From the Department of Orthopaedics and Traumatology
Helsinki University Central Hospital
University of Helsinki
Finland

THE EFFECTS OF LOW-INTENSITY ULTRASOUND IN BIOABSORBABLE SELF-REINFORCED POLY-L-LACTIDE- FIXED CANCELLOUS BONE FRACTURE

An experimental and a clinical study by

Lauri Handolin

Academic Dissertation

To be presented, with permission of the Medical Faculty of the University of Helsinki, for public discussion in Auditorium of the Töölö Hospital, Helsinki University Central Hospital, on April 28th, 2006 at 12 o'clock noon.

Helsinki 2006

Supervised by: Adjunct Professor Esa K. Partio, M.D., Ph.D.
Dextra Medical Center and
University of Helsinki, Finland

and

Adjunct Professor Ilkka Arnala, M.D., Ph.D.
Department of Orthopaedics and Traumatology
Kanta-Häme Central Hospital and
University of Kuopio, Finland

Reviewed by: Adjunct Professor Ilkka Kiviranta, M.D., Ph.D.
Department of Orthopaedics and Traumatology
Jyväskylä Central Hospital, Finland

and

Adjunct Professor Harri Pihlajamäki, M.D., Ph.D.
Department of Orthopaedic Surgery and
Research Institute of Military Medicine
Central Military Hospital
Helsinki, Finland

Opponent: Professor Pekka Jalovaara, M.D., Ph.D.
Orthopaedics and Trauma Surgery
Oulu University Hospital
University of Oulu, Finland

ISBN 952-92-0078-1 (paperback)
ISBN 952-10-3049-6 (PDF)
http://ethesis.helsinki.fi/

Yliopistopaino
Helsinki 2006

ABSTRACT

Aims

The purpose of the present study was to investigate the effects of low-intensity ultrasound on bioabsorbable self-reinforced poly-L-lactide (SR-PLLA) screws and on fracture healing after SR-PLLA device fixation in experimental and clinical cancellous bone fracture.

Materials and methods

The present study consists of a set of five experimental and clinical studies. In the first experimental study, the assessment of the mechanical strengths of the SR-PLLA screws was performed after one, three, six, nine, and 12 weeks of daily 20-minute ultrasound exposure in vitro. In the second experimental study, 32 male Wistar rats with an experimental distal femur osteotomy fixed with an SR-PLLA rod were exposed for daily low-intensity ultrasound treatment for 21 days. The effects on the healing bone were assessed by plain radiographs and histological, microradiographical, oxytetracycline fluorescence, and histomorphometrical analyses three, six, and 12 weeks after the primary operation.

The clinical studies consist of three prospective, randomized, and placebo-controlled series of dislocated lateral malleolar fractures fixed with one SR-PLLA screw. The total number of the patients in these series was 52. Half of the patients were provided randomly with a sham ultrasound device. The patients underwent ultrasound therapy 20 minutes daily for six weeks. Radiological bone healing was assessed both by radiographs at two, six, nine, and 12 weeks and by multidetector computed tomography (MDCT) scans at two weeks, nine weeks, and 18 months. Bone mineral density was assessed by dual-energy X-ray absorptiometry (DXA) postoperatively, at 12 weeks, and at 18 months. The clinical outcome was assessed by both Olerud-Molander scoring and clinical examination of the ankle.

Results

Low-intensity ultrasound had no effects on the mechanical properties and degradation behaviour of the SR-PLLA screws in vitro. There were no obvious signs of low-intensity ultrasound-induced enhancement in the bone healing in SR-PLLA-rod-fixed metaphyseal distal femur osteotomy in rats. The biocompatibility of low-intensity ultrasound treatment and SR-PLLA was found to be good, both with SR-PLLA rods in the experimental distal femur osteotomy study and with SR-PLLA screws in the clinical lateral malleolar fracture series.

In the clinical series, low-intensity ultrasound was observed to have no obvious effects on the bone mineral density of the fractured lateral malleolus. In general, the bone mineral density tended to increase slightly during the follow-up regardless of the low-intensity ultrasound. There were no obvious differences in

the radiological bone healing times of the SR-PLLA-screw-fixed lateral malleolar fractures after low-intensity ultrasound treatment. Low-intensity ultrasound did not have any effects on radiological bone morphology, bone mineral density or clinical outcome in the SR-PLLA-screw-fixed lateral malleolar fractures 18 months after the injury.

Conclusions

The biocompatibility of bioabsorbable SR-PLLA fixation devices and low-intensity pulsed ultrasound is good and they can be safely combined in clinical practice. There were no obvious findings in the present study to support the hypothesis that low-intensity pulsed ultrasound enhances bone healing in SR-PLLA-rod-fixed experimental metaphyseal distal femur osteotomy in rats or in clinical SR-PLLA-screw-fixed lateral malleolar fractures. The slight increase in bone mineral density was symmetrical both in the active and sham ultrasound group, and the six-week ultrasound treatment had no effect on the increase. It is important to limit the conclusions of the present set of studies only to lateral malleolar fractures fixed with an SR-PLLA screw. Studies of low-intensity ultrasound also in other types of fractures with longer healing times and more problematic prognosis of healing are needed in the future.

LIST OF ORIGINAL ARTICLES

The present study is based on the following articles:

I. Handolin L, Pohjonen T, Partio EK, Arnala I, Törmälä P, Rokkanen P: The effects of low-intensity pulsed ultrasound on bioabsorbable self-reinforced poly-L-lactide screws. Biomaterials 23: 2733-2736, 2002

II. Handolin L, Partio EK, Arnala I, Pajarinen J, Pätiälä H, Rokkanen P: The effect of low-intensity pulsed ultrasound on bone healing in SR-PLLA rod fixed experimental distal femur osteotomy in rat. J Mater Sci: Mater Med (submitted)

III. Handolin L, Kiljunen V, Arnala I, Pajarinen J, Partio EK, Rokkanen P: The effect of low intensity ultrasound and bioabsorbable self-reinforced poly-L-lactide screw fixation on bone in lateral malleolar fractures. Arch Orthop Trauma Surg 125: 317-321, 2005

IV. Handolin L, Kiljunen V, Arnala I, Kiuru MJ, Pajarinen J, Partio EK, Rokkanen P: Effect of ultrasound therapy on bone healing of lateral malleolar fractures of ankle joint fixed with bioabsorbable screws. J Orthop Sci 10: 391-395, 2005

V. Handolin L, Kiljunen V, Arnala I, Kiuru MJ, Pajarinen J, Partio EK, Rokkanen P: No long- term effects of ultrasound therapy on bioabsorbable screw-fixed lateral malleolar fracture. Scan J Surg 94: 239-242, 2005

The above articles are referred to in the text by their Roman numerals.

ABBREVIATIONS

ALP	alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
CT	computed tomography
DXA	dual-energy X-ray absorptiometry
DVT	deep venous thrombosis
PDS	polydioxanone
PGA	polyglycolic acid
PLA	polylactic acid
PDLA	poly-D-lactic acid
PLLA	poly-L-lactic acid
M	molarity
MPR	multiplanar reconstruction
MRI	magnetic resonance imaging
mW/cm^2	milliwatts/square centimeter
NaCl	sodium chloride
Na_2HPO_4	sodium hydrogen phosphate
NaH_2PO_4	sodium di-hydrogen phosphate
SAFH	Sonic Accelerated Fracture Healing System™
SR	self-reinforced
SR-PGA	self-reinforced polyglycolide
SR-PLLA	self-reinforced poly-L-lactic acid
SATA	Spatial Average – Temporal Average
THM	treatment head module
US	ultrasound

1. INTRODUCTION

Open reduction and internal fixation form a general standard in the current treatment of many types of displaced fractures. The operative treatment aims at achieving anatomical reduction and ensuring early and active mobilization, which is crucial especially when treating fractures involving joints (Müller et al. 1991). The traditional metallic implants have shown problems such as corrosion and release of metallic ions into surrounding tissues (Cohen and Wulf 1972, Barbosa 1991, Galante et al. 1991). Also the stress-shielding phenomenon, i.e. osteopenia and reduced strength of bone due to an influence of a rigid metallic implant, increases the risk of refractures (Uhthoff and Dubuc 1971, Tonino et al. 1976, Woo et al. 1976, Paavolainen et al. 1978, Slätis et al. 1978, Rosson et al. 1991). In addition, metallic implants interfere in computed tomography (CT) and magnetic resonance imaging (MRI) by causing artifacts.

Sometimes metallic implants need to be removed in a second operation. Soft-tissue irritation and pain caused by metallic implants are common reasons for the removal procedure (Jacobsen et al. 1994). Removal operations cause increase in the total costs of fracture treatment (Böstman 1996, Juutilainen et al. 1997b) and unnecessary inconvenience to the patients involved. The replacement of metallic implants by bioabsorbable material eliminates the need for a removal operation. To avoid the disadvantages of metallic implants, bioabsorbable polymers have played the main role in the development of alternative fixation devices since the late 1960's (Schmitt and Polistina 1969). Bioabsorbable implants used for fracture fixation maintain initially the stability of fixation during the period of healing and resorb after that. The group of alpha-hydroxy acids, such as polyglycolide (PGA), polylactide (PLA), and polydioxanone (PDS) and their copolymers, has been used as raw material for absorbable sutures since the 1970's (Frazza and Schmitt 1971, Ray et al. 1981). The first studies using PLA implants were reported in maxillofacial surgery (Cutright et al. 1971, Cutright and Hunsuck 1972, Getter et al. 1972).

The modest strength values of the early implants required further development before they could be extensively used in orthopaedic and orthopaedic trauma surgery. The true break-through was achieved in the 1980's by Törmälä and his co-workers with the introduction of a new manufacturing method, the so-called self-reinforcing (SR) technique for PGA and PLA implants. In this new technique the bioabsorbable polymeric matrix was reinforced with oriented, fibrous reinforcing elements which had the same chemical composition as the matrix (Törmälä et al. 1987, Törmälä 1992). Bioabsorbable implants have been in clinical use for ankle fracture fixations since 1984 (Rokkanen et al. 1985). SR-PLLA screws came into the clinical use in July 1988. Since then, the implants have been greatly improved resulting in good outcome in clinical ankle fracture series (Partio et al. 1992a, Bucholz et al. 1994).

Wolff (1892) demonstrated a phenomenological relationship between the architecture of cancellous bone and the inferred locomotory forces acting upon the skeleton. That phenomenon was later referred to as "Wolff's law". The acoustic

pressure waves generated by the ultrasound signal, at least in theory, represent a non-invasive means of influencing the healing of fractures by providing a surrogate for the forces at work in Wolff's law. Two prospective and randomized studies (Heckman et al. 1994, Kristiansen et al. 1997) suggest that ultrasound has an enhancing effect on fresh bone fracture healing also in clinical settings.

It has been desirable to develop methods and appliances which would eliminate associated costs and decrease the total costs of medical treatment. The main benefit of the use of absorbable implants is the avoidance of a removal procedure reducing the total costs and the length of sick-leave (Partio et al. 1992a). There are no previous studies on low-intensity ultrasound treatment combined with bioabsorbable fixation devices. The present thesis, consisting of five sets of studies, was launched to find out if the above-mentioned goals could be met in the cancellous bone fracture treatment after bioabsorbable SR-PLLA fixation.

2. REVIEW OF THE LITERATURE

2.1. POLY-L-LACTIDE

2.1.1. Chemical properties of poly-L-lactide

Polylactide is a synthetic crystalline thermoplastic polymer of cyclic diesters (Gilding and Reed 1979). Polylactic acid is a hard, pale-colored, semi-crystalline polymer with thermoplastic properties. The lactic acid is asymmetric. Thus, polylactic acid exists in two enantiomeric forms; poly-L-lactic acid (PLLA) and poly-D-lactic acid (PDLA). Polymers with high molecular weights suitable for orthopaedic devices are exclusively produced by the anionic ring opening polymerization of the cyclic lactides under the influence of a low catalyst (inorganic metal salt) concentration (Lowe 1954, Schneider 1955, Cutright et al. 1974, Vert et al. 1981, 1984, Jamshidi 1984, Bendix 1998). PLLA is a stereoregular homopolymer derived from pure L-lactide. Poly-L-lactide retains its strength longer than poly-D-lactide, which has made it more popular as orthopaedic implant material. A high crystalline PLLA with a molecular weight of over 100 000 has its melting point at 174-184 ^{o}C with a glass transition temperature of 57-58 ^{o}C (Vert et al. 1981, Jamshidi 1984, Hollinger and Battistone 1986, Törmälä et al. 1998).

2.1.2. Biodegradation of poly-L-lactide

Polylactide undergoes a two-stage degradation process in the living tissue. In the first phase water splits the chemical bonds of PLLA chains to lactide, mainly by a non-specific hydrolysis. In the second phase, lactide becomes incorporated into the mitochondrial citric acid cycle and is subsequently excreted by the lungs as carbon dioxide and through the urine as water (Miller et al. 1977, Williams 1981, Williams 1981, 1982, Hollinger and Battistone 1986). At the cellular level, polylactide first degrades to small particles. The particles trigger the phagocytocis reaction, and both macrophages and multinuclear giant cells digest the polymer fragments (Woodward et al. 1985, Bos et al. 1991, Rozema et al. 1994, Laitinen et al. 2002, Böstman et al. 2005).

The strength of the SR-PLLA implant is of the level of cancellous bone in 36 weeks (Majola et al. 1992), but remnants of PLLA have been detected in human tissue even after five years of implantation (Bergsma et al. 1995, Tams et al. 1996, Suuronen et al. 1998). An in vitro degradation study on SR-PLLA screws showed that the implant degrades slowly and still retains 90 % of its initial bending strength after 26-week immersion in saline at 37°C (Pohjonen et al. 1997). An experimental sheep study revealed a total degradation of the SR-PLLA screw, used for subcapital femoral head osteotomy fixation, at seven years (Jukkala-Partio et al. 2002).

2.1.3. Biocompatibility of poly-L-lactide

PGA seems to be immunologically inert, leading only to slight non-specific lymphocyte activation (Santavirta et al. 1990). However, a mild inflammatory or foreign-body reaction was observed already in the early studies of PGA sutures (Herrman et al. 1970, Gammelgaard and Jensen 1983). Clinically a foreign-body reaction occurs from eight to 16 weeks after the implementation of the PGA implant (Rokkanen et al. 2000). Partio et al. (1992a) observed that the rate of soft tissue reactions was lower in PGA implants without dye. The incidence of the foreign-body reaction and accumulation of fluid at the area of the implant has recently been under 2 % and has hardly ever occurred with the use of polylactide implants (Rokkanen et al. 2000).

Clinically manifest adverse inflammatory reactions have been reported to accompany mainly the use of PGA devices, but, in some cases, late foreign-body reactions have also been reported after fixation with PLLA devices (Böstman et al. 1990, Barfod and Svendsen 1992, Hoffmann et al. 1992, Bucholz et a. 1994, Bergsma et al. 1995, Böstman et al. 1995). The biocompatibility of PLLA materials has been investigated in several experimental and clinical studies (Majola et al. 1991, Pihlajamäki et al. 1992, Pihlajamäki et al. 1994, Matsusue et al. 1997, Viljanen et al. 1997, Nordström et al. 1998, Nordström et al. 2001). The tissue tolerance and degradation of the PLLA implants in displaced ankle fractures were studied by Böstman et al. (1995) in a clinical trial of 51 patients with a mean follow-up time of 52 months. They observed a foreign-body reaction to PLLA screws in the ankle fracture only in one patient at 22 months after the operation, the incidence being thus less than 2 %. Voutilainen et al. (2002) reported SR-PLLA-implant-related clinical soft tissue swelling to appear generally relatively late, 40-115 months after the primary surgery. The first 1043 clinical operations carried out with SR-PLLA implants at the Department of Orthopaedics and Traumatology, University of Helsinki, were analysed by Juutilainen et al. (2002). There were no clinical sinus formations, but three patients out of 1043 had soft tissue fluid accumulations in the area of the inserted implant.

The infection rate between the metallic and absorbable fixation devices in displaced ankle fractures in a total of 3111 patients was observed to be equal with both fixation methods (Sinisaari et al. 1996). The bioabsorbable devices studied were made of PGA, PLLA or their copolymers. The rate of deep infection was 0.4 %, and the infections were mostly caused by micro-organisms of the Staphylococcus species (Sinisaari et al. 1996). In another study consisting of PLLA fixation devices in different kinds of fractures, arthrodeses, and osteotomies, the infection rate was found to be 0,7 % (Sinisaari et al. 1995).

2.1.4. Mechanical properties of poly-L-lactide

The first polylactide implants were manufactured with traditional techniques such as melt-extrusion, compression moulding, and injection moulding. However, bioabsorbable polymers as such are either too brittle or weak and flexible for safe clinical use. For the non-reinforced PLLA, the bending strength values are

between 113-145 MPa, the shear strength values between 53-68 MPa, and the bending modulus between 3-5 GPa (Törmälä 1992, Pohjonen and Törmälä 1996). These values are below those measured for cortical bone: bending strength of 180-190 MPa, shear strength of 68 MPa, and bending modulus of 9,5-11 GPa (Reilly and Burstein 1975, Tonino et al. 1976).

The self-reinforcing (SR) technique for ultra-high strength bioabsorbable implants has been developed by a Finnish research group under the leadership of professors Pentti Rokkanen and Pertti Törmälä (Törmälä et al. 1987, Törmälä 1992). In this method, fibers of PLLA are sintered together at a high temperature and pressure leading to an implant, in which the matrix and the reinforcing fibers are of the same material (SR-PLLA). SR-PLLA screws were introduced into clinical use in 1988. For the SR-PLLA, the bending strength values are between 200-300 MPa, the shear strength values between 94-220 MPa, and the bending modulus between 7-10 GPa (Törmälä 1992, Majola et al. 1992). These figures are highly comparable to those of cortical bone, making the strength of SR-PLLA implants well sufficient for bone fixation.

2.2. LOW-INTENSITY ULTRASOUND

2.2.1. Medical applications of ultrasound

Ultrasound, a form of mechanical energy that is transmitted through and into biological tissues as an acoustic pressure wave, is widely used in medicine as therapeutic, operative, and diagnostic tool (Ziskin 1987, Maylia and Nokes 1999). The frequency of ultrasound is more than 20 000 Hz being above the limit of human hearing. Therapeutic and some operative ultrasound devices use intensities as high as 1-5 W/cm^2 which may cause heating in living tissues. Intensities up to 300 W/cm^2 are needed for using ultrasound as surgical instrument, and with very high intensities the tissue heating can be considerable. The rise of temperature in tissues is due to transformation of one form of energy (sound) to another (heat). Ultrasound is propagated via a series of compression and rarefaction waves induced in the molecules of the medium through which it passes. At high power the rarefaction cycle may exceed the attractive forces of the molecules and cavitation bubbles will be formed. The succession of positive and negative pressures (acoustic pressure wave) can cause oscillatory motions of bubbles, which relates to a process known as acoustic cavitation (ter Haar and Daniels 1981). This can result in cell membrane disruption and even release of free radicals (Price et al. 1997).

