were analyzed by electrophoresis on a 1.0% agarose gel (GelRed Agarose LE, Cat.41029-50G, Biotium, USA), in TAE buffer (Cat.161–0743, Bio-Rad, USA). A DNA marker 100 bp DNA ladder (Cat.G210A, Promega, Madisom, WI, USA) was used. The gels were then visualized using a ChemiDoc MP Imaging System (Bio-Rad, USA).

2.8 | Histomorphometry analysis using micro-computed tomography

The isolated mandible was scanned using a micro-computed tomography (micro-CT) system (MiLabs U-CT, Netherlands) with the following settings: ultra-focus, accurate mode, total rotation of 360°; step size of 0.25°; source settings of 50 kV and 240 μA; and exposure time of 75 ms per step. Images were reconstructed as three-dimensional images at 10–12 μm voxel size with Imalytics Preclinical software version 2.1 (Gremse-IT, Germany).[21]

ABL was evaluated as the distance from cemento-enamel junction to alveolar bone crest, using a perpendicular line drawn from the cemento-enamel junction with the highest score recorded at five different sites (Site 1—mesial, Site 2—buccal surface, Site 3—interproximal, Site 4—lingual, and Site 5—bifurcation) as shown in Figure S1P,Q. Measurements were taken around the left first mandibular molar (ligated site) as well as around the right first mandibular molar (control molar) of the same animal, where no intervention occurred.

For bone microarchitectural parameters, regions of interest (ROIs) were confined to trabecular bone areas. ROIs in the mandible were selected from two regions on both the left (ligated first molar) and right (first molar without a ligature) sides. The first ROI was located in the trabecular bone area of the interradicular septum of the first molar, following the method adapted from Lee et al. (Figure S1R).[22] The second ROI was acquired from the mandibular body, positioned below the first molar and separated manually by a straight line drawn between the mesial and distal roots (Figure S1R), as suggested by Lee et al[22] The following parameters were measured within the defined ROIs using the 'bone statistics' function: bone volume fraction, bone mineral density, trabecular thickness, and trabecular separation.

2.9 | Determination of the optimal material and time for the periodontal bone lesion formation

Twenty-week-old female SD rats were randomly assigned to two groups: wire ligature ($n=10$) and silk ligature ($n=10$). On day 0, ligatures were placed around the first left mandibular molar as previously described. Starting from day 1, periodontal pocket irrigation with bacterial suspension was performed every other day in all rats. During each irrigation, ligature stability and retention were assessed, with ligature loss leading to exclusion and pre-terminal euthanasia.

On days 6 and 10 ($n=3$/group) and day 14 ($n=4$/group), rats were euthanized via CO_2 exposure. Mandibles were extracted, and ligature retention and position were evaluated (Figure S1N). The ligatures were then placed in 0.2 mL bacterial broth. Within 15 min of sample collection, 0.1 mL was plated on blood agar and incubated under aerobic and anaerobic conditions. The samples of mandibles were stored at −30°C for further micro-CT examination. The sequence of events is shown in Figure 1A.

2.10 | Investigation of the stability of alveolar bone defects following ligature removal and the persistence of periopathogenic bacterial microflora in induced periodontal lesions

Twenty-four-week-old female SD rats ($n=22$) had a silk ligature placed around the first left mandibular molar. They were then randomly assigned to two groups: a control group ($n=11$), which received periodontal pocket irrigation with the BHI broth containing 5% yeast extract and 10 g/L CMC, and a test group ($n=11$), which received irrigation with the same broth containing human periodontal pathogenic bacteria.

For the next 14 days, periodontal pocket irrigation was performed every second day. On day 14, the rats were anesthetized with isoflurane, the ligature was removed, and micro-CT imaging was conducted to assess ABL caused by the ligature. Post-ligature removal, periodontal pocket irrigation continued every second day for the next 7 days and was performed once more on day 24.

Rats were euthanized via CO_2 exposure on days 28, 35 ($n=3$/group), and 42 ($n=5$/group), corresponding to the 14th, 21st, and 28th days after ligature removal. Periodontal pocket curettage material was collected in 0.15 mL of bacterial broth. Within 15 min of collection, 0.15 mL of each sample was plated on blood agar and incubated under anaerobic conditions. Mandibular samples were stored at −30°C for further micro-CT analysis. The sequence of events is shown in Figure 1B.

