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ORIGINAL ARTICLE

Effect of local hIL-10 gene therapy on experimental periodontitis in ovariectomized rats

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ABSTRACT

Objective: To investigate the effect of local hIL-10 gene therapy on experimental periodontitis in rats and to elucidate the mechanism underlying this effect.

Material and methods: Experimental periodontitis was induced in ovariectomized (OVX) rats using a silk ligature. We then injected 5 μg of hIL-10 plasmid with 5 μl of liposomes or 5 μg of vector plasmid with 5 μl of liposomes into the palatal side of the gingival mucosa of the upper left second molar once every two days. The rats were killed 48 hours after the seventh injection. The body weight; bone mineral density of the whole body, pelvis and spine; resorption of the alveolar bone; and number of cytokine-positive cells were measured to determine the effects of hIL-10 on the periodontal tissue.

Results: hIL-10 was expressed in periodontal tissues after local gene delivery. The expressed hIL-10 protein inhibited alveolar bone resorption and downregulated IL-1β, IL-6, TNF-α, RANKL and MMP-8 in the periodontal ligament in the root furcation region.

Conclusions: Local hIL-10 gene transfer suppressed alveolar bone resorption in OVX rats, and this effect was probably associated with the decline in the expression of pro-inflammatory cytokines in the periodontal tissues.

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Cytokine; IL-10; osteoporosis; periodontal tissues; gene transfer

Introduction

Periodontitis and osteoporosis are frequently seen in middle-aged and elderly women, especially post menopause. Periodontitis is a chronic inflammatory disorder in the periodontal supporting tissues that occurs as a result of accumulation of microorganisms in dental plaque biofilms. It is commonly characterized by inflammation of the periodontal tissue and alveolar bone resorption [1]. Osteoporosis is a systemic bone metabolic disorder characterized by histological abnormalities in the bone, enhanced bone fragility, low bone mass, and consequent increase in fracture risk [2]. Postmenopausal osteoporosis (PMO) is associated with the decline in oestrogen in postmenopausal women and mainly affects the cancellous bones. In PMO, because of the decline in ovarian function, reduction of oestrogen secretion, loss of bone mass, and decrease in bone mineral density (BMD), patients may develop back pain, deformities of the spine or limbs, or even fractures.

Upon in-depth study on the periodontal tissue and the physiology and pathology in postmenopausal women, PMO has been increasingly recognized as a systemic contributor to periodontitis [3–5]. Moreover, the loss of bone mass and osteoporosis are considered as additional contributing factors for alveolar bone resorption [6]. Payne et al. [7] studied the incidence of periodontitis in patients with systemic bone mass loss and history of osteoporosis and fractures and found that loss of bone mass and osteoporosis are associated with periodontitis. Animal studies have also shown that oestrogen deficiency in ovariectomized (OVX) rats caused decline of not only the BMD of the femur but also bone mass and BMD in the alveolar bone. A positive correlation has been shown between oestrogen levels and BMD, which indicates that alveolar bone could develop osteoporosis under the conditions of PMO [8]. In addition, OVX can also lead to progressive alveolar bone loss; upregulate interleukin (IL)-6, osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL); and downregulate interleukin (IL)-10 in periodontal ligaments in the root furcation region [9].

IL-10 is a cytokine of the interleukin family. Its main biological function is the inhibition of inflammatory responses and the regulation of the proliferation and differentiation of immune cells such as natural killer cells, B cells and T cells. IL-10 also mediates pathological and physiological processes in many diseases [10]. It halts the progression of periodontitis and is required for periodontal health and stability [11,12]. IL-10 deficiency accelerates alveolar bone resorption and reduces bone formation [13–17]. The anti-inflammatory function of IL-10 in periodontitis has also been demonstrated in knockout animal models [18]. Goutoudi et al. [19] showed that after periodontal non-surgical treatment, local inflammation in periodontal tissue was reduced and IL-10

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expression was increased, which indicates that IL-10 is involved in the periodontal repair process. As IL-10 can maintain bone mass through inhibition of osteoclastic bone resorption and regulation of osteoblastic bone formation, we hypothesized that IL-10 may have therapeutic potential in periodontitis. However, recombinant IL-10 protein has a short half-life *in vivo* and is costly, which can limit its clinical implications. To achieve therapeutic efficiency, the concentration of IL-10 has to be maintained at a certain level *in vivo* within a proper time frame.

Local gene therapy can help to maintain relatively high levels of target proteins and thus enhance the overall treatment outcome. In addition, gene therapy can also reduce systemic toxicity and side effects by reducing the exposure of the non-target cells and organs to the protein. Therefore, in the present study, we used local gene transfer to deliver the hIL-10 gene to the periodontal tissue and investigated the effect of hIL-10 on experimental periodontitis (EP) induced in OVX rats by immunohistochemistry and histopathological analysis. Our findings suggest that hIL-10 is an attractive therapeutic option for suppressing the production of proinflammatory cytokines following perio-pathogenic bacterial challenge.

Material and methods

Animals

All animal care and study protocols were approved by the Animal Care and Use Committee of Fujian Medical University. In total, 24 three-month-old female virgin Sprague–Dawley (SD) rats (220–260 g) were purchased from an Animal Resource Center (SLAC Laboratory Animal Co. Ltd., Shanghai, China) with the license number SCXK (SH) 2003-0003.

The rats were maintained in separate cages with 12 h day–night cycles at an ambient temperature of 20 ± 1 °C. The rats were fed with a standard rodent diet. After one week of acclimatization, the rats were subjected to experimentation.

Methods

Plasmids

The hIL-10 recombinant eukaryotic expression plasmid EX-A0007-M03 and its corresponding vector plasmid EX-EGFP-M03 (GeneCopoeia, Rockville, MD) were extracted and purified as stipulated in the Qiagen Endotoxin-free Giga Prep Kit manual (Hilden, Germany).

