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Original Article

Progesterone affects periodontitis in perimenopausal women and in an experimental rat model

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KEYWORDS

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Abstract *Background/purpose:* Progesterone (PG) is sex steroid hormone that commonly used to control menopausal symptoms, but its exact role in periodontitis remains unclear. This study aimed to investigate the effects of PG on periodontitis in perimenopausal women and in an experimental rat model.

Materials and methods: Total 412 perimenopausal women with periodontitis and a history of PG deficiency-induced uterine dysfunctional bleeding were enrolled, among which 209 women had been treated with PG. The alveolar bone height (ABH) and bone mineral density (BMD) were measured by cone beam computed tomography in the full-mouth. Additionally, a ligation-induced rat model of periodontitis was established. After treated with PG, the alveolar bone was evaluated by micro-computed tomography, and the expression of osteogenic and inflammatory markers was evaluated by immunohistochemistry. The levels of inflammatory markers were further measured by enzyme linked immunosorbent assay.

Results: In perimenopausal women with periodontitis, significantly lower maximum mesial ABH and higher minimum lingual/palatal BMD were revealed in the PG group than in the control group. Compared with the control group, the mean values of BMD around all teeth were

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significantly higher in the PG group. In a rat model of periodontitis, the intervention of PG significantly improved the quality of alveolar bone, up-regulated osteogenic markers, and down-regulated inflammatory markers.

Conclusion: PG is associated with the remission of alveolar bone loss in perimenopausal women with periodontitis. PG may contribute to the remission of periodontitis through inhibiting alveolar bone loss and inflammation.

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Introduction

Perimenopause is a transition period from a reproductive stage to menopause in women, indicating the beginning of reproductive senescence.^{1,2} Women at perimenopausal period are accompanied by a variety of physiological, psychological, and affective changes.³ Clinically, perimenopause-related hormonal fluctuation contributes to the onset of a variety of symptoms, such as menstrual irregularities, hot flushes, sleep disturbance, mood issues, vasomotor symptoms, musculoskeletal disorders, and urogenital tract atrophy.^{3,4} Of note, perimenopause is also associated with the progression of periodontitis. As a common inflammatory disease in the mouth, periodontitis is characterized by alveolar bone resorption and destruction of supporting periodontal tissues.⁵ Evidence has determined that the hormones changes in women are involved in the regulation of bone resorption, bone mineral density (BMD), and inflammatory response.^{6–9} Therefore, how to manage periodontitis at perimenopausal period is of great significant.

Progesterone (PG) is an important steroid hormone in females, which can physiologically antagonize the effects of estrogen under normal menstrual cycle.^{10,11} When the lack of corpus luteum limits PG production, estrogen alone may stimulate the proliferation of endometrium, leading to endometrial hyperplasia and subsequent abnormal uterine bleeding (AUB). Evidence has determined that the supplement of PG is effective in controlling AUB, depressive symptoms, and vasomotor symptoms.^{12–16} Interestingly, PG connects the ovaries and skeleton, and is related to the proliferation and differentiation of human osteoblasts.^{17,18} PG, being considered as a bone-trophic hormone is positively associated with bone formation,¹⁷ and contributes to the prevention and treatment of osteoporosis in women.¹⁹ As an anti-inflammatory drug and immunomodulator,²⁰ PG also exerts a crucial regulatory role in several inflammatory processes, such as chronic airway inflammation,²¹ brain inflammation,²² placentalitis,²³ temporomandibular joint inflammation,²⁴ hepatic inflammation,²⁵ etc. These osteogenic and anti-inflammatory effects of PG prefigure its potential in alleviating periodontitis. However, the knowledge on the effects of long-term use of PG in periodontitis remains limited.

In this study, alveolar bone loss including alveolar bone height (ABH) and BMD were evaluated in perimenopausal women with periodontitis who had been or not been treated with PG. Subsequently, a rat model of periodontitis

was established to further explore the effects of PG on alveolar bone loss and inflammation. This study aimed to reveal whether PG has the potential to alleviate periodontitis.

Materials and methods

Subjects

Total 203 women (42–47 years old) with periodontitis, who had a medication history of PG for more than 36 months within 5 years were screened from our Hospital between January 2022 and January 2024. For these patients, PG was used to treat PG deficiency-induced UDB. In details, didroxyprogesterone tablet (10 mg) was orally taken beginning from the 15th day of menstruation for twice a day and 10 days/month. Correspondingly, 209 women (42–47 years old) with periodontitis but without PG medication within 5 years during the same period were enrolled as the controls. The inclusion criteria include: 1) meet the diagnostic criteria of periodontitis in accordance with the Classification of Periodontal and Peri-Implant Diseases and Conditions described in 2017;²⁶ 2) with a history of PG deficiency-induced UDB; 3) estrogen >20 pg/ml. All subjects met the following criteria were excluded from this study: 1) history of periodontal therapy within 1 year; 2) medication history of antibiotics and/or immunosuppressants within 6 months; 3) history of diabetes, hypertension, osteoporosis, rheumatoid arthritis, bone metabolic diseases, and/or malignant tumors; 4) teeth number <20. The baseline characteristics of participants were shown in Table 1, and no significantly differences were revealed between the PG and control groups. This study was approved by the local Ethics Committee in accordance with the Declaration of Helsinki, and written informed consents were obtained from all cases (Q/ZXY-YWB-LL201819).