Much lower magnitudes of intensities, 1-50 mW/cm^2, are used in non-invasive medical diagnostic devices, such as used in imaging the structures of vital organs, peripherial blood flow, and fetal development. The intensity level used for imaging, which is of the magnitude below that used for surgery, is regarded as non-thermal and non-destructive (Brown 1984). Nevertheless, low-intensity ultrasound of 30 mW/cm^2 delivered by Sonic Accelerated Fracture Healing System (SAFH) 2 device (Exogen Inc., NJ, USA) used in the present study, is a mechanical force holding a potential to influence bone mass and morphology through the strong sensitivity of bone tissue to physical stimuli.

2.2.2. Biological effects of low-intensity ultrasound

The ultrasound stimulator used in this study functions by producing a low-intensity pulsed ultrasonic signal conducted to tissues as mechanical energy. The molecular and chemical means by which this mechanical stimulus may be translated into biologic response in living tissues is unknown. In 1892 Wolff demonstrated a phenomenological relationship between the architecture of cancellous bone and the inferred locomotory forces acting upon the skeleton (Wolff's law). Wolff's law suggests that the form and architecture of bone adapt to the mechanical environment by remodeling to accommodate the magnitude and direction of the applied stress. Therefore, the acoustic pressure waves generated by the ultrasound signal, at least in theory, represent a non-invasive means of influencing the healing of fractures by providing a surrogate for the forces at work in Wolff's law.

There are reports on the biological effects caused by the static mechanical forces (Binderman et al. 1988) and pressure waves of the mechanical perturbation of the ultrasound (Chapman et al. 1980, Ryaby et al. 1991). These pressure waves may mediate biological activity directly by mechanical deformation of the cell membrane or indirectly by an electrical effect caused by cell deformation. In an in vitro study low-intensity ultrasound stimulated osteogenesis in cell cultures by increased calcium incorporation in chondrocytes (Ryaby et al. 1989). Intracellular concentration of calcium is also reported to be elevated in ultrasound-stimulated rat chondrocytes, implying that the ultrasound-stimulated synthesis of the cell matrix proteoglycan may be mediated by intracellular calcium signaling (Parvizi et al. 2002). Another in vitro study revealed that low-intensity ultrasound had a stimulatory effect on endochondral ossification, possibly due to the stimulation of bone cell differentiation and calcified matrix production (Korstjens et al. 2004). An in vitro human periosteal cell study indicated that low-intensity ultrasound stimulated periosteal cell proliferation and differentiation toward osteogenic lineage (Leung et al. 2004). The effects were seen in increased alkaline phosphatase (ALP) activity and osteocalsin secretion, and the responses were observed to be dose-dependent. Increased ALP activity and osteoblast cell count have also been reported in laboratory tests after low-intensity ultrasound stimulation of rat calvaria osteoblasts (Sun et al. 2001).

2.3. PREVIOUS STUDIES

2.3.1. Bioabsorbable fixation and fracture healing

2.3.1.1. Bioabsorbable implants in experimental fracture settings

Ideal devices in operative fracture treatment should support the fracture long enough and then decompose gradually transferring the stress to the healing bone, thus preventing the loss of mineral content and strength of bone tissue. There are several trials of different PLLA implants used for both cortical and cancellous bone fracture fixation experimentally with favorable results. Viljanen et al. (1995) suggested that distal femoral osteotomy fixed with SR-PLLA screws showed a

more rapid and better healing than when fixed with metallic screws. They assessed the healing with CT and MRI. In another study Viljanen et al. (1997) observed that the stress-protection effect of intramedullary fixation on the femoral diaphyse may be avoided by using an absorbable SR-PLLA rod instead of a metallic one. Miettinen et al. (1992) reported on SR-PLLA-rod-fixed femoral diaphyse osteotomy in the growing rabbit; the SR-PLLA rod did not cause any disturbance to the longitudinal growth of the femur. SR-PLLA implants have also been found appropriate for fixation of mandibular body and condyle osteotomies in the sheep experimental model (Suuronen et al. 1992a, Suuronen et al. 1992b).

Nordström et al. (2001) conducted a trial of SR-PLLA-fixed distal femoral osteotomies in the rat showing that the biocompatibility of SR-PLLA was good and they also observed the same type of signs of osteostimulatory effects of SR-PLLA as published earlier by Hollinger (1983) with a copolymer of PLA and PGA. The possible osteogenic capacity of bioabsorbable materials has been further studied by Hollinger (1983), Pihlajamäki et al. (1994b), Ikada et al. (1996), and Juutilainen et al. (1997). The so-called piezoelectric effect is the external potential difference generated directly by straining polarizable materials. This effect is ascribed to the change of the microscopic orientation of crystals having fundamentally an asymmetrical structure (Cady 1964, Korostoff 1979). An outstanding feature of piezoelectric materials is the automatic generation of electric fields which is achieved simply by applying mechanical strains to the materials without an external power supply. These strains may have biologic significance (Basset 1968). Ikada et al. (1996) suggested that the promotion of fracture healing in experimental diaphyseal tibial fracture in the cat fixed with an intramedullary SR-PLLA rod can be ascribed to the piezoelectric current generated by the strains accompanying the leg movement.

2.3.1.2. Bioabsorbable implants in clinical fracture settings

In several clinical studies biodegradable fixation devices have been proved to be suitable to be used for different kinds of fractures and osteotomies: ankle fractures (Rokkanen et al. 1985, Partio et al. 1992a, Bucholz et al. 1994), chevron osteotomies (Hirvensalo et al. 1991), radial head fractures (Hirvensalo et al. 1990, Pihlajamäki 1992, Pelto et al. 1994), hand fractures (Kumta et al. 1992), fractures of the distal radius (Casteleyn et al. 1992, Hoffman et al. 1992), fractures of the humeral capitellum (Hirvensalo et al. 1993), humeral medial epicondylar fractures (Partio et al. 1996), transphyseal fractures of the humeral capitellum in children (Böstman et al. 1989), distal femoral epiphyseal fractures (Partio et al. 1997), olecranon fractures (Hope et al. 1991), patella fractures (Juutilainen et al. 1995), and talocrural joint arthrodeses (Partio et al. 1992b).

2.3.1.3. Bioabsorbable implants in ankle fractures

Bioabsorbable implants have been used for ankle fracture fixation since 1984 (Rokkanen et al. 1985). During these decades the implants have been greatly improved resulting in good outcome in clinical ankle fracture series (Böstman et al. 1987, Böstman 1989b, Partio et al. 1992a, Bucholz et al. 1994, Pihlajamäki et al.

1994a). However, biodegradable implants have been found incompatible with ankle fractures of alcoholics due to poor co-operation. Kankare et al. (1995) reported that eight patients out of 16 treated with bioabsorbable self-reinforced polyglycolide (SR-PGA) fixation had postoperative redisplacement of the fracture and six had to be re-operated. On the other hand, the results of ankle fractures of the elderly patients treated with biodegradable implants are well comparable with those with metallic ones (Kankare et al. 1996).

The outcome of all 1202 fractures of the ankle operated at the Department of Orthopaedics and Traumatology (Helsinki) between 1984 and 1994 with biodegradable implants made of polyglycolide-polylactide copolymer or SR-PGA and/or SR-PLLA was analysed by Pelto-Vasenius et al. (1997). They observed that the total redisplacement rate was 2.5 %, being 0.9 % in the simple ankle fractures and 8.2 % in the severe ankle fractures. The conclusion was that bioabsorbable implants seem to provide a secure fixation in the majority of ankle fractures, but the use of these implants showed unsatisfactory results in unstable and comminuted fractures (Pelto-Vasenius et al. 1997).

2.3.2. Low-intensity ultrasound

2.3.2.1. Low-intensity ultrasound in experimental fracture healing

Pilla et al. (1990) reported significant acceleration in the healing of fibular osteotomy in rabbits after 20-minute daily exposure to low-intensity ultrasound. Wang et al. stated in 1994 that endochondral ossification may be stimulated by increased chondrocyte aggrecan gene expression due to the acoustic pressure waves of ultrasound and reported an increase in the bending strength of the rat femoral fracture model. The increased mechanical properties of the rat femur fracture model after low-intensity ultrasound stimulation were also reported by Yang et al. (1996) and Azuma et al. (2001).

Gebauer et al. (2002) evaluated the effects of daily low-intensity ultrasound application on mid-diaphyseal femoral fractures in rats with and without diabetes mellitus. They reported significantly greater torque to failure and stiffness in the ultrasound-treated diabetes mellitus rats compared to the non-diabetes mellitus rats at six weeks post-fracture, stating that the application of ultrasound clearly results in improved mechanical properties during the late phases of healing (Gebauer et al. 2002). There is even a report of the use of a diagnostic ultrasound device with an intensity of 11.8 mW/cm^2, which is one-third of the intensity normally used (30 mW/cm^2) in studies of low-intensity ultrasound, in the rat femur fracture model showing signs of accelerated fracture healing (Heybeli et al. 2002). Aynaci et al. (2002) observed an increased rate and quality of spinal fusion using muscle-pediculated bone grafts in conjunction with low-intensity ultrasound in rabbits.

Callus distraction is an orthopaedic procedure used mainly for long bone transportation techniques to lengthen the bone in case of bone loss. There are animal studies on the use of low-intensity ultrasound with attempts to shorten the healing time of newly formed distracted bone. Mayr et al. (2001) reported a

significantly accelerated maturation of the regenerate in the sheep metatarsus distraction model. Eberson et al. (2003) had positive results on femur distraction in rats reporting an increase in the mechanical strength, while Tis et al. (2002) reported an increased size of callus but no effect on the mechanical strength of tibiae distraction in rabbits. On the contrary, Uglow et al. (2003) reported no effect of low-intensity ultrasound on the distraction osteogenesis of rabbits with respect to the bone mineral content, cross-sectional area in quantitative CT or mechanical strength. Histologically no differences were observed in the bone volume fraction either; however ultrasound-treated regenerates appeared to have fewer trabeculae of increased thickness and fewer osteoclasts (Uglow et al. 2003).

One possible reason behind the potentially faster bone healing process may be the change or increase in vascularity during healing. Rawool et al. (2003) reported that power Doppler sonography showed an increase in the vascular flow around the fracture site and surrounding soft tissue in the dog ulna osteotomy model.

2.3.2.2. Low-intensity ultrasound in clinical fracture healing

Five original articles on low-intensity ultrasound exposure in fresh fracture treatment have been published before the present study: two on cortical bone (tibial diaphyse) and three on cancellous bone healing (distal radius, scaphoid, base of the fifth metatarsal). The number of reviews, including case reports and meta-analyses, is greater than that of original articles. The use of ultrasound for treatment of fresh fractures has been evaluated in two multicenter, prospective, double-blind, and placebo-controlled clinical trials (Heckman et al. 1994, Kristiansen et al. 1997). Heckman et al. (1994) performed a trial of 67 closed or grade-I open and non-operatively treated tibial fractures to evaluate the effect of ultrasound on the healing of cortical fractures. They reported a 24 % reduction in the time to clinical healing after daily 20-minute ultrasound exposure with an intensity of 30 mW/cm^2 as well as a 38 % decrease in the time to overall (clinical and radiological) healing. Kristiansen et al. (1997) performed a trial of 61 dorsally angulated fractures of the distal radius to determine the effect of ultrasound on the healing of fractures in areas consisting primarily of trabecular bone. The time to union was reported to be 38 % shorter for the fractures treated with 20-minute daily ultrasound, and the ultrasound treatment was also observed to be associated with a significantly smaller loss of reduction during the healing process.

Cook et al. (1997) performed a further analysis of the data of Heckman et al. (1994) and Kristiansen et al. (1997) and reported that the use of low-intensity ultrasound was associated with a significant reduction in the healing time of fractures of the tibia and the distal radius in smoking patients. Mayr et al. (2000) reported the results of a prospective, randomized, and controlled study on 30 fresh scaphoid fractures treated with 20-minute daily low-intensity ultrasound exposure. They stated that computed tomography-assessed fracture healing was 30 % faster in the ultrasound group. Strauss et al. (1999) performed a trial of 20 patients with a fresh fracture at the base of the fifth metatarsal (Jones fracture) treated with the standard orthopaedic technique and showed that the healing time was shorter in the ultrasound group.

In contrast to Heckman et al. (1994), Emami et al. (1999) noticed no difference in the radiographically assessed healing times of intramedullary-fixed tibial fractures after 75 days of low-intensity ultrasound treatment. The study protocol was placebo-controlled and randomized with the total number of patients being 32. The fractures were closed or grade-I open diaphyseal fractures treated with a reamed and locked intramedullary nail.

There are also reports of the influence of low-intensity ultrasound treatment in delayed unions and non-unions with a wide array of sites (Frankel et al. 1998, Nolte et al. 1999, Gebauer et al. 2000, Mayr et al. 2000), but none of them has a randomized and controlled study setting. Nolte et al. (2001) reported a series of 29 non-union cases treated with daily 20-minute low-intensity ultrasound. They concluded that the ultrasound therapy can be useful in the treatment of challenging, established non-unions. The efficacy of the treatment was assessed retrospectively on the within-patient, self-paired control basis.

2.3.2.3. Low-intensity ultrasound and biodegradable materials

There are no studies on the mechanical effects of low-intensity ultrasound on bioabsorbable bone fixation devices, such as SR-PLLA screws or rods. Neither are there studies combining low-intensity ultrasound and metallic screw fixation devices. However, bioabsorbable materials have been used in terms of developing controlled drug-release methods to achieve the release of drugs in vivo over a prolonged period of time. To achieve this, released drugs are often incorporated in bioabsorbable polymers which are then surgically or otherwise placed in the desired compartment of the body. Release of the drugs takes place at a steady rate, and, in some cases, it would be advantageous to have additional drug release on demand. There are reports on ultrasound exposure in terms of increasing the biodegradation rate of the polymers used as a “container” (Kost et al. 1989, Agrawal et al. 1994), thus increasing the drug release rate.

Kost et al. (1989) reported up to five-fold reversible increases in the degradation rate of polyanhydrides, polyglycolides, and polylactides in vivo. The release rate of molecules incorporated within polymers increased in proportion to the intensity of ultrasound which was increased up to 5 W/cm^2 being hundred-folds of the magnitude used in the present study (30 mW/cm^2). Instead, Agrawal et al. (1994) observed that 1.5 W/cm^2 ultrasound exposures had a three-fold increase in the release of incorporated proteins from 50-50 % copolymer of PLA and PGA and both the ultrasound frequency and the signal duration affected the molecular weight loss and mass loss.

Lin et al. (1994) attempted to speed up the bone regeneration rate and bioglass absorption rate with low-intensity ultrasound. They reported that ultrasound with an intensity of 0.5 W/cm^2 had a profound effect on the rate of both the bone regeneration and bioglass absorption rate in the rabbit femur condyle model. The intensity used by Lin et al. (1994) was ten-fold in magnitude compared to the one used in the present study.

2.3.2.4. Low-intensity ultrasound and bone mineral density

Bone is a dynamic tissue with a well-balanced homeostasis preserved by both formation and resorption of bone. The normal turnover of bone can be disturbed by either increased osteoclast activity or decreased osteoblast function; either mechanism alone or together may result in a net loss of bone. Li et al. (2003) demonstrated that ultrasound with an intensity of 600 mW/cm^2 could enhance osteoblast population in vivo and concluded that ultrasound stimulation might be a good method to prevent bone loss due to osteoporosis. The intensity Li et al. (2003) used was 20-fold in magnitude compared to the one in the present study.

Shimazaki et al. (2000) reported that treatment with ultrasound with an intensity of 30 mW/cm^2 for 20 minutes daily resulted in increased bone mineral density (BMD) during bone maturation in distraction osteogenesis in rabbits. On the contrary, Spadaro and Albanese (1998) used the same intensity 20 minutes daily for growing femoral and tibial bone for four weeks but were not able to detect any difference in the bone mineral density between the ultrasound and control group. They suggested that physeal bone growth is far less sensitive to low-intensity ultrasound.

Lower extremity bone loss is common sequelae of immobilization and non-weight-bearing due to spinal cord injury. A clinical and controlled trial of 15 patients was performed by Warden et al. (2001) who used low-intensity ultrasound of 30 mW/cm^2 20 minutes daily for six weeks on the calcaneal bone in spinal cord injury patients. They observed that ultrasound, at least with the intensity they used, is not a beneficial intervention for osteoporosis induced by a spinal cord injury; there were no differences between the actively and in-actively treated groups in any skeletal measure. Warden et al. (2001) also concluded that their finding may primarily relate to the inability of ultrasound to penetrate the outer cortex of bone due to its acoustic properties.

3. THE PRESENT STUDY

3.1. AIMS

The aims of the present study were to find answers to the following questions:

1. Does low-intensity ultrasound have an effect on the mechanical properties of SR-PLLA screws in vitro (I)?

2. Does low-intensity ultrasound have an effect on bone healing in SR-PLLA-rod-fixed experimental distal femur osteotomy in rats (II)?

3. Can SR-PLLA fixation be safely combined with low-intensity ultrasound in experimental (II) and clinical settings (III-V)?

4. Does low-intensity ultrasound have an effect on bone mineral density in SR-PLLA-screw-fixed lateral malleolar fractures (III)?

5. Does low-intensity ultrasound have an effect on bone healing in lateral malleolar fractures fixed with an SR-PLLA screw (III-IV)?

6. Does low-intensity ultrasound have a long term effect on healing bone, bone mineral density or clinical outcome in SR-PLLA-screw-fixed lateral malleolar fractures (V)?

3.2. GENERAL REMARKS ON THE PRESENT STUDY

The use of bioabsorbable implants for different kinds of fractures, osteotomies, and arthrodeses is well documented. There is also some data of low-intensity ultrasound therapy as adjunctive to fracture treatment. However, there are no studies of bioabsorbable fracture fixation combined with low-intensity ultrasound therapy either in experimental or clinical settings. The present trial consists of five separate studies creating a general view of the combined use of low-intensity ultrasound and bioabsorbable fixation devices.

As no basic data existed on the combined use of low-intensity ultrasound and poly-L-lactide acid screws, the first study comprising a total of 96 SR-PLLA screws was aimed to assess the biocompatibility of SR-PLLA with low-intensity ultrasound. After that, it was possible to continue with an experimental animal study. In the second study a total of 32 male Wistar rats were operated on with one SR-PLLA rod in each. The animal study was approved by the Ethics Committee of Helsinki University Central Hospital.