Animal grouping

The rats were weighed and anesthetized by abdominal administration of ketamine at 20 mg/kg body weight. Twelve rats underwent OVX, and the remaining 12 underwent a sham operation (SHAM) [20]. In the SHAM group, the ovaries were exposed, and equal volumes of the fatty tissue adjacent to each ovary were excised. All 24 rats were then divided into four groups: SHAM + hIL-10 (abbreviated as S + I), SHAM + VECTOR (abbreviated as S + V), OVX + hIL-10 (abbreviated as O + I) and OVX + VECTOR (abbreviated as O + V). In the S + I and S + V groups, at 12 weeks after the sham operation, silk ligatures were placed in the left upper second molars of the rats to establish EP (SHAM + hIL-10 + EP and SHAM + VECTOR + EP groups, respectively; abbreviated as S + I+E and S + V+E), whereas no ligatures were placed on the right molars, which were then used as controls (SHAM + hIL-10 + C and SHAM + VECTOR + C, abbreviated as S + I+C and S + V+C). In the preliminary experiment, four doses of hIL-10 (a complex of 1, 3, 5 or 7 μg of hIL-10 plasmid with 1, 3, 5 or 7 μl of liposomes) were injected into the palatal side of the gingival mucosa in four SD rats. The injection was performed once every two days, and seven times in total. The rats were then killed by an overdose of ether anaesthesia. The palatal gingival mucosa was dissected, fixed, decalcified, dehydrated and then embedded in paraffin blocks, and consecutive 5-μm mesial-distal sections were prepared. The sections were randomly selected for immunohistochemical staining for IL-10. The expression of hIL-10 in the fibroblasts, epithelial cells, endothelial cells and inflammatory cells of the gingival connective tissue in the 5 μg and 7 μg groups was strong, but the 1 μg and 3 μg groups did not show hIL-10 expression. Therefore, a complex of 5 μg of hIL-10 plasmid with 5 μl of liposomes for S + I rats and 5 μg of vector plasmid with 5 μl of liposomes for S + V rats was injected into the palatal side of the gingival mucosa of the upper second molars on both the left and the right sides. The injection was performed once every two days, and seven times in total.

In the O + I and O + V groups, 12 weeks after the excision of both ovaries, silk ligatures were placed on the left upper second molars of the rats to establish EP [21] (OVX + hIL-10 + EP and OVX + VECTOR + EP, abbreviated as O + I+E and O + V+E). At the same time, complexes of 5 μg of hIL-10 plasmid with 5 μl of liposomes for the O + I rats and 5 μg of vector plasmid with 5 μl of liposomes for the O + V rats were injected into the palatal side of the gingival mucosa of the upper second molars on the left side. The injection was performed once every two days, and seven times in total.

Drug administration

As shown in Figure 1, the drugs were administered as described by Kanzaki et al. In brief, the plasmid DNA/liposome complex was drawn using a 25-μl microinjector. The microinjector was inserted into the palatal mucosa at the intersection between the coronal line (3 mm from the interproximal surface of the first molar) and the sagittal line (1–2 mm from its palatal side). The microinjector was inserted 8–10 mm into the periosteal layer. When the microinjector reached the gingival mucosa of the second molar, the complex was slowly injected into the tissue (taking 1–2 min). The microinjector was pulled out 1–2 min after the injection [22,23].

Analysis

Weight measurement

The body weight of all the rats was measured before and after the experiments.

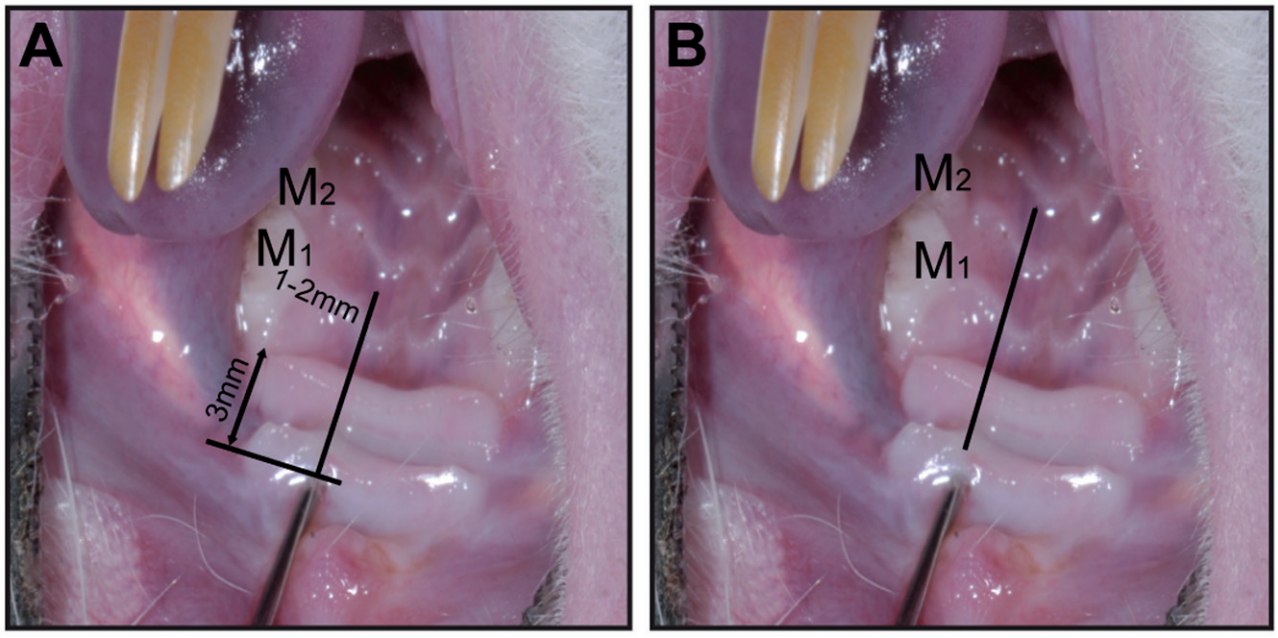

Figure 1. Local gene transfer in periodontal tissue. (A) The microinjector was inserted into the palatal mucosa at the intersection between the coronal line (3 mm from the interproximal surface of the first molar) and the sagittal line (1–2 mm away from its palatal side). (B) The microinjector was advanced approximately 8–10 mm into the periosteum layer until it reached the palatal side of the gingival mucosa of the second molar (M2).

BMD measurement

At 48 h after the seventh gene transfer, all the rats were weighed and anesthetized using ketamine. Dual-energy X-ray absorptiometry (DEXA) (GE Healthcare, Chicago, IL) was used to measure the BMD of the whole body, pelvis and spine.

Serum preparation and histological examination

The rats were killed by an overdose of ether anaesthesia. Blood samples were taken after the rats were sacrificed. The serum was separated by centrifugation for 5 min at 3000 rpm. Tissue samples from the three molars including the left maxillary were dissected and fixed for 48 h using 4% paraformaldehyde. The samples were then washed with running water overnight and decalcified for 24 h in a rapid decalcification solution (RapidCal.Immuno^{TM}). After gradient alcohol dehydration, the specimens were embedded in paraffin blocks, and consecutive 5 μm mesial-distal sections were prepared. Only the sections that included the distal root of the first molar in the left maxillary and the mesial and distal roots and furcation region of the second molar were selected. Three sections from each rat were randomly selected for haematoxylin and eosin staining. Seven other sections were randomly selected for immunohistochemical staining for IL-1β, IL-6, IL-10, TNF-α, RANKL, OPG and MMP-8. The soft tissue on the femur was quickly removed and placed in 4% paraformaldehyde for 48 h. The femur tissue samples were then washed overnight under running water and decalcified for 24 h. When the tissue offered no further resistance to the needles, the samples were trimmed and the distal femoral metaphysis was retained. After dehydration, the samples were embedded in paraffin blocks, and 5-μm-thick consecutive sections were prepared for haematoxylin and eosin staining. The gingival tissue in the injection region of the S + I+C and S + V+C rats was also dissected and placed in 4% paraformaldehyde for 48 h. These samples were dehydrated and embedded in paraffin, and five sections of gingival tissue and the mandible were used for IL-10 immunohistochemical staining.

