Available online at www.sciencedirect.com
ScienceDirect
Ultrasonics 48 (2008) 330–338
Ultrasonics
www.elsevier.com/locate/ultras

Low intensity pulsed ultrasound for fracture healing: A review of the clinical evidence and the associated biological mechanism of action

Neill M. Pounder *, Andrew J. Harrison

Orthopaedic Trauma and Clinical Therapies, Smith and Nephew, Inc., Memphis, TN 38116, United States

Received 13 June 2007; received in revised form 15 November 2007; accepted 28 February 2008
Available online 27 March 2008

Abstract

Low intensity pulsed ultrasound is used in the clinical treatment of fractures and other osseous defects. Level I clinical studies demonstrate the ability of a specific ultrasound signal (1.5 MHz ultrasound pulsed at 1 kHz, 20% duty cycle, 30 mW/cm^2 intensity (SATA)) to accelerate the healing time in fresh tibia, radius and scaphoid fractures by up to 40%. Additionally, the same ultrasound signal has been shown to be effective at resolving all types of nonunions of all ages, following a wide range of fracture types and primary fracture management techniques.

Recently, significant efforts have resulted in a more comprehensive understanding of the biological mechanism of action that produces the documented clinical outcomes. Low intensity pulsed ultrasound has been demonstrated to accelerate in vivo all stages of the fracture repair process (inflammation, soft callus formation, hard callus formation). In particular, accelerated mineralisation has been demonstrated in vitro with increases in osteocalcin, alkaline phosphatase, VEGF and MMP-13 expression. Integrins, a family of mechanoreceptors present on a wide range of cells involved in the fracture healing process, have been shown to be activated by the ultrasound signal. Downstream of the integrin activation, focal adhesions occur on the surface of cells with the activation of multiple signalling pathways, including the ERK, NF-κβ, and PI3 kinase pathways. These pathways have been directly linked to the production of COX-2 and prostaglandin, which are key to the processes of mineralisation and endochondral ossification in fracture healing.

Keywords: Bone; Fracture healing; Low intensity pulsed ultrasound; Mechanism of action

Contents

1. Introduction

Ultrasound may be applied clinically in a number of different product formats with a range of different medical

* Corresponding author.
E-mail address: neill.pounder@smith-nephew.com (N.M. Pounder).

0041-624X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ultras.2008.02.005

outcomes ranging from surgical excision using high intensity focused ultrasound (HIFU), through medium intensity physiotherapy ultrasound, to low intensity, short burst ultrasound for diagnostic imaging. The phrase "therapeutic ultrasound" is commonly used for all of these intensities which aim to produce an effect on the body, as opposed to those which are purely used for imaging or monitoring. Therapeutic ultrasound may be further divided into those signals which are destructive, such as HIFU, and those which have a less obvious, but nevertheless beneficial, effect on tissue. Physiotherapy ultrasound and ultrasound for fracture repair are less clearly distinguished. Busse et al.[1] found that some orthopaedic surgeons and physiotherapy students believe therapeutic ultrasound to be contra-indicated or harmful to healing bone, which indeed is true for physiotherapy ultrasound.

One form of ultrasound signal has been demonstrated clinically to be effective in the treatment of fractures and other osseous defects. It has been shown to accelerate healing of fresh fractures, to minimize delayed healing, and to stimulate healing of established nonunions. This ultrasound signal consists of 1.5 MHz ultrasound wave pulsed at 1 kHz with a 20% duty cycle at an intensity of 30 mW/cm^2 I_{SATA} (spatial average temporal average) applied for 20 minutes per day. A medical device incorporating this ultrasound signal was approved for the acceleration of certain fresh fractures in 1994 and for nonunions in 2000 in the United States by the Food & Drug Administration (Exogen 4000+, Smith & Nephew, Inc., Memphis, TN) (Fig. 1). This combination of parameters will hereafter be referred to as LIPUS (low intensity pulsed ultrasound).

Malizos et al. [2] reviewed the clinical literature supporting the use of LIPUS. They concluded that level 1 clinical evidence exists for accelerating the time to heal conservatively treated closed or grade-I open, cortical tibial fractures, and cancellous radial fractures by 38% [3,4].