After two experimental studies, the trial continued with clinical studies on lateral malleolar fractures of the ankle. The lateral malleolar fracture is a cancellous bone fracture with a good healing tendency. A relatively short follow-up period (12 weeks) for fracture-healing assessment was chosen, since the consolidation of the lateral malleolar fracture usually occurs within six weeks. On the other hand, the final clinical outcome was assessed at 18 months. The present clinical study of 52 fractures consisted of three series of 52 patients altogether. All of the studies were prospective, randomized, and placebo-controlled series of dislocated lateral malleolar fractures fixed with one SR-PLLA screw. Altogether 52 SR-PLLA screws were used for the clinical study. All clinical and radiographic analyses were performed blind to the ultra-sound treatment, the code indicating whether it was an active or sham ultrasound device was broken after that.

The clinical study was approved by the Ethics Committee of Helsinki University Central Hospital. The patients were informed of the new method of combining low-intensity ultrasound with bioabsorbable fracture fixation and of the possible adverse effects, after which a written consent was obtained. Patients aged 18-65 years were included. The patients with compromised co-operation, such as alcoholics and mentally disturbed persons, were excluded. Also the patients with diabetes mellitus were excluded. The patients who refused to participate in the study were not included and they were treated with metallic implants in a normal way. The data of the refused patients were not recorded.

3.3. IMPLANTS

The bioabsorbable SR-PLLA rods and screws used in the present study were manufactured by Bionx Ltd. (at present Linvatec Biomaterials Ltd.), Tampere, Finland, with the self-reinforcing technique (Törmälä et al. 1987, Törmälä 1992). The implants were sterilized by using a minimum dose of 25 kGy gamma irradiation. All implants were manufactured without dye (Partio et al. 1992a).

The SR-PLLA rods used in the experimental animal study had a diameter of 2.0 mm and length of 15 mm. They were straight and cylindrical in shape. The bending strength of the SR-PLLA rods was 200-300 MPa, the shear strength 100-180 MPa, and the bending modulus 6 GPa. The SR-PLLA screws used in the first experimental study had a diameter of 4.5 mm and a length of 35 mm (48 screws) and 70 mm (48 screws), and in the clinical study the diameter was 4.5 mm and length 30-45 mm. The screws were fully threaded having an outer diameter of 4.5 mm and an inner diameter of 3.2 mm. The bending strength of the SR-PLLA screws was 200-250 MPa, the shear strength 110 MPa, and the bending modulus 5-6 GPa.

3.4. LOW-INTENSITY ULTRASOUND DEVICE

The low-intensity pulsed ultrasound treatment used in the present study was performed with a Sonic Accelerated Fracture Healing System (SAFH) 2 device (Exogen Inc., NJ, USA). The ultrasound pressure wave signal was composed of a pulse burst width of 200 microseconds containing approximately 300 sine wave pressure pulses, each of the duration of approximately 6.7 nanoseconds (frequency of 1.5 megahertz). The 200-microsecond burst of pressure pulses was followed by an “off-time” of 800 microseconds and was therefore repeated every millisecond (repetition rate of 1 kilohertz). The intensity of the pressure wave applied to the skin at the fracture site was 30 mW/cm^2 presented as the Spatial Average – Temporal Average (SATA). SATA is defined as the maximum intensity averaged over time and the spatial area of the transducer. The treatment time was 20 minutes a day.

All patients were provided with an ultrasound device and instructed to use it daily. The first treatment was performed during the two weeks out-patient clinics together with the investigator. Detailed instructions over the general use of the device and the placement of the treatment head module (THM) were given to the patients. Half of the devices were active (US-group) and half were sham (non-US group). The designated length of the treatment period was six weeks (42 days). The patients were randomly provided with either an active or a sham ultrasound device in a double-blind manner.

All patients performed the ultrasound treatment daily without knowing whether the device was active or sham. It was not possible for the patients to sense the activity or non-activity of the device. After the THM was fastened into a designated place over the fracture line, the device was switched on manually by pressing a button.

The automatics of the device took care of the rest by telling the remaining time and the end of the 20-minute treatment session by switching itself automatically off. It was possible to switch the device on again only after 12 hours had passed since the last 20-minute session, which prevented any bias caused by an inappropriate over-use of the device. The devices were returned to the manufacturer after the treatment for downloading the log in order to obtain the data and control the compliance to designated daily treatments.

3.5. EXPERIMENTAL ULTRASOUND EXPOSURE ON BIOABSORBABLE SCREWS

3.5.1. Materials and methods

A total of 96 SR-PLLA screws with a diameter of 4.5 mm were used for the study. Forty-eight screws (length 70 mm) were used for the bending tests and 48 screws (length 35 mm) for the shear tests. The molecular weight was investigated on the 70 mm screws after the bending tests (12 screws). The SR-PLLA screws were immersed in Na_2HPO_4 (2.87 g/l) - NaH_2PO_4 (0.67 g/l) buffered saline (NaCl 5.9 g/l) at pH = 7.4 and M = 0.127 and kept at 37 ^oC. No antibiotics were used. Buffered saline was replaced every two weeks.

The ultrasound exposure was performed with an SAFH 2 device. The daily ultrasound exposure lasted 20 minutes. The intensity of the pressure wave applied to the screws in buffered saline was 30 mW/cm2. For the ultrasound exposure the screws were placed in a metallic container filled up with similar buffered saline. The treatment head module was placed on the bottom of the container through a hole (Fig. 1). The ultrasound exposure was performed from day one on seven times a week. The control screws were kept in buffered saline at 37 ^oC until the investigations. Changes in the mechanical and molecular weight were determined at 0, 1, 3, 6, 9, and 12 weeks.

The bending strength of the SR-PLLA screws before and after the ultrasound exposure was measured by the three-point bending method, using a Lloyd 6000R Materials Testing Machine (Lloyd Instruments PLC, U.K.) at room temperature (22-23 ^oC). Four samples of both the ultrasound-exposed and control screws were tested at 0, 1, 3, 6, 9, and 12 weeks (Fig. 2). The bending strength (b) was calculated using the following equation: $b = 8FmL/D3$, where Fm = maximum force recorded (in Newtons), L = distance between the lines of support (mm) and D = inner diameter of the screw (mm).

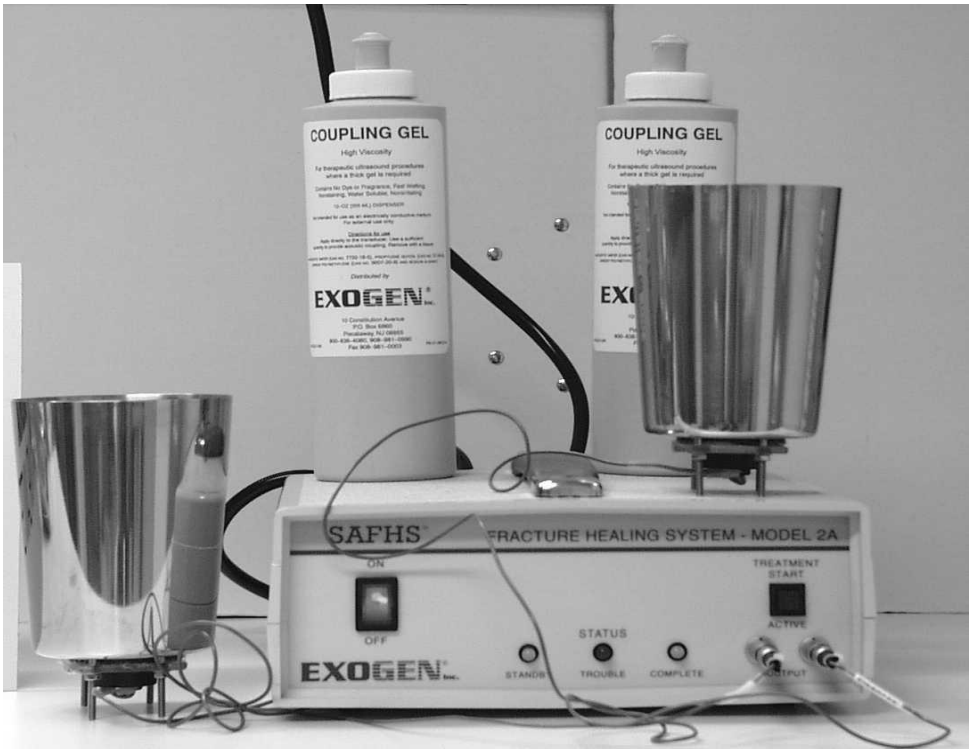

Figure 1. Sonic Accelerated Fracture Healing System (SAFH) 2 device with treatment head modules attached on the bottom of two metallic containers.

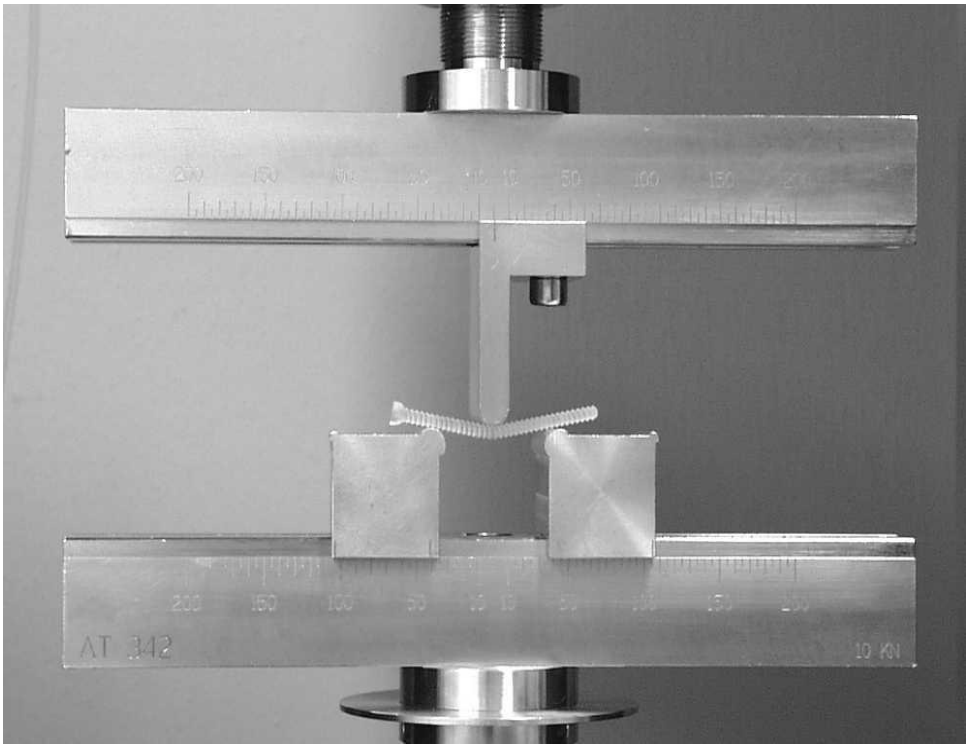

Figure 2. Three-point bending method used for testing the bending strength.

The shear strength of the SR-PLLA screws was measured by means of a tool constructed by modifying the standard BS (British Standard) 2782, Method 340 B (1978). The tool consisted of two parts which were joined together by the implant. The parts were pulled apart during the test using a Lloyd 6000R Materials Testing Machine so that the implant resting in a drill hole was cut into pieces perpendicular to the long axis of the screw (Fig. 3). The shear strength (s) was calculated using the following equation s = F / 2A, where F = force at the fracture (N) and A = cross-sectional area of the test screw.

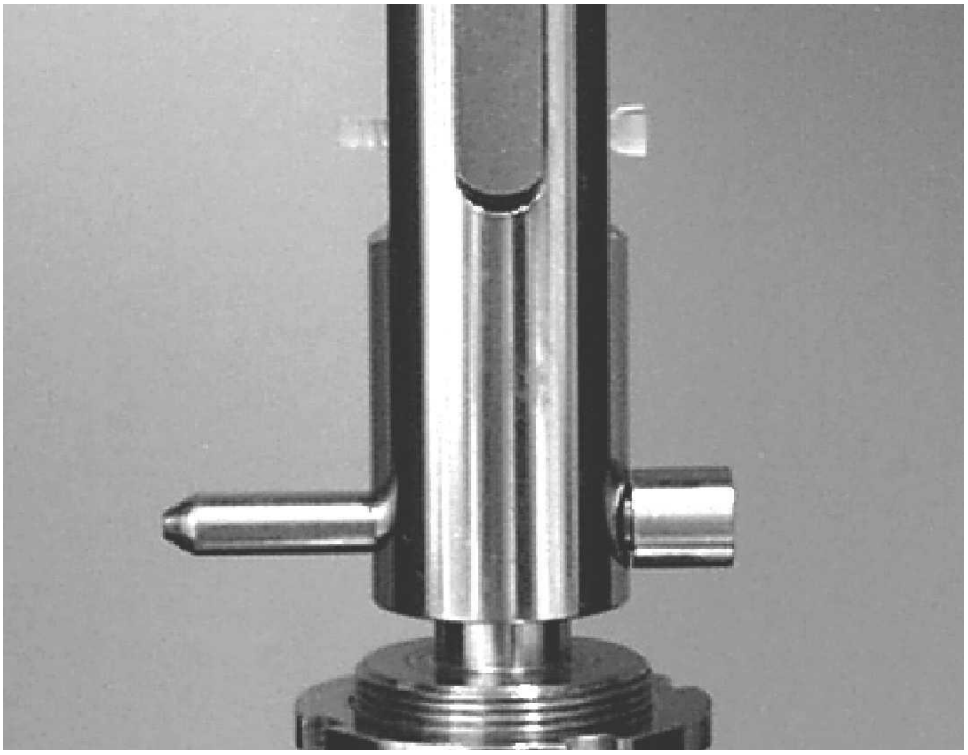

Figure 3. Two parts of the shear-strength-testing tool joined together by the implant.

The solution viscosities {according to ASTM (American Society for Testing and Materials) 445 - 88} of the SR-PLLA screws before and after the ultrasound exposure were measured in chloroform at 25 ^{o}C with an Ubbelohde capillary viscometer (type 0a according to ASTM 446). The intrinsic viscosities (n in g/dl) were obtained by the linear regression analysis from the dilution series (0,1 g/dl, 0,2 g/dl, 0,3 g/dl and 0,5 g/dl), and the viscosity-average molecular weights (in g/mol) were calculated using the Mark-Houwink equation and parameters determined by Schindler et al. (1979): [n] = 5,45 x 10 – 4 x MV0,73 ([n] = intrinsic viscosity dl/g).

The statistical calculations were performed using the analysis of variance. The numeric values are presented as means with a standard deviation (SD). P-values <0.05 were considered statistically significant.

3.5.2. Results

In the bending strength tests there were no signs of statistically significant diminishing during the 12-week immersion time, neither inside the two groups nor between the two groups. There was, however, a slight tendency towards improvement in the bending strength during the 12-week ultrasound exposure. In the shear strength tests there was a statistically significant slight diminishing in both groups during the 12-week immersion time. On the other hand, there were no differences between the control group (non-US-group) and the ultrasound exposure group.

In the molecular weight tests there was a statistically significant reduction in both groups during the 12-week immersion time. The decrease in the molecular weight in both groups was due to normal hydrolysis of the SR-PLLA screws. On the other hand, there were no statistically significant differences between the control group and the ultrasound exposure group. The results of the measurements are given in Table 1.

	Bending strength (MPa)				Shear strength (MPa)				Molecular weight (g/mol: 1000)			
	Non-US-group		US-group		Non-US-group		US-group		Non-US-group		US-group	
Wk.	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	182.6	(4.5)	181.0	(3.9)	115.6	(6.5)	117.5	(2.0)	62.8	(2.9)	59.3	(2.3)
1	182.9	(8.7)	183.3	(3.1)	114.5	(3.6)	110.4	(1.3)	55.4	(2.7)	53.8	(2.1)
3	184.6	(5.2)	184.2	(7.1)	110.9	(6.7)	110.4	(4.2)	54.7	(2.1)	58.9	(1.7)
6	176.9	(1.9)	189.2	(3.6)	107.9	(2.0)	112.0	(3.4)	50.8	(1.0)	55.5	(3.5)
9	181.7	(2.8)	185.8	(2.3)	113.4	(6.0)	107.0	(2.8)	49.3	(2.5)	49.0	(2.6)
12	173.1	(5.2)	187.3	(4.1)	108.6	(5.7)	111.5	(2.9)	44.8	(0.1)	48.4	(0.5)
All	180.3	(6.2)	185.1	(4.7)	111.8	(5.6)	111.5	(4.1)	53.0	(6.1)	54.1	(4.8)

Table 1. The bending strength, shear strength, and molecular weight of a 4.5 mm SR-PLLA screw after 0-12 weeks of low-intensity ultrasound exposure, presented as means and standard deviation (SD)(analysis of variance with no statistical significances (p<0.05) between the groups) . Wk. = duration of ultrasound exposure in weeks.

3.5.3. Comments

There was a minor tendency towards improvement in the bending strength of the SR-PLLA screws during 12-week daily ultrasound exposure. In practice this finding has no clinical relevance, since the decrease of the molecular weights is symmetrical in both of the examined groups indicating that the material degrades in a similar manner. Also an earlier in vitro degradation study on SR-PLLA screws showed that the implant degrades slowly and still retains 90 % of its initial bending strength after 26-week immersion in buffered saline at 37°C (Pohjonen et al. 1997).

It seems that ultrasound exposure does not impair the bending strength of 4.5 mm SR-PLLA screws during a period of 12 weeks. It also seems that ultrasound exposure does not impair the shear strength or accelerate the normal diminishing of the molecular weight of these screws, but the effects of normal hydrolysis can be seen after 12 weeks. The present study shows that low-intensity pulsed ultrasound does not affect significantly the mechanical properties and degradation behaviour of SR-PLLA screws in vitro. This provides a safe starting-point to clinical trials on biodegradable fixation combined with low-intensity ultrasound.

3.6. EXPERIMENTAL DISTAL FEMORAL FRACTURE IN RATS

3.6.1. Experimental animals, operative care, and ultrasound exposure

A total of 32 male Wistar rats with a mean weight of 407 g (range 326-497 g) were operated on at the age of 20 weeks. The rats were randomly divided into two groups prior to the operation, 16 rats to the ultrasound (US) group and 16 to the control group. The US- and control groups were further divided into three follow-up subgroups, five rats into the three- and six-week subgroups, and six rats into the 12-week subgroup. The rats were anesthetized with subcutaneous injections of ketamine (Ketalar, Parke-Davis, Spain) 75 mg/kg and medetomidine (Domitor, Orion-Pharmos, Finland) 0.5 mg/kg. A single subcutaneous dose of 100.000 IU procaine penicillin (Procapen, Orion, Finland) was administered preoperatively for infection prophylaxis.