Cone beam computed tomography

ABH and BMD were measured on the full mouth (without wisdom teeth) by cone beam computed tomography (CBCT). The center point was selected on the horizontal plane: the center point of the maximum diameter of the proximal and distal dental crown in the coronal plane (the axis is parallel to the long axis of the tooth); the center point of the maximum diameter of the buccal/lingual and palatal sides of dental crown in the sagittal plane (the axis

Table 1 The baseline characteristics of participants.

Variables		Periodontitis		P-value
		Control (N = 203)	PG (N = 209)	
Age (years)		44.73 ± 1.64	44.98 ± 1.60	0.118
BMI (kg/m ²)		22.75 ± 2.58	23.07 ± 2.74	0.223
Gravida (N)	1	84	90	0.636
	2–3	91	85	
	≥4	28	34	
Parity (N)	0	6	4	0.570
	1	69	78	
	≥2	106	100	
Drinking	Yes	13	10	0.474
	No	190	199	
Smoking	Yes	9	12	0.546
	No	194	197	
Stage	I	59 (29.06%)	58 (27.75%)	
	II	75 (36.95%)	90 (43.06%)	
	III/IV	69 (33.99%)	61 (29.19%)	
Tooth brushing	<2 times/day	96	90	0.389
	≥2 times/day	107	119	
Use of dental floss	Everyday	38	41	0.639
	Nearly everyday	72	65	
	Occasionally	93	103	

PG, progesterone; BMI, body mass index.

is parallel to the long axis of the tooth). ABH represented the distance between the cemento-enamel junction and the point adjacent to the root surface at the base of the defect (parallel to the longitudinal axis of tooth) at the mesial and distal sides. BMD was measured at 3 mm from alveolar ridge to the root orientation (parallel to the longitudinal axis of tooth) at buccal and lingual/palatal sides (Fig. S1). Sites with unclear cemento-enamel junction were excluded. CBCT was performed by the same radiologist who was blinded to the treatment (PG) using CBCT instrument (3D eXam, KaVo, Biberach, Germany) and OnDemand 3D dental software (KaVo). The setting parameters included: 16 × 13 cm field of view, 120 kV tube voltage, 8.9 s scan time, and 0.3 mm voxel size.

Animal modeling

Wistar rats (6 months) were purchased from the Animal Experiment Center of Yangzhou University (Yangzhou University, Yangzhou, China). All rats were fed in a stable environment at 25 °C, 60% relative humidity, and 12/12 h day/night cycle, with free access to water and food. Rats were randomly divided into three groups, including the control, model, and PG groups (N = 6 for each group). A rat model of periodontitis was established by ligating the maxillary first molar. Simply, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium, and the molar was fastened using a ligature wire (0.2 mm steel wire and 4-0 suture). Six weeks later, 50 mg/g didroxyprogesterone loaded in carboxymethyl chitosan sustained-release hydrogel (CMC-SH) was administered to the periodontal pocket once a week in the PG group.²⁷ In the model group, rats were ligated and treated with empty CMC-SH. In

the control group, rats were not ligated and treated with empty CMC-SH. After 8 weeks of medication, rats were euthanized and the maxillary samples were collected for subsequent experiments. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (20211215).

Micro-computed tomography

The alveolar bone in the target site (maxillary first molar) was evaluated in rats of different groups using micro-computed tomography (Micro-CT) (SkyScan 1176, Bruker, Luken, Germany). The scanning parameters included 18 µm scanning resolution, 65 kV scanning voltage, 385 µA scanning current, and 340 ms exposure time. CTVOX software was used for 3D reconstruction, and CTAn software was used for morphometric analysis of BMD, bone volume, cement enamel junctions to alveolar bone crest (CEJ-ABC), trabecular separation, trabecular number, and trabecular thickness.

Immunohistochemistry

The positive expression of osteogenic markers (bone sialoprotein (BSP), runt-related transcription factor 2 (Runx2), and osteocalcin (OCN)) and inflammatory markers (tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6) were measured in rats of different groups by immunohistochemistry (ICH). Simply, paraffin-embedded tissue samples were sliced at 4 µm, dewaxed in xylene, and dehydrated with graded ethanol. After microwave irradiated in sodium citrate (Beyotime, Shanghai, China) for 15 min at 95 °C, the sections were incubated in 3% H₂O₂ for

15 min and blocked in 5% goat serum for 15 min. Subsequently, the sections were incubated with specific primary antibody (anti-BSP, -Runx2, -OCN, -TNF- α , -IL-1 β , and -IL-6; Affinity Biosciences, Jiangsu, China) for 12 h at 4 °C, washed with phosphate buffer saline, and further incubated with horseradish peroxidase-conjugated secondary antibody (Abcam, Cambridge, England) for 30 min at 37 °C. Finally, the sections were visualized using Diaminobenzidine kit (Beyotime), counter-stained with Hematoxylin (Solarbio, Beijing, China), and captured under microscope (BX53, Olympus, Tokyo, Japan).