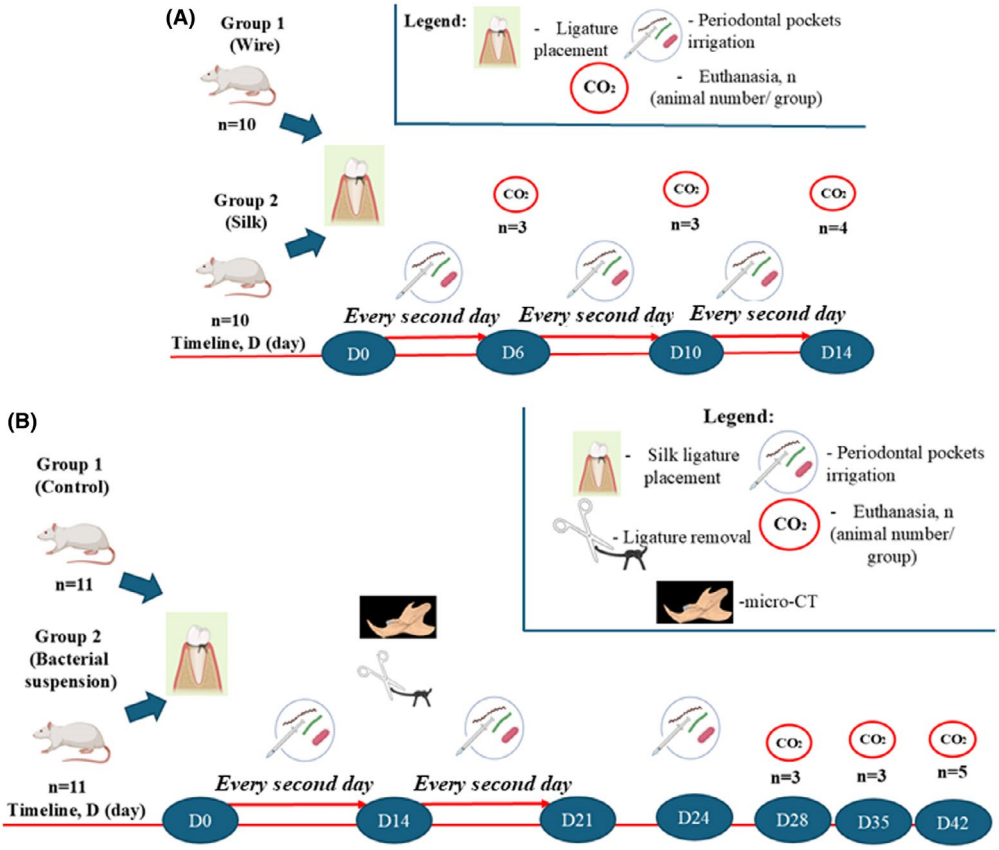


FIGURE 1 Flowchart illustrating the experimental design. (A) Two groups of rats ($n = 10$ per group) received either silk or wire ligatures, Following ligature placement, periodontal pocket irrigation with a bacterial suspension containing *P. gingivalis*, *P. intermedia*, and *S. gordonii* was performed every other day. Rats were euthanized on days 6 ($n = 3$/group), 10 ($n = 3$/group, and 14 ($n = 4$/group). (B) Two groups of rats ($n = 11$/group) received a silk ligature for 14 days. Following ligature placement, periodontal pocket irrigation with a bacterial suspension containing *P. gingivalis*, *P. intermedia*, and *S. gordonii* was performed every second day in group 2 (bacterial suspension), In group 1 (control), irrigation was performed with both. The ligature was removed on the 14th day, and micro-CT was conducted to assess alveolar bone loss. Afterwards, rats were observed for 14 ($n = 3$/group), 21 ($n = 3$/group), and 28 ($n = 5$/group) days to monitor changes in alveolar bone loss.

2.11 | Statistics

GraphPad PRISM 8.0.1 (Dotmatics) was used for statistical data analysis. All the results are presented as mean ± standard deviation unless stated otherwise. A one-way ANOVA followed by Tukey's multiple comparison test was used to analyze data within a single group across different time points. A multiple t test was performed to assess statistical significance and p-value of <0.05 was considered significant.

3 | RESULTS

3.1 | Experimental periodontitis did not compromise animal well-being

Animal well-being remained unaffected throughout the intensive monitoring period, as evidenced by stable body weight measurements (Figures S3A and S4A). No animals were prematurely euthanized due to ligature loss, as the ligatures remained securely in place throughout the entire observation period until the rats reached the scientific endpoint (Figure S1N). Only one rat did not recover after ketamine/xylazine anesthesia, which was accounted for in the attrition rate.

3.2 | Periopathogens remain viable under long-term storage in suspension for periodontal pocket irrigation

P. gingivalis, *P. intermedia*, and *S. gordonii* in the bacterial suspension for periodontal pocket irrigation remained viable when prepared under anaerobic conditions and stored at +4°C for up to 14 days. The addition of 10 g/L CMC to the BHI broth, supplemented with 5 g/L yeast extract, did not affect bacterial viability (Figure S2A–C). Additionally, even when exposed to oxygen, these bacteria remained viable, as confirmed by seeding the remaining bacterial suspension onto agar after the periodontal pocket irrigation procedure (Figure S1O).