Measurement of alveolar bone loss

Alveolar bone loss was evaluated as described by Luo et al. [9]. In brief, alveolar bone resorption was evaluated using the following indices: (A) area of the periodontal ligament in the root furcation of the upper second molar with a vertical distance of 1 mm to the top of the furcation (which represents the alveolar bone resorption in the root furcation region) and (B) distance from the cement–enamel junction (CEJ) to the alveolar bone crest (ABC). The results were analysed using Image-Pro Plus 6.0 (Image-Pro Plus, Media Cybernetics, Inc., Rockville, MD).

Cytokine-positive cell counting

Digital photos of the root furcation region of the second molar were taken using the photomicrography system. As shown in Figure 2, three fields (50 μm × 50 μm) were randomly selected from the root furcation region of the upper second molar. The cells that were positive for IL-1β, IL-6, IL-10, TNF-α, RANKL, OPG and MMP-8 were counted. The number of cytokine-positive cells (/2500 μm^2) was determined as the average number of positive cells from three different fields [24].

Enzyme-linked immunosorbent assays (ELISAs)

The serum levels of hIL-10 were assayed by ELISA using a commercial kit (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations.

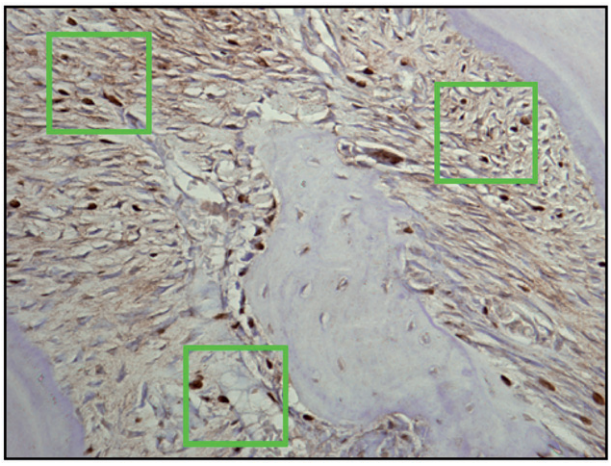

Figure 2. Number of cytokine-positive cells. Cytokine-positive cells were counted inside the green square area (50 μm × 50 μm), which represents the periodontal ligament in the root furcation region.

Table 1. Body weight in all four groups before and after gene transfer (g, $n = 6$).

Groups	Weight before transfer	Weight after transfer
S + V	311.7 ± 12.8	319.5 ± 12.3
S + I	319.0 ± 7.2	327.2 ± 9.1
O + V	343.3 ± 19.4[a]	353.5 ± 11.9[a]
O + I	354.5 ± 12.6[b]	361.8 ± 6.2[b]

O + V group: ovariectomized rats that received only the vector plasmid; O + I group: ovariectomized rats that received only hIL-10 gene transfer.
Values are expressed as the mean ± SD.
[a] $p < .05$ versus S + V group (sham-operated rats that received only the vector plasmid).
[b] $p < .05$ versus S + I group (sham-operated rats that received hIL-10 gene transfer).

Statistical analysis

All data such as the body weight, BMD, alveolar bone resorption in the root furcation region, CEJ–ABC distance, and cytokine-positive cell numbers (including IL-1β-, IL-10-, TNF-α-, RANKL-, OPG- and MMP-8-positive cells) were expressed as the mean ± SD. All of the data were subjected to paired *t*-tests using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL), and differences were considered significant when $p < .05$.

Results

Gross observation

Table 1 shows the body weights of all four groups. There was no significant difference between the S + I group and S + V group or between the O + I group and the O + V group ($p > .05$). Local hIL-10 gene transfer had no effect on the body weight of the SHAM and OVX rats. However, the O + I rats and O + V rats were significantly heavier than the S + I rats and S + V rats, respectively ($p < .05$).

BMD

The BMD values of the whole body, pelvis and spine are shown in Table 2. The BMD values of the whole body, pelvis and spine were higher in the SHAM-hIL-10 group than in the O–I group. The BMD values of the whole body, pelvis and spine were higher in the S + V group than in the O + V group. All of the differences were statistically significant ($p < .05$). Ovariectomy affected the BMD values of the whole body, pelvis and spine. After hIL-10 gene transfer, the BMD of the whole body, pelvis and spine did not differ significantly between the S + I and S + V group and between the O + I and O + V groups ($p > .05$). This indicated that local hIL-10 gene transfer had no effect on the BMD of the SHAM and OVX rats.

Table 2. Bone mineral density (BMD) values of the whole body, pelvis and spine in the four groups after gene transfer (g/cm^2, $n = 6$).

Groups	Whole body	Pelvis	Spine
S + V	0.169 ± 0.006	0.166 ± 0.005	0.154 ± 0.005
S + I	0.170 ± 0.004	0.168 ± 0.004	0.159 ± 0.004
O + V	0.155 ± 0.005[a]	0.153 ± 0.006[a]	0.139 ± 0.006[a]
O + I	0.151 ± 0.006[b]	0.148 ± 0.007[b]	0.134 ± 0.005[b]

S + V group: sham-operated rats that received only the vector plasmid; S + I group: sham-operated rats that received hIL-10 gene transfer; O + V group: ovariectomized rats that received only the vector plasmid; O + I group: ovariectomized rats that received only hIL-10 gene transfer.
Values are expressed as the mean ± SD.
[a] $p < .05$ versus S + V group.
[b] $p < .05$ versus S + I group.

Table 3. Detection of serum cytokines in the four groups of rats.

Groups	hIL-10 (ng/L)
S + V	0
S + I	26.8 ± 6.4[a]
O + V	0
O + I	31.6 ± 5.7[b]

S + V group: sham-operated rats that received only the vector plasmid; S + I group: sham-operated rats that received hIL-10 gene transfer; O + V group: ovariectomized rats that received only the vector plasmid; O + I group: ovariectomized rats that received only hIL-10 gene transfer.
Values are expressed as the mean ± SD.
[a] $p < .05$ versus S + V group.
[b] $p < .05$ versus O + V group.

Detection of serum hIL-10

At 48 h after the seventh gene transfer, hIL-10 was detected using ELISA in the S + I and O + I groups, but could not be detected in the S + V and O + V groups. The hIL-10 levels were higher in the group that received the gene transfer than in the S/+V group, and all differences were statistically significant ($p < .05$) (Table 3). These results indicate that local transfection of the hIL-10 gene resulted in the release of a small amount of hIL-10 protein into the circulation.