The right hind leg was shaved and scrubbed with an antiseptic fluid (Neo-Amisept, Orion-Pharmos, Finland), and a medial parapatellar incision with a lateral dislocation of the patella was performed. The femoral medullary canal was opened through the intercondylar region and drilled up to the depth of 20 mm with a 2.0 mm pneumatic drill, and a transverse transcondylar osteotomy was created with an oscillating saw. The osteotomy was reduced exactly and fixed with one 15 mm (diameter 2 mm) SR-PLLA rod (Bionx Ltd, Tampere, Finland). The incision was closed in layers with 3-0 polyglycolide sutures (Dexon, Davis and Geck, United Kingdom). Postoperative radiographs were taken in antero-posterior and lateral views. After the recovery from anesthesia, the rats were returned to their gages,

two rats in each. They were fed ad libitum with a regular laboratory animal diet and water. No external support of the operated limb was used.

The ultrasound exposure was performed with an SAFH 2 device. The daily 20-minute ultrasound exposure was started on the second postoperative day for a period of 21 days (US-group). For exposure, the rats were anesthetized with subcutaneous injections of medetomidine (Domitor, Orion-Pharmos, Finland) 0.15 mg/kg and immobilized in a ventral position with the right hind leg abducted and externally rotated. The treatment head module applied with coupling gel was fastened over the medial aspect of the distal femur and knee. The control-group rats were anesthetized and immobilized similarly to the ultrasound-group rats for 20 minutes from the second postoperative day on for a period of 21 days without ultrasound exposure. This was done to avoid any bias caused by the effect of immobilization or nutritional intake on bone healing during the daily anesthesia and recovery period.

3.6.2. Tissue sampling techniques

The rats were killed with an overdose of sodium pentobarbital (Mebunat, Orion, Finland) 60 mg/kg at three weeks (five ultrasound and five control rats), six weeks (five ultrasound and five control rats), and 12 weeks (six ultrasound and five control rats) postoperatively. A dose of 50 mg/kg of hydrochloride oxytetracycline (Terramycin, Pfizer, Belgium) was injected intramuscularly three days before killing for oxytetracycline labeling studies. Both femora were dissected free, and radiographs were taken. The osteotomy was determined as united if 3/4 or 4/4 cortices were united in the antero-posterior and lateral radiographic views. Any signs of infection or other complication were visually observed. The specimens were fixed in a series of ethanol immersions with increasing concentrations (70-99 %) and embedded in methylmethacrylate.

For histological and histomorphometrical evaluation, 5-µm-thick longitudinal sections were cut with a Polycut S microtome (Reichert-Jung, Germany). The sections were stained using the Masson-Goldner trichrome method (Goldner 1938). For microradiographic and oxytetracycline labeling studies, 80-µm-thick longitudinal sections were cut with a Leitz Saw Microtome 1600 (Leitz, Germany). The microradiographs were made using the Faxitron Xray system Model 43855A (Hewlett Packard, USA) and high resolution ultraflat plates type 1 A (Imtec Products, USA). The histologic, oxytetracycline fluorescence, and microradiographic specimens were studied with a Diaplan microscope (Leitz, Germany). The fluorescence microscopic analysis was performed using an HBO 220 ultraviolet lamp (Osram, Germany) and a BG 812/6 primary filter (Leitz, Germany).

For semiautomatic quantitative histomorphometrical analysis, a Leitz microscope was linked via a video camera (Color View II, Soft Imaging System GmbH, Münster, Germany) to a computer (Dell Precision 340, Ireland). Magnifications of 13.0 x and 78.8 x were used. The image-analysing software was AnalySIS docu 3.2 (Soft-Imaging Software GmbH, Münster, Germany). Both femora were analysed in each rat, the left femur acting as control. Four standardized sample

fields were determined in each femur around the lateral implant channel 6.0 mm from the distal joint level of the lateral condyle and 1.5 mm apart in the horizontal direction (Fig. 4). The AnalySIS-program was used in the determination of the corresponding sample field. Within the 1.46 mm x 1.08 mm (1.57 mm2) sample fields, the histomorphometrical variables were analysed. The variables were as follows: total tissue area, total area of trabecular bone, total length of the trabecular bone circumference, total length of the osteoid, and total length of the osteoblast lines (Frost 1983). The ongoing calcification of the osteoid was confirmed by fluorescence microscopy and microradiography (Jowsey et al. 1965).

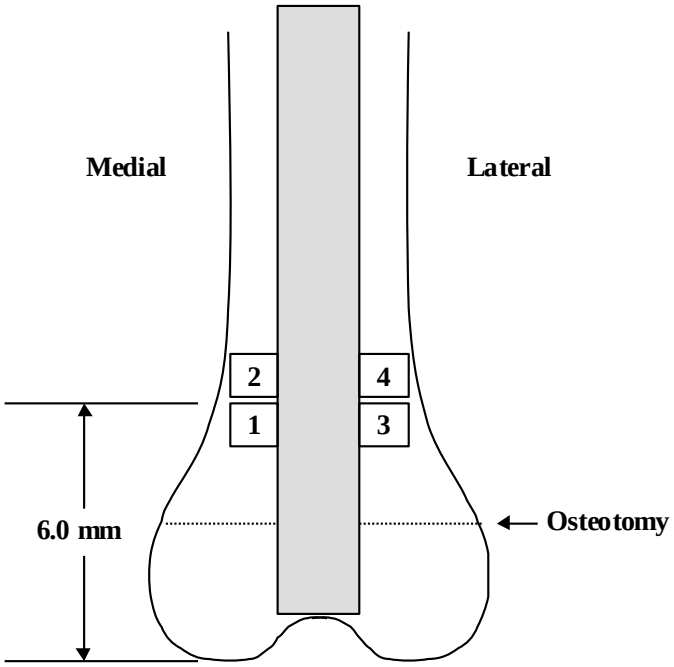

Figure 4. Schematic anterior view of the distal femur showing the position of the pin and the four standardized sample fields (1, 2, 3, 4), each measuring 1.46 mm x 1.08 mm.

All assessments of the sections were done by one investigator to avoid any bias caused by interobserver variation. The differences of means between the treatment groups, at given times, were compared using the two-tailed independent samples t-test.

3.6.3. Results

3.6.3.1. Clinical and macroscopical results

One 12-week control group rat had to be killed on the second postoperative day due to a primary operation-related iatrogenic femoral diaphyseal fracture. The remaining rats tolerated the three-week daily anesthesia, resulting in 31 rats with a complete follow-up. However, the three-week daily anesthesia was observed to be a major exertion and stress for the rats. It was seen in the alteration of the body weights during the follow-up; during the first 21 days the rats lost 14 % of their initial weight (mean weight loss 54 g, range 29-75 g), but after that they started to gain weight again. Compared to the initial body weights (mean 407 g, range 326-497 g), at six weeks the mean body weight increased by 5 % (mean 449 g, range 361-517 g) and at 12 weeks by 13 % (mean 458, range 393-523 g). The alteration in the body weights was symmetrical in the ultrasound and control groups. One three-week control-group rat and one 12-week ultrasound-group rat were observed to have a displaced and non-united osteotomy due to a technical failure at the primary operation resulting in an intercondylar fracture. No signs of wound healing problems, such as superficial infections or fistulas, were observed. Mild synovitis with osteoarthrotic lesions of the femur condyle was seen in four rats (two in the six-week ultrasound group, and one in the six-week and one in the 12-week control group).

3.6.3.2 Radiological, histological, microradiographical, and oxytetra-cycline fluorescence results

At three weeks, radiologically all five osteotomies seemed united in the ultrasound group compared to two out of four osteotomies in the control group. No difference was observed in the visualization of the osteotomy line between the groups. Histologically at three weeks solid bony unions through the osteotomy line were seen in two out of five osteotomies in the US-group compared to one out of four osteotomies in the control group. Microradiographically there was periosteal callus formation in all cases, but solid bony unions through the osteotomy line were observed in the same cases as seen in the histological analyses. There was strong oxytetracycline uptake periosteally and endosteally in both groups. The beginning of the bony rim around the implant was also seen in all cases.

After six weeks, radiologically all osteotomies seemed united and the fracture lines were almost faded out both in the ultrasound and control group (Fig. 5). An external radiological callus formation was noted in three cases in both groups. Histologically at six weeks a solid bony union through the osteotomy line was seen in four out of five osteotomies in both groups (Fig. 6). Microradiographically there was periosteal callus formation in all cases, and one osteotomy in both groups was not completely united (Fig. 7). The periosteal oxytetracycline uptake was almost similar to that of the three-week uptake and it was even in the US- and control group. A clearly visible bony rim around the implant was observed in all specimens.

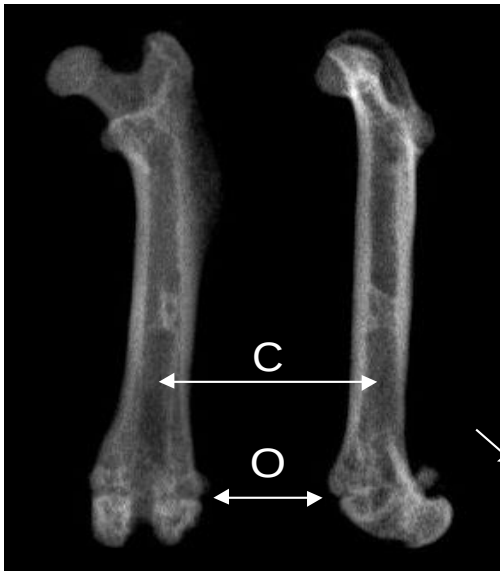

Figure 5. Antero-posterior and lateral radiographs of the control-group-rat (no ultrasound) femur at six weeks. The implant channel (white letter C) is seen clearly. The osteotomy line (white letter O) has faded and is hardly seen.

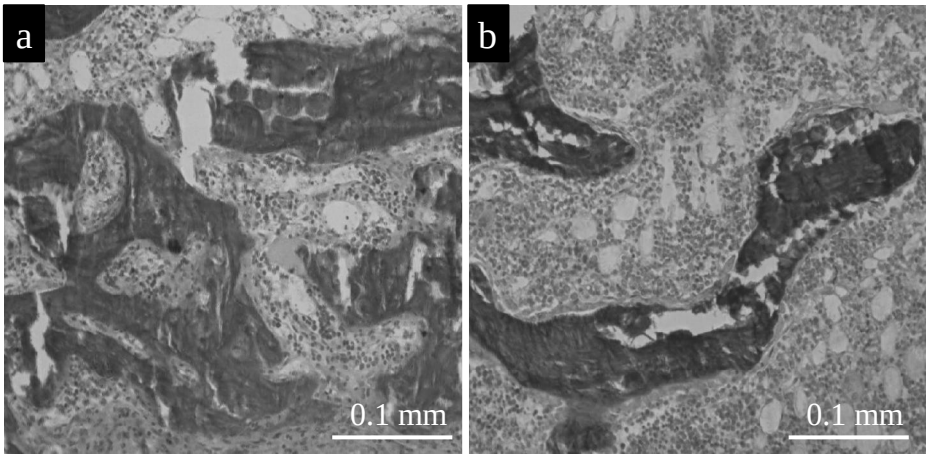

Figure 6. (a) Photomicrograph of the osteotomized site after 21-day ultrasound exposure at six weeks. The osteotomy is consolidated. (b) Photomicrograph of the same area in a control-group rat (no ultrasound) at six weeks. The bone structure is similar in both groups (original magnification X 125).

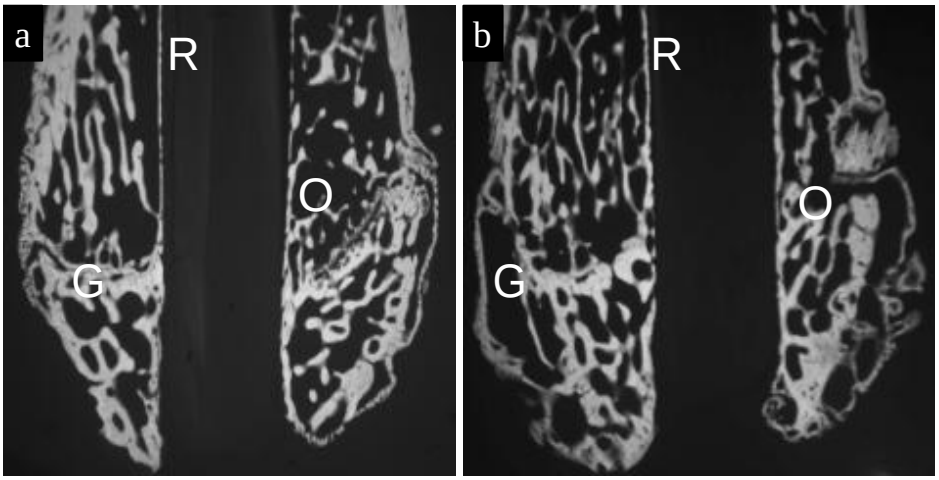

Figure 7. (a) Microradiograph of the osteotomized site after 21-day ultrasound exposure at six weeks. The osteotomy (white letter O) is consolidated. Remnant of the growth cartilage (white letter G) is seen. The bony rim around the implant channel is seen clearly (white letter R). (b) The bone structure and consolidation are similar to those of the control group (no ultrasound) (original magnification X 20.6).

At 12 weeks, all the osteotomies seemed radiologically united both in the ultrasound and control group, excluding one osteotomy with the above-mentioned iatrogenic and, later on, dislocated intercondylar femoral fracture in the US-group. External radiological callus formation was seen in four US-group specimens, but in none of the control group. Histologically a solid bony union through the osteotomy line was seen in three and four out of five osteotomies in the US- and control group, respectively. The bony rim around the implant was clearly seen in both groups. Microradiographically the new bone was mineralized as normal bone. Two osteotomies in the US-group and one in the control group were observed only partially united. The oxytetracycline uptake around the osteotomy line was symmetrically smaller in both groups compared to six weeks.

3.6.3.3. Histomorphometrical results

In the US-group the mean total trabecular bone area fraction over the total tissue area was slightly decreased during the follow-up period. However, there was no statistical difference between the US-group and the control group (non-US-group) at three, six or 12 weeks. The mean trabecular bone circumference fraction over the total tissue area rose slightly up to six weeks and then started to diminish towards 12 weeks. The alteration was symmetrical in both study groups, and no statistical differences were observed. The total osteoid length fraction over the total tissue area was statistically higher in the control group at three weeks (difference of means 0.463, 95 % confidence interval 0.870 – 0.056). At six weeks it was higher in the US-group and at 12 weeks in the control group, but the differences were not statistically significant. Also the total length of the osteoblast

line over the total tissue area was statistically higher in the control group at three weeks (difference of means 0.662, 95 % confidence interval 0.884 – 0.439). At six and 12 weeks it was higher in the US-group, but the difference was of no statistical significance. The histomorphometrical results are presented in Table 2.

Follow-up (weeks)	Total trabecular bone area fraction over the total tissue area (%)		Trabecular bone circumference fraction over the total tissue area (%)		Total osteoid length over the total tissue area (%)		Total osteoblast line length over the total tissue area (%)	
	US-group	Non-US-group	US-group	Non-US-group	US-group	Non-US-group	US-group	Non-US-group
3	0.27 (0.19)	0.24 (0.10)	4.18 (1.90)	4.98 (2.13)	0.52 (0.52)*	0.98 (0.58)	0.04 (0.08)*	0.07 (0.10)
6	0.20 (0.14)	0.23 (0.07)	4.23 (2.22)	5.07 (1.20)	0.67 (0.90)	0.58 (0.41)	0.07 (0.17)	0.02 (0.04)
12	0.21 (0.14)	0.23 (0.13)	3.87 (1.46)	3.54 (0.98)	0.50 (0.75)	0.80 (0.70)	0.03 (0.06)	0.00 (0.00)

*Table 2. Results of the histomorphometric analysis of the tissue-implant interface in the SR-PLLA-rod-fixed distal femoral osteotomy in rats after 21-day ultrasound exposure (US-group) or without it (non-US-group) at three, six, and 12 weeks (mean and SD). t-test with two-tailed interpretation, * ($p<0.05$).*

3.6.4. Comments

The rats had to be anesthetized for the ultrasound exposure, as otherwise it would have been impossible to hold them still for 20 minutes. Generally it took about two to three hours for the rats to recover from the anesthesia after the daily ultrasound exposure. During the recovery period the rats did not eat much and moved only a little. Therefore also the control-group rats were anaesthetized every day during the three postoperative weeks, since a daily immobilization period and cessation in the nutritional intake caused by the anesthesia during the recovery phase probably had an effect on the normal bone healing process. The observed changes in the mean body weights were symmetrical in the ultrasound and control group; therefore it is assumed that the groups were comparable regarding mobilization and nutritional intake.

The biocompatibility of SR-PLLA and ultrasound was found to be good. No foreign-body reactions were noted. Four rats had mild synovitis with osteoarthrotic lesions of the femur condyle which were more probably related to the arthrotomy and the operation itself than to poor biocompatibility. In the radiological and histological assessments there was a slight tendency for enhanced fracture healing in the ultrasound group at three weeks. After six weeks no differences were observed, since the distal femoral osteotomy is generally united by that time. Mobilization and weight bearing of the operated limb cause torque and micro-movement between the osteotomy lines until the consolidation occurs, which may

increase callus formation. However, ultrasound had no clear effect on the callus formation in the consolidation phase during the first three weeks. After six weeks, there was a tendency for increased callus formation in the ultrasound group, which is controversial to the assumption that micro-movement stimulates callus formation, since the fractures were already consolidated by that time.

Oxytetracycline labeling results in deposition of a fluorochrome label on bone surfaces undergoing active mineralization at the time of the labeling. In the present study, the oxytetracycline fluorescence was observed to be strong at three and six weeks, indicating that there was an intense ongoing mineralization. At 12 weeks, the rate of active mineralization was lower. However, the amount of the oxytetracycline uptake was observed to be even in the ultrasound and control groups during the follow-up, which suggests that ultrasound exposure had no obvious effect on the rate of mineralization around the healing osteotomy line or the adjacent periosteum. The histomorphometrical results showed that at 12 weeks the active osteoid formation was slow both in the US- and control group, indicating that bone healing had occurred within normal time. However, the active osteoid formation was found to be faster in the control group at three weeks. Later on no difference was seen between the groups, indicating that ultrasound had no effect on the rate of new bone formation.

The results of the present study show that there were no obvious findings to support the hypothesis that low-intensity pulsed ultrasound enhances bone healing in SR-PLLA-rod-fixed metaphyseal distal femur osteotomy in rats. On the other hand, the observed good biocompatibility provides a safe basis for clinical trials on biodegradable fixation combined with low-intensity ultrasound.