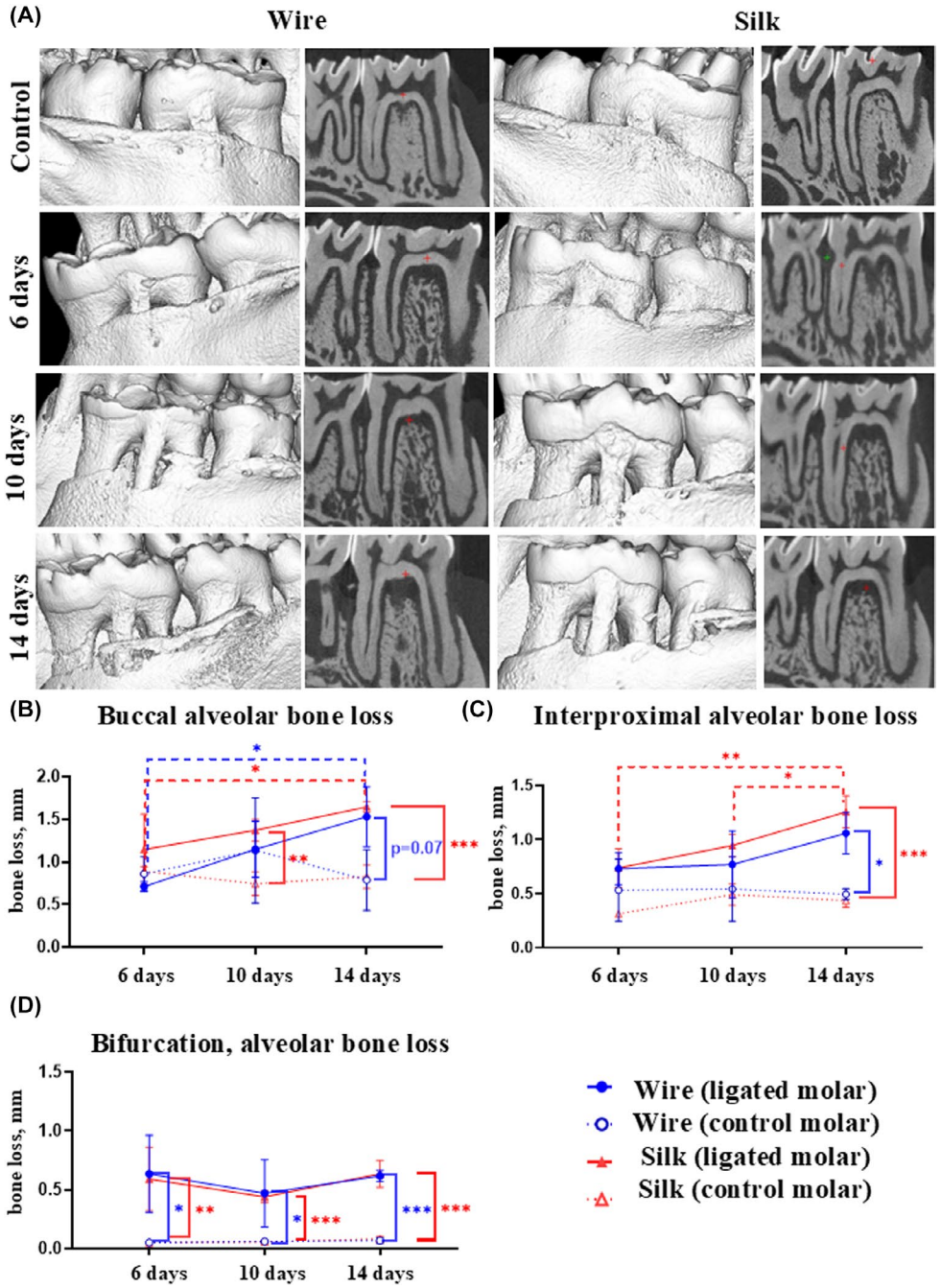
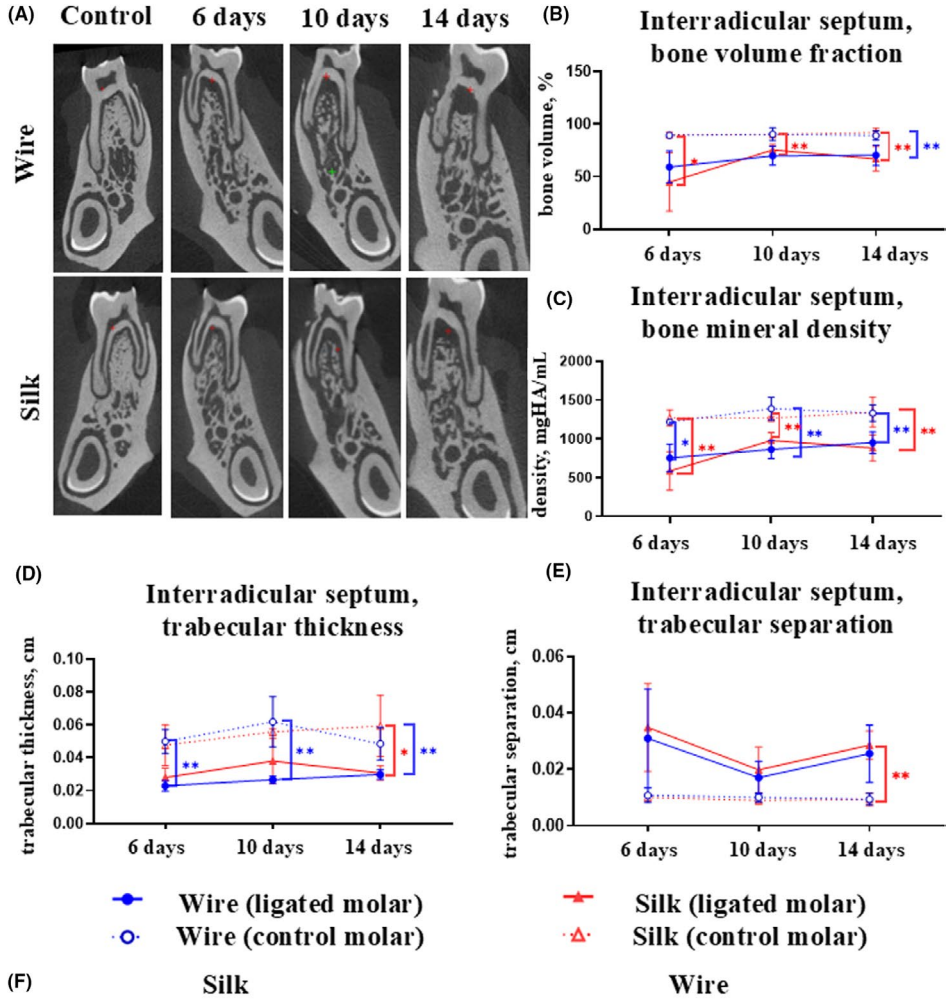


FIGURE 2 Silk and wire ligatures induce alveolar bone defects in a time-dependent manner over 6, 10, and 14 days. (A) Representative 3D reconstructions and sagittal tomographic sections were obtained from micro-CT scans of control rat molars and at 6, 10, and 14 days following the placement of either silk or wire ligatures. (B–D) Alveolar bone loss on the buccal (B), interproximal (C) surfaces, and bifurcation (D) was assessed following the placement of wire and silk ligatures at different time points (solid line) in comparison to control molar measurements (dashed line). Statistical significance was determined using a one-way ANOVA to evaluate the effect of ligation duration on alveolar bone loss and a Student's *t* test to compare differences between the two groups under varying conditions. Data are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.3 | Both silk and wire ligatures effectively induce ABL, which progresses over time and supports the survival of periopathogens

ABL occurs on the 6th day after silk or wire ligature placement, specifically in the interradicular alveolar bone beneath the bifurcation, as confirmed by linear and volumetric measurements (Figures 2A,D and 3A,B). However, bone volume fraction revealed significant ABL only in the silk ligature group on the 6th day. On the 10th day, the ABL on the buccal and interproximal surfaces showed a tendency to increase, with significant ABL observed in the silk ligature group at the buccal surface and bifurcation site (Figures 2A-D and 3A,B).



(G)

Days \ Ligature type	Wire		Silk	
	P. gingivalis	*P. intermedia*	*P. gingivalis*	*P. intermedia*
6	3/3	0/3	3/3	0/3
10	3/3	2/3	3/3	3/3
14	4/4	3/4	4/4	4/4

FIGURE 3 Wire and silk ligatures alter trabecular bone microarchitectural parameters of the interradicular septum in a time-dependent manner and promote bacterial colonization of periodontal pockets by human periopathogens. (A) Representative tomograms in the coronal plane obtained from micro-CT scans illustrate changes in the interradicular septum of the first mandibular molar induced by wire and silk ligatures of control rat molars and at 6, 10, and 14 days after the ligature placement. (B–E) Changes in bone volume fraction (B), bone mineral density (C), trabecular thickness (D), and trabecular separation (E) of the interradicular septum of the ligated first left molar (solid line) were assessed in comparison to control molar (dashed line) using micro-CT analysis in rats on the 6th, 10th and 14th day after wire of silk ligature placement. (F) Representative images of viable bacteria cultured under anaerobic conditions for 9 days from silk and wire ligature samples removed on the 14th day after ligature placement. (G) Summary of viable bacterial strains of *P. gingivalis* and *P. intermedia* identified by PCR analysis of colonies grown on blood agar after 9 days of anaerobic cultivation in animals with silk or wire ligatures at the indicated time points. Statistical significance was determined using a one-way ANOVA to evaluate the effect of ligation duration on alveolar bone loss and a Student's *t*-test to compare differences between the two groups under varying conditions. Data are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$.

