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Influence of low-intensity pulsed ultrasound on osteogenic tissue regeneration in a periodontal injury model: X-ray image alterations assessed by micro-computed tomography

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ABSTRACT

Objective: This study was conducted to evaluate, with micro-computed tomography, the influence of low-intensity pulsed ultrasound on wound-healing in periodontal tissues.
Methods: Periodontal disease with Class II furcation involvement was surgically produced at the bilateral mandibular premolars in 8 adult male beagle dogs. Twenty-four teeth were randomly assigned among 4 groups (G): G1, periodontal flap surgery; G2, periodontal flap surgery + low-intensity pulsed ultrasound (LIPUS); G3, guided tissue regeneration (GTR) surgery; G4, GTR surgery plus LIPUS. The affected area in the experimental group was exposed to LIPUS. At 6 and 8 weeks, the X-ray images of regenerated teeth were referred to micro-CT scanning for 3-D measurement.
Results: Bone volume (BV), bone surface (BS), and number of trabeculae (Tb) in G2 and G4 were higher than in G1 and G3 ($p < 0.05$). BV, BS, and Tb.N of the GTR + LIPUS group were higher than in the GTR group. BV, BS, and Tb.N of the LIPUS group were higher than in the periodontal flap surgery group.
Conclusion: LIPUS irradiation increased the number, volume, and area of new alveolar bone trabeculae. LIPUS has the potential to promote the repair of periodontal tissue, and may work effectively if combined with GTR.

1. Introduction

Periodontal disease is a chronic bacterial inflammatory disease, characterized by the destruction of periodontal tissue and the loss of teeth. Class II furcation involvement, characterized by massive loss of periodontal tissue and deep periodontal pockets, poses a challenge for local plaque control. Routine treatment such as root scaling is insufficient, and guided tissue regeneration (GTR) is needed. However, GTR requires strict and specific indications, and the regeneration of periodontal tissues is slow and localized, limiting the application of GTR.

Low-intensity pulsed ultrasound (LIPUS) is a safe and non-invasive therapeutic technique [1–4]. Previous studies [5,6,7–9] have shown that ultrasound might have a positive application in the repair of osseous defects. LIPUS has been successfully applied for the clinical treatment of fracture and non-union of bone [1–3,10,11]. Previous studies demonstrated that LIPUS could induce osteoblast differentiation, extracellular matrix proliferation, accelerated calcium deposition, and improved local microcirculation [12,13]. Ikai et al. [14] reported that osteoblasts, as well as cells in periodontal ligament and gingival epithelium, respond to mechanical stress loaded by LIPUS, and accelerate periodontal wound-healing and bone repair. They suggested that, theoretically, LIPUS may have the potential to restrain inflammation, promote alveolar regeneration and differentiation, and improve the effect of GTR.

Micro-computed tomography (micro-CT) has been widely applied in the medical field for its extraordinary high resolution, up to 10 μm, especially for hard-tissue imaging. It can also be used for 3D measurement [15,16]. The progression of periodontal disease is characterized by the destruction and restoration of alveolar bone, which has high density in contrast to that of surrounding tissues. Micro-CT can measure bone density quantitatively and three-dimensionally [17–19], which is of high value in periodontal treatment.

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In this study, experimental teeth in different groups were referred to micro-CT for 3-D reconstruction and quantitative measurement of the effect of the GTR technique, combined with LIPUS, on periodontal lesions. This study may provide experimental evidence for the use of the LIPUS-assisted GTR technique in periodontal treatment.

2. Materials and methods

Eight healthy male beagle dogs (age ranging from 12 to 18 months; weight ranging from 10 to 15 kg) were included in this study. The dogs were kept in runs and were fed standard dog food regularly, with water available *ad libitum*. The experiment began after one week's adaptive feeding. The complete experimental plan was approved by the Ethics Committee of Chongqing Medical University.

A Class II furcation involvement model of mandibular premolars was established. The dogs' second, third, and fourth mandibular premolars were selected. By removing alveolar bone rapidly, ligating teeth with stainless steel wire, and feeding the dogs a high-sugar and high-viscosity diet, we established the Class II furcation involvement model [14] (Fig. 1).