Enzyme linked immunosorbent assay

The concentrations of inflammatory markers (TNF- α , IL-1 β , and IL-6) in tissue supernatants in rats of different groups were detected using commercial enzyme linked immunosorbent assay (ELISA) kits (TNF- α : Mlbio, Shanghai, China; IL-1 β and IL-6: Elabscience, Wuhan, China) according to the manufacturer's instructions. The optical density at 450 nm was measured by a microplate reader (DR-3518G, Hiwell Diatek, Wuxi, China) and used for calculation according to the standard curves.

Statistical analysis

Statistical analyses were performed using statistical product and service solutions (SPSS) software (version 21.0; SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean $\pm$ standard deviation. The differences between two groups were analyzed by independent samples *t*-test, and the differences among multiple-groups were analyzed by one-way analysis of variance followed by least significant difference post hoc test. A *P* value less than 0.05 was considered as statistical significant.

Results

The differences of ABH and BMD in women with periodontitis who had or had not received PG

To reveal the responses of alveolar bone to PG in perimenopausal women with periodontitis, ABH and BMD were measured in the full-mouth by CBCT. As shown in Table 2, some teeth had significantly lower mesial ABH in the PG group than in the control group, including teeth 15/25, 16/26, 17/27, 35/45, 36/46, and 37/47 ($P < 0.05$). Compared with that in the control group, significantly lower distal ABH was observed in teeth 14/24, 15/25, 31/41, 32/42, and 36/46 in the PG group ($P < 0.05$). The maximum mesial ABH was significantly lower in the PG group than in the control group ($P < 0.05$).

Additionally, the buccal BMD (3 mm) was significantly higher in many teeth (11/12, 12/22, 16/26, 17/27, 34/44, and 36/46) in the PG group than in the control group ($P < 0.05$). The lingual/palatal BMD (3 mm) in teeth 11/21, 13/23, 16/26, 17/27, 34/44, 35/45, and 36/46 was also significantly higher in the PG group than in the control group ($P < 0.05$). In the PG group, the mean values of BMD at buccal and lingual/palatal sides (all teeth) were both significantly higher than those in the control group ($P < 0.01$). Only minimum lingual/palatal BMD was revealed to be significantly higher in the PG group than in the control group ($P < 0.001$) (Table 3). Of note, teeth 36/46 simultaneously have lower ABH at mesial and distal sides, and higher BMD at buccal and lingual/palatal sides in the PG group ($P < 0.05$).

PG inhibits alveolar bone loss in a rat model of periodontitis

A rat model of periodontitis was established to further explore the action mechanisms of PG on alveolar bone.

Table 2 The ABH in the control and PG groups.

Tooth position	Mesial ABH			Distal ABH		
	Control (N = 203)	PG (N = 209)	<i>P</i> -value	Control (N = 203)	PG (N = 209)	<i>P</i> -value
11/12	2.93 $\pm$ 0.76	2.90 $\pm$ 0.78	0.686	2.89 $\pm$ 0.92	2.87 $\pm$ 0.80	0.741
12/22	2.99 $\pm$ 0.85	2.94 $\pm$ 0.81	0.551	2.98 $\pm$ 0.89	2.97 $\pm$ 0.81	0.871
13/23	3.05 $\pm$ 0.90	3.02 $\pm$ 0.80	0.652	3.10 $\pm$ 1.16	3.02 $\pm$ 0.84	0.315
14/24	3.07 $\pm$ 1.04	3.01 $\pm$ 0.79	0.463	3.15 $\pm$ 1.25	2.95 $\pm$ 0.87	0.032*
15/25	3.18 $\pm$ 1.17	2.94 $\pm$ 0.85	0.006*	3.28 $\pm$ 1.15	3.06 $\pm$ 1.03	0.013*
16/26	3.41 $\pm$ 1.26	3.09 $\pm$ 0.87	<0.001*	3.38 $\pm$ 1.19	3.27 $\pm$ 1.33	0.291
17/17	3.52 $\pm$ 1.55	3.20 $\pm$ 1.11	0.005*	3.51 $\pm$ 1.23	3.36 $\pm$ 1.22	0.128
31/41	3.43 $\pm$ 1.44	3.27 $\pm$ 0.98	0.086	3.36 $\pm$ 1.52	3.06 $\pm$ 0.95	0.003*
32/42	3.50 $\pm$ 1.34	3.36 $\pm$ 1.06	0.129	3.24 $\pm$ 1.26	3.05 $\pm$ 1.04	0.029*
33/43	3.33 $\pm$ 1.29	3.16 $\pm$ 0.89	0.051	3.09 $\pm$ 1.03	3.01 $\pm$ 0.89	0.250
34/44	3.16 $\pm$ 1.21	3.03 $\pm$ 0.95	0.127	3.19 $\pm$ 1.18	3.02 $\pm$ 1.09	0.096
35/45	3.25 $\pm$ 1.37	2.92 $\pm$ 0.76	0.001*	3.03 $\pm$ 0.98	2.99 $\pm$ 1.00	0.645
36/46	3.39 $\pm$ 1.50	3.13 $\pm$ 0.92	0.013*	3.31 $\pm$ 1.04	3.13 $\pm$ 1.03	0.046*
37/47	3.65 $\pm$ 1.76	3.21 $\pm$ 1.07	0.001*	3.51 $\pm$ 1.35	3.47 $\pm$ 1.29	0.727
Mean	3.27 $\pm$ 1.14	3.10 $\pm$ 0.78	0.072	3.21 $\pm$ 1.01	3.09 $\pm$ 0.86	0.205
Maximum	4.48 $\pm$ 1.68	4.16 $\pm$ 1.30	0.032*	4.59 $\pm$ 1.59	4.36 $\pm$ 1.47	0.126