The highest ABL was observed on the 14th day in the wire and silk groups in the buccal (0.618 ± 0.04 mm vs. 0.635 ± 0.115 mm) and interproximal surfaces (1.06 ± 0.19 mm vs. 1.25 ± 0.148 mm) (Figure 2A-C). In contrast, ABL at the bifurcation site remained stable, with no significant changes compared to the 6th or 10th day (Figures 2A,D and 3A,B). The silk ligature group exhibited greater and more predictable levels of ABL on the buccal and interproximal surfaces, as well as at the bifurcation site, which are characteristic patterns for periodontitis. In contrast, the ABL on the lingual and mesial surfaces remained unchanged in both groups across all time points (Figure S3B,C). Interradicular septum trabecular bone experienced a loss in mineral density and changes in the bone microarchitectural parameters, further confirming the activation of bone resorption (Figure 3C–E). In contrast, the microarchitectural parameters of trabecular bone in the mandibular body remained unaffected in both groups across all the observation time points (Figure S3D–G).

Culturing bacteria retained on the ligatures revealed the presence of viable anaerobic, black-pigmented bacteria growing on blood agar, which were identified by PCR as *P. gingivalis* and *P. intermedia* (Figure 3F). *P. gingivalis* was detected in both groups at all time points, whereas *P. intermedia* colonies appeared only after the 10th day, predominantly in the silk ligature group (Figure 3G).

Both materials induced sufficient ABL; however, silk ligatures resulted in more predictable ABL, particularly in the interproximal area, which is typical for periodontitis. Additionally, silk showed better bacterial retention. The optimal period for establishing a bone defect was determined to be 14 days, with silk selected as the best ligature material.

3.4 | The presence of viable periopathogens is essential for sustaining progressive ABL following ligature removal

Periodontal pocket irrigation with a suspension of *P. gingivalis*, *P. intermedia*, and *S. gordonii* resulted in a progressive increase in ABL over the observation period, with significantly greater ABL observed on day 28 after ligature removal at the interproximal surface (1.076 ± 0.22 mm vs. 2.012 ± 0.374 mm; $p = 0.003$) and bifurcation site (0.484 ± 0.185 mm vs. 0.818 ± 0.202 mm; $p = 0.04$) (Figure 4A,C,D). In contrast, the extent of ABL on the buccal surface did not differ between the two groups or across the observation time points (Figure 4B). At the same time, the ABL was unaffected on the mesial and lingual surfaces (Figure S4B,C). These linear measurements were further supported by a gradual decrease in interradicular septum bone volume fraction, change in mineral density, and the microarchitectural parameters, related to the chronicity of inflammation over the observation period (Figure 5A–E). In contrast, the aforementioned parameters of the mandibular body remained unchanged (Figure S4D–G). The culturing of curettage samples revealed the presence of viable *P. gingivalis* and *P. intermedia* in the test group, whereas these periodontopathogens were not detected in the control group (Figure 5F,G).

4 | DISCUSSION

This study established and characterized a simple and effective rat model of experimental periodontitis that does not compromise animal well-being. The model utilizes a 14-day silk ligature placement to rapidly induce a periodontal defect and create favorable conditions for human periodontopathogen colonization. The presence of viable pathogens was found to be essential for sustaining disease progression rather than allowing regeneration to occur after the mechanical factor (ligature) was removed. Moreover, we introduced and validated methods for bacterial suspension preparation and a highly reproducible technique for ligature placement, making this model standardized and easy to replicate.

One of the challenges in implementing this model was the technical difficulty of ligature placement. We were unable to place the silk ligature directly through the interdental space using forceps due to tight interproximal contacts between the teeth, as reported in prior works.[23,24] To overcome this problem, some researchers have performed interproximal area separation, which we found impractical due to limited reproducibility, and possible dentin or pulp exposure, which negatively affects animal welfare, increasing biofilm accumulation, and potentially resulting in the exclusion of animals from the study.[25,26] Instead, we developed an alternative, reliable method for ligature placement that overcomes these challenges, as detailed in the Methods section.

We posit that extending the ligation period beyond 14 days is unnecessary, as both wire and silk ligatures tend to loosen after 10–14 days due to extensive alveolar bone resorption, resulting in loss of tight attachment to the tooth (Figure S1N). While the ligature may remain in place beyond this period, we argue that it can only function effectively as a reservoir for bacterial colonization when it maintains close contact with the gingiva. From a clinical perspective, ligature placement in localized periodontitis models simulates mechanical factors such as overhanging restorations, poorly fitted prosthetic constructions or improper interdental contacts, which promote food impaction and serve as sites for increased bacterial biofilm formation—conditions that typically involve close contact with the tooth and surrounding periodontal tissues. The removal of the ligature corresponds to the clinical resolution of these factors, such as the restoration of proper interdental contact or the replacement of an overhanging restorations or prosthetic constructions.[27] In a mouse model of experimental periodontitis induced by silk ligatures, the inflammatory response initiates around day 4, peaks by day 8, and continues to intensify up to day 14 of ligation.[28] Maintaining a loose ligature beyond the initial period is not meaningful, as 14 days are sufficient for the formation of a biofilm, which will continue to sustain inflammation within the periodontium. Moreover, in our model, the ligature serves to rapidly induce a gingival wound that facilitates the colonization of periopathogens and leads to progressive ABL, in contrast to the oral gavage model where bacterial colonization may occur but does not typically result in intensive ABL in rodents.[14]