3.7. CLINICAL ANKLE FRACTURE STUDIES

3.7.1. Patients, operative technique, and ultrasound treatment

The present study comprising three clinical series included altogether 52 patients. The operations were performed between October 2001 and March 2003 at the Department of Orthopaedics and Traumatology, Helsinki University Central Hospital. The inclusion criterion was a grossly dislocated (both lateral and rotational dislocation, and shortening) Weber-B lateral malleolar fracture due to a low-energy injury in a patient aged 18-65 years. The exclusion criteria were multiple injuries, widening of the distal tibio-fibular joint space (combined syndesmotic injury), open fracture, and poor overall co-operation.

The patients were operated on with the similar technique in supine position under tourniquet. All operations were performed by two orthopaedic trauma surgeons. The fracture was exposed through a lateral incision. After reduction it was fixed with one 4.5 mm SR-PLLA screw (Bionx Ltd, Tampere, Finland) placed anteriorly. The protruding screw head was removed with an oscillating saw, and the wound was rinsed before closure. Postoperatively the ankle was immobilized for six weeks with a removable brace made out of Soft Cast® (3M, St. Paul, MN, USA).

Weight-bearing was not allowed for the first two weeks and only partially for the next two weeks. After four weeks the patients were allowed to bear full weight.

The patients were provided randomly in a double-blind manner with either an active (US-group, 26 patients) or a sham (non-US-group, 26 patients) SAFH 2 device (Exogen®, NJ, USA). The patients were instructed to use the device for six weeks (42 days), 20 minutes daily, starting from the third postoperative week to the end of the eighth week. Afterwards every individual device was returned to the manufacturer for downloading the report of its daily use. The target area for the ultrasound treatment was on the lateral side of the ankle directly over the fracture line. For daily treatment, the patients fastened the treatment head module with adjustable straps exactly on the right place using the scar and certain suture holes as landmarks. Coupling gel was used to secure contact to the skin. The brace was removed for the period of ultrasound treatment and replaced after that.

The statistical calculations were performed with SPSS for Windows using the independent samples test and Fisher's exact test. The numeric values are presented as means, with a standard deviation (SD) and range. When the differences of means are compared between the ultrasound and non-ultrasound group, they are presented with 95 % confidence intervals (CI). Intervals excluding 0 and p-values <0.05 were considered statistically significant.

3.7.2. Follow-up

3.7.2.1. Thirty patients assessed by radiographs and bone mineral density measurements

The first clinical series consisted of 30 patients assessed with radiographs and bone mineral density measurements. The patients were seen by the same investigator at out-patient clinics on four different appointments after the operation. Every patient had a follow-up of 12 weeks. Fracture healing was assessed by anterior and lateral radiographs taken immediately, two, six, nine, and 12 weeks postoperatively. A visualization of the fracture line and the first appearance of external callus were assessed visually by three orthopaedic surgeons working in consensus. BMD in the lateral malleolus was measured with DXA scans (Hologic QDR-4500W, Hologic Inc., Bedford MA, USA) using the forearm software (version 8.26) both immediately and 12 weeks postoperatively. For measuring purely the lateral malleolar region, the ankle was placed in a specially manufactured rack to provide a slightly oblique AP-view to prevent the tibio-fibular overlapping in the distal syndesmotic region. The area of interest in the distal fibula was determined to be 50 mm, starting 10 mm from the tip of the lateral malleolus (Fig. 8).

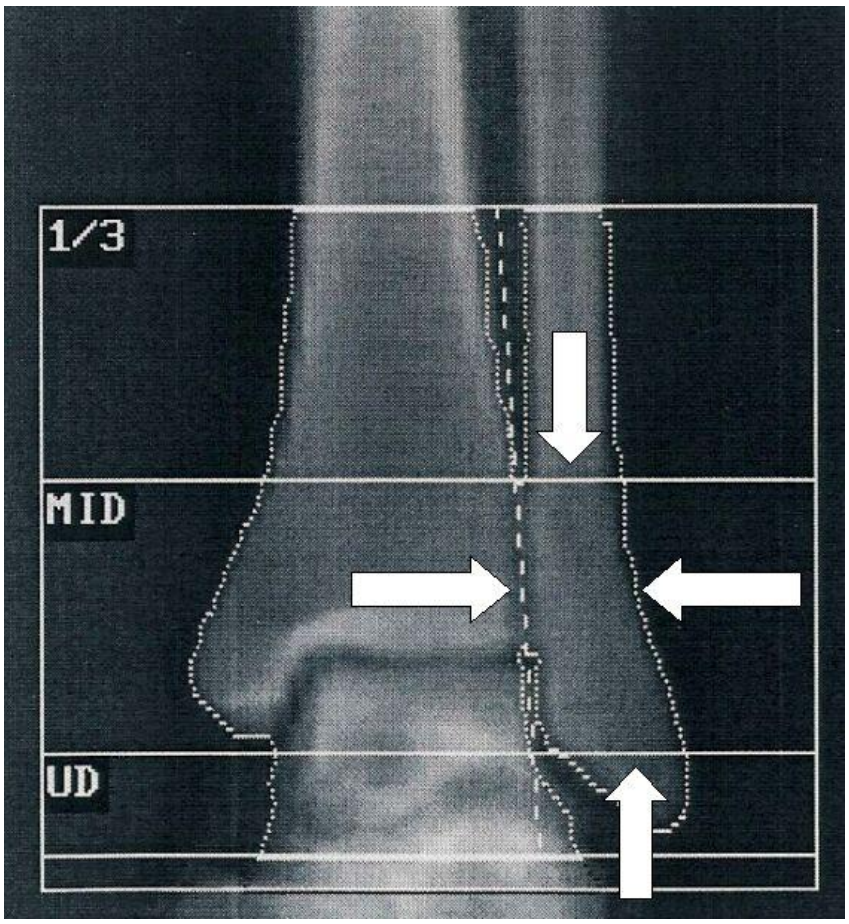

Figure 8. Bone mineral density in the lateral malleolus was measured with dual-energy X-ray absorptiometry. The area of interest (white arrows) in the distal fibula was determined to be 50 mm, starting 10 mm from the tip of the lateral malleolus.

3.7.2.2. Twenty-two patients assessed by computed tomography scans

The second clinical series consisted of 22 patients assessed by radiographs and computed tomography. The patients were seen by the same investigator at out-patient clinics on four different appointments after the operation. Every patient had a follow-up of 12 weeks. Fracture healing was assessed by both plain radiographs and CT scans (LightSpeed QX/i; GE Medical Systems, Milwaukee, USA). Plain anterior and lateral radiographs were taken immediately, two, six, nine, and 12 weeks postoperatively. A visualization of the fracture line and the first appearance of external callus were assessed visually by three orthopaedic surgeons working in consensus. Routine CT examinations of the ankle were performed two and nine weeks after the operation as follows: 4 x 1.25 mm collimation, interval 0.62 mm, gantry rotation time 1.0 s, pitch 3, table feed 3.75 mm, kV 100, mA 100, approximately total exposure time 10 s. Routine multiplanar reconstructions (MPR) were done in three planes (coronal, axial, and sagittal): slice thickness of 1.0 mm and reconstruction increment of 1.0 mm. Endosteal bone healing in all three planes was assessed by a radiologist measuring the share of the united fracture

line to the non-united line in the slice representing the central part of the fracture line. Endosteal bone healing is presented as the mean share of these three planes.

3.7.2.3. Sixteen patients followed-up for 18 months

The third clinical study consisted of 16 consecutive patients from the beginning of the first series with an extended follow-up of 18 months. At 18 months, the clinical outcome was assessed by both Olerud-Molander scoring (Olerud and Molander 1984) (Table 3) and clinical examination of the ankle (wound, range of movements, stability). The fracture site was assessed by both plain radiographs and CT scans (LightSpeed QX/i; GE Medical Systems, Milwaukee, USA) at 18 months by a musculoskeletal radiologist. Routine MPRs were done in three standard planes (coronal, axial, and sagittal). The BMD in the lateral malleolus was measured at 18 months with DXA scans (Hologic QDR-4500W, Hologic Inc., Bedford MA, USA) using the forearm software (version 8.26). The 18-month BMD assessments were combined with the ones achieved by the same method during the primary follow-up of 12 weeks, resulting in three separate BMD measurements for each patient. The area of interest in the distal fibula was determined to be 50 mm, starting 10 mm cranially from the tip of the lateral malleolus as previously shown in Figure 8. All the analyses were performed blind to the ultra-sound treatment, the code indicating whether it was an active or sham device was broken only afterwards at 18 months.

Parameter	Degree	Score
I Pain	None	25
	While walking on uneven surface	20
	While walking on even surface outdoors	10
	While walking indoors	5
	Constant and severe	0
II Stiffness	None	10
	Stiffness	0
III Swelling	None	10
	Only evenings	5
	Constant	0
IV Stair-climbing	No problems	10
	Impaired	5
	Impossible	0
V Running	Possible	5
	Impossible	0
VI Jumping	Possible	5
	Impossible	0
VII Squatting	No problems	5
	Impossible	0
VIII Supports	None	10
	Taping, wrapping	5
	Stick or crutch	0
IX Work, activities of daily life	Same as before injury	20
	Loss of tempo	15
	Change to a simpler job/part-time work	10
	Severely impaired work capacity	0
Maximum		100

Table 3. Scoring system for symptom evaluation after malleolar fractures according to Olerud and Molander (1984).

3.7.3. Results

3.7.3.1. Radiographical and bone mineral density results

The patient characteristics and operational data of the first series (30 patients) are presented in Table 4. The groups were comparable. The only statistically significant difference between the two groups was the length of the used SR-PLLA screw (mean length 40.3 mm US vs. 36.3 mm non-US, difference of means 4.0, 95 % CI: 0.7 – 7.3, p=0.019).

	US-group (n=15)		Non-US-group (n=15)		difference of means	95 % confidence interval	p
	mean	range	mean	range			
Age	41.4	(19-65)	39.4	(18-59)	1.9	-8.8 – 12.4	0.709
Sex	male 5 /	female 10	male 8 /	female 7			0.462
Hospital stay / days	2.6	(2-4)	2.8	(1-7)	-0.2	-1.1 – 0.7	0.658
Operation time / min.	31.5	(19-50)	33.9	(19-60)	-2.5	-10.1 – 5.1	0.511
Screw length / mm	40.3	(35-45)	36.3	(30-45)	4.0	0.7 – 7.3	0.019*
US device used / days	40.7	(18-47)	39.9	(30-45)	0.7	-3.8 – 5.3	0.745

Table 4. Patient characteristics and operational data of 30 patients assessed by plain radiographs and bone mineral density measurements.

All except two reductions of the fractures were categorised as excellent. All fractures united uneventfully, and no re-displacements were observed during the follow-up. In the plain radiographs, the fracture line was usually most visible at two weeks post-operatively and faded with progressing consolidation of the fracture during the following weeks. No difference was observed between the groups. A callus formation, most often dorsally at the proximal part of the fracture line, was seen in five, 11, and 14 US-patients vs. three, nine, and 12 non-US-patients at six, nine, and 12 weeks, respectively (Fig. 9).

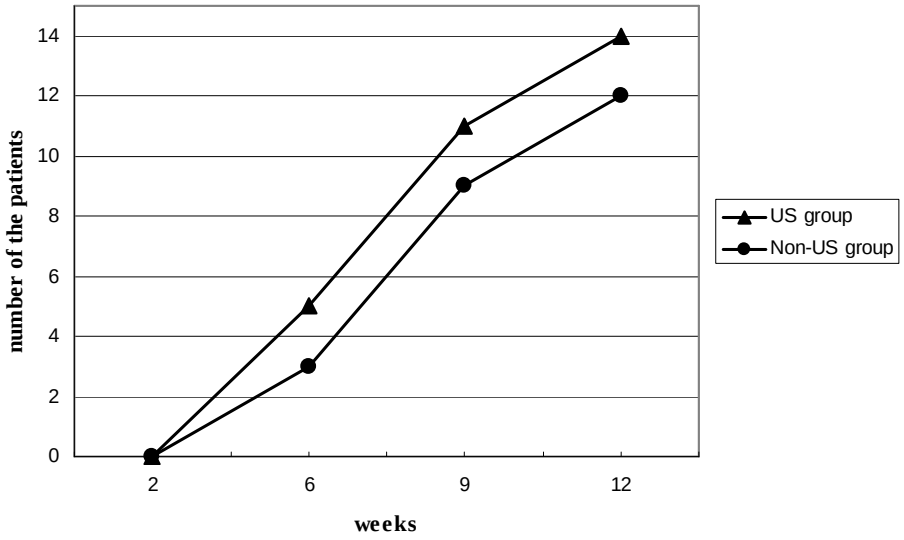

Figure 9. Callus formation at the site of the fracture line on the posterior cortex of the fibula in the lateral-view radiographs in 30 patients. The figure shows the number of the patients with observed callus during the follow-up.

The postoperative BMD of the lateral malleolus was 0.511 g/cm2 in the US-group (range 0.383 – 0.723 g/cm2) and 0.538 g/cm2 in the non-US-group (range 0.423 – 0.663 g/cm2). During the follow-up of 12 weeks, there was a slight increase in the BMD in both groups. However, the difference between the groups was not statistically significant (difference of means -0.001, 95 % CI: -0.049 – 0.046, p=0.952) (Fig. 10).

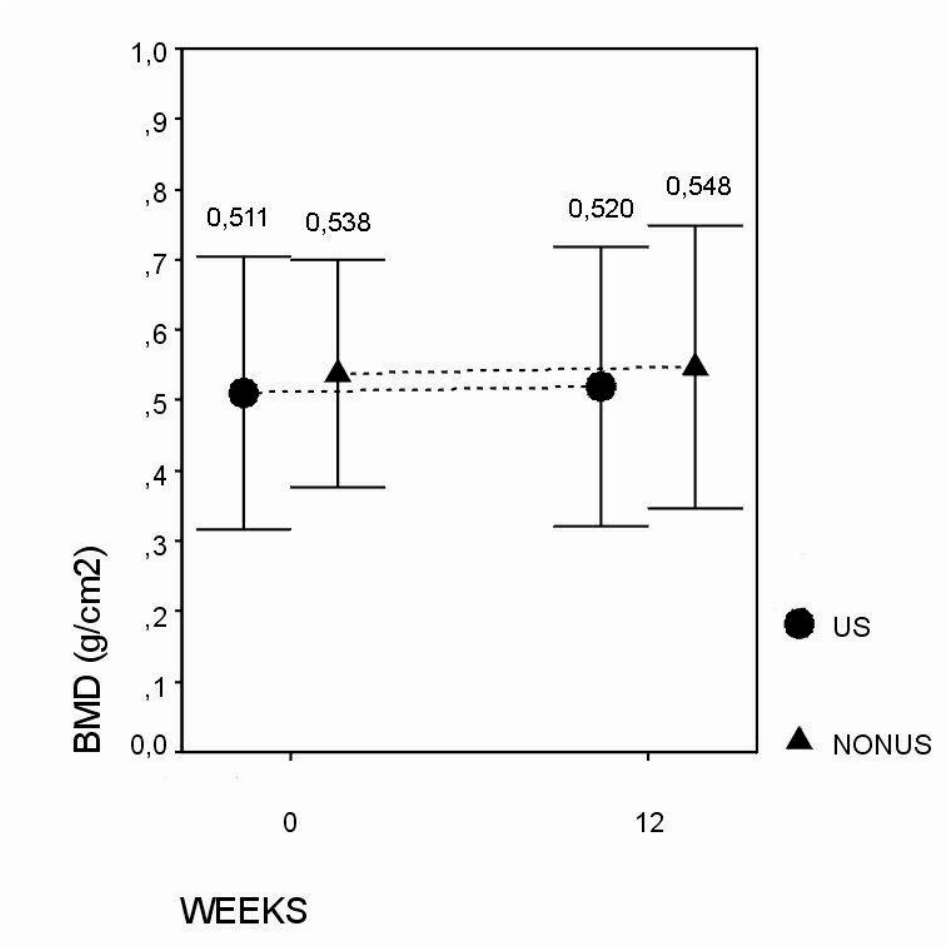

Figure 10. Bone mineral densities g/cm^2 (mean and standard deviation) of the lateral malleolar region in the ultrasound (US) and non-ultrasound (NONUS) groups on the first postoperative day and 12 weeks postoperatively.

3.7.3.2. Computed tomography results

The patient characteristics and operational data of the second series (22 patients) are presented in Table 5. The characteristics of the groups were well comparable. One patient was excluded from the series due to a new injury which caused a fixation failure in the initial fracture leading to a re-operation. The re-operation was carried out with metal implants.

	US-group (n=11)		Non-US-group (n=11)		difference of means	95 % confidence interval	p
	mean	range	mean	range			
Age	37.5	(18-54)	45.5	(26-59)	-8.0	-17.5 – 1.5	0.095
Sex	male 9 / female 2		male 6 / female 5				0.361
Hospital stay / days	2.2	(2-3)	2.1	(2-3)	0.1	-0.2 – 0.4	0.557
Operation time / min.	24.2	(14-39)	24.3	(14-35)	-0.1	-6.7 – 5.9	0.975
Screw length / mm	36.4	(35-40)	38.2	(35-45)	-1.8	-4.8 – 1.1	0.211
US device used / days	35.6	(14-42)	40.2	(38-42)	-4.6	-10.1 – 1.0	0.100

Table 5. Patient characteristics and operational data of 22 patients assessed by computed tomography scans.

The results of CT-assessed bone healing at two and nine weeks are presented in Table 6. Despite some amount of external callus formation, no true cortical bone healing could be observed in either group on the CT images (Fig. 11). At 18 months, no difference was observed between the groups in the fracture-site bone morphology: All fractures were fully healed, and no differences were seen in the radiological bone morphology at the fracture or screw site between the groups.

	US-group (n=11)		Non-US-group (n=11)		Difference of means between the groups	95 % confidence interval	p
	mean	range	mean	range			
Bone healing							
two weeks	0.000	0.00 – 0.00	0.000	0.00 – 0.00	0.000		
nine weeks	0.425	0.00 – 0.916	0.388	0.00 – 0.773	0.038	-0.290 – 0.365	0.812
Difference of means within the group	0.425		0.388				
95 % confidence interval	0.700 – 0.151		0.166 – 0.609				
p	0.007		0.003				

Table 6. Endosteal bone healing at two and nine weeks was assessed by measuring the share of the united fracture line to the non-united fracture line in coronal, axial, and sagittal planes after ultrasound exposure (US-group) or without it (non-US-group). Bone healing is presented as the mean share (0.00 – 1.00) of these three planes.