ABH, alveolar bone height; PG, progesterone.

* $P < 0.05$ vs. Control.

Table 3 The BMD at 3 mm in the control and PG groups.

Tooth position	Buccal BMD (3 mm)			Lingual/palatal BMD (3 mm)		
	Control (N = 203)	PG (N = 209)	P-value	Control (N = 203)	PG (N = 209)	P-value
11/12	833.52 ± 185.25	870.02 ± 176.68	0.014*	775.20 ± 137.79	819.27 ± 167.56	0.001*
12/22	821.30 ± 175.68	848.97 ± 162.43	0.042*	819.12 ± 168.87	844.32 ± 158.08	0.057
13/23	788.44 ± 176.58	793.44 ± 130.40	0.715	769.82 ± 146.85	816.01 ± 141.02	<0.001*
14/24	835.58 ± 151.27	840.14 ± 134.32	0.684	826.48 ± 144.90	839.19 ± 120.04	0.236
15/25	840.72 ± 210.35	863.96 ± 143.23	0.087	829.01 ± 131.12	847.89 ± 145.92	0.094
16/26	696.04 ± 114.54	732.08 ± 120.28	<0.001*	686.15 ± 128.04	730.23 ± 164.69	0.003*
17/17	693.92 ± 126.11	726.30 ± 163.49	0.035*	679.17 ± 111.96	711.71 ± 115.84	0.001*
31/41	904.26 ± 123.26	911.68 ± 152.18	0.508	872.10 ± 120.68	877.14 ± 151.14	0.674
32/42	907.49 ± 119.99	909.23 ± 131.11	0.860	844.07 ± 163.39	848.41 ± 156.54	0.741
33/43	897.87 ± 155.49	900.21 ± 171.06	0.883	853.01 ± 142.80	860.24 ± 193.61	0.623
34/44	854.29 ± 152.78	908.53 ± 155.98	<0.001*	836.64 ± 124.77	894.06 ± 158.02	<0.001*
35/45	719.53 ± 105.56	728.53 ± 103.62	0.259	758.68 ± 158.70	783.53 ± 133.78	0.031*
36/46	805.72 ± 138.42	876.84 ± 144.27	<0.001*	810.12 ± 126.92	835.83 ± 117.94	0.004*
37/47	700.79 ± 131.01	710.49 ± 124.00	0.389	741.36 ± 137.28	753.29 ± 167.06	0.323
Mean	807.55 ± 72.31	835.64 ± 67.22	<0.001*	795.76 ± 61.05	818.54 ± 74.01	0.001*
Minimum	593.51 ± 32.54	594.85 ± 57.89	0.773	612.81 ± 38.85	592.43 ± 72.69	<0.001*

BMD, bone mineral density; PG, progesterone.

* $P < 0.05$ vs. Control.

Micro-CT showed that the CEJ-ABC and trabecular separation were both significantly higher in the model group than in the control group ($P < 0.001$). The intervention of PG significantly decreased the CEJ-ABC and trabecular separation in the model rats ($P < 0.05$). Additionally, significantly lower BMD, bone volume, trabecular thickness, and trabecular number were revealed in the model group compared with those in the control group ($P < 0.001$). The changes of the above parameters in the model rats were partially reversed by the intervention of PG ($P < 0.05$) (Fig. 1).

PG promotes osteogenesis in a rat model of periodontitis

The protein expression of osteogenic markers, including BSP, Runx2, and OCN were subsequently detected in rat models by ICH. As shown in Fig. 2, the positive expression of BSP, Runx2, and OCN were weaker in the model group than in the control group ($P < 0.001$). The intervention of PG partially reversed the down-regulation of BSP, Runx2, and OCN in the model rats, evidenced by more positive cells in the PG group than in the model group ($P < 0.001$).