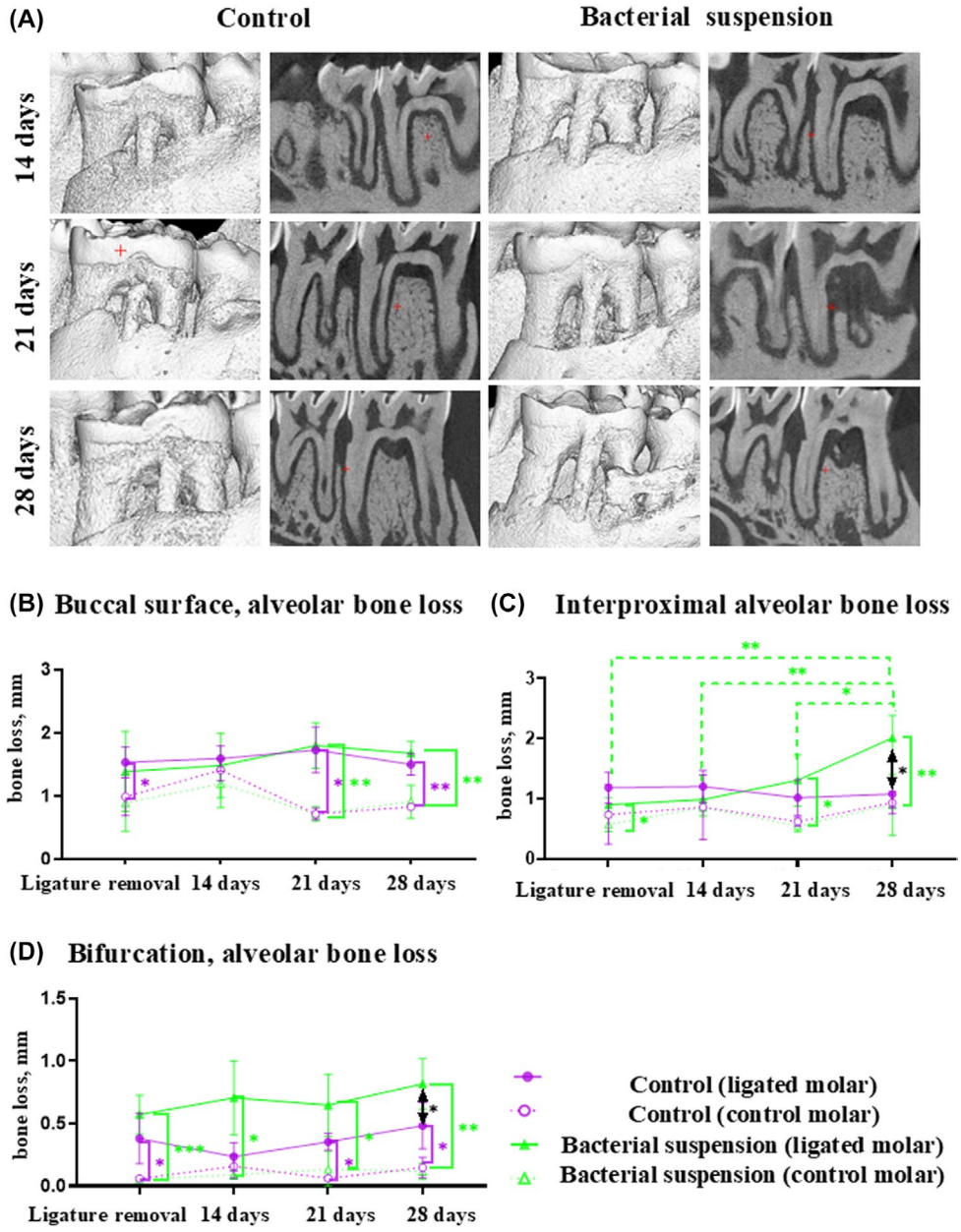
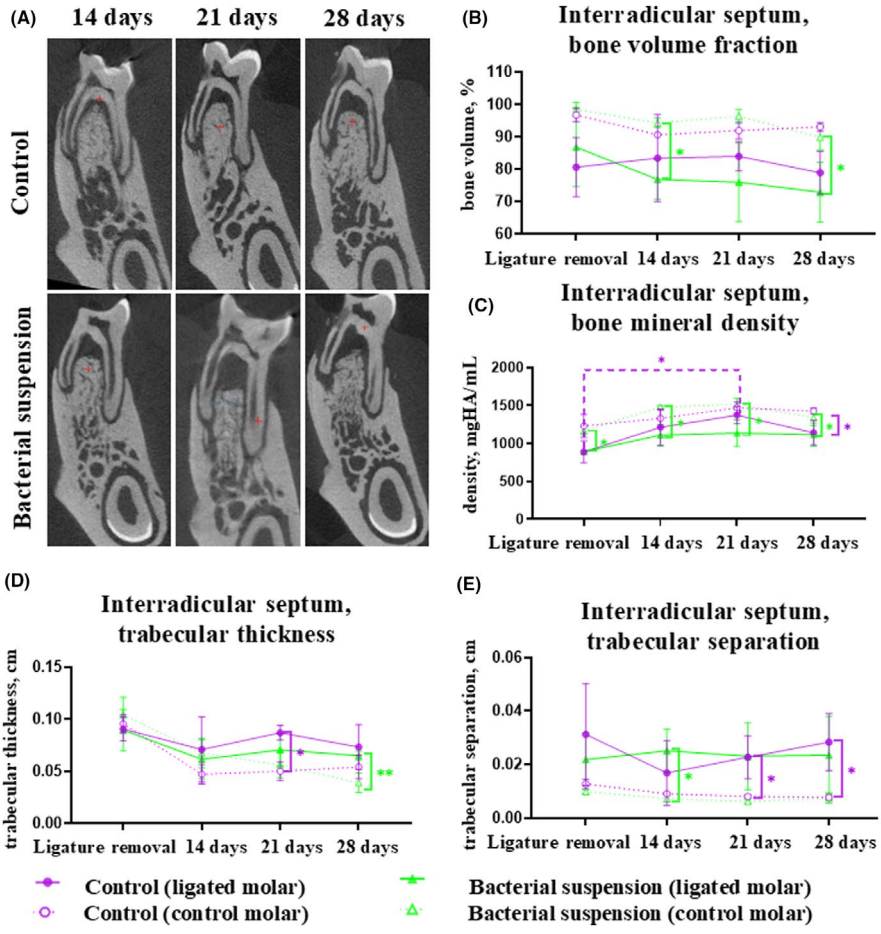
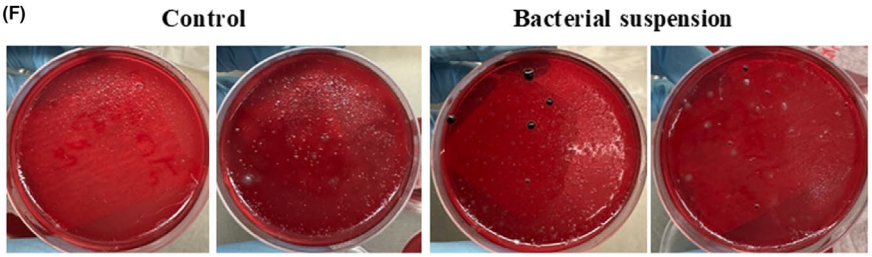