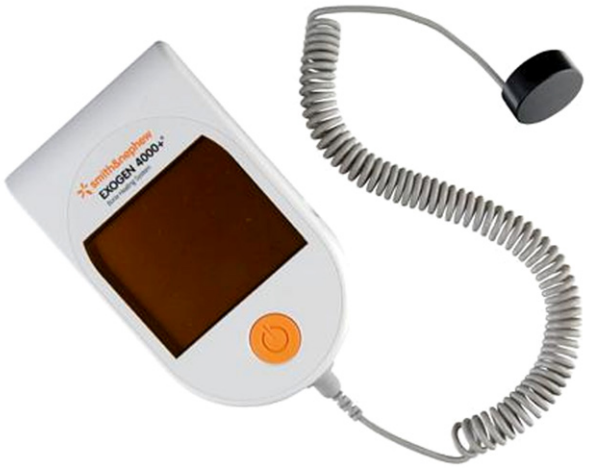

Fig. 1. Commercially available low intensity pulsed ultrasound device used for fracture healing (Exogen 4000+, Smith & Nephew, Inc., Memphis, TN, USA).

However, the data supporting the use of LIPUS for surgically treated tibia fractures is equivocal. Emami et al. [5] showed no beneficial effect for the use of LIPUS for closed or grade-I open tibial fractures treated with an intramedullary (IM) nail, whereas Leung et al. [6] demonstrated acceleration of at least 40% in all clinical and radiographic assessments when LIPUS was used in open and high energy tibial fractures stabilized with an IM nail or external fixator. Accelerated healing of scaphoid fractures was reported by Mayr et al. [7], but this review concluded lack of placebo control and blinding diminish the findings. Overall, Malizos et al. [2] reached the conclusion that some evidence existed for accelerating fresh fracture healing. Malizos et al. [2], following their review of the clinical evidence for LIPUS in the treatment of nonunions [8–10], concluded that LIPUS promoted healing, removing the need for a further surgical operation. However, the average time to healing remained significant at approximately 5 months. To conclude the review, Malizos et al. [2] reported that evidence, in vivo, of LIPUS causing callus maturation following distraction osteogenesis was inconclusive. However, LIPUS applied only during the consolidation phase (after distraction had ceased) on hemicallotasis after high tibial ostetomy, significantly enhanced the mineralisation of the callus in a randomized clinical study [11]. Two further papers support this finding with more clinically relevant measures. El-Mowafi et al. [12] in a randomized, placebo controlled study and Gold et al. [13] in a controlled case series showed healing (judged by removal time of the external fixator) to be accelerated.

The clinical data reviewed by Malizos et al. [2] is generally supportive of the use of LIPUS in treating fresh fractures and nonunions. However, the mechanism of action has not been fully elucidated. Claes et al. [37] reviewed some of the in vitro biological effects, reaching the conclusion that the mechanism was nonthermal, with increases in cellular activity and influences on the cell membrane. The aim of this review is to build on these biological effects, and provide proposals for a potential mechanism of action that links to fracture repair.

2. Biological mechanism of action

2.1. Physical interaction with biological material

The LIPUS ultrasound signal, previously described, is delivered via a transducer which is coupled to the skin with water-based gel. Longitudinal pressure waves are emitted by the transducer and in the clinical scenario pass through the soft tissue to the bone. It is deduced from theory that ultrasound waves undergo modal conversion at the interface between soft tissue and bone when they are not incident along the normal to the surface. The longitudinal ultrasound waves incident on the bone surface are converted into both longitudinal and shear waves that travel into the bone. Critical-angle reflectometry has been used to measure the speeds of sound in bone and therefore