PG inhibits inflammation in a rat model of periodontitis

To further explore the effects of PG on periodontal inflammation, inflammatory markers, including TNF- α , IL-1 β , and IL-6 were detected. ICH showed the expression of TNF- α , IL-1 β , and IL-6 was stronger in the model group than in the control group ($P < 0.001$). The up-regulation of TNF- α , IL-1 β , and IL-6 were all weakened by the intervention of PG in the model rats ($P < 0.001$) (Fig. 3A). Consistently, ELISA showed that the intervention of PG also significantly

weakened the elevation of TNF- α , IL-1 β , and IL-6 levels in the model rats ($P < 0.001$) (Fig. 3B).

Discussion

Periodontitis is a common oral disease with a high prevalence in the world, and is a leading cause of tooth loss that seriously affects the quality of life.²⁸ The dynamic interaction between host inflammatory/immune responses and pathogens is the main pathogenesis of periodontitis.²⁹ Due to their influence on periodontal tissue and alveolar bone, sex hormones are also involved in the progression of periodontitis. PG is a sex steroid hormone in females that commonly administered in controlling perimenopausal symptoms in women, such as AUB, sleep disturbance, vasomotor symptoms, and depressive symptoms.^{12–16,30} Since the effects of PG on periodontitis are rarely reported yet, this study is designed to reveal this issue. As a result, PG is associated with the remission of alveolar bone loss in perimenopausal women with periodontitis. Additionally, PG inhibited both alveolar bone loss and inflammation in a rat model of periodontitis.

Alveolar bone loss is a key characteristic of periodontitis, which directly contributes to tooth loss. PG connects the ovaries and bone in women, and its negative correlation with bone loss has been reported by many clinical studies. Starrach et al. found that the reduction of ovulatory rate (low PG) in perimenopausal period is related to the loss of bone density in healthy women.¹⁸ Lydeking-Olsen et al. revealed that transdermal PG improves the loss of bone mineral content and density in lumbar spine in postmenopausal women with risk of osteoporosis.³¹ Seifert-Klauss et al. summarized that PG is negatively related to bone resorption and positively related to bone formation in perimenopausal women.¹⁷ However, the responses of alveolar bone loss to PG were rarely known. Here, in