FIGURE 4 The presence of human periodontal pathogens accelerates alveolar bone loss following silk ligature removal and inhibits bone regeneration during the 14, 21, and 28-day observation period. (A) Representative 3D reconstructions and sagittal plane tomograms were obtained from micro-CT scans of rats on the 14th, 21st, and 28th days after ligature removal. These scans were conducted for rats with periodontal pocket irrigation performed with either broth (control) or bacterial suspension. (B–D) Alveolar bone loss on the buccal (B), interproximal (C) surfaces, and bifurcation (D) was assessed after the removal of the ligature and on the 14th, 21st and 28th day (solid line) in comparison to control molar measurements (dashed line). Statistical significance was determined using a one-way ANOVA to evaluate the effect of ligation duration on alveolar bone loss and a Student's *t*-test to compare differences between the two groups under varying conditions. Data are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Meanwhile, another study on rats has reported a significantly lower ABL in the interproximal area after 24 and 35 days of different ligature types, including various metal wires and silk ligatures, but without human periopathogen exposure.[29] In contrast, our findings demonstrated greater ABL after just 14 days of ligation combined with bacterial irrigation, highlighting the critical role of human periodontopathogens in ABL, which was confirmed by linear, volumetric and bone parameter measurements by micro-CT, which is as informative as histological measurements.[18] We showed that the ABL in rats with viable periodontal pathogens does not heal but instead progresses over time after ligature removal. This contrasts with other models that produced either regeneration or stabilization of the ABL following ligature removal,[12,13,24,25] and underscores the critical role of viable human periodontopathogens in disease progression, rather than attributing disease solely to the direct mechanical trauma caused by the





(G)

Ligature type / Days	Control		Bacterial suspension	
	P. gingivalis	*P. intermedia*	*P. gingivalis*	*P. intermedia*
14	0/3	0/3	2/3	1/3
21	0/3	0/3	3/3	1/3
28	0/4	0/4	5/5	4/5

FIGURE 5 The presence of viable human periopathogens in periodontal pockets impedes bone defect healing. (A) Representative tomograms in the coronal plane obtained from micro-CT scans illustrate changes, such as reactive sclerosis, related to chronicity of inflammation, in the interradicular septum of the first mandibular molar induced by silk ligatures on the 14th, 21st, and 28th days of observation after ligature removal. (B–E) Changes in bone volume fraction (B), bone mineral density (C), trabecular thickness (D), and trabecular separation (E) of the interradicular septum of the ligated first left molar (solid line) were assessed in comparison to control molar (dashed line) using micro-CT analysis in rats on the 14th, 21st and 28th days after the ligature removal. (F) Representative images of viable bacteria cultured from curettage material under anaerobic conditions for 9 days, obtained from control and bacterial suspension samples collected from rats on the 28th day of observation after ligature removal. (G) Summary of viable *P. gingivalis* and *P. intermedia* bacterial strains identified by PCR analysis of colonies grown on blood agar after 9 days of anaerobic cultivation in animals that underwent periodontal pocket irrigation with broth (control group) or bacterial suspension at the indicated time points (days after ligature removal). Statistical significance was determined using a one-way ANOVA to evaluate the effect of ligation duration on alveolar bone loss and a Student's *t*-test to compare differences between the two groups under varying conditions. Data are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$.

ligature and endogenous biofilm accumulation, as highlighted by de Molon et al[14,25]

In the proposed model, periodontitis is considered established after ligature removal on the 14th day, as this eliminates the mechanical pressure exerted by the ligature, which may otherwise contribute to periodontal destruction. Both wire and silk produce comparable outcomes in terms of ABL and are good substrates for colonization by bacteria, as reported previously.[30] However, in our study, silk ligatures showed superior periopathogen colonization and induced significantly greater ABL in a split-mouth design. Therefore, silk was selected as the preferred material for ligature placement. Our model does not require prior suppression of the rats' oral endogenous microflora through antibiotic administration to establish colonization of periodontal pockets with human periopathogens.[31–33] This approach also minimizes the potential impact of antibiotics on the reproducibility of the model. Among all known human bacteria associated with periodontitis, *P. gingivalis* was chosen as it is a key pathogenic species involved in the initiation and progression of the disease. It possesses multiple virulence factors and can alter the endogenous microflora, contributing to dysbiosis and disease progression, as discussed elsewhere.[10,34] *S. gordonii*, as an 'accessory pathogen', is essential for facilitating the colonization of *P. gingivalis* because, on its own, the latter fails to establish itself in periodontal pockets.[35,36] Moreover, the combined presence of *S. gordonii* and *P. gingivalis* in animal models has been shown to lead to a significantly greater ABL than either organism alone through multiple molecular mechanisms, which have been discussed elsewhere.[10,34,37] *P. intermedia* is a periodontopathogen associated with severe periodontitis and necrotizing forms of periodontal disease, particularly in patients with systemic chronic conditions such as diabetes, respiratory and cardiovascular diseases.[38] It utilizes a heme uptake system, in which the heme-binding lipoprotein HmuY plays a dominant role, facilitating heme acquisition and enhancing the virulence of *P. gingivalis*, as well as other mechanisms which have been discussed elsewhere.[38,39] Laboratory rodents do not naturally harbor human periopathogens, as demonstrated in our control group. Therefore, the exogenous introduction of these pathogens is essential to replicate the pathogenesis of human periodontitis in a rat model, as ligature placement alone is insufficient to reproduce the complex disease observed in humans.

We demonstrated the persistence of viable *P. gingivalis* and *P. intermedia* in the periodontal pockets of rats. In previous studies, their presence was confirmed using PCR, which does not indicate bacterial viability.[14,33] We also demonstrated that bacteria in the microbial suspension for periodontal pocket irrigation remained viable for up to 14 days, ensuring consistency in bacterial concentration during each irrigation and reducing the time required for daily suspension preparation.