Histology of the distal femur

Resorption of the cancellous bone was more obvious in the distal femurs of rats in the OVX group (O + I and O + V groups) than in those in the SHAM groups (S + I and S + V groups). The volume of the trabecular bone and the number of trabeculae were lower in the OVX group than in the SHAM group. The trabecular separation in the OVX group was more pronounced than that in the SHAM group; thus,

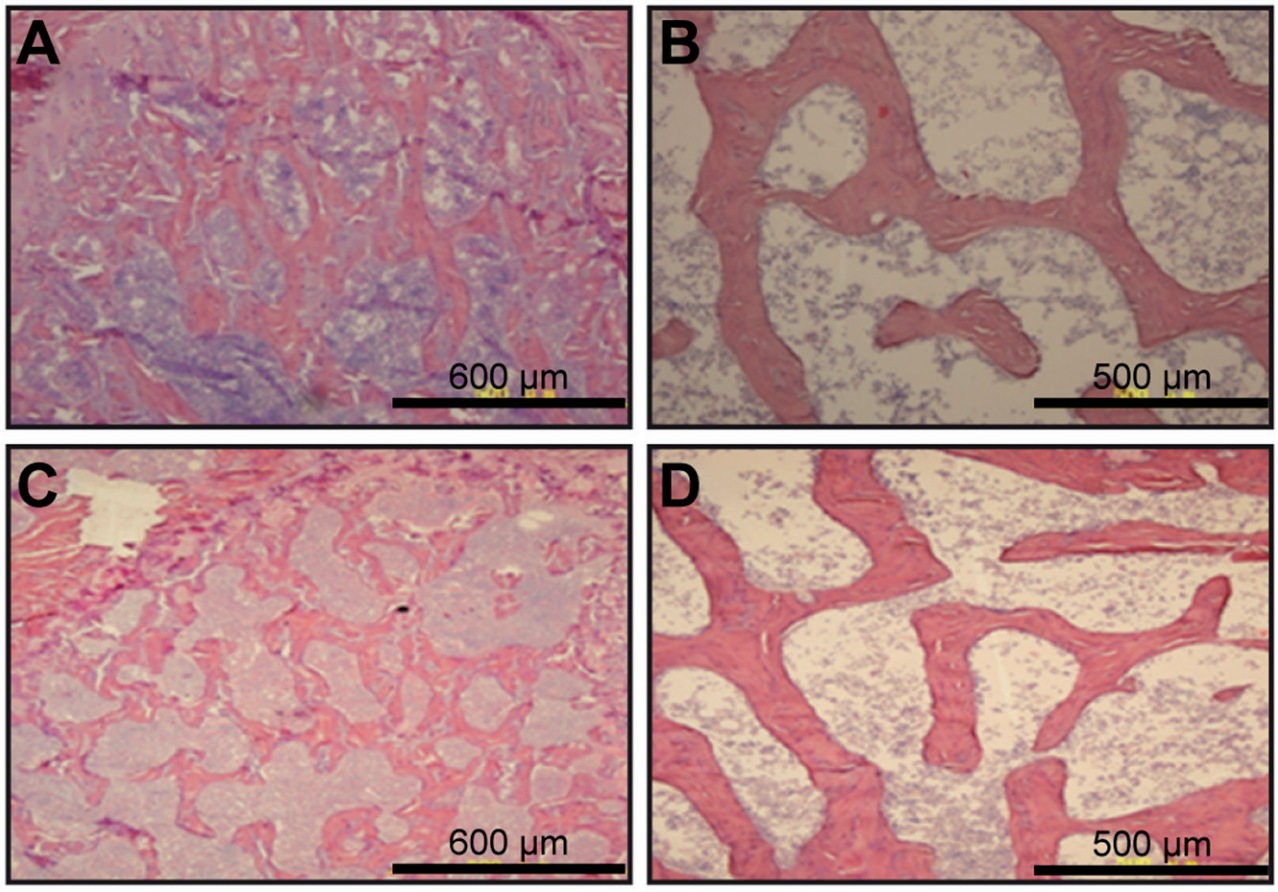

Figure 3. Histology of the femur after hIL-10 gene transfer in the four groups (haematoxylin and eosin staining, scale bar =500 μm). (A) S + I group: sham-operated rats that received hIL-10 gene transfer, (B) O + I group: ovariectomized rats that received hIL-10 gene transfer, (C) S + V group: shame-operated rats that received only the vector plasmid and (D) S + I group: shame-operated rats that received hIL-10 gene transfer.

the bone marrow expanded and formed a large marrow space. However, there was no marked difference in the histology of the cortical bones between the groups (Figure 3).

Histometric results of the alveolar bone

As shown in Table 4 and Figures 4 and 5, the alveolar bone resorption area in the root furcation region and the CEJ–ABC distance were both significantly greater in the S + I+C group than in the S + V+C group ($p < .05$). The alveolar bone resorption area in the root furcation region and the CEJ–ABC distance were significantly smaller in the S + I+E group and O + I+E group than in the S + V+E and O + V+E groups, respectively ($p < .05$). These data indicate that the locally transferred hIL-10 genes suppressed the EP-associated alveolar bone resorption to varying degrees in the SHAM and OVX rats.

Table 4. Comparison of alveolar bone loss in the four groups.

Group	Area of the periodontal ligament in the root furcation (mm^2)	Distance from the CEJ to the ABC (mm)
S + V+C	0.28 ± 0.05	0.50 ± 0.06
S + I+C	0.35 ± 0.08[a]	0.67 ± 0.10[a]
S + V+E	0.36 ± 0.07	0.77 ± 0.12
S + I+E	0.30 ± 0.05[b]	0.51 ± 0.08[b]
O + V+E	0.45 ± 0.11	0.85 ± 0.11
O + I+E	0.36 ± 0.07[c]	0.69 ± 0.07[c]

CEJ to the ABC: the distance from the cement–enamel junction (CEJ) to the alveolar bone crest (ABC); S + V+C: sham-operated rats that received only the vector plasmid and did not receive the silk ligatures; S + I+C: sham-operated rats that received hIL-10 gene transfer, but did not receive the silk ligatures; S + V+E: sham-operated rats that received only the vector plasmid, but received silk ligatures; S + I+E: sham-operated rats that received hIL-10 gene transfer and the silk ligatures; O + V+E: ovariectomized rats that received only the vector plasmid, but received the silk ligatures; O + I+E: ovariectomized rats that received hIL-10 gene transfer and the silk ligatures.
Values are expressed as the mean ± SD.
[a]$p < .05$ versus S + V+C group.
[b]$p < .05$ versus S + V+E group.
[c]$p < .05$ versus O + V+E group..

