Effect of low-intensity pulsed ultrasound stimulation on gap healing in a rabbit osteotomy model evaluated by micro computed tomography-based 3-dimensional cross-sectional moment and cross-sectional moment of inertia

INTRODUCTION

Low-intensity pulsed ultrasound stimulation (LIPUS) reportedly enhances restoration of strength at fracture healing sites according to previous experimental studies [1]. However, evaluation of strength by mechanical testing is limited to only one direction, with either bending or torsion. Quantitative micro computed tomography (µCT) is able to acquire 3-dimensional (3D) histomorphometric data and density distributions of hard tissues, from which strength-related parameters can be calculated to allow strength analysis of the tissue. Strength-related parameters such as cross-sectional moment of inertia (CSMI) and cross-sectional moment of inertia (CSM) have been used to evaluate strength of the fracture healing site and reportedly correlate well with measured strength from mechanical testing [2]. However, previous studies have performed 2-dimensional (2D) analyses, and 3D evaluations have not been described. The purpose of this study was thus to investigate the effects of LIPUS on osteotomy healing using conducting 3D analyses of strength-related parameters of CSM and CSMI derived from µCT of the osteotomy gap.

MATERIALS AND METHODS

Surgical Procedures and LIPUS Treatment

A total of 42 skeletally mature between 21 and 23-week-old male Japanese white rabbits (Kitayama Labes, Nagano, Japan), weighing 3.4-4.0 kg, were used for this study. Under general anesthesia, four transfixation pins (diameter, 2 mm; length, 50 mm) were inserted at the metaphyseal regions of the tibia in the frontal plane using a custom-made surgical pin driver. Transverse osteotomy was performed using a T-saw (blade thickness, 0.36 mm) with continuous irrigation with saline solution across the mid-shaft of the tibia at 12 mm distal to the tibio-fibular junction. The osteotomy with a 2-mm gap was immobilized with four pins fixed to an external fixator with double side bars. The LIPUS system (model SAFHS-200, Teijin Pharma, Tokyo, Japan), which transmits 200-µsec burst of 1-MHz sine waves repeated at 1kHz with an average intensity of 30mW/cm², was used. After postoperative day 3, LIPUS was continued under general anesthesia for both the treatment group (n=7/group/time point) and the control group (n=7/group/time point). The transducer was placed onto the anterior surface of the operated leg with ultrasound coupling gel, for 20 min, six times/week, for 4, 6, or 8 weeks. The control group also received a sham inactive transducer under exactly the same condition as the LIPUS group.

µCT Analysis

All animals were euthanized and the entire tibia was removed. The harvested tibia was scanned by µCT system (Scan X mate-E090, Comscantecno, Kanagawa, Japan). The scan was performed along the long axis of the diaphysis, with a voltage of 60 kVp and a current of 80 mA. Scan range covered 5 mm proximal and 5 mm distal to the center of the gap, with a resolution of 28.57 µm/voxel size. The region of interest (ROI) was set at the callus healing area (Fig. 1) defined by the gap filled with callus in 2D CT and extended 0.5 mm proximally and distally to the center of the osteotomy gap with a total of 36 CT axial scans. 3D reconstruction of mineralized tissue was performed using a TRI-BONE system (Ratoc System Engineering, Tokyo, Japan). A threshold for newly formed mineralized callus was set as 200 mg/cm³. Morphometric parameters were used for evaluation of mineralized callus volume (BV, cm³) and mineralized callus contents (BMC, mg) calculated from the contoured ROI in 3D images, and volumetric bone mineral density of mineralized tissue comprising the callus (mBMD, mBMD = BMC/BV, mgHA/cm³).

Center of gravity for the ROI was calculated automatically. The Z (polar) axis was defined to coincide with the long axis of the tibia. The Y axis was defined as parallel to the transfixation pins, which were also parallel to the posterior surface of the tibia (mediolateral (ML) direction). The X axis was defined as perpendicular to the YZ plane, and was directed anteroposterior (AP) on the tibia (Fig. 2).

Three-dimensional CSM and CSMI

An optional line (l) can be drawn in this XYZ coordinate. The angle of the Z axis (θ) was measured, and also the degree of angle of the X axis (φ) was measured (Fig. 3). The 3D CSM [I (θ, φ)] around this line was calculated as shown. 3D CSM was calculated using the following equation: I (θ, φ) = ∫ r²dm, where r is the distance of a voxel to the center of gravity (mm) and dv (BV/voxel) is the area of a voxel (mm²).

The transducer was placed onto the anterior surface of the operated leg in bending in AP and ML planes, as well as torsion.

REFERENCES

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2. JL Ferretti et al, J Muscolskel Neurom Interact, 2001

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