The limitations of this study include a relatively small sample size and the inability to fully replicate the complex microbial environment characteristic of human periodontitis, highlighting the need for future validation in larger cohorts. Additionally, the specific mechanisms underlying chronic, progressive ABL following the colonization of rat periodontal pockets with human periopathogens remain unclear. Further research is warranted to elucidate the inflammatory pathways involved in the pathogenesis of periodontitis. It is yet to be determined whether this occurs due to shifts in the native rat microbiota toward a more pathogenic state mediated by *P. gingivalis* and *P. intermedia* or through their direct influence. Later observation time points, such as 6 and 8 weeks, could be included to better evaluate the dynamics of defect progression over time. A potential source of measurement bias in this study arises from the use of the split-mouth design. Although this approach is widely accepted in dental research, the untreated contralateral site may not adequately represent a true control.[40] Localized periodontitis on one side of the jaw can induce systemic inflammation, which may in turn affect ABL and periodontal status on the opposite side. To mitigate this potential bias, the inclusion of a separate control group comprising completely untreated animals is recommended, allowing for a more reliable baseline comparison. Another potential source of bias relates to the lack of hormonal monitoring, as the study used female rats, and fluctuations in estrogen levels are known to influence periodontal inflammation and bone metabolism. This effect is particularly relevant in older or ovariectomised animals.[41] However, in our study, we used young, skeletally mature female rats, which are known to exhibit regular estrous cycles and stable hormone levels.[42] Therefore, while we acknowledge this as a theoretical limitation, we believe that the absence of estrous cycle monitoring is unlikely to have significantly impacted the outcomes of our study. Future studies should address these limitations.

5 | CONCLUSION

The proposed model successfully induced an initial alveolar bone defect by combining silk ligature-induced periodontitis, which mechanically triggered rapid ABL while promoting biofilm accumulation and the retention of exogenous human periodontopathogens. The persistence of viable periodontopathogens was essential for sustained disease progression, enabling the investigation of their pathogenic mechanisms, interactions with the immune system, and microbial dynamics within periodontal pockets. This model may serve as a platform for evaluating host modulation or regenerative therapies in established periodontitis.

AUTHOR CONTRIBUTIONS

Maksym Skrypnyk: Conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing – original draft. **Chun Xu:** Resources; supervision; writing – review and editing. **Shlomo Berkovsky:** Conceptualization; supervision; writing – review and editing. **Thilini Jayasinghe:** Conceptualization; resources; supervision; writing – review and editing. **Axel Spahr:** Conceptualization; funding acquisition; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

All data relevant to this research are included in the publication.

ETHICS STATEMENT

This study was approved by the University of Sydney Animal Ethics Committee (project number 2024/2444) and animals cared for within animal ethics committee guidelines, ensuring consideration of animal welfare as per the NHMRC code.

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REFERENCES

1. Trindade D, Carvalho R, Machado V, Chambrone L, Mendes JJ, Botelho J. Prevalence of periodontitis in dentate people between 2011 and 2020: a systematic review and meta-analysis of epidemiological studies. *J Clin Periodontol*. 2023;50(5):604-626. doi:10.1111/jcpe.13769
2. Wang Y, Zhuo L, Yang S, Dong C, Shu P. Burden of periodontal diseases in young adults. *Sci Rep*. 2025;15(1):6653. doi:10.1038/s41598-025-88249-0
3. Skrypnyk M, Petrushanko T, Neporada K, Petrushanko V, Skrypnyk I. Changes in the periodontium against the background of systemic vascular reactions in young individuals with obesity. *Dent Med Probl*. 2024;61(2):197-207. doi:10.17219/dmp/157294
4. Schaefer AS, Nibali L, Zoheir N, Moutsopoulos NM, Loos BG. Genetic risk variants implicate impaired maintenance and repair of periodontal tissues as causal for periodontitis—a synthesis of recent findings. *Periodontol*. 2025:1-18. doi:10.1111/prd.12622
5. Oz HS, Puleo DA. Animal models for periodontal disease. *J Biomed Biotechnol*. 2011;2011:1-8. doi:10.1155/2011/754857
6. Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: a review. *Open Dent J*. 2010;4(1):37-47. doi:10.2174/1874210601004010037
7. Messer JG, La S, Kipp DE, et al. Diet-induced generalized periodontitis in Lewis rats. *Comp Med*. 2019;69(5):384-400. doi:10.30802/AALAS-CM-18-000113
8. Rojas C, García MP, Polanco AF, et al. Humanized mouse models for the study of periodontitis: an opportunity to elucidate unresolved aspects of its immunopathogenesis and analyze new immunotherapeutic strategies. *Front Immunol*. 2021;12:12. doi:10.3389/fimmu.2021.663328
9. Graves DT, Fine D, Teng YA, Van Dyke TE, Hajishengallis G. The use of rodent models to investigate host–bacteria interactions related to periodontal diseases. *J Clin Periodontol*. 2008;35(2):89-105. doi:10.1111/j.1600-051X.2007.01172.x
10. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol Oral Microbiol*. 2012;27(6):409-419. doi:10.1111/j.2041-1014.2012.00663.x
11. Liu R, Bal HS, Desta T, et al. Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *J Dent Res*. 2006;85(6):510-514. doi:10.1177/154405910608500606
12. Zhao YJ, Li Q, Cheng BX, Zhang M, Chen YJ. Psychological stress delays periodontitis healing in rats: the involvement of basic fibroblast growth factor. *Mediat Inflamm*. 2012;2012:1-13. doi:10.1155/2012/732902
13. Lin P, Niimi H, Ohsugi Y, et al. Application of ligature-induced periodontitis in mice to explore the molecular mechanism of periodontal disease. *Int J Mol Sci*. 2021;22(16):8900. doi:10.3390/ijms22168900
14. de Molon RS, Mascarenhas VI, de Avila ED, et al. Long-term evaluation of oral gavage with periodontopathogens or ligature induction of experimental periodontal disease in mice. *Clin Oral Investig*. 2016;20(6):1203-1216. doi:10.1007/s00784-015-1607-0
15. Wiebe CB, Adkins CA, Putnins EE, Hakkinen L, Larjava HS. Naturally occurring periodontal bone loss in the wild deer mouse, genus *Peromyscus*. *J Periodontol*. 2001;72(5):620-625. doi:10.1902/jop.2001.72.5.620
16. Glowacki AJ, Yoshizawa S, Jhunjhunwala S, et al. Prevention of inflammation-mediated bone loss in murine and canine periodontal disease via recruitment of regulatory lymphocytes. *Proc Natl Acad Sci*. 2013;110(46):18525-18530. doi:10.1073/pnas.1302829110
17. Georgiev GZ. Sample Size Calculator. https://www.gigacalculator.com/calculators/power-sample-size-calculator.php [Accessed Date: 21 Feb, 2025]
18. Bueno CRE, Sumida DH, Duarte MAH, et al. Accuracy of radiographic pixel linear analysis in detecting bone loss in periodontal disease: study in diabetic rats. *Saudi Dent J*. 2021;33(8):987-996. doi:10.1016/j.sdentj.2021.07.004
19. Sotoudeh N, Namavar MR. Optimisation of ketamine-xylazine anaesthetic dose and its association with changes in the dendritic spine of CA1 hippocampus in the young and old male and female Wistar rats. *Vet Med Sci*. 2022;8(6):2545-2552. doi:10.1002/vms3.936
20. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):e3000410. doi:10.1371/journal.pbio.3000410
21. Gremse F, Stärk M, Ehling J, Menzel JR, Lammers T, Kiessling F. Imalytics preclinical: interactive analysis of biomedical volume data. *Theranostics*. 2016;6(3):328-341. doi:10.7150/thno.13624
22. Lee C, Lee JH, Han SS, et al. Site-specific and time-course changes of postmenopausal osteoporosis in rat mandible: comparative study with femur. *Sci Rep*. 2019;9(1):14155. doi:10.1038/s41598-019-50554-w