Expression of locally transferred hIL-10 in the periodontal tissue

As shown in Figure 6, hIL-10 was strongly expressed in the fibroblasts, epithelial cells, endothelial cells and inflammatory cells in the gingival connective tissue and moderately expressed in the periodontal ligaments in the root furcation region in the S + I+C group. In the S + V+C group, there was no hIL-10 expression in the gingival connective tissue and periodontal ligament in the root furcation region. These results indicate that the locally transferred hIL-10 had been successfully expressed in the periodontal tissue.

Immunohistochemical expression of cytokines

Immunohistochemical staining of the periodontal ligament in the root furcation region is shown in Figure 7. The results of

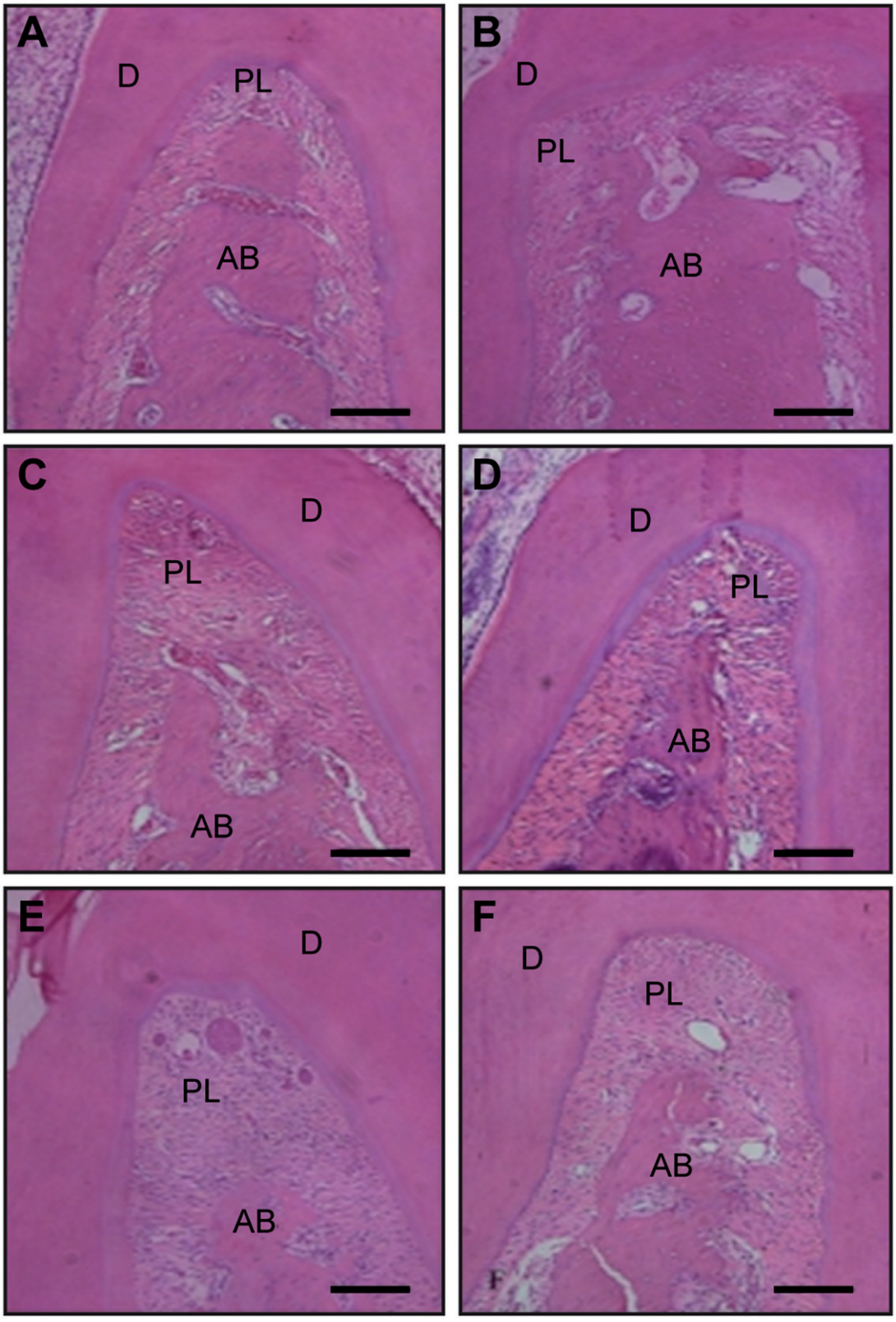

Figure 4. Area of the periodontal ligament in the root furcation region (scale bar =200 μm). Dentin (D); periodontal ligament area (PL); alveolar bone (AB). (A) S + V+C: sham-operated rats that received only the vector plasmid and did not receive the silk ligatures, (B) S + I+C: sham-operated rats that received hIL-10 gene transfer but did not receive the silk ligatures, (C) S + V+E: sham-operated rats that received only the vector plasmid, but received silk ligatures, (D) S + I+E: sham-operated rats that received hIL-10 gene transfer and the silk ligatures, (E) O + V+E: ovariectomized rats that received only the vector plasmid, but received the silk ligatures and (F) O + I+E: ovariectomized rats that received hIL-10 gene transfer and the silk ligatures.

