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Expression of collagen I, collagen III and MMP-1 on the tension side of distracted tooth using periodontal ligament distraction osteogenesis in beagle dogs

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ABSTRACT

Objective: To investigate the expression changes of collagen types I and III (Coll I and III) and matrix metalloproteinase-1 (MMP-1) in the periodontal ligament on the tension side of the distracted tooth using periodontal ligament distraction osteogenesis in beagle dogs.
Methods: Twenty Beagle dogs were randomly divided into five groups of four dogs each according to their experimental periods. Dogs in each group were subjected to five days (T1), 10 days (T2), or 15 days (T3) of force loading, or 15 days of force loading with 10 days (T4) or 90 days (T5) of retention. For dogs in each group, the first premolar movement was achieved in the lower arch on one side (Exp) using periodontal distraction osteogenesis and on the other side (Con) using the conventional orthodontic method. The orthodontic movements of the first and third premolars in each group were measured at indicated time. Coll I and III expressions were determined by Picrosirius red staining and HE staining while MMP-1 expression was examined by immuno-histochemical staining.
Results: Fifteen days of force loading resulted in faster movement of the first premolar on the Exp side, but no difference in the third premolar movements between the Exp and Con sides. Periodontal ligament tissue on the tension side was remodelled faster on the Exp side than on the Con side. The quality of the newly formed bone was acceptable. Coll III and MMP-1 in the periodontal ligament tissue reached a higher expression peak at an earlier time point on the Exp side than on the Con side. Significant difference also existed in the Coll I expression between the Exp and Con sides ($P < 0.05$). During force loading, the Coll I expression first decreased and then increased on the Exp side, but increased continuously on the Con side. After 90 days' retention, collagen expressions showed no significant difference between the two sides ($P > 0.05$) and the expression level of MMP-1 returned to normal on the two sides.

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Conclusion: Rapid tooth movement accompanied with local upregulation of Coll I, Coll III, and MMP-1 was achieved in the early stage of periodontal distraction osteogenesis. The rapid tooth movement is in accordance with the physiological remodelling process.

1. Introduction

In recent years, distraction osteogenesis has been widely used for correction of dental deformities and has achieved good therapeutic effects.[1] In 1998, Liou first proposed the theory of periodontal ligament distraction osteogenesis.[2] He combined the principle of distraction osteogenesis with bone suture distraction and elicited a rapid canine movement of 6 mm within 3 weeks. Since then, a large number of clinical studies have been performed on periodontal ligament distraction osteogenesis. Sayin and Kumar achieved rapid canine movement by using resistance reduction and canine retraction.[3–5] Clinical measurements and radiography images showed that the newly formed bone was rapidly deposited without any defect on the tension side of the canine, suggesting the feasibility of periodontal ligament distraction osteogenesis. Biomechanical studies have revealed that different mechanical stresses induce different types of bone formation. The histological and molecular mechanisms of endochondral ossification and intramembranous ossification in distraction osteogenesis are similar to those that regulate fracture healing.[6]

Periodontal tissue remodelling during orthodontic treatment is a complex mechanical and biomechanical reaction process. The dynamic remodelling of periodontal ligament plays critical roles in mediation of external forces and alveolar bone remodelling and in maintenance of periodontal tissue homeostasis.[7] Degradation and synthesis of extracellular matrix (ECM) is a key process in regulation of bone remodeling.[8,9] Collagen fibres are principal components of periodontal ligament ECM, and collagen types I and III play important parts in periodontal tissue remodeling.[10] Matrix metalloproteinases (MMPs), as collagen-degrading enzymes, are critical to renovation and reconstruction of ECM.[11] MMP-1, an interstitial collagenase, can specifically degrade collagen and plays an important role in tissue remodeling.[12] Periodontal tissue remodelling of the orthodontic tooth is mostly realized by regulation of MMP-1 expression in periodontal ligament cells.[13] Since mechanic force is directly exerted on periodontal ligament during orthodontic treatment, periodontal ligament cells undergo great changes in morphology and function, leading to active degradation and synthesis of extracellular matrix. Therefore, studies on the mechanism underlying rapid periodontal tissue remodelling during orthodontic tooth movement may provide clues for clinical application of periodontal ligament distraction.

In the present study, we aimed to investigate the biological effect of periodontal tissue remodelling in periodontal distraction osteogenesis. We achieved rapid tooth movement in a previously established dog model[14] by using periodontal distraction osteogenesis and further examined the expression changes of collagens and MMP-1 in the periodontal ligament on the tension side of the distracted tooth.

2. Materials and methods

2.1. *Experimental animals and establishment of rapid tooth movement*

Twenty 8–18-month-old male Beagle dogs (weight 8.5–10 kg) were purchased from Xi'an DLP Biological Resource Development Co. Ltd (Xi'an, China). All animal procedures were approved by the Ethics Committee of Xi'an Jiaotong University. The dogs were randomly divided into five groups of four dogs each according to their experimental periods. Dogs in each group were subjected to 5 days (T1), 10 days (T2), or 15 days (T3) of force loading, or 15 days of force loading with 10 days (T4) or 90 days (T5) of retention. For dogs in each group, the first premolar movement was achieved in the lower arch on one side (Exp) using periodontal distraction osteogenesis and on the other side (Con) using the conventional orthodontic method. Periodontal distraction osteogenesis and conventional orthodontic treatment were performed as previously described.[14] Briefly, the two mandible second premolars were extracted while the mandible first premolars were moved distally using the third premolar as the anchorage. On the Exp side, a tooth-borne, custom-made, intraoral distraction device was placed to distract the first premolar distally into the second premolar extraction space. The cast crowns were made for the first and third premolars and modified screws were welded to the cast crowns. A 360° activation of the screw produced 0.5 mm of distal movement of the first premolar. After extraction of the second premolar, the interseptal bone distal to the first premolar on the Exp side was undermined and thinned by half to about 1.5 mm. Two vertical grooves were made at the buccal and lingual sites and extended toward the base of the interseptal bone to weaken the resistance (Fig. 1). After that, a distractor was cemented in place to attain an advancement of 0.25 mm (0.125 mm each time; twice a day). On the Con side, the first premolar was subjected to conventional orthodontic treatment for tooth movement without resistance reduction. The orthodontic spring was fixed between the first and third premolars using ligating wire and flow resin (Z350, 3M ESPE, US). The traction force was 100 g. All the dogs were closely monitored during orthodontic tooth movement. Dogs were sacrificed by the end of their respective orthodontic treatments (T1–T5).

2.2. *Tissue preparation*

The mandibular segments containing the first premolar and the extraction socket were dissected. The segments were fixed with 4 g/L PFA for 48 h, decalcified with 15% EDTA for 3 months, and embedded in paraffin wax. Serial sections with a thickness of 5 μm were cut along the sagittal plane of the first