23. Abe T, Hajishengallis G. Optimization of the ligature-induced periodontitis model in mice. *J Immunol Methods*. 2013;394(1–2):49-54. doi:10.1016/j.jim.2013.05.002
24. Wichienrat W, Surisaeng T, Sa-Ard-Iam N, Chanamuangkon T, Mahanonda R, Wisitrasameewong W. Alveolar bone loss in a ligature-induced periodontitis model in rat using different ligature sizes. *Eur J Dent*. 2024;18(3):933-941. doi:10.1055/s-0044-1779426
25. de Molon RS, Park CH, Jin Q, Sugai J, Cirelli JA. Characterization of ligature-induced experimental periodontitis. *Microsc Res Tech*. 2018;81(12):1412-1421. doi:10.1002/jemt.23101
26. Munar-Bestard M, Villa O, Ferrà-Cañellas M d M, Ramis JM, Monjo M. Induction of periodontitis via a combination of ligature and lipopolysaccharide injection in a rat model. *J Vis Exp*. 2023;(192):e64842. doi:10.3791/64842
27. Kwon T, Lamster IB, Levin L. Current concepts in the Management of Periodontitis. *Int Dent J*. 2021;71(6):462-476. doi:10.1111/idj.12630
28. Yoon H, Jung BH, Yoo KY, et al. Temporal changes of periodontal tissue pathology in a periodontitis animal model. *J Periodontal Implant Sci*. 2023;53(4):248. doi:10.5051/jpis.2203420171
29. Chatzaki N, Stavropoulos A, Denes B, Cancela J, Kiliaridis S, Giannopoulou C. Induced periodontitis in rats with three ligature types: an exploratory study. *Clin Exp Dent Res*. 2024;10(4):e946. doi:10.1002/cre2.946
30. Li D, Feng Y, Tang H, et al. A simplified and effective method for generation of experimental murine periodontitis model. *Front Bioeng Biotechnol*. 2020;8:444. doi:10.3389/fbioe.2020.00444
31. Zhang W, Ju J, Rigney T, Tribble G. Porphyromonas gingivalis infection increases osteoclastic bone resorption and osteoblastic bone formation in a periodontitis mouse model. *BMC Oral Health*. 2014;14(1):89. doi:10.1186/1472-6831-14-89
32. Saadi-Thiers K, Huck O, Simonis P, et al. Periodontal and systemic responses in various mice models of experimental periodontitis: respective roles of inflammation duration and *Porphyromonas gingivalis* infection. *J Periodontol*. 2013;84(3):396-406. doi:10.1902/jop.2012.110540
33. Ai R, Li D, Shi L, et al. Periodontitis induced by orthodontic wire ligature drives oral microflora dysbiosis and aggravates alveolar bone loss in an improved murine model. *Front Microbiol*. 2022;13:875091. doi:10.3389/fmicb.2022.875091
34. How KY, Song KP, Chan KG. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. *Front Microbiol*. 2016;7:53. doi:10.3389/fmicb.2016.00053
35. Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe*. 2011;10(5):497-506. doi:10.1016/j.chom.2011.10.006
36. Whitmore SE, Lamont RJ. The pathogenic persona of community-associated oral streptococci. *Mol Microbiol*. 2011;81(2):305-314. doi:10.1111/j.1365-2958.2011.07707.x
37. Daep CA, Novak EA, Lamont RJ, Demuth DR. Structural dissection and *in vivo* effectiveness of a peptide inhibitor of *Porphyromonas gingivalis* adherence to *Streptococcus gordonii*. *Infect Immun*. 2011;79(1):67-74. doi:10.1128/IAI.00361-10
38. Zhang S, Zhao Y, Lalsiamthara J, et al. Current research progress on *Prevotella intermedia* and associated diseases. *Crit Rev Microbiol*. 2024;51(4):1-562. doi:10.1080/1040841X.2024.2390594
39. Byrne DP, Potempa J, Olczak T, Smalley JW. Evidence of mutualism between two periodontal pathogens: co-operative haem acquisition by the HmuY haemophore of Porphyromonas gingivalis and the cysteine protease interpain A (InpA) of *Prevotella intermedia*. *Mol Oral Microbiol*. 2013;28(3):219-229. doi:10.1111/omi.12018
40. Lesaffre E, Philstrom B, Needleman I, Worthington H. The design and analysis of split-mouth studies: what statisticians and clinicians should know. *Stat Med*. 2009;28(28):3470-3482. doi:10.1002/sim.3634
41. Yousefzadeh N, Kashfi K, Jeddi S, Ghasemi A. Ovariectomized rat model of osteoporosis: a practical guide. *EXCLI J*. 2020;19:89-107. doi:10.17179/excli2019-1990
42. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil Res Pract*. 2020;6(1):5. doi:10.1186/s40738-020-00074-3

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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