to calculate the elastic properties of that material [14–16]. Antich et al. [15] and Mehta et al. [16] demonstrated experimentally that mode conversion occurs when a wave impinges on the interface between materials of different acoustic impedance (water and bone) at an oblique angle of incidence. PZ-Flex (Weidlinger Associates), a finite element modeling package, has been used to model in two dimensions a cross sectional of the human thigh, consisting of muscle, cortical femoral bone, and bone marrow [17]. The model demonstrated that when the ultrasound wave reaches the bone surface, there is reflection at the surface, but some longitudinal pressure waves are refracted through the bone, and mode conversion occurs with shear waves traveling around within the bone. On the furthest side of the femur, the acoustic pressure was primarily in the form of shear waves. Intuitively, one could consider that acceleration of fracture healing may be linked to the level of acoustic pressure. Therefore it may be expected that the fracture cortex closest to where the ultrasound transducer was positioned would heal first. Leung et al. [6] specifically commented on the position of the ultrasound transducer in the open and high energy tibial fracture study. They reported the formation of fracture callus on the anteromedial surface of the tibia, but also on the posterolateral surface where there was less likely to be a direct effect from the ultrasound transducer. This indicated that the stimulatory effect of ultrasound was not localized just to the area of direct stimulation, which may suggest that the clinical efficacy is not dependent just on longitudinal waves.

2.2. Biological response in fracture healing

In the majority of cases, fractures treated surgically or conservatively heal through a combination of bone-on-bone apposition and endochondral ossification. The repair process is divided into four stages: inflammation, soft callus formation, hard callus formation and remodeling. Azuma et al. [18] have shown that LIPUS increases the rate of fracture healing at each stage of the process, with the biggest impact occurring when used throughout all of the stages. A rat closed femoral fracture model was used, with the right femur exposed to LIPUS, and the left femur used as a control. Rats were divided into four groups according to timing and duration of treatment. The animals in each group were treated with LIPUS as follows: LIPUS treatments were performed in the Phase 1 group for 8 days, from day 1 to 8 after fracture; in the Phase 2 group for 8 days, from day 9 to 16 after fracture; in the Phase 3 group for 8 days, from day 17 to 24 after fracture; and in the T (throughout) group for 24 days, from days 1 to 24 throughout the healing process. Animals were euthanized on day 25. Radiographs and torsional biomechanical testing were used to assess fracture healing. Fig. 2 shows the maximum torque for each of the groups. The maximal torque and stiffness in torsion of the fractured femur on the LIPUS-treated side was significantly higher than that of the contralateral control side in all groups. This indicated that the partial treatment with LIPUS during Phase 1, 2, or 3 improved the mechanical properties of the fracture callus, as did the treatment throughout the 24 days. Furthermore, the maximal torque for the LIPUS-treated side in the T group was significantly higher than that for the other three groups. The authors concluded that these data suggest that LIPUS acts on cellular reactions involved in each phase of the healing process such as inflammatory reaction, angiogenesis, chondrogenesis, intramembranous ossification, endochondral ossification, and bone remodeling. Other in vivo studies have also reported on the positive effect of LIPUS on the inflammation and soft callus phases of fracture healing, with increased biomechanical strength [19–22], but not in bone remodeling. LIPUS has further been demonstrated to accelerate the process of endochondral ossification during the hard callus phase of fracture repair in an ex vivo model of fetal mouse metatarsal rudiments [23,24].

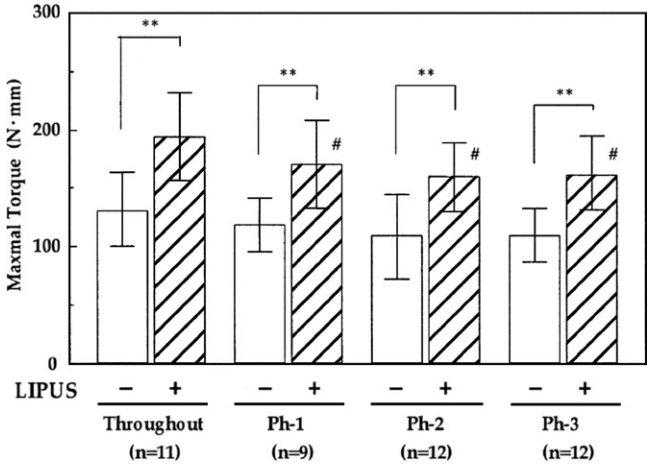

Fig. 2. Maximum torque of the LIPUS-treated femurs was significantly greater than the placebo controls at each phase of fracture healing. The maximum torque of the group treated throughout the repair process was significantly higher than the LIPUS groups treated for a single phase (Ph1, Ph2, Ph3) alone. ($^{**}p < 0.01$, $\#p < 0.05$) (Azuma et al. [18]).

