Ultrasound in Med. & Biol., Vol. 38, No. 10, pp. 1744–1758, 2012

0301-5629/$ - see front matter

doi:10.1016/j.ultrasmedbio.2012.05.001

● *Original Contribution*

COMPUTED RADIOGRAPHIC AND ULTRASONIC EVALUATION OF BONE REGENERATION DURING TIBIAL DISTRACTION OSTEOGENESIS IN RABBITS

Hon Kit Luk,* Yau Ming Lai,* Ling Qin,† Yan-Ping Huang,‡ and Yong-Ping Zheng‡

*Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, P. R. China; †Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, P. R. China; and ‡Interdisciplinary Division of Biomedical Engineering, Faculty of Engineering, The Hong Kong Polytechnic University, Hong Kong, P. R. China

(*Received* 6 *January* 2012; *revised* 28 *April* 2012; *in final form* 1 *May* 2012)

Abstract—Computed radiography (CR) and a combined ultrasound (US) approach involving two-dimensional (2-D) and three-dimensional (3-D) ultrasonography with ultrasonometry were employed to evaluate their respective efficacies in monitoring bone regeneration during rabbit tibial distraction osteogenesis (DO). Results demonstrated that 2-D and 3-D ultrasonography depicted bone callus growth changes during distraction while CR could not. Evaluation of callus speed of sound, acoustic reflection and attenuation showed significant linear changes over time during early DO stage ($p < 0.05$). However, surrogate measure of callus density by CR only showed such significant linear changes during consolidation ($p < 0.05$). Also, callus speed of sound and acoustic reflection during early DO stage showed strong predictions to the bone mineral density and microstructural properties (adjusted-R^2 = 0.43–0.67) of consolidated bone callus measured at the treatment end-point by microcomputed tomography. Findings of the present study indicated a preferred use of the combined US approach over CR in the early monitoring of bone regeneration during DO treatment. (E-mail: yauming.lai@polyu.edu.hk) © 2012 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Tibial distraction osteogenesis, Bone regeneration, Computed radiography, Ultrasonography, Ultrasonometry.**

INTRODUCTION

Self-regeneration of bone tissues has been evident in distraction osteogenesis (DO) (Samchukov et al. 2008), which is commonly applied to orthopaedic patients for limb reconstruction (Chan and Leung 2008). Treatment complications are associated with an inappropriate distraction protocol (Li et al. 2000). This may end up undesirable outcomes such as delayed union or non-union at the distraction site (Paley 1990). In the study of canine tibial DO model, there was a significant *in vivo* difference in the bone callus axial stress between the union group (47 N/cm^2) and the non-union group (<27 N/cm^2) after 3 weeks of the distraction period (Aronson and Harp 1994). The result indicates that *in vivo* assessment of callus growth condition at the early DO stage can inform the treatment progress and the prognosis of the treatment outcome. Optimization of the distraction protocol or remedial measure can be initiated for alleviating undesirable outcomes as early as possible during the treatment (Aronson and Shin 2003).

Conventional radiography is a routine to assess the bone callus growth condition during DO treatment. The detection of subtle callus bone mineral density (BMD) changes during distraction period is a challenge to the plain-film radiography because of its sigmoid-shaped detector response (Aronson 2007; Eyres et al. 1993). Computed radiography (CR), capable of digital processing and dynamic linear detector response, is introduced for monitoring the callus growth during consolidation period (Fink et al. 2002; Hazra et al. 2008; Kolbeck et al. 1999). Nevertheless, limited studies evaluate the ability of CR in detecting the new bone formation during the distraction period. Assessment of BMD changes using radiography, however, is indirect because calibrated aluminum step-wedge thickness only serves as a surrogate measure, without correcting the extra- and intra-osseous soft tissues contribution. Callus BMD changes can also be measured by dual-energy X-ray absorptiometry (DXA) (Maffulli et al. 1997; Saran and Hamdy 2008) but the measured areal BMD is subject to the projectional bias (Babatunde et al. 2009).

Address correspondence to: Dr. Yau Ming Lai, Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, P. R. China. E-mail: yauming.lai@polyu.edu.hk

Furthermore, multiple soft-tissue components may confound the BMD measurement using DXA. Deviation from the two-component constraints of DXA would result in systematic errors as inherent in DXA methods (Bolotin 1998; Sorenson 1990). Although quantitative computed tomography (QCT) can evaluate the callus volumetric BMD and microstructural properties, the presence of external distractors causes metallic artifacts. It results in an inaccurate measurement of bone properties (Aronson and Shin 2003).