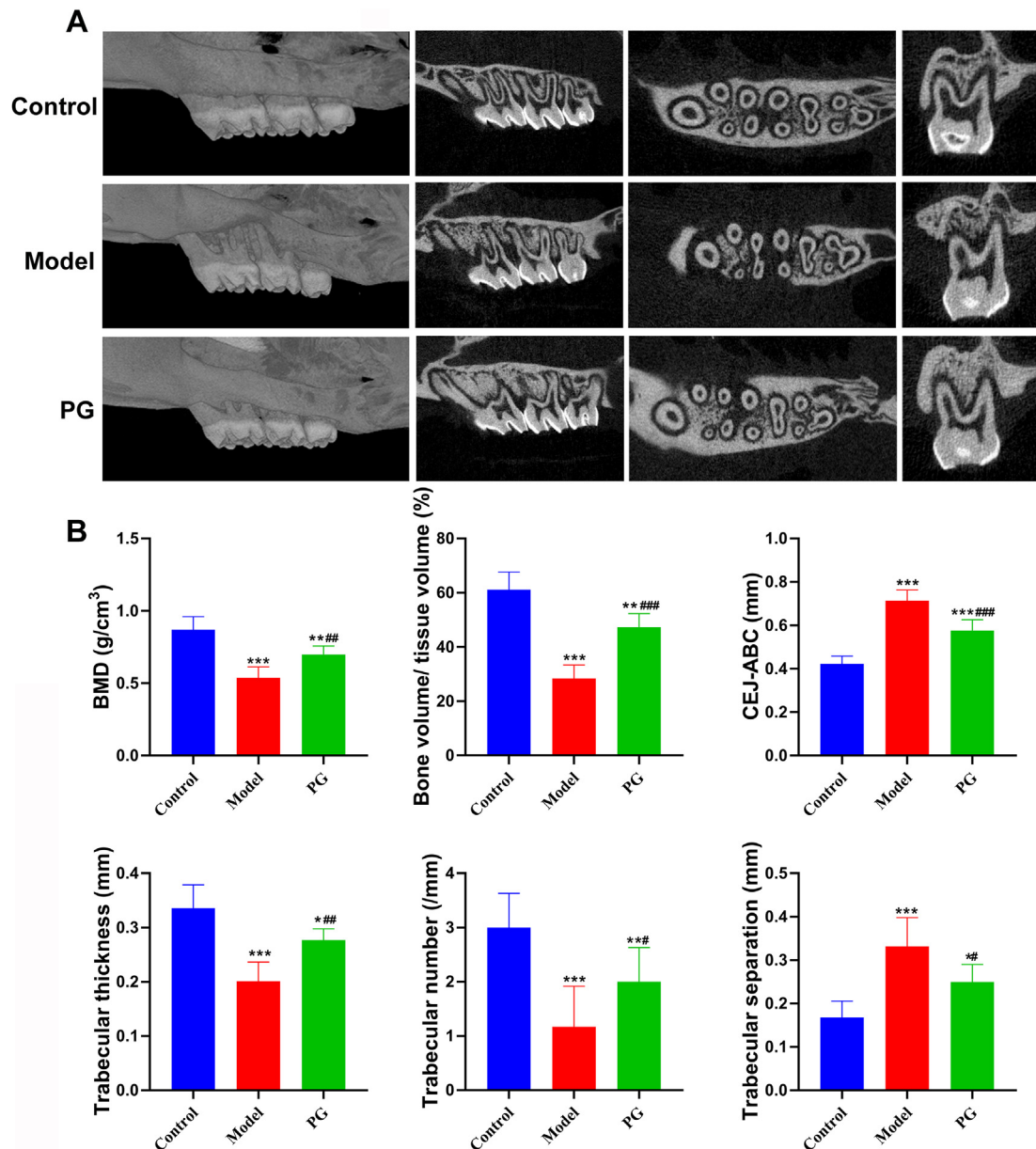


Figure 1 Micro-CT of alveolar bone in a rat model of periodontitis. (A) Micro-CT images. (B) Quantification of BMD, bone volume, CEJ-ABC, trabecular thickness, trabecular number, and trabecular separation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. Model. Micro-CT, micro-computed tomography; BMD, bone mineral density; CEJ-ABC, cement enamel junctions to alveolar bone crest; PG, progesterone.

perimenopausal women with periodontitis, CBCT showed significantly lower maximum mesial ABH, higher minimum lingual/palatal BMD (3 mm), and higher mean BMD in the PG group than in the control group. These results are similarly with previous studies and support that the improvement of alveolar bone loss in periodontitis is associated with the use of PG. Although ABH exhibited a lower trend and BMD exhibited a higher trend in all paired teeth in the PG group, the differences were not all significant in every position. Interestingly, teeth 36/46 simultaneously had significantly lower ABH and higher BMD in the PG group. However, the specific reasons for these phenomena remain unclear, which may be related to structural morphology, function,

pathological change, microenvironment, etc. Additionally, potential confounding factors also affect the assessment of PG therapy in humans, as the progression of periodontitis is associated with multiple factors. Therefore, more detailed research on the therapeutic potential of PG on periodontitis is still needed in the future.

The potential protective effects of PG on alveolar bone were further explored in a rat model of periodontitis. Similarly with CBCT results in humans, PG intervention improves alveolar bone loss in the model rats, evidenced by the elevation of BMD, bone volume, trabecular number, and trabecular thickness, as well as the reduction of CEJ-ABC and trabecular separation under Micro-CT. These results