In vivo studies are appropriate for identifying overall biological effects, but mechanisms and cellular action are better studied in vitro. It has been suggested that the increase in mechanical strength reported in vivo is due to an acceleration of callus mineralization, i.e. hard callus formation. In vitro studies support this hypothesis. Leung et al. [25] demonstrated LIPUS stimulated human periosteal cell differentiation. The osteogenic markers osteocalcin and alkaline phosphatase were measured in the culture media after 2 and 4 days ultrasound stimulation. After 4 days of 20 minutes per day stimulation osteocalcin, alkaline phosphatase and VEGF expression in the culture media was significantly increased compared to untreated control cultures. In addition alizarin red staining showed significant increases in calcium nodule formation (representing mineralization) in cultures after 4 weeks of daily stimulation. Unsworth et al. [26] have shown that over a

10 day period the level of alkaline phosphatase in MC3T3 cultures (a murine pre-osteoblastic cell line) increased, but this increase was significantly enhanced by daily stimulation with LIPUS. Significant differences ($p < 0.05$) were achieved on days 2, 4, 6 and 8 with the controls reaching the same levels as LIPUS stimulated cells by day 10 (Fig. 3). In addition to the acceleration in alkaline phosphatase synthesis, MMP13 mRNA levels in ultrasound-stimulated cultures followed the same temporal pattern that was seen in untreated controls but at a higher expression level. MC3T3 cultures stimulated for up to 25 days showed a significant increase in the degree of mineralization as determined by colorimetric analysis of alizarin red staining.

In vitro studies have reported on the cellular effects, as a result of LIPUS stimulation, supporting the fracture processes of chondrogenesis and endochondral ossification. Chondrogenesis, the replacement of fibrovascular tissue with a cartilage-specific matrix, has been demonstrated by increased proteoglycan synthesis in LIPUS stimulated chondrocytes [22,31]. VEGF is a key growth factor and a crucial regulator of angiogenesis and endochondral ossification (the replacement of cartilage with woven bone). Elevated levels of VEGF have been observed in human osteoblasts and periosteal cells following LIPUS stimulation [25,32]. Both chondrogenesis and endochondral ossification require the differentiation of mesenchymal cells into chondrocytes and osteoblasts. Accelerated differentiation in both cell types has been reported by Ebisawa et al. [27], and by Ridgway et al. [28]. Sena et al. [29] and Naruse et al. [30] also showed differentiation of mesenchymal cells to osteoblasts, with increased expression of early response genes (c-jun,c-myc, Egr-1, TSC-22), osteonectin, osteopontin and IGF-1. This evidence of osteoblast stimulation was further supported with studies demonstrating upregulation of bone markers Cbfa1, HIF-1α and prostaglandin E2 by LIPUS [32–34].

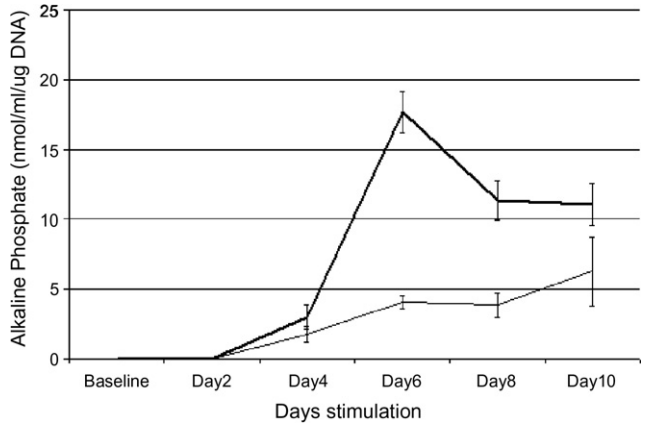

Fig. 3. Alkaline phosphatase activity of MC3T3-E1 cultures at days 2, 4, 6, 8 and 10. Ultrasound treatments were given 20 minutes per day. LIPUS stimulated cultures (bold line) showed significantly greater activity at days 6, 8, and 10 than control cultures (thin line). ($^{**}p < 0.05$) (Unsworth et al. [26]).