After the modeling, experimental teeth were divided into four groups (G): G1, periodontal flap surgery; G2, periodontal flap surgery + low intensity pulsed ultrasound (LIPUS); G3, guided tissue regeneration (GTR) surgery; and G4, GTR surgery plus LIPUS. To avoid the influence of LIPUS, if one tooth was included in the LIPUS group, teeth on the same side were excluded from the non-LIPUS group. Periodontal examinations were conducted on the 6th and 8th weeks after treatment, respectively.

Experimental teeth were scaled one week before the GTR. Dogs were given general anesthesia and fixed on a sterile experimental table, with their mouths gagged. Experimental teeth were manually scaled, and flushed by 2% aqueous hydrogen peroxide solution and physiological saline. Each dog's oral cavity was flushed post-operatively with 1.5% hibitane solution and physiological saline once per day for one week.

One week after scaling, periodontal flap surgery was conducted with the dogs under general anesthesia. Strict sterilization was maintained throughout the operation, which was performed by an experienced doctor.

For full exposure of the experimental teeth, a full-thickness mucoperiosteal flap was extended from the distal gingiva of the 1st molar to the mesial gingiva of the 2nd premolar on the same side. The papilla was kept intact by careful operation with a gum lancet.

The root surfaces were planed, then flushed by 2% aqueous hydrogen peroxide solution and physiological saline.

Fig. 1. The Class II furcation involvement modeling.

The coronal extensions of alveolar bone were marked horizontally on root surfaces by means of a high-speed fissure bur, with physiological saline cooling.

For the GTR operation, a bone graft was taken from the mandibular 1st molar alveolar bone (including cortical and spongy bone) by means of an osteotome and fissure bur, with physiological saline cooling. Mixed with physiological saline, the bone graft was smashed by scissors. The root surfaces were treated with tetracycline solution applied by cotton for 1 min and were flushed by physiological saline for 3 min. The bone graft was then implanted into the furcation. Bio-Gide collagen membranes were properly shaped to cover the defect with a 2- to 3-mm margin, and applied to the bone graft. The flaps were then reset and sutured with 4–0 silk sutures.

Post-surgery, the dogs were prescribed intramuscular injections of penicillin (800,000 iu/day for one week), and fed soft dog food. The oral cavity was flushed daily with 1.5% hibitane solution and physiological saline. The sutures were removed on the 7th post-surgical day.

Dogs were given general anesthesia and fixed on a sterile experimental table. The experimental teeth chosen for LIPUS irradiation were flushed by physiological saline. The LIPUS probe with ultrasonic complants was set in contact with furcation area. A retort stand was fixed to the rear of the probe to maintain stability. The affected area on the experimental side of the mandible was exposed to low-intensity pulsed ultrasound which was manufactured by China National Engineering Research Center of Ultrasound Medicine (a 200-ls burst sine wave of 1.5 MHz repeated at a frequency of 1.0 kHz, with intensity of 30 mW/cm^2) [14,20]. In a preliminary study, we found that the transmittance of Bio-Gide collagen membranes was 46.5%; so the intensity of GTR combined with LIPUS groups was 65 mW/cm^2. Control groups were flushed by physiological saline only. The experimental teeth were irradiated once a day, 20 min each time.

After 6-week irradiation, all 8 dogs underwent general anesthesia. Periodontal and X-ray examinations were carried out.

Four dogs in the 6-week group were euthanized by anesthesia with an overdose of xylazine hydrochloride. On the 8th week, the other four dogs were euthanized by the same method. Mandibles were taken as specimens and fixed in 4% neutral formaldehyde solution.

The samples were referred for micro-CT scanning (μCT80, Scanco Medical, Bassersdorf, Switzerland),with the following scan parameters: voxel size resolution, 37 μm; tube voltage, 55 kV; and tube current, 145 μA. Regions of Interest (ROI) were 3-D-reconstructed and analyzed by means of an HP Integrity 64-bit workstation. Parameters selected were: bone volume, BV; bone surface/bone volume, BS/BV; trabecular number, Tb.N; trabecular spacing, Tb.Sp; and trabecular thickness, Tb.Th.

The statistical analysis was conducted with Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS (Release 13.0, standard version; SPSS, Chicago, IL, USA).

3. Results

3.1. Post-operative general observations

The weight of the beagle dogs increased significantly after 4 days ($p < 0.05$). However, a t-test showed that there were no statistical differences in height and length ($p > 0.05$) (Table 1).

3.2. Clinical observation and X-ray examination

Before modeling, we inspected the experimental teeth and found no gingival swelling, bleeding, or attachment loss. Eight