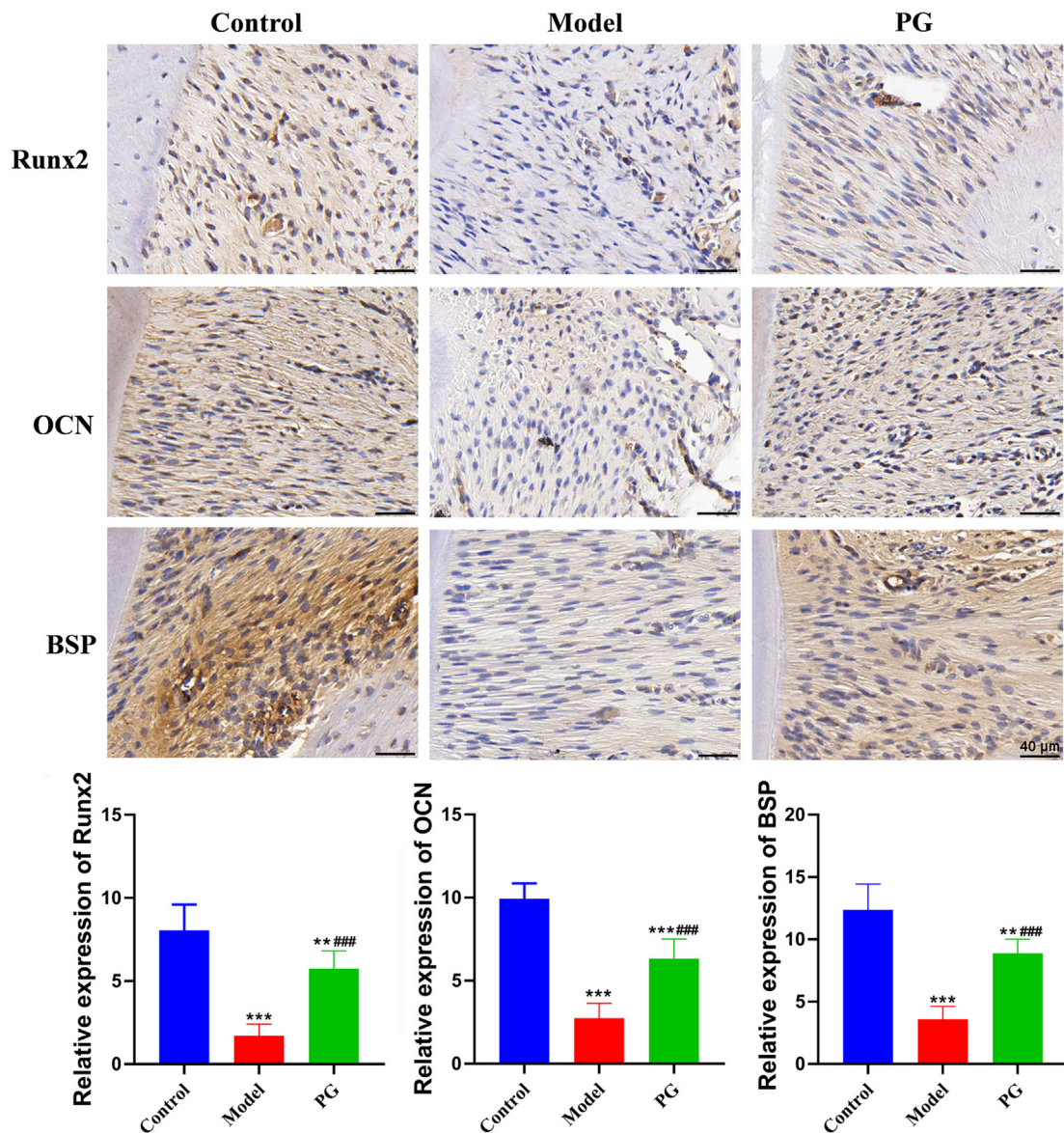


Figure 2 The positive expression of BSP, Runx2, and OCN was detected by IHC in a rat model of periodontitis ($40\times$). $**P < 0.01$, $***P < 0.001$ vs. Control; $###P < 0.001$ vs. Model. IHC, immunohistochemistry; BSP, bone sialoprotein; Runx2, runt-related transcription factor 2; OCN, osteocalcin; PG, progesterone.

illustrate an inhibitory role of PG on alveolar bone loss under periodontitis. Moreover, the down-regulation of osteogenic markers, including BSP, Runx2, and OCN in the model rats was partially reversed by the intervention of PG. Our findings support the evidence that PG is an osteo-anabolic hormone that can induce the proliferation and the differentiation of osteoblasts.¹⁸ For a mechanistic perspective, PG may enhance bone formation directly by engaging osteoblast receptor or indirectly by composing glucocorticoid osteoblast receptor.³² In a word, PG may contribute to the remission of periodontitis through inhibiting alveolar bone loss.

In addition to osteogenesis, inflammation is also an important regulatory target of PG. As a key sex hormone, PG not only functions in improving utero-placental circulation, reducing uterine contractility, and providing luteal

phase support, but also participates in the regulation of immune and inflammatory responses.³³ Until now, more and more basic studies have reported that PG is effective in inhibiting the inflammation of different organs. For example, Gutzeit et al. found that PG alleviates lipopolysaccharide-induced brain inflammation in pregnant and non-pregnant mice.²² Fei et al. revealed that PG dose-dependently reduces IL-1 β , IL-6, IL-8, and IL-17A levels, and inhibits chronic ozone-induced airway inflammation in mice.²¹ Teraoka et al. reported that PG down-regulates IL-1 β , IL-8, TNF- α , and Cyclooxygenase (COX)-2, and relieves the inflammation of fetal membrane in *Porphyromonas gingivalis*-infected mice (a model of preterm birth caused by chronic inflammation).³⁴ Similarly, PG is also able to inhibit temporomandibular joint inflammation,²⁴ placentitis,²³ sepsis-induced systemic inflammation,³⁵ cadmium-

