

S2. Pre-experiment of periodontitis model construction in rats

Animals

Twelve rats were placed in an airtight container and injected with 4% (v/v) isoflurane until completely anesthetized, then a 0.20 mm diameter orthodontic wire ring was lapped at the neck of the upper first molar. Check the ligature daily to ensure it is firmly in place and apply *Porphyromonas gingivalis* suspension to the ligature wire daily. Probing depth (PD)、bleeding on probing (BOP) and gingival index (GI) were measured respectively before ligation, at the end of 1 week and 3 week. The rats were euthanized at the end of 1 week and 3 week, and the maxilla was collected for Micro-CT scanning.

Periodontal Examination

A 0.245N (25g) force was applied to probe the buccal, lingual, mesial and distal periodontal sites of the first molar of rats, and probing depth (PD, the distance from the gingival margin to the bottom of the gingival groove) was measured. The percentage of each periodontal BOP positive site in the total inspected site was recorded. Gingival index (GI) was used to measure the extent of inflammation and was scored as follows: 0, normal gingiva; 1, mild inflammation (slight change in hue and edema, no bleeding on probing); 2, moderate inflammation (redness, edema, and bleeding on probing); and 3, severe inflammation (noticeable redness, edema,

ulceration, and tendency of spontaneous bleeding).

Micro-CT

Same as the test method in the text

Statistical analysis

GraphPad Prism 9.5 software (GraphPad Software, Inc., La Jolla, CA, USA) was applied for statistical analysis. All data were found to be normally distributed and were shown as mean $\pm$ standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) and post hoc test (Tukey test) for differences among multiple groups. $p < 0.05$ was considered statistically significant. * $P < 0.05$, reference Ctrl.

Results

Results of the periodontal examination

After 1 week of ligation, the percentage of PD、GI and BOP positive sites in rats increased compared with the control group, but only BOP and GI showed statistical difference. After 3 weeks of ligation, there were significant differences in the percentage of PD、GI and BOP positive sites compared with the control group (T2).

Table 2. PD、BOP and GI at baseline、1week and 3weeks (n=4)

Group	PD/mm, $\bar{x} \pm s$				BOP	GI
	Medial	Distal	Palatal	Buccal	+/%	

Ctrl	0.62±0.27	0.49±0.18	0.85±0.33	0.51±0.26	0	0.66±0.19
L(1w)	0.86±0.35	1.07±0.23	1.66±0.45	1.02±0.39	39.5	1.32±0.15
P	0.5747	0.0618	0.0844	0.2204	0.042	0.0135
L(3w)	2.13±0.31	2.08±0.35	2.67±0.42	2.17±0.40	84.7	2.17±0.26
P	0.0019	0.0006	0.0027	0.0023	<0.0001	0.0002

PD, probing depth; GI, gingival index; BOP+, the percentage of each periodontal BOP positive site in the total inspected site; L, ligature. Data are presented as mean ± SEM (n = 4) and p < 0.05 indicate significant differences, reference to Ctrl.