Ultrasound has significant roles in the evaluation of bone healing. Clinical studies report that two-dimensional (2-D) ultrasonography can assess the early bone formation during DO treatment (Maffulli et al. 1992; Richter et al. 1996). The planar sonographic images, however, may limit the demonstration of the callus condition such as its alignment and interior architecture. Instead, a serial ultrasound (US) scans across the distracting callus may allow quality three-dimensional (3-D) volume rendering and image reformation in different planes (Parmar et al. 2010), which optimizes the demonstration of callus growth changes. Subjective semiquantitative evaluation of echogenic features have been the cases for bone healing evaluation (Bruno et al. 2008; Kaban et al. 2003; Selim et al. 2009) but objective assessment of *in vivo* callus condition can be accomplished by quantitative acoustic parameters (ultrasonometry). Animal and clinical studies have demonstrated that the propagation velocity across fractured site can be used as an indicator of bone healing (Machado et al. 2011; Machado et al. 2010; Protopappas et al. 2008). *In vitro* trabecular bone specimen studies showed that ultrasonometry, such as speed of sound (SoS), acoustic reflection and attenuation, highly correlated to trabecular BMD (Hakulinen 2006; Hakulinen et al. 2005; Karjalainen et al. 2009), whereas acoustic backscattering significantly related to the trabecular microstructure (Chaffai et al. 2002; Deligianni and Apostolopoulos 2007; Hakulinen et al. 2005). Also, ultrasound penetration depth (UPD) within the distraction gap at the end of the consolidation period had strong associations with the maximum load and torsional stiffness of consolidated callus (Bail et al. 2002). There are scant studies using ultrasonometry to assess the callus growth condition during DO treatment. Acoustic reflection and backscattering, which possibly reflect the callus surface property and microstructural changes, respectively, are not examined in the previous DO studies (Bail et al. 2002; Daniel et al. 1994). These acoustic parameters measured at the early treatment stage may provide the prognosis of the treatment outcome such as the accretion of BMD and improvement of microstructural properties of consolidated callus.

In this study, the efficacies of CR and a combined US approach, which involved 2-D and 3-D ultrasonography with ultrasonometry, for monitoring the *in vivo* bone callus growth condition were evaluated by using a rabbit tibial DO model. With the microcomputed tomographic (μCT) evaluation of consolidated callus properties at the end of DO treatment, predictive values of parameters acquired from CR and the combined US approach measured in the distraction period were determined, respectively.

MATERIAL AND METHODS

Animals

Seven skeletally mature male New Zealand White rabbits (7 and 8 months old, 3.0–4.0 kg) were used. The rabbits were housed separately in standard cages in a temperature controlled room (22°C) with free access to food and water. The experiment was approved by the local Department of Health ([09–83] in DH/HA&P/8/2/4) and the university ethics committee (ASESC 09/40 & 10/012/MIS-5).

Surgery

Rabbit tibial lengthening was performed according to previous studies (Chan et al. 2006; Tis et al. 2002). Temgesic® (Schering-Plough, Hertfordshire, UK) (0.05 mg/kg) was given to all rabbits half an hour before the surgery. They were then generally anesthetised using IM injection of a mixture of ketamine (75 mg/kg) and xylazine (10 mg/kg). The right tibia was surgically incised at the anteriomedial surface. Periosteum and tibial muscles were carefully preserved and the medial tibial surface was exposed for bicortical drilling. Four pairs of pin-holes were drilled with a 1.5 and 2.0 mm drill bit at the tibial mid-diaphyseal region. A unilateral external distractor acted as a template for inserting four self tapping 2.0–2.5 mm pins (Orthofix International, Verona, Italy) into the pin-holes. Next, periosteum and soft tissues were protected. Using a low-energy oscillating saw (Synthes International, Umkirch bei Freiburg, Germany), transverse osteotomy was performed under saline irrigation between the second and third pin position, which was just below the tibiofibular junction. After sawing, bone misalignment was reduced before loading the external distractor. All soft tissues were retrieved to their original positions before the wound closure using biodegradable suture (Vicryl Plus; Johnson & Johnson, Somerville, NJ, USA). Postoperation and over the following 3 days, Temgesic® (s.c. 0.05 mg/kg, twice a day) was given to all rabbits for pain relief. Their health and behaviour were monitored daily.

Distraction and imaging protocol

Temgesic® (s.c. 0.04 mg/kg) was given to rabbits 30 min before each distraction. To achieve a distraction length of 12 mm, the distraction was started from day 8 to day 19 postoperation, with a distraction rate of 1 mm/day (0.5 mm in two increments with 7 h apart).