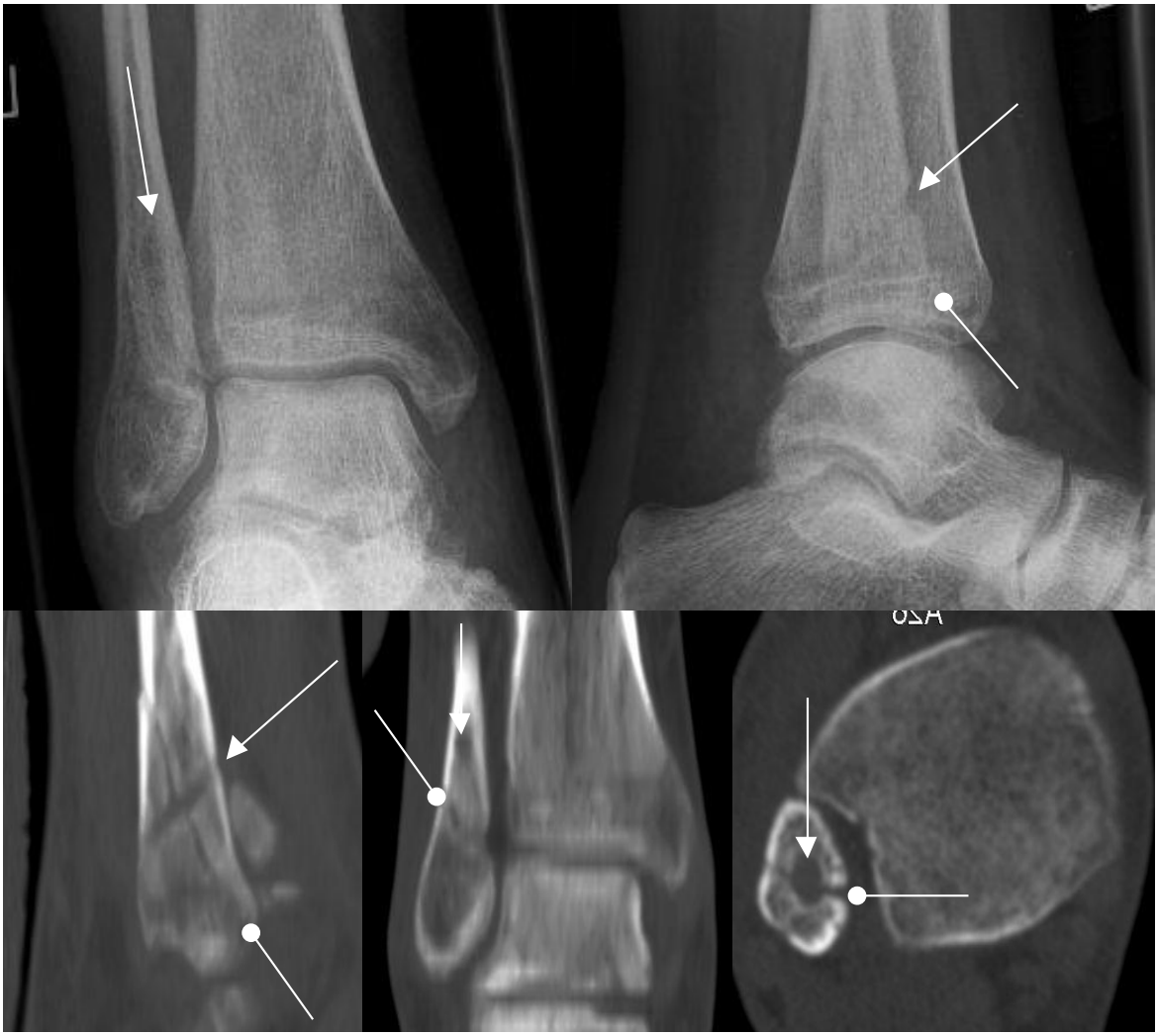

Figure 11. Plain radiographs (a, b) and CT-reconstructions (c, d, e) of lateral malleolus fracture fixed with one bioabsorbable screw at nine weeks (non-US-group). CT-reconstructions (c, d, e) reveal most of the fracture line (white ball with a stem) being still non-united. The drill hole and the screw are marked with a white arrow.

3.7.3.3. Clinical outcome results

Thirty-six patients were followed-up for 12 weeks and 16 patients for 18 months. All wounds of all 52 patients healed uneventfully, and no foreign-body reactions were observed during the course of the follow-up. Six patients had slight hypo-sensation of the skin distally to the wound (one US-patient and five non-US-patients). That was likely due to the peroperative distraction of the *nervus cutaneus lateralis dorsalis*. No one had a complete loss of skin sensation indicating a complete iatrogenous cut-off of the nerve. Deep venous thromboses (DVT) were not screened actively, but ultrasonography of the veins of the lower extremity was performed on demand in case of clinical suspicion. There were

altogether five deep venous thromboses diagnosed with ultrasonography among 52 patients, one in the US-group (below the knee) and four in the non-US-group (two below and two above the knee).

Sixteen patients were followed-up for 18 months. All of the fractured ankles were stable in the talar tilting test as well as in both the anterior and lateral drawer test at the end of the 18-month follow-up. The mean Olerud-Molander score (Olerud and Molander 1984) in the US-group was 99.4 preoperatively versus 95.0 at 18 months, in the non-US-group 98.8 versus 96.3, respectively. The reasons for decreased Olerud-Molander score at the end of the 18-month follow-up were the following: Three patients (one in the non-US and two in the US-group) had a mild restriction in the dorsal flexion of the ankle, and four patients (one in the non-US and three in the US-group) suffered from mild antero-lateral pain while walking on an uneven surface and one (non-US-group) from mild swelling in the evenings.

3.7.4. Comments

On passing through the tissues, the ultrasound energy is absorbed at different rates depending on the density of the tissue. In this study, the treatment head module was laid on the lateral side of the lateral malleolus in the area of very thin subcutis, thus delivering the ultrasound energy directly into the fracture line. The occurrence of a non-infectious foreign-body reaction following biodegradable fixation of ankle fracture has been earlier observed approximately in 2 % of the patients operated on with polyglycolide implants (Böstman et al. 1995). In the present study, the compatibility of ultrasound treatment and SR-PLLA screw fixation was found to be good. No foreign-body reactions were observed in either group, suggesting indirectly that low intensity pulsed ultrasound did not have a clinically significant effect on the biodegradation rate of the SR-PLLA screw implanted in the human body.

Regarding the post-treatment downloads of the individual ultrasound devices, the overall compliance to the daily ultrasound treatments was observed to be good in the present study. Thus, the results may be considered reliable in terms of the daily use of ultrasound both within and between the groups. The risk of misplacing the treatment head module (THM) was minimized by indicating an exact place using the scar and certain suture holes as landmarks. Thus, in the vast majority of the cases the THM was obviously placed properly. The lateral side of the distal fibula is slightly convex, which may cause connection problems between the THM and the skin. However, that cause of error was minimized by using coupling gel to fill all the empty spaces between the surface of the THM and the skin to secure optimal ultrasound conduction to the target area.

In the present study no malreduction was observed in either group, and all fractures were clinically united at six weeks allowing patients to mobilize the ankle and bear weight freely. Metaphyseal fractures, such as lateral malleolus fracture, tend to heal endosteally with new bone being formed on the existing trabeculae; the fading of the fracture line seen in the radiograph is a sign of endochondral ossification during the fracture healing process. Lateral malleolar fracture is a

benign cancellous bone fracture with a good healing tendency. In the present study, there was a slight tendency to more frequent callus formation on the posterior cortex of the fibula in the ultrasound group, but with the present number of patients the difference is not significant. On the other hand, no differences were observed between the groups regarding the fracture line visualization in the radiographs. It was not possible to detect differences in the healing times of these fractures, since fracture line visualization does not completely correlate to the clinical finding of fracture consolidation which usually occurs within six weeks.

Ingle et al. (1999) reported that the decrease in BMD was most obvious at six weeks after the fracture and recovered at one year in patients with a stable and non-operatively treated ankle fracture. In contrast to the results published by Ingle et al. (1999), the BMD of the fractured lateral malleolus tended to increase slightly in the present study during the 12-week follow-up. It was probably due both to the use of a removable brace that allowed patients to mobilize the ankle a few times a day and to the relatively early weight-bearing. The slight increase in BMD seemed to continue to the end of the 18-month follow-up, but it was symmetrical in both present groups and the six-week ultrasound treatment had no effect on it. This, however, has no correlation to the fracture line healing or fracture line mineralization, but reflects the overall bone mineral density in the whole lateral malleolar region during the bone healing process.

CT is an accurate method to assess fracture line healing, enabling the detection of early changes in normal bone healing (Grigoryan et al. 2003). In the present study, the bioabsorbable screws used for osteosynthesis enabled accurate assessment of CT scans, which is almost impossible due to artifact if metal implants were used. Although all the fractures in the present study were clinically united at six weeks, it was found that at nine weeks, on average, less than half (approximately 40 %) of the length of the fracture line was firmly endosteally united. The result was a slight surprise, because normally in plain radiographs the fracture line is almost fading away at nine weeks and the share of the united fracture line is easily supposed to be greater. However, there was no significant effect of low-intensity ultrasound on CT-assessed fracture line healing in the present study. Grigoryan et al. (2003) reported that the CT-assessed mean time for a radiological union in malleolar fractures was eight weeks.

4. GENERAL DISCUSSION

The ideal osteosynthesis material should have approximately the same rigidity as bone, the rigidity should diminish with time at an appropriate speed allowing the healing bone to be loaded gradually, and the fixation material should disappear from the living tissue after the bone has healed. The material should also be biocompatible with no side-effects, and the results have to be comparable to those of metallic implants. In theory, bioabsorbable implants meet all these requirements. Implants made of bioabsorbable SR-PLLA show several of the ideal characteristics.

In the first of the present studies the safety aspects regarding the effects of low-intensity ultrasound on the mechanical properties of SR-PLLA screws were investigated. The results suggested that ultrasound did not impair the bending strength or shear strength, neither did it affect the molecular weight, even after 12 weeks of daily exposure. Only the effects of the normal hydrolysis affecting the mechanical strength and molecular weight could be seen, as earlier reported by Pohjonen et al. (1997); the in vitro degradation study on SR-PLLA screws showed that the implant degrades slowly and still retains 90 % of its initial bending strength after 26 weeks of immersion in buffered saline at 37°C. Thus, it was safe to continue the investigation towards the experimental study on rats.

In the present experimental study on rats the effects of low-intensity ultrasound on bone healing in SR-PLLA-rod-fixed distal femur osteotomy were investigated. Previous experimental animal studies have shown that the biocompatibility of SR-PLLA is acceptable for internal fixation (Kulkarni et al. 1966, Nordström et al. 1998). In the present study the biocompatibility of SR-PLLA and ultrasound was found to be good, and no harmful tissue responses were observed; only some mild osteoarthritic lesions in the knee joints, possibly related rather to the arthrotomy and the operation itself than to poor biocompatibility, were found. On the other hand, no obvious findings supporting the hypothesis that low-intensity pulsed ultrasound enhances bone healing in SR-PLLA-rod-fixed metaphyseal distal femur osteotomy in rats were found in the present study. Still, the observed good biocompatibility of low-intensity ultrasound and bioabsorbable SR-PLLA fixation material gave a justification to continue the studies with a clinical ankle fracture series.

Ankle fracture is not a typical osteoporotic fracture (Ingle and Eastell 2002), but the majority of malleolar fractures are indirect injuries resulting from subluxation of the talus out of the ankle mortise. Certain fracture patterns are sometimes associated with ligamentous disruptions or their equivalent avulsion fractures of the ligamentous attachments. Those associated injuries have to be often surgically repaired together with osteosynthesis of the main fracture. The associated injuries may also affect the clinical outcome, and, at least to some extent, they may directly or indirectly affect the bone healing after the injury. Thus, a variety of different ankle fracture patterns and associated ligamentous injuries would have resulted in a possible bias in assessing certain fracture healings. To avoid that, it was determined that only isolated lateral malleolar fractures of the ankle were

included in the clinical studies investigating the use of low-intensity ultrasound in cancellous bone fractures fixed with a bioabsorbable screw.

The fracture line of the lateral malleolus lies normally beyond the relatively thin subcutis, and also almost the whole length of the fracture line can be covered by the area of the treatment head module. Thus, in the present study, it can be concluded that the ultrasound energy was delivered directly into the area of interest. Reliable evaluation of the effectiveness of the treatment also requires compliance from the patients involved in the study to follow the instructions given by the investigators. In the present study, the daily use of the ultrasound device was controlled by downloading the log of each individual ultrasound device after the treatment period. It revealed that the overall compliance to use the device daily as instructed was good and that the study groups were comparable. That enables to draw conclusions with confidence to that extent as well.

In the present ankle fracture studies, no malreduction was observed in either group, and all fractures were clinically united at six weeks. There was a slight tendency to more frequent callus formation on the posterior cortex of the fibula in the ultrasound group. But, on the other hand, no differences were observed between the groups regarding the fracture line visualization in the radiographs. The bioabsorbable screws used for osteosynthesis enabled accurate assessment of the CT scans, which is almost impossible due to artefact if metal implants were used. In the present study, the CT images at nine weeks showed the share of the endosteal united fracture line to the non-united one to be slightly higher after ultrasound exposure than without it. However, that result was not statistically significant.

When low bone mass is associated with architectural deterioration of the bone, decreased bone strength, and increased susceptibility to fractures, the condition is called osteoporosis (Silver and Einhorn 1995). Injury with subsequent immobilization and disuse of the injured extremity leads to a rapid loss of bone (Finsen and Haave 1987, Finsen and Benum 1989, Ulivieri et al. 1990, Sievänen et al. 1994). Post-traumatic bone loss is described as a high-turnover condition with both increased bone formation and increased bone resorption, increased bone resorption overcoming bone formation and thus resulting in a net bone loss (Nilsson 1966, Obrant and Nilsson 1984). The severity of bone loss caused by post-traumatic immobilization is influenced at least by the length of disuse and non-weight-bearing of the injured extremity, loss of normal muscle function, severity of the injury, treatment method of the injury, and the total functional recovery of the patient (Uhthoff and Jaworski 1978, Andersson and Nilsson 1979, Minaire 1989, Van der Wiel et al. 1994). After a lateral malleolar fracture, the normal immobilization period is six weeks with no allowance to bear any weight during the first two weeks, resulting in a minor risk for BMD loss. Juutilainen et al. (1997) compared the effect of SR-PGA and SR-PLLA to that of metallic fixation devices on the BMD of ankle fracture patients distal the tibia and distal fibula. They reported that there was no difference in the BMD after SR-PLLA fixation compared to metallic fixation.

Several techniques are available for measurement of the bone mineral content (BMC) and BMD, including single photon absorptiometry, single- and dual-energy

X-ray absorptiometry (DXA), and quantitative computed tomography. With each of these techniques, BMD is determined by the measurement of the attenuation of a beam of energy passing through the bones. In the present study, the BMD in the lateral malleolus was measured with DXA scans. DXA is the preferred method for assessment of the bone mineral status in clinical practice due to accuracy, precision, stability, and a low dose of radiation as well as to the speed and ease of scanning (Compston 1995, Compston et al. 1995, Sievänen et al. 1996). In the present study, the bone mineral density of the fractured lateral malleolus tended to increase slightly during the first 12 weeks, and the slight increase seemed to continue towards the 18-month follow-up. However, ultrasound was observed to have no effect on it. The possible explanation for BMD not to be decreased in the present study during the first 12 weeks after the operation may be both the use of a removable brace that allowed the patients to mobilise the ankle a few times a day and the relatively early weight-bearing, only two weeks post- operatively.

In the present clinical studies, no significant effects of low-intensity ultrasound on lateral malleolar fracture healing were observed. However, the present clinical studies are, to some extent, limited due to the small number of patients. With a small number of patients, the risk of missing the effect when it really was there is increased due to a possibility of statistical Type II error. Consequently, the rate of false negatives is increased since the confidence interval is wider and is therefore more likely to overlap zero. The planning of a future prospective series should start with a statistical power analysis in order to increase the statistical quality. It is important to calculate the minimum number of patients under the significance level of 0.05 and power of 70-80 %. A small difference of means and a relatively big standard deviation between the study groups may lead to a huge number of patients when aiming to high study power. For example, it should be recognized that an alfa error of 0.05 and a study power of 0.80 would have necessitated a study population of 2218 patients in order to exclude a significant difference in the present study of CT-assessed bone healing between the groups. This huge number is a consequence of the small difference of means between the groups and of a very big standard deviation. However, the figures used to calculate the necessary study population for a certain study power were known only after the study. Thus, the study population in the present study may be too small to draw reliable conclusions on the basis of some of the statistical results. On the other hand, to conduct a study comprising 2218 patients would not be realistic. It should also be recognized that when the present study was initiated and planned four years ago, it was not yet a general custom to perform a power analysis in randomized studies, and this, of course, is a weakness which is frequently seen in articles published today. However, that trend is gradually changing, and it certainly increases the validity of published studies.

Considering the previously reported results on the effects of low-intensity ultrasound in non-operatively-treated tibial shaft fractures and distal radial fractures (Heckman et al. 1994, Kristiansen et al. 1997), it is important to limit the conclusions of the present set of studies only to lateral malleolar fractures fixed with an SR-PLLA screw. Studies of low-intensity ultrasound also in other types of fractures with longer healing times and a more problematic prognosis of healing are needed in the future.

5. CONCLUSIONS

Recalling the aims of the present prospective set of studies, the results can be summarised in the following conclusions:

1. Low-intensity ultrasound does not affect the mechanical properties and degradation behaviour of SR-PLLA screws in vitro. The effects of degradation caused by the normal hydrolysis can be seen on the mechanical properties after 12 weeks.

2. There were no obvious signs of low-intensity ultrasound induced enhancement in bone healing in SR-PLLA-rod-fixed metaphyseal distal femur osteotomy in rats.

3. Low-intensity ultrasound and SR-PLLA implants can be safely combined in fracture treatment. The biocompatibility of low-intensity ultrasound treatment and SR-PLLA was found to be good, both with SR-PLLA rods in the experimental distal femur osteotomy study in rats and with SR-PLLA screws in the clinical lateral malleolar fracture series.

4. Low-intensity ultrasound had no obvious effects on the bone mineral density of the fractured lateral malleolus fixed with an SR-PLLA screw. In general, the bone mineral density tended to increase slightly during the follow-up regardless of the low-intensity ultrasound.

5. There were no obvious differences in the radiological bone healing times of the SR-PLLA-screw-fixed lateral malleolar fractures after low-intensity ultrasound treatment.

6. Low-intensity ultrasound did not have any effects on radiological bone morphology, bone mineral density or clinical outcome in the SR-PLLA-screw-fixed lateral malleolar fractures 18 months after the injury.

6. ACKNOWLEDGEMENTS

The present study was carried out at the Department of Orthopaedics and Traumatology, Helsinki University Central Hospital and at the Institute of Biomaterials, Tampere University of Technology, in 1999-2005. I wish to thank all who have helped and supported me during these years of the study.