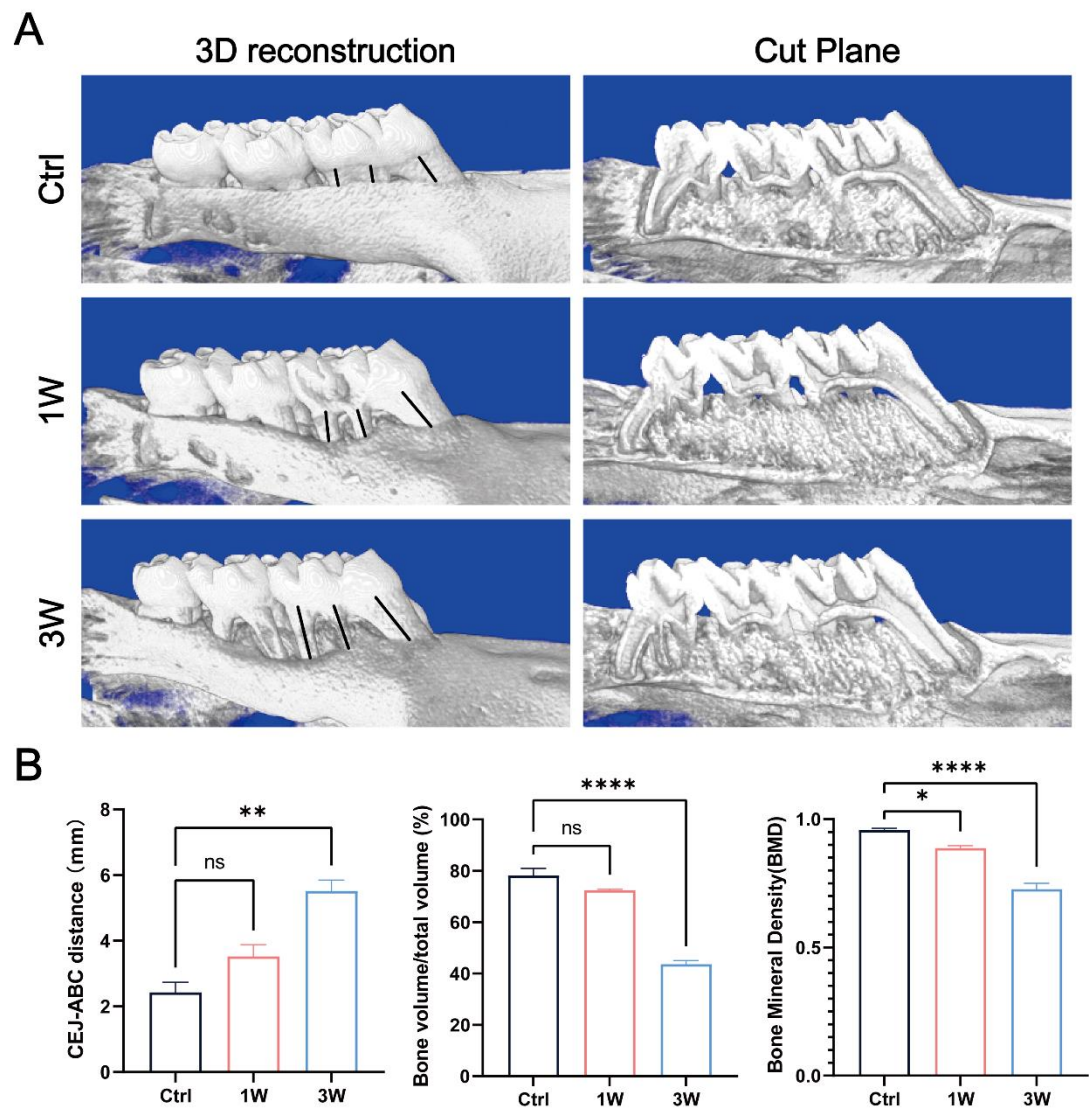
Micro-CT imaging and bone parameter analysis

Both CEJ-ABC distance and BV/TV (%) in the 1W group tended to decrease compared with the Ctrl group, but the difference was not statistically significant, whereas a significant decreasing trend could be observed at 3 weeks (Fig S1.B). And there was a highly significant difference between the decreasing trend of BMD in the 3W group.

Conclusion

Taken together with the above analysis of periodontal examination and CT results, we believe that 3-week is an appropriate choice as the node for stable modeling of periodontitis in the ligation method of this experiment.

Figure S1



Micro-CT analysis of rats modeled with periodontitis at 1 and 3 weeks. (A) Micro-CT scan imaging. (B) The linear distance (mm) from CEJ-ABC of the first molar in rats, the percentage of bone volume /total volume of the standardised region of interest (ROI) and bone mineral density (BMD) of the standardised region of interest (ROI).