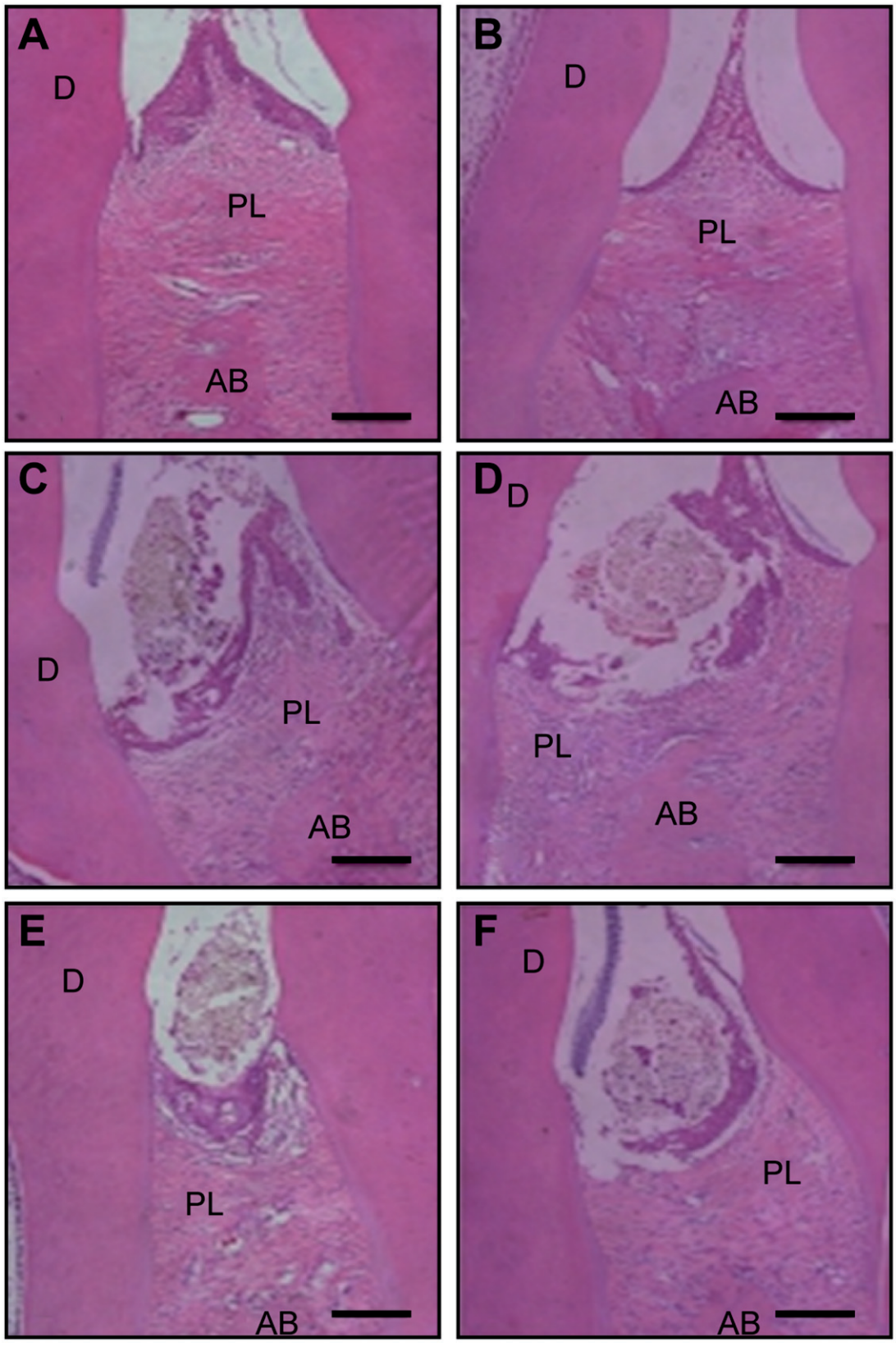

Figure 5. Distance from the CEJ to the ABC (scale bar =200 μm). Dentin (D); periodontal ligament area (PL); alveolar bone (AB). CEJ to the ABC: the distance from the cement–enamel junction (CEJ) to the alveolar bone crest (ABC). (A) S + V+C: sham-operated rats that received only the vector plasmid and did not receive the silk ligatures, (B) S + I+C: sham-operated rats that received hIL-10 gene transfer, but did not receive the silk ligatures, (C) S + V+E: sham-operated rats that received only the vector plasmid, but received silk ligatures, (D) S + I+E: sham-operated rats that received hIL-10 gene transfer and the silk ligatures, (E) O + V+E: ovariectomized rats that received only the vector plasmid, but received the silk ligatures and (F) O + I+E: ovariectomized rats that received hIL-10 gene transfer and the silk ligatures.

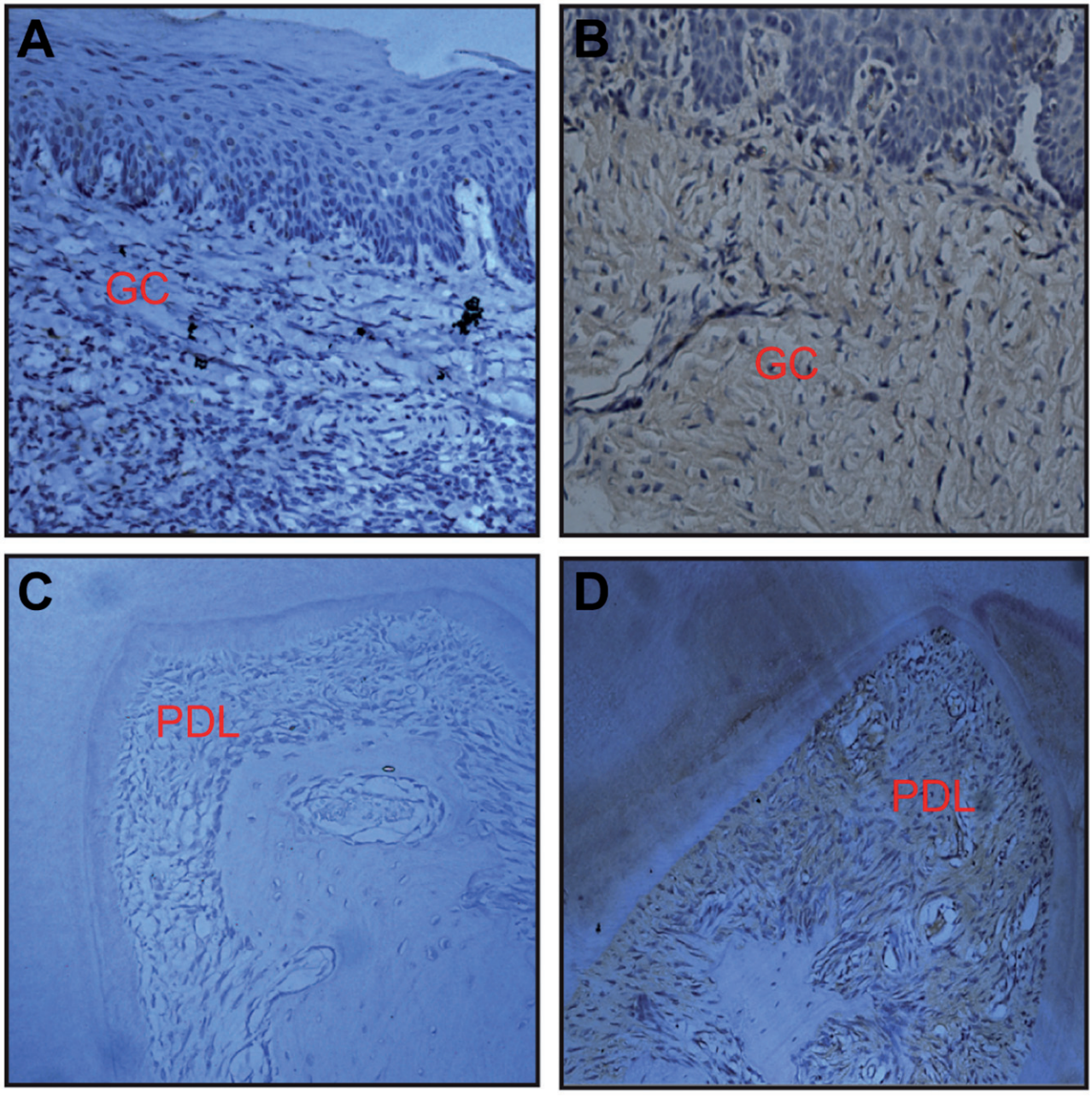

Figure 6. Distribution of hIL-10 in the periodontal tissue (×200). (A) No hIL-10 was expressed in the gingival connective tissue (GC) in the S + V+C group (sham-operated rats that received only the vector plasmid and did not receive the silk ligatures); (B) hIL-10 was strongly expressed in the GC in the S + I+C group (sham-operated rats that received hIL-10 gene transfer but did not receive the silk ligatures); (C) no hIL-10 was expressed in the periodontal ligament (PDL) in the S + V+C group; (D) hIL-10 was moderately expressed in the PDL in the S + I+C group.