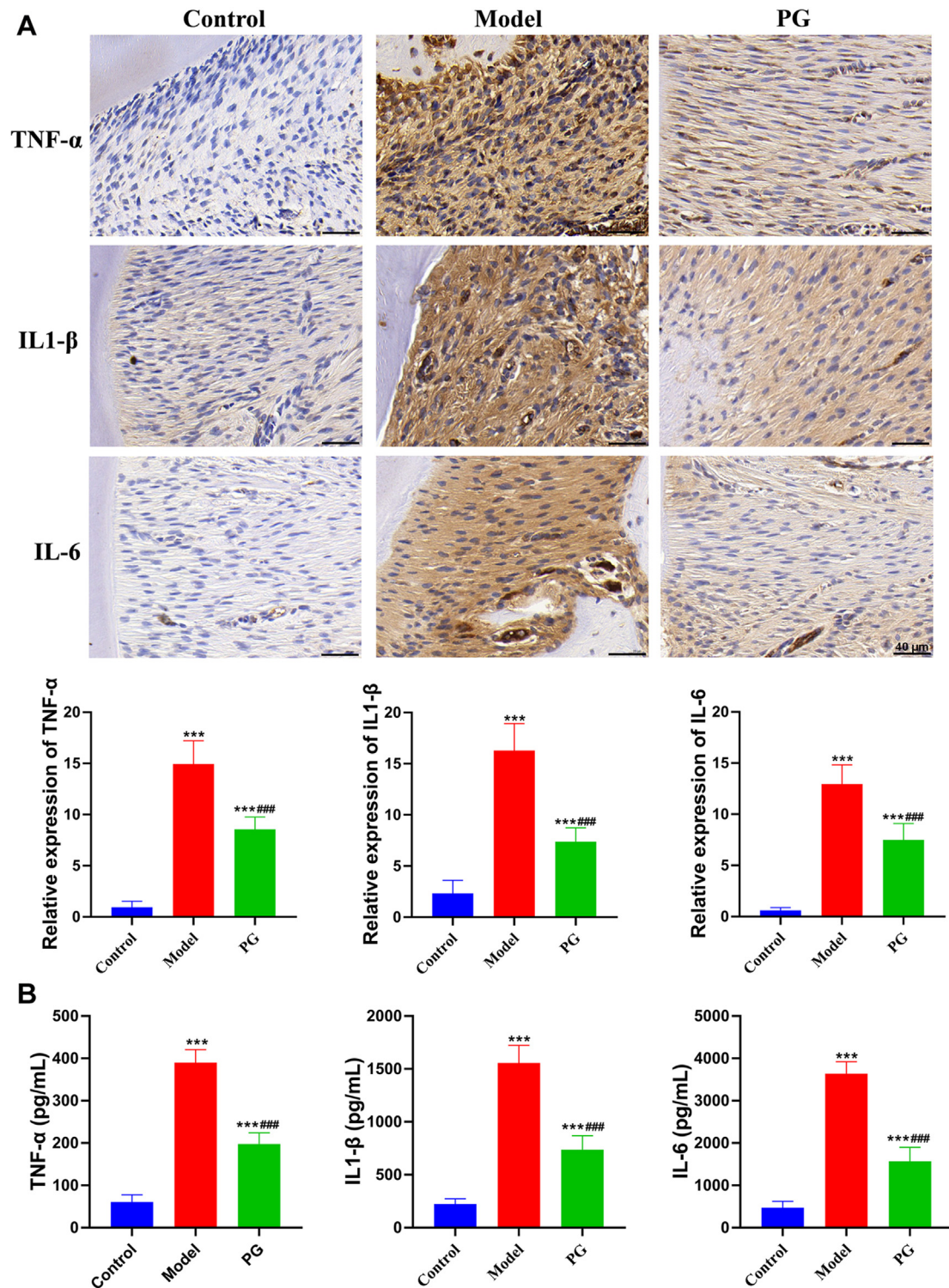


Figure 3 The expression/levels of TNF- α , IL-1 β , and IL-6 were detected by IHC (40 $\times$, A) and ELISA (B) in a rat model of periodontitis. *** $P < 0.001$ vs. Control; ### $P < 0.001$ vs. Model. IHC, immunohistochemistry; ELISA, enzyme linked immunosorbent assay; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; PG, progesterone.

induced hepatic inflammation,²⁵ post-ischemic stroke systemic inflammation,³⁶ *Neisseria gonorrhoeae* infection induced-inflammation,³⁷ etc. However, the anti-inflammatory role of PG in periodontitis is rarely reported. In this study, three inflammatory markers, including

TNF- α , IL-1 β , and IL-6 were detected in a rat model of periodontitis. ICH and ELISA both showed that the intervention of PG significantly weakened the elevation of TNF- α , IL-1 β , and IL-6 in the model rats. These results are consistent with previously studies, and illustrate that PG is

effective in inhibiting periodontal inflammation. The underlying action mechanisms of PG may be specifically related to T-cell activation, pro-/anti-inflammatory cytokines, and immune tolerance, or non-specifically related to Nuclear factor kappa-B, COX, and prostaglandin synthesis.²⁰ Since inflammation-mediated bone loss is a characteristic of periodontitis, the remission of inflammation is also beneficial for preventing bone loss. Moreover, the results of animal experiments also illustrate that CMC-SH is an effective carrier for local administration of PG. However, the effectiveness of CMC-SH-loaded PG on controlling periodontitis is only confirmed in a rat model, and further clinical research is needed in the future.

In conclusion, the intervention of PG is associated with the improvement of alveolar bone loss in perimenopausal women with periodontitis. Additionally, PG inhibits the alveolar bone loss and inflammatory response in a rat model of periodontitis. The administration of PG may relieve periodontitis through inhibiting alveolar bone loss and inflammation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jds.2024.05.020>.

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