Above all, I wish to express my deepest gratitude to my respected teachers and supervisors; Adjunct Professor Esa K. Partio M.D., Ph.D. and Adjunct Professor Ilkka Arnala M.D., Ph.D. Adjunct Professor Esa K. Partio presented the initial idea of the study to me and, together with Adjunct Professor Ilkka Arnala, introduced me to the world of science. It has been a privilege for me to be navigated through all this under their warm and prompt guidance.

I also feel very privileged to have a mental co-supervisor Emeritus Professor Pentti Rokkanen, M.D., Ph.D., Ph.D. (Hon. Vet. Med.). He is the former Head and Surgeon-in-Chief of the Department of Orthopaedics and Traumatology and the absolute authority in the field of research of bioabsorbable devices. He also placed excellent working facilities at my disposal with his encouraging and inspiring attitude.

I wish to thank Timo Pohjonen M.Sc. (Eng), the Institute of Biomaterials, Tampere University of Technology, for his expertise concerning polymer technology and for the help to conduct the mechanical testing; it would not have been possible to carry out the tests without him.

I wish to thank Adjunct Professor Eero Hirvensalo, M.D., Ph.D, the Chief of Töölö Hospital and Surgical Hospital, and Adjunct Professor Jarkko Pajarinen, M.D., Ph.D, the present Head of the Department of Orthopaedics and Traumatology, for giving me the possibility to bring this thesis to its conclusion.

My deepest thanks are due to my colleague Veikko Kiljunen, M.D., for his marvellous input on the lateral malleolar fracture operations we performed. I also thank Martti J. Kiuru, M.D., Ph. D., for the excellent radiographical analyses, and Adjunct Professor Hannu Pätiälä, M.D., Ph.D, for providing the facilities to carry out the second experimental study.

I also owe my gratitude to Adjunct Professor Matti Kataja, Dr. Tech. and Adjunct Professor Jarkko Pajarinen, M.D., Ph.D., for their help in the world of statistics, and to Mrs. Ilona Pihlman, L.F.Ph., for her professional revision of the English language.

I am greatly indebted to laboratory technicians Mrs. Taina Hutko, HuC, Ms. Ritva Sohlman, and Mrs. Johanna Virri, M.Sc., for their excellent work in preparing the histological, microradiographic, and OTC-fluorescence specimens. Mrs. Johanna Virri, M.Sc., also helped me in performing the semiautomatic quantitative histomorphometrical analysis, for which I thank her warmly. Many thanks are also due to the staff of the Experimental Laboratory for taking good care of the animals.

My warmest thanks go to Mrs. Mia Kalervo (Siitonen) for her kind help in office work and for her help in instructing the patients in the clinical ankle fracture series.

I wish to thank all the authors of the original articles of this thesis.

My deepest thanks are due to Adjunct Professor Ilkka Kiviranta and Adjunct Professor Harri Pihlajamäki, appointed by the Faculty of Medicine, University of Helsinki, for their constructive criticism and valuable help and advice after reviewing the manuscript.

Collectively I wish to express my warm thanks to all colleagues at Helsinki University Central Hospital with whom I have worked. I also owe my warmest thanks to the highly skilled staff of the emergency department, radiological department, operative department, in-patient wards, and out-patient clinics for kindly helping me in taking care of the patients in the clinical series.

I thank Exogen Inc. for providing the ultrasound devices for this study. I also thank the Finnish representatives of Exogen ultrasound devices, Mr. Matti Tarkkonen from Innovation Sports Finland, and Mr. Jani Toukosalo from Smith and Nephew Finland, for taking care of the logistics of shipping devices between the manufacturer and Finland.

In addition, I wish to thank all my patients who kindly accepted the extra visits and examinations at the out-patient clinics due to the study. The positive atmosphere could be recognized throughout the follow-ups.

My deepest thanks go also to my dear parents for their understanding and unconditional support in every field of this work and my life.

Finally, I owe my most loving thanks to my three J's; Jere, Juho, and Johanna. My dear Johanna has been the ultimate source of strength during the final steps of this work; she made me realize that there is no mountain I cannot climb with her. My dear sons Juho and Jere are the light of my life; I thank them from the bottom of my heart for the happiness they bring. Without their absolute devotion to everything we do together, I may have accomplished this thesis in half of the time it took now. But I am sure it would not have been worth it.

This study has been supported by grants from the Academy of Finland, the Foundation for Orthopaedical and Traumatological Research in Finland, the Finnish Medical Foundation, and Helsinki University Central Hospital.

Hyvinkää, March 2006

Lauri Handolin

7. REFERENCES

Agrawal C, Kennedy M, Micallef D: The effects of ultrasound irradiation on a biodegradable 50-50% copolymer of polylactic and polyglycolic acids. J Biomed Mater Res 28: 851-859, 1994

Andesson SM, Nilsson BE: Changes in bone mineral content following tibial shaft fractures. Clin Orthop 144: 226-229, 1979

Aynaci O, Önder C, Piskin A, Özoran Y: The effect of ultrasound on the healing of muscle-pediculated bone graft in spinal fusion. Spine 27: 1531-1535, 2002

Azyma Y, Ito M, Harada Y, Takagi H, Ohta T, Jingushi S: Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on various cellular reactions in the fracture callus. J Bone Miner Res 16: 671-680, 2001

Barbosa MA: Corrosion mechanisms of metallic biomaterials. In: MA Barbosa, ed. Biomaterials Degradation. Elsevier Science Publisher, Amsterdam, 1991

Barfod G, Svendsen RN. Synovitis of the knee after intraarticular fracture fixation with Biofix. Report of two cases. Acta Orthop Scand 63: 680-681, 1992

Basset CAL: Biologic significance of piezoelectricity. Calcified Tissue Research 1: 252-272, 1968

Bendix D: Chemicals synthesis of polylactide and its copolymers for medical applications. Polymer Degradation and Stability 59: 129-135, 1998

Bergsma JE, deBruijn W, Rozema F, Bos RR, Boering G: Late degradation tissue response to poly(L-lactide) bone plates and screws. Biomaterials 16: 25-31, 1995

Binderman I, Zor U, Kaye AM, Shimshoni Z, Harell A, Somjen D: The transduction of mechanical force into biomechanical events in bone cells may involve activation of phospholipase A2. Calsif Tissue Internat 42:261-266, 1988

Bos R, Rozema F, Boering G, Nijenhuis A, Pennings A, Verwey A, Nieuwenhuis P, Jansen H: Degradation of and tissue reaction to biodegradable poly(L-lactide) for use as internal fixation of fractures: astudy on rats. Biomaterials 12: 32-36, 1991

Brown St John R: How safe is diagnostic ultrasonography? J Can Med Assoc 131: 307-311, 1984

Bucholz RW, Henry S, Henley MB: Fixation with bioabsorbable screws for the treatment of fracture of the ankle. J Bon Joint Surg Am 76: 319-324, 1994

Böstman O, Vainionpää S, Hirvensalo E, Mäkelä A, Vihtonen K, Törmälä P, Rokkanen P: Biodegradable internal fixation for malleolar fractures. A prospective randomized trial. J Bone Joint Surg Br 69: 615-619, 1987

Böstman O, Mäkelä EA, Törmälä P, Rokkanen P: Transphyseal fracture fixation using biodegradable pins. J Bone Joint Surg Br 71: 706-797, 1989a

Böstman O, Hirvensalo E, Vainionpää S, Mäkelä A, Vihtonen K, Törmälä P, Rokkanen P: Ankle fractures treated using biodegradable internal fixation. Clin Orthop 238: 195-203, 1989b

Böstman O, Hirvensalo E, Mäkinen J, Rokkanen P. Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. J Bone Joint Surg Br 72: 592-596, 1990

Böstman O, Pihlajamäki H, Partio EK, Rokkanen P: Clinical biocompatibility and degradation of polylevolactide screws in the ankle. Clin Orhop 320: 101-109, 1995

Böstman O: Metallic or absorbable fracture fixation devices. A cost minimization analysis. Clin Orthop 329: 233-239, 1996

Böstman O, Laitinen O, Tynninen O, Salminen S, Pihlajamäki H. Tissue restoration after resorption of polyglycolide and poly-laevo-lactic acid screws. J Bone Joint Surg Br 87: 1575-1580, 2005

Cady WG: Piezoelectricity: An introduction to the theory and applications off electromechanical phenomena in crystals. Dover, New York, 1964

Casteleyn PP, Handelberg F, Haentjens P: Biodegradable rods versus Kirschner wire fixation of wrist fractures. A randomized trial. J Bone Joint Surg Br 74: 858-861, 1992

Chapman IV, Mac Nally NA, Tucker S: Ultrasound-induced changes in rates of influx and efflux of potassium ions in rat thymocytes in vitro. Ultrasound Med Biol 6:47-58, 1980

Cohen J, Wulf J: Clinical failure caused by corrosion of a vitallium plate. Case report, new testing methods for crevice corrosion, and new techniques for fashioning cobolt chromium alloys to be used in surgical implants. J Bone Joint Surg Am 54: 617-628, 1972

Compston JE: Bone density : BMC, BMD or corrected BMD? Bone 16: 5-7, 1995

Compston JE, Cooper C, Kanis JA: Bone densitometry in clinical practice. BMJ: Brittish Med J 310: 1507-1510, 1995

Cook S, Ryaby JP, McCabe J, Frey J, Heckman J, Kristiansen T: Acceleration of tibia and distal radius fracture healing in patients who smoke. Clin Ortop 337: 198-207, 1997

Cutright DE, Hunsuck EE, Beasley JD: Fracture reduction using a biodegradable material, polylactic acid. J Oral Surg 29: 393-397, 1971

Cutright DE, Hunsuck EE: The repair of fractures of the orbital floor using biodegradable polylactic acid. Oral Surg Oral Med Oral Pathol 33: 28-34, 1972

Cutright DE, Perez B, Beasley JD, Larson WJ, Posey WR: Degradation rates of polymers and copolymers of polylactic and polyglycolic acids. Oral Surg Oral Med Oral Pathol 37: 142-152, 1974

Eberson C, Hogan K, Moore D, Ehrlich M: Effect of low-intensity ultrasound stimulation on consolidation of the regenerate zone in a rat model of distraction osteogenesis. J Ped Orthop 23: 46-51, 2003

Emami A, Petren-Mallmin M, Larsson S: No effect of low-intensity ultrasound on healing time of intramedullary fixed tibial fractures. J Orthop Trauma 13: 252-257, 1999

Finsen V, Haave O: Changes in bone-mass after tibial shaft fracture. Acta Orthop Scand 58: 369-371, 1987

Finsen V, Benum P: Osteopenia after ankle fractures. The influence of early weight bearing and muscle activity. Clin Orthop 245: 261-268, 1989

Frankel V: Results of prescription use of pulse ultrasound therapy in fracture management. In: Szabo Z, Lewis JE, Frantini GA, Salvagi RS, eds. Surgical technology international VII. Universal Medical Press, San Francisco, pp. 389-394, 1998

Frazza EJ, Schmitt EE: A new absorbable suture. J Biomed Mater Res Symposium 1: 43-58, 1971

Frost HM: Bone histomorphometry: Analysis of trabecular bone dynamics. In: RR R ed. Bone histomorphometry: Techniques and interpretation. CRC Press, Boca Raton, 109-131, 1983

Galante JO, Lemons J, Spector M, Wilson PD Jr, Wright TM: The biologic effects of implant materials. Rewiew. J Orthop Res 9: 760-775, 1991

Gammelgaard N, Jensen J: Wound complications after closure of abdominal incisions with Dexon or Vicryl. A randomized double blind study. Acta Chir Scand 149: 505-508, 1983

Gebauer D, Mayer E, Orthner E, Heppenstall RB, Frey J, McCabe JM, Ryaby JP: Nonunions treated by pulsed low-intensity ultrasound. J Orthop Trauma 14: 154, 2000

Gebauer G, Lin S, Beam H, Vieira P, Parsons JR: Lo-intensity pulsed ultrasound increases the fracture callus strength in diabetic BB Wistar rats but does not affect cellular proliferation. J Orthop Res 20: 587-592, 2002

Getter L, Cutright DE, Bhaskar SN, Augsburg JK: A biodegradable intraosseous appliance in the treatment of manbibular fractures. J Oral Surg 30: 344-348, 1972

Gilding DK, Reed AM: Biodegradable polymers for use in surgery – polyglycolic/polylactic acid homo- and copolymers:1. Polymer 20: 1459-1464, 1979

Goldner J: A modification of the Masson trichrome for routine laboratory purposes. Am J Pathol 14: 237-243, 1938

Grigoryan M, Lynch JA, Fierlinger AL, Guermazi A, Fan B, MacLean DB, MacLean A, Genant H: Quantitative and qualitative assessment of closed fracture healing using computed tomography and conventional radiography. Acad Radiol 10: 1267-1273, 2003

Heckman J, Ryaby JB, McCabe J, Frey J, Kilcoyne R: Acceleration of tibial fracture-healing by non-invasive, low-intensity ultrasound. J Bone Joint Surg Am 76: 26-34, 1994

Herrman R, Kelly RJ, Higgins GA: Polyglycolic acid sutures. Laboratory and clinical evaluation of a new absorbable suture material. Arch Surg 100: 486-490, 1970

Heybeli N, Yesildag A, Oyar O, Gülsoy U, Tekinsoy M, Mumcu E: Diagnostic ultrasound treatment increase the bone fracture-healing rate in an internally fixed rat femoral osteotomy model. J Ultrasound Med 21: 1357-1363, 2002

Hirvensalo E, Böstman O, Rokkanen P: Absorbable polyglycolide pins in fixation of displaced fractures of the radial head. Arch Orthop Trauma Surg 109: 258-261, 1990

Hirvensalo E, Böstman O, Törmälä P, Vainionpää S, Rokkanen P: Chevron osteotomy fixed with absorbable polyglycolide pins. Foot & Ankle 11: 212-218, 1991

Hirvensalo E, Böstman O, Partio E, Törmälä P, Rokkanen P: Fracture of humeral capitellum fixed with absorbable polyglycolide pins. 1-year follow-up of 8 adults. Acta Orthop Scand 64: 85-86, 1993

Hoffmann R, Krettek C, Hetkamper A, Haas N, Tscherne H: Osteosynthese distaler Radiusfrakturen mit biodegradablen Frakturstiften. Zweijahresergebnisse. Unfallchirurg 95: 99-105, 1992

Hollinger J: Preliminary report on osteogenic potential of a biodegradable copolymer of polylactide (PLA) and polyglycolide (PGA). J Bio Med Mater Res 17: 71-82, 1983

Hollinger J, Battistone G: Biodegradable bone repair materials. Synthetic polymers and ceramics. Clin Orthop 207: 71-82, 1986

Hope PG, Williamson DM, Coates CJ, Cole WG: Bioderadable pin fixation of elbow fractures in children. A ramdomised trial. J Bone Joint Surg Br 73: 965-968, 1991

Ikada Y, Shikinami Y, Hara Y, Tagawa M, Fukada E: Enhancement of bone formation by drawn poly(L-lactide). J BioMed Mater Res 30: 553-558

Ingle BM, Hay S, Bottjer H, Eastell R: Changes in bone mass and bone turnover following ankle fracture. Osteoporosis Int 10: 408-415, 1999

Ingle BM, Eastell R: Site-specific bone measurements in patients with ankle fracture. Osteoporosis Int 13: 342-347, 2002

Jacobsen S, Honnens de Lichtenberg M, Jensen CM, Torholm C: Removal of internal fixation – the effect on patients' complaints: a study of 66 cases of removal of internal fixation after malleolar fractures. Foot Ankle 15: 170-171, 1994

Jamshidi K: Synthesis and properties of polylactides. Thesis Kyoto University, 1984

Jowsey J, Kelly PJ, Riggs BL, Bianco AC, Scholzt DA, Gershon-Cohen J: Quantitative microradiographic studies of normal and osteoporotic bone. J Bone Joint Surg Am 47: 785-806, 1965

Jukkala-Partio K, Laitinen O, Vasenius J, Partio EK, Toivonen T, Tervahartiala P, Kinnunen J, Rokkanen P: Healing of subcapital femoral osteotomies fixed with self-reinforced poly-L-lactide screws: an experimental long-term study in sheep. Arch Orthop Trauma Surg 122: 360-364, 2002

Juutilainen T, Pätiälä H, Rokkanen P, Törmälä P: Biodegradable wire fixation in olecranon and patella fractures combined with biodegradable screws or plugs and compared with metallic fixation. Arch Orthop Trauma Surg 114: 319-323, 1995

Juutilainen T, Hirvensalo E, Majola A, Partio EK, Pätiälä H, Rokkanen P: Bone mineral density in fractures treated with absorbable or metallic implants. Ann Chir Gynaecol 86: 51-55, 1997a

Juutilainen T, Pätiälä H, Ruuskanen M, Rokkanen P: Comparison of costs in ankle fractures treated with absorbable or metallic fixation devices. Arch Orthop Trauma Surg 166: 204-208, 1997b

Juutilainen T, Hirvensalo E, Partio EK, Pätiälä H, Törmälä P, Rokkanen P: Complications in the first 1043 operations where self-reinforced poly-L-lactide implants were used solely for tissue fixation in orthopaedics and traumatology. Int Orthop 26: 122-125, 2002

Kankare J, Hirvensalo E, Rokkanen P: Malleolar fractures in alcoholics treated using biodegradable internal fixation. 6/16 reoperations in a randomized study. Acta Orthop Scand 66: 524-528, 1995

Kankare J, Partio E, Hirvensalo E, Böstman O, Rokkanen P: Biodegradable self-reinforced polyglycolide screws and rods in the fixation of displaced malleolar fractures in the elderly. A comparison with metallic implants. Ann Chir Gyn 85: 263-270, 1996

Korostoff E: A linear piezoelectric model for characterizing stress generated potentials in bone. J Biomech 12: 335-347, 1979

Korstjens C, Nolte P, Burger E, Albers G, Semeins C, Aartman I, Goei S, Klein-Nulend J: Stimulation of bone cell differentiation by low-intensity ultrasound – a histomorphometric in vitro study. J Orthop Res 22: 495-500, 2004

Kost J, Leong K, Langer R: Ultrasound-enhanced polymer degradation and release of incorporated substances. Proc Natl Acad Sci 86: 7663-7666, 1989

Kristiansen T, Ryaby JP, McCabe J, Frey J, Roe L: Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. J Bone Joint Surg Am 79: 961-973, 1997

Kulkarni RK, Pani KC, Neuman C, Leonard F: Polylactic acid for surgical implants. Arch Surg 93: 839-843, 1966

Kumta SM, Spinner R, Leung PC: Absorbable intramedullary implants for hand fractures. Animal experiments and clinical trial. J Bone Joint Surg Br 74: 563-566, 1992

Laitinen O, Pihlajamäki H, Sukura A, Böstman O. Transmission electron microscopic visualization of the degradation and phagocytosis of a poly-L-lactide screw in cancellous bone: a long-term experimental study. J Biomed Mater Res 61: 33-39, 2002