cell counting and statistical analyses are shown in Figure 8. After transfer of the hIL-10 gene, the number of cytokine-positive cells in the S + I+C, S + I+E and O + I+E groups was significantly higher than that in the S + V+C, S + V+E and O + V+E groups, respectively ($p < .05$). There were significantly fewer IL-1β-positive cells in the periodontal ligament in the root furcation region in the S + I+C group than in the S + V+C group ($p < .05$), but the number of RANKL-positive cells was higher in the S + I+C group ($p < .05$). There were significantly fewer IL-1β-, IL-6-, TNF-α- and RANKL-positive cells in the periodontal ligaments in the S + I+E group than in the S + V+E group ($p < .05$). The number of IL-1β-, IL-6-, RANKL- and MMP-8-positive cells in the O + I+E group was also lower than that in the O + V+E group ($p < .05$).

Discussion

There are profound similarities in anatomy and metabolism between rats and humans [25]. Rats also share some characteristics of the molar periodontal tissue structure and PMO with humans. Therefore, rats are most commonly used as the animal model for studying periodontitis and osteoporosis [26,27]. Removal of both ovaries from rats is widely used to simulate human PMO. Bone density measurement, bone histological analysis and biomechanical parameter measurement are the major criteria used to determine the successful establishment of an osteoporosis animal model. In the current study, we showed that after removal of both ovaries, the rats gained weight and the

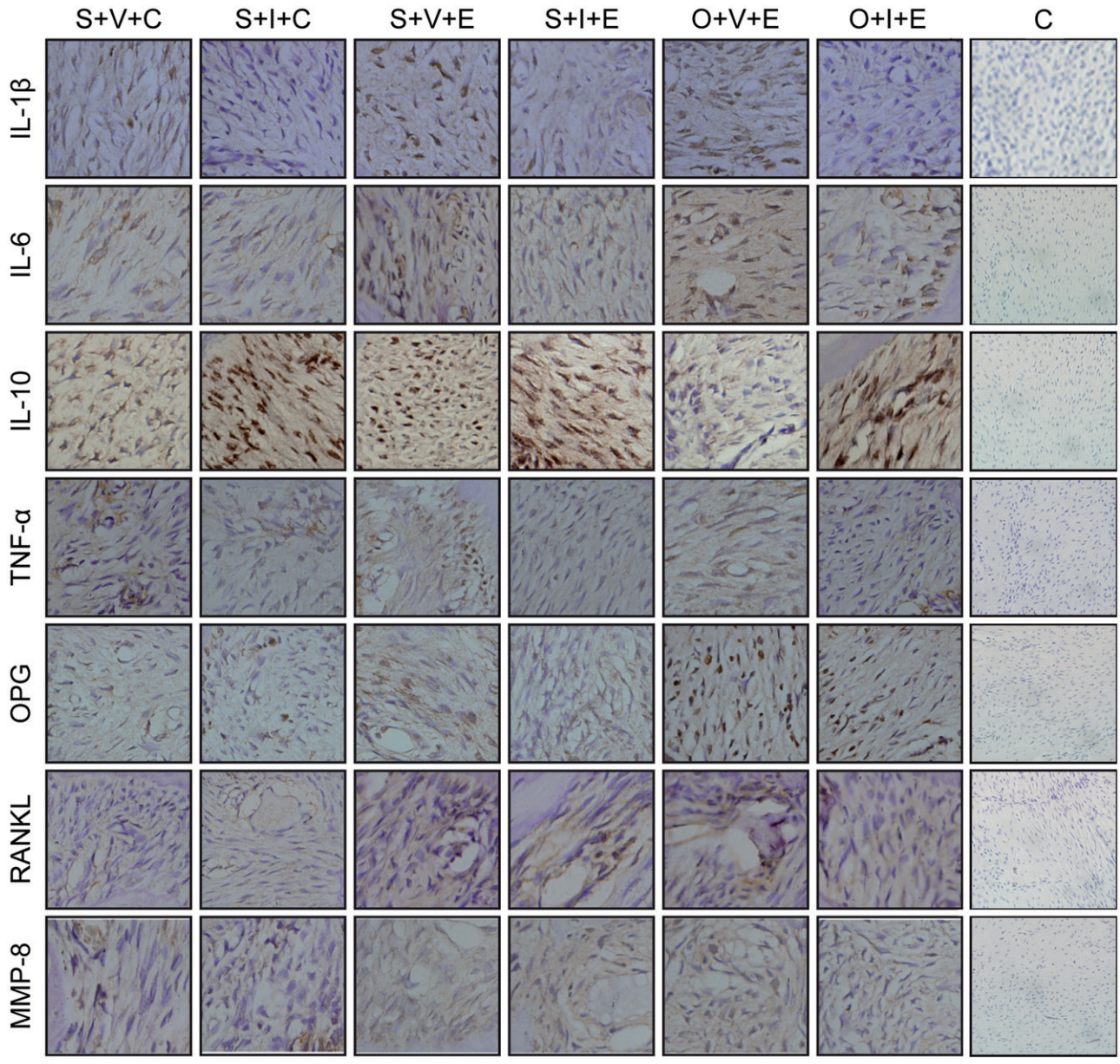

Figure 7. Immunohistochemical staining images of the periodontal ligament in the root furcation region (×400).

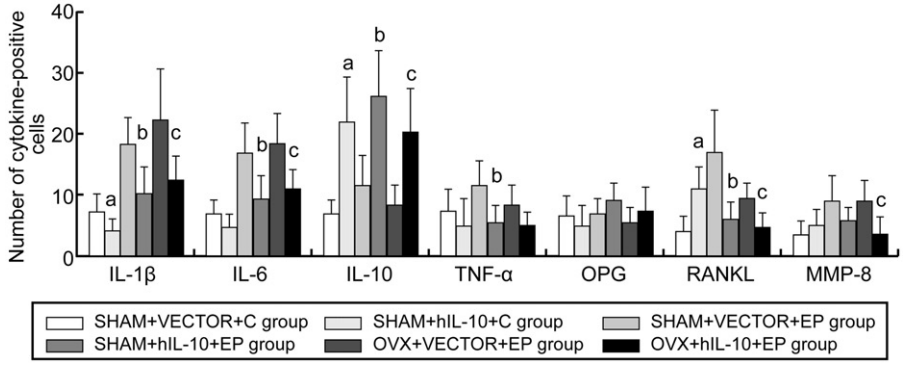

Figure 8. Cytokine-positive cells in the periodontal ligament in the root furcation region.

BMD of the whole body, pelvis and spine declined dramatically. Femur tissues, especially the cancellous bone, became sparse, which indicated that the model had been established successfully.

Alveolar bone resorption is one of the major clinical manifestations of periodontitis. There are two ways to control alveolar bone loss: reducing alveolar bone resorption and increasing bone formation [28–30]. Oestrogen replacement

therapy and selective oestrogen receptor modulators have been shown to aid in the treatment of periodontitis or alveolar bone resorption caused by osteoporosis [28–30]. However, as drug concentrations in the blood decline rapidly, the drug must be taken daily, which is inconvenient for patients and can cause systemic side effects. In recent years, gene therapy has seen marked progress and can be used experimentally to treat periodontitis and osteoporosis [31,32]. If exogenous genes can be delivered *in vivo*, their safe, long-term, effective expression can be achieved with minimal toxicity and side effects. In such cases, gene therapy may be expected to replace regular medication. In our study, hIL-10 was injected into the palatal mucosa to investigate the effect of local hIL-10 gene therapy on EP in rats. The palatal area provided more access for injection than the buccal side because of the lack of interference by the lips and buccal tissues. Moreover, the injection area was distant from the lesions to prevent trauma in the process of plasmid application.

Recent studies have shown that serum IL-10 levels are significantly higher in postmenopausal osteoporotic women than in healthy postmenopausal women [33]. Low levels of IL-10 are not sufficient to inhibit pro-inflammatory cytokines and collagenase, which may affect the development of osteoporosis [13]. Animal studies have also shown that IL-10 deficiency can lead to the loss of femur tissue [14,15] and alveolar bone resorption [15,16,34]. In lytic oral bone diseases, such as periapical lesions and periodontitis, IL-10 has been shown to be a critical regulator of alveolar bone [15,17,18,35]. The purpose of the present study was to study the effect of local hIL-10 gene transfer on the initiation and development of EP in OVX rats. We expect that IL-10 gene transfection will eventually be applied clinically. Therefore, in our study, hIL-10 was injected into the palatal mucosa of SD rats as a proof of concept. The human and rat IL-10 genes show 81% homology in their nucleotide sequences, whereas the amino acid sequences of the proteins show 73% homology. This allowed the rat cells to express functional human IL-10 protein after the injection. Our results indicated that after local hIL-10 gene therapy, the endothelial cells, fibroblasts, epithelial cells and inflammatory cells of the gingival connective tissue in the transferred area all expressed the hIL-10 protein. Liu et al. explored the effect of IL-10 on the apoptosis of the vascular smooth muscle cells (VSMCs) in rats receiving allograft arterial transplantation. The isolated donor vasculature was transduced with an adenovirus carrying the hIL-10 gene through immersion. The transgene expression of hIL-10 was identified by ELISA, RT-PCR and immunohistochemistry [36]. In the present study, after delivery of the plasmids into the target cells, the hIL-10 gene was not incorporated into the host genome. The exogenous gene could only be transiently expressed, and its expression would decrease rapidly. For this reason, the hIL-10 gene was injected every other day to ensure that the levels of hIL-10 protein in the target region would be maintained.

After local transfer of the hIL-10 gene, the expressions of RANKL, TNF-α, IL-1β and IL-6 in the periodontal tissue in the S + I+E group decreased dramatically. There were also significantly fewer IL-1β-, IL-6-, RANKL- and MMP-8-positive cells in the periodontal ligaments in the O + I+E group. In addition, alveolar bone resorption was greatly reduced in both groups, which further confirmed the strong association between IL-10 levels and periodontal health. IL-10 is an important regulator of periodontal inflammation and alveolar bone resorption [37,18]. However, in S + I+C rats, alveolar bone resorption was far more pronounced, IL-1β expression was lower, and RANKL expression was higher than that in the controls. One possible explanation for this is that the acceleration of alveolar bone resorption does not depend solely on the expressions of IL-1β, TNF-α, RANKL, IL-10 or OPG but rather on the ratios of IL-1β/IL-10 and OPG/RANKL. A deviation in the ratios from the normal range is not conducive to the health of the periodontal tissue. Therefore, overexpression of IL-10 in the periodontal tissue microenvironment may also not be conducive to the preservation of the tissue, and this phenomenon has been confirmed in other studies [38,39]. As IL-10 inhibits the secretion of interferon-gamma (IFN-γ) by Th1 cells, low levels of IFN-γ are not sufficient to remove periodontal pathogens [40]. In addition, IFN-γ can block the bypass signalling pathway of osteoclast differentiation; therefore, low levels of IFN-γ can increase the number of osteoclasts. As the anti-inflammatory cytokine IL-10 participates in both chronic and acute inflammation, it is important to note that it may play a differential role in distinct inflammatory responses. Our current finding that alveolar bone resorption in the S + I+C group was higher than that in the S + V+C group indicated that inhibition of IL-10 expression may decrease the capability of the host to generate a robust inflammatory response to chronic and low-level pathogenic stimulation and that such decreased inflammation might have contributed to the increased alveolar bone resorption observed in the hIL-10-transfected non-ligated mice.

After local gene transfection, limited expression of hIL-10 was observed in the serum, possibly because it is difficult for IL-10 to enter the circulation through capillaries. The locally transferred hIL-10 genes had no effect on other bone tissues except at the transfection sites. In addition, there was no significant difference in the BMD of the whole body, pelvis and spine between the SHAM and OVX rats after local gene transfection.

In summary, locally transferred hIL-10 genes were expressed effectively in the periodontal tissues of OVX rats in our study. Experimental periodontitis-associated alveolar bone resorption was greatly reduced in both sham and OVX rats after successful hIL-10 gene transfer. The expression of pro-inflammatory cytokines was also reduced to varying degrees, which may be the mechanism underlying the effect of hIL-10 gene transfer on the periodontitis induced in these rats. Due to the high prevalence of periodontitis and the effect of periodontal infection on other major health problems, it is important to find new therapeutic approaches to control this inflammatory disease. Our findings indicate that hIL-10 gene transfer may be a promising technique for the treatment of periodontitis. In future studies, we will try to determine the optimal dose of hIL-10. The application of this technique needs to be evaluated in clinical trials in the future.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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