Leung K, Cheung W, Zhang C, Lee K, Lo H: Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. Clin Orthop 418: 253-259, 2004

Li JK, Chang WH, Lin JC, Ruaan RC, Liu HC, Sun JS: Cytokine release from osteoblasts in response to ultrasound stimulation. Biomaterials 24: 2379-2385, 2003

Lin FH, Lin CC, Lu CM, Liu HC, Wang CY: The effects of ultrasonic stimulation on DP-bioglass bone substitute. Med Eng Phys 17: 20-26, 1994

Lowe CE: Preparation of high molecular weight polyhydroxyacetic ester. U.S. Patent 2 668 162, 1954

Majola A: Fixation of experimental osteotomies with absorbable polylactic acid screws. Ann Chir Gyn 80: 274-281,1991

Majola A, Vainionpää S, Rokkanen P, Mikkola HM, Törmälä P: Absorbable self-reinforced polylactide (SR-PLLA) composite rods for fracture fixation; strength and strength retention in the bone and subcutaneous tissue of rabbits. J Mater Sci Mater Med 3: 43-47, 1992

Matsusue Y, Hanafusa S, Yamamuro T, Shikinami Y, Ikada Y: Tissue reaction of bioabsorbable ultra high strength poly (L-lactide) rod. A long-term study in rabbits. Clin Orthop 317: 246-253, 1995

Mayr E, Frankel V, Rüter A: Ultrasound – an alternative healing method for nonunions? Arch Orthop Trauma Surg 120: 1-8, 2000

Mayr E, Rudzki MM, Rudzki M, Borchardt B, Haüsser H, Rüter A: Beschleunigt niedrig intensiver, gepulster Ultraschall die Heilung von Skaphoidfrakturen? Handchir Mikrochir Plast Chir 32: 115-122, 2000

Mayr E, Laule A, Suger G, Rüter A, Claes L: Radiographic results of callus distraction aided by pulsed low-intensity ultrasound. J Orthop Trauma 15: 407-414, 2001

Maylia E, Nokes LD: The use of ultrasonics in orthopaedics – a review. Technol Health Care 7: 1-28, 1999

Miettinen H, Mäkelä EA, Vainio J, Rokkanen P, Törmälä P: The effect of an intramedullary self-reinforced poly-L-lactide (SR-PLLA) implant on growing bone with special reference to fixation properties. An experimental study on growing rabbits. J Biomater Sci Polymer Edn 3: 443-450, 1992

Miller RA, Brady JM, Cutright DE: Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res 11: 711-719, 1977

Minaire P: Immobilization osteoporosis: a rewiew. Clin Rheumatol, 8 (supl 2): 95-103, 1989

Müller ME, Allgöver M, Schneider R, Willenegger H: Manual of internal fixation. Techniques recommended by the AO-ASIF group. 3rd ed. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1991

Nilsson BE: Post-traumatic osteopenia. A quantitative study of the bone mineral mass in the femur following fracture of the tibia in man using americium-241 as a photon source. Acta Orthop Scand, supl 91, 1966

Nolte P, Albers RG, Patka P, Janssen IM, van der Krans A: An effective therapy for nonunions – low-intensity ultrasound. In proceedings of the Sixth Meeting of the International Society for Fracture Repair, Strasbourg. J Orthop Trauma 13: 309, 1999

Nolte P, van der Krans A, Patka P, Janssen I, Ryaby JP, Albers GH: Low-intensity ultrasound in the treatment of nonunions. J Trauma 51: 693-703, 2001

Nordström P, Pihlajamäki H, Toivonen T, Törmälä P, Rokkanen P: Tissue response to polyglycolide and polylactide pins in cancellous bone. Arch Orthop Trauma Surg 117: 197-204, 1998

Nordström P, Pihlajamäki H, Toivonen T, Törmälä P, Rokkanen P: Tissue response to polyglycolide and polylevolactide pins in osteotomized cancellous bone. Clin Orthop 382: 247-257, 2001

Obrant KJ, Nilsson BE: Histomorphologic changes in the tibial epiphysis after diaphyseal fracture. Clin Orthop 185: 270-275, 1984

Olerud C, Molander H: A scoring scale for symptom evaluation after ankle fracture. Arch Orthop Trauma Surg 103: 190-194, 1984

Paavolainen P, Karaharju E, Slätis P, Ahonen J, Holmström T: Effect of rigid plate fixation on structure and mineral content of cortical bone. Clin Orthop 136: 287-293, 1978

Partio EK, Böstman O, Hirvensalo E, Vainionpää S, Vihtonen K, Pätiälä H, Törmälä P, Rokkanen P: Self-reinforced absorbable screws in the fixation of displaced ankle fractures: A prospective Clinical Study of 152 patients. J Orthop Trauma 6: 209-215, 1992a

Partio E, Hirvensalo E, Partio E, Pelttari S, Jukkala-Partio K, Böstman O, Hänninen A, Törmälä P, Rokkanen P: Talocrural arthrodesis with absorbable screws. 12 cases followed for 1 year. Acta Orthop Scand 63:170-172, 1992b

Partio EK, Hirvensalo E, Böstman O, Rokkanen P: A prospective controlled trial of the fracture of the humeral medial epicondyle – how to treat? Ann Chir Gyn 85: 67-71, 1996

Partio EK, Tuompo P, Hirvensalo E, Böstman O, Rokkanen P: Totally absorbable fixation in the treatment of fractures of the distal femoral epiphysis. A prospective clinical study. Arch Orthop Trauma Surg 116: 213-216, 1997

Parvizi J, Parpura V, Greenleaf J, Bolander M: Calcium signaling is required for ultrasound-stimulated aggrecan synthesis by rat chondrocytes. J Orthop Res 20: 51-57, 2002

Pelto K, Hirvensalo E, Böstman O, Rokkanen P: Treatment of radial head fractures with absorbable polyglycolide pins: A study on the security of the fixation in 38 cases. J Orthop Trauma 8: 94-98, 1994

Pihlajamäki H, Böstman O, Hirvensalo E, Törmälä P, Rokkanen P. Absorbable pins of self-reinforced poly-L-lactic acid for fixation of fractures and osteotomies. J Bone Joint Surg Br 74: 853-857, 1992

Pihlajamäki H, Böstman O, Hirvensalo E, Törmälä P, Rokkanen P: A biodegradable expansion plug for fixation of fractures of the medial malleolus. Ann Chir Gyn 83: 49-54, 1994a

Pihlajamäki H, Böstman O, Manninen M, Päivärinta U, Rokkanen P: Tissue-implant interface at an absorbable fracture fixation plug made of polylactide in cancellous bone of distal rabbit femur. Arch Orthop Trauma Surg 113: 101-105, 1994b

Pilla AA, Mont MA, Nasser PR, Khan SA, Figueiredo M, Kaufman JJ, Siffert RS: Non-invasive low-intensity pulsed ultrasound accelerates bone healing in the rabbit. J Orthop Trauma 4: 246-253, 1990

Pohjonen T, Törmälä P: In vitro hydrolysis of self-reinforced polylactide composites. Medical engineering & computing 34 Suppl 1: 127-128, 1996

Pohjonen T, Helevirta P, Törmälä P, Koskikare K, Pätiälä H, Rokkanen P: Strength retention of self-reinforced poly-L-lactide screws. A comparison of compression moulded and machine cut screws. J Mater Sci Mater Med 8: 311-320, 1997

Price GJ, Duck FA, Dibgy M, Holland W, Berryman T: Measurement of radical production as a result of cavitation in medical ultrasound fields. Ultrason Sonochem 4: 165-171, 1997

Ray JA, Doddi N, Regul D, Williams JA, Melveger A: Polydioxanone (PDS), a novel monofilament synthetic absorbable suture. Surg Gynecol Obstet 153: 497-507, 1981

Reilly DT, Burstein AH: The elastic and ultimate properties of compact bone tissue. J Biomechanics 8: 393-405, 1975

Rozema FR, Bergsma JE, Bos RRM, Boering G, Nijenhuis AJ, Pennings AJ, de Bruijn WC: Late degradation simulation of poly(L-lactide). J Mater Sci Mater Med 5: 575-581, 1994

Rokkanen P, Böstman O, Vainionpää S, Vihtonen K, Törmälä P, Laiho J, Kilpikari J, Tamminmäki M: Biodegradable implants in fracture fixation; early results of treatment of fractures of the ankle. Lancet: 1422-1424, 1985

Rokkanen P, Böstman O, Hirvensalo E, Mäkelä EA, Partio EK, Pätiälä H, Vainionpää S, Vihtonen K, Törmälä P: Bioabsorbable fixation in orthopaedic surgery and traumatology. Biomaterials 21: 2607-2613, 2000

Rosson JW, Egan J, Shearer JR, Monro P: Bone weakness after the removal of plates and screws: cortical atrophy or screw holes? J Bone Joint Surg Br 73: 283-286, 1991

Ryaby JT, Bachner EJ, Bendo JA, Dalton PF, Tannenbaum S, Pilla AA: Low-intensity pulsed ultrasound increases calsium incorporation in both differentiating cartilage and bone cell cultures. Trans Orthop Res Soc 14: 15, 1989

Ryaby JT, Matthews J, Pilla AA: Low intensity pulsed ultrasound modulates adenylate cyclase activity and transforming growth factor beta synthesis. In: Brighton CT, Pollock SR, eds. Electromechanics in Biology and Medicine. San Francisco Press, San Francisco, pp. 95-100, 1991

Santavirta S, Konttinen YT, Tomoyuki S, Grönblad M, Parti E, Kemppinen P, Rokkanen P: Immune response to polyglycolic acid implants. J Bone Joint Surg Br 72: 597-600, 1990

Schmitt EE, Polistina RA: Polyglycolic acid prosthetic devices. U.S. Patent 3 463 158, 1969

Shimazaki A, Inui K, Azuma Y, Nishimura N, Yamano Y: Low-intensity pulsed ultrasound accelerates bone maturation in distraction osteogenesis in rabbits. J Bone Joint Surg Br 82: 1077-1082, 2000

Schindler A, Harper DJ: Polylactide. II. Viscosity-molecular weight relationship and unperturbed chain dimensions. J Polym Sci 17: 2593-2599, 1979

Schneider AK: Polymers of high melting lactide. U.S. Patent, 2 703 316, 1955

Sievänen H, Kannus P, Heinonen A, Oja P, Vuori I: Bone mineral density and muscle strength of lower extremities after log-term strength training, subsequent knee ligament injury and rehabilitation: a unique 2-year follow-up of a 26-year-old female student. Bone 15: 85-90, 1994

Sievänen H, Kannus P, Heinonen A, Oja P, Vuori I: Estimation of various mechanical characteristics of human bones using dual energy x-ray absorptiometry: methodology and precision. Bone 18 (supl 1): 17-26, 1996

Silver JJ, Einhorn TA: Osteoporosis and aging. Current update. Clin Orthop 316: 10-20, 1995

Sinisaari I, Pätiälä H, Böstman O, Mäkelä EA, Hirvensalo E, Partio EK, Törmälä P, Rokkanen P: Wound infections associated with absorbable or metallic devices used in the fixation of fractures, arthrodeses and osteotomies. Eur J Orthop Surg Traumatol 5: 41-43, 1995

Sinisaari I, Pätiälä H, Böstman O, Mäkelä EA, Hirvensalo E, Partio EK, Törmälä P, Rokkanen P: Metallic or absorbable implants for ankle fractures. Acta Orthop Scand 67: 16-18, 1996

Slätis P, Karaharju E, Holmström T, Ahonen J, Paavolainen P: Structural changes in intact tubular bone after application of rigid plates with and without compression. J Bone Joint Surg Am 60: 516-522, 1978

Spadaro J, Albanese S: Application of low-intensity ultrasound to growing bone in rats. Ultrasound Med Biol 24: 567-573, 1998

Strauss E, Ryaby JP, McCabe JM: Treatment of Jones' fractures of the foot with adjunctive use of low-pulsed ultrasound stimulation. In proceedings of the Sixth Meeting of the International Society for Fracture Repair, Strasbourg. J Orthop Trauma 13: 310, 1999

Sun J, Hong R, Chang W, Chen L, Lin F, Liu H: In vitro effects of low-intensity ultrasound stimulation on the bone cells. J Biomed Mater Res 57: 449-456, 2001

Suuronen R, Pohjonen T, Vasenius J, Vainionpää S: Comparison of absorbable self-reinforced multilayer poly-L-lactide and metallic plates for the fixation of mandibular body osteotomies: An experimental study in sheep. J Oral Maxillofac Surg 50: 255-262, 1992a

Suuronen R, Wessman L, Mero M, Törmälä P, Vasenius J, Partio E, Vihtonen K, Vainionpää S: Comparison of shear strength of osteotomies fixed with absorbable self-reinforced poly-L-lactide and metallic screws. J Mater Sci Mater Med 3: 288-292, 1992b

Suuronen R, Pohjonen T, Hietanen J, Lindquist C: A 5-year in vitro and in vivo study of the biodegradation of poly-L-lactide plates. J Oral Maxillofac Surg 36: 604-614, 1998

Tams J, Rozema RF, Bos RRM, Roodenburg JLN, Nikkels PGJ, Vermey A: Poly(L-lactide) bone plates and screws for internal fixation and mandibular swing osteotomies. J Oral Maxillofac Surg 25: 20-24, 1996

ter Haar G, Daniels S: Evidence for ultrasonically induced cavitation in vivo. Phys Med Biol 26: 1145-1149, 1981

Tis J, Meffert R, Inoue N, McCarthy E, Machen M, McHale K, Chao E: The effect of low-intensity pulsed ultrasound applied to rabbit tibiae during the consolidation phase of distraction osteogenesis. J Orthop Res 20: 793-800, 2002

Tonino AJ, Davidson CL, Klopper PJ, Linclau LA: Protection from stress in bone and its effects. Experiments with stainless steel and plastic plates in dogs. J Bone Joint Surg Br 58: 107-113, 1976

Törmälä P, Vainionpää S, Kilpikari J, Rokkanen P: The effects of fibre reinforcement and gold plating on the flexural and tensile strength of PGA/PLA copolymer materials in vitro. Biomaterials 8: 42-45, 1987

Törmälä P: Biodegradable self-reinforced composite materials; manufacturing structure and mechanical properties. Clin Mater 10: 29-34, 1992

Törmälä P, Pohjonen T, Rokkanen P: Bioabsorbable polymers: materials technology and surgical applications. Proc Inst Mech Eng H 212: 101-111, 1998

Uglow M, Peat R, Hile M, Bilston L, Smith E, Little D: Low-intensity ultrasound stimulation in distraction osteogenesis in rabbits. Clin Orthop Rel Res 417: 303-312, 2003

Uhthoff HK, Dubuc FL: Bone structure changes in the dog under rigid internal fixation. Clin Orthop 81: 165-170, 1970

Uhthoff HK, Jaworski ZGF: Bone loss in response to long-term immobilization. J Bone Joint Surg Br 60: 420-429, 1978

Ulivieri FM, Bossi E, Azzoni R, Ronzani C, Trevisan C, Montesano A, Ortolani S: Quantification by dual photon absorptiometry of local bone loss after fracture. Clin Orthop 250: 291-296, 1990

Van der Wiel HE, Lips P, Nauta J, Patka P, Haarman HJ, Teule GJ: Loss of bone in the proximal part of the femur following unstable fractures of the leg. J Bone Joint Surg Am 76: 230-236, 1994

Vert M, Chabot F, Leray J, Christel P: Stereoregular bioresosbable polyesters for orthopaedic surgery. Makromol Chem Suppl 5: 30-41, 1981

Vert M, Christel P, Chabot F, Leray J: Bioresorbable plastic materials for bone surgery. In: Hastings GW, Ducheyne P, eds. Maxillomolecular biomaterials. CRC Press, Boca Raton, pp. 119-142, 1984

Viljanen J, Kinnunen J, Bondestam S, Majola A, Rokkanen P, Törmälä P: Bone changes after experimental osteotomies fixed with absorbable self-reinforced poly-L-lactide screws or metallic screws studied by plain radiographs, quantitative computed tomography and magnetic resonance imaging. Biomaterials 16: 1353-1358, 1995

Viljanen J, Pihlajamäki H, Törmälä P, Rokkanen P. Comparison of the tissue response to absorbable self-reinforced polylactide screws and metallic screws in the fixation of cancellous bone osteotomies: an experimental study on the rabbit distal femur. J Orthop Res 15: 398-407, 1997

Viljanen J, Kinnunen J, Bondestam S, Rokkanen P: Intramedullary fixation of distal femoral diaphyseal osteotomies with absorbable self-reinforced poly-L-lactide and metallic intramedullary rods assessed by plain radiographs, quantitative tomography, and magnetic resonance imaging: An experimental study in rabbits. J Biomed Mat Res 39: 222-228, 1998

Voutilainen N, Hess MW, Toivonen TS, Krogerus L, Partio EK, Pätiälä H: A long-term clinical study on dislocated ankle fractures fixed with self-reinforced polylevolactide (SR-PLLA) implants. J Long-term Eff Med Implants 12: 35-53, 2002

Wang S-J, Lewallen D, Bolander M, Chao E, Illstrup D, Greenleaf J: Low-intensity ultrasound treatment increases strength in a rat femoral fracture model. J Orthop Res 12: 40-47, 1994

Warden S, Bennel K, Matthews B, Brown D, McMeeken J, Wark J: Efficacy of low-intensity pulsed ultrasound in the prevention of osteoporosis following spinal cord injury. Bone 29: 431-436, 2001

Williams DF: Enzymic hydrolysis of polylactic acid. Eng in Med 10: 5-7, 1981

Williams DF: Enzymic hydrolysis of surgical polymers. J Mat Sci 17:1233-1246, 1982

Wolff J: Das Gesetz der Transformation der Knochen. Verlag von August Hirschwald, Berlin, 1892

Woo SL-Y, Akeson WH, Coutis RD, Rutherford L, Doly D, Jemmot GF, Amiel D: A comparison of cortical bone atrophy secondary to fixation with plates with large differences in bending strength. J Bone Joint Surg Am 58: 190-195, 1976

Woodward SC, Brewer PS, Moatamed F, Schindler A, Pitt CG: The intracellular degradation of poly(epsilon-caprolactone). J Biomed Mater Res 19: 437-444, 1985

Yang K-H, Parvizi J, Wang S-J, Lewallen D, Kinnick R, Greenleaf J, Bolander M: Exposure to low-intensity ultrasound increases aggrecan gene expression in a rat femur fracture model. J Orthop Res 14: 802-809, 1996

Ziskin MC: Applications of ultrasound in medicine – comparison with other modalities. In: Rapacholi MH, Grandolfo M, Rindi A, eds. Ultrasound: Medical applications, biological effects, and hazard potential. Plenum Press, New York, pp. 49-59, 1987

8. ORIGINAL ARTICLES