Although the majority of the biological effects have been reported with the LIPUS signal, other ultrasound signals have been investigated. Reher et al. [35] stimulated nitric oxide and prostaglandin E2 production in primary osteoblasts using 45 kHz continuous ultrasound at intensities between 5 and 50 mW/cm^2, and with pulsed 1 MHz ultrasound between 0.1 and 1 W/cm^2 SAPA (20–200 mW/cm^2 SATA). Harle et al. [36] reported increased alkaline phosphatase and osteopontin mRNA levels of osteoblastic like cells (MG63) after exposure to 3 MHz continuous ultrasound for 10 min with intensities between 120 mW/cm^2 and 1.49 W/cm^2.

2.3. Transduction processes

The demonstrated beneficial effects throughout the fracture healing process would suggest that one specific mechanism of action, and thereby a single transduction pathway is unlikely. Claes et al. [37], in his review article, commented that minimal heating would occur at such low intensities, indicating the mechanism of action would be nonthermal in nature. Greenleaf et al. [38] have reported that tissue and bone motion can be generated by LIPUS in a cadaveric forearm model. A surgical opening was created in a cadaver to expose the distal radius. An osteotomy was then created in the radius. Ultrasound was applied to the underside of the arm and a laser interferometer targeted on the exposed radius to measure the motion of the distal and proximal edges of the bone fracture. The results showed movement occurred at a frequency of 1 kHz, matching the pulse of the signal. It appears that a demodulation of the pulsed ultrasound signal occurred, causing an effect due to radiation pressure. However, this cannot be confirmed as measurements were not taken at the 1.5 MHz carrier frequency. At 1 kHz, the velocity of the bone ends and soft tissue ranged from 1 μms^{-1} to 3.5 μms^{-1}, equating to 0.15 to 0.55 nm displacement, respectively. These levels of motion are approximately 1000 times less than "micromotion" [39,40] where accelerated fracture healing occurred with displacements between 0.5 mm and 2 mm. This work suggests that LIPUS may be providing motion on a nanometer scale, indicating a mechanism of action independent of fixation methods such as casting or external fixation where millimeter levels of motion can occur [41].

Other potential mechanistic theories were discussed by Claes et al. [37]. It has been suggested that the acoustic pressures generated by LIPUS may provide surrogate forces following Wolff's Law [42]. However, if this were the case, one may expect bone adaptation to enhance remodeling at the site of ultrasound application. Leung et al. [43] reported no increase in bone mineral density in osteoporotic patients treated with LIPUS at the distal radius. The generation of cavitation as a result of ultrasound application has also been proposed. Cavitation can enhance acoustic streaming, resulting in shear forces applied to cells. Cavitation by LIPUS has not been con-

firmed, and is unlikely given the low mechanical index (0.08 for LIPUS).

The influence of LIPUS on shear forces may occur, but by other mechanisms. McCormick et al. [44] investigated the ability of LIPUS and physiological shear stress levels to regulate bone cell function in osteoblastic cells (SaOS-2). They reported no change in cell morphology after 20 minutes exposure to LIPUS, suggesting no direct cell deformation. The application of LIPUS or shear stress alone had no effect on cell alignment. However, application of LIPUS followed by shear stress significantly enhanced cell alignment, but inhibited shear stress induced cell elongation. The exposure of LIPUS prior to shear stress significantly increased BMP-4 mRNA levels compared to the application of each stimulus alone. In addition, LIPUS had no effect on Caveolin-1 mRNA levels, which has previously been suggested as a shear stress mechanotransduction pathway [45]. From these data, the authors propose interdependence between the two forces with LIPUS sensitizing cells to shear stress, rather than LIPUS directly generating shear stresses at the cell membrane.

2.4. Integrins transduce the ultrasound signal

Integrins are a family of transmembrane cell adhesion molecules comprised of one alpha and one beta chain. These molecules are thought to respond to mechanical stimuli by initiating intra cellular signaling and organization of the cell cytoplasm. One of their key characteristics is the capacity to switch between low and high-affinity states. When in the high-affinity state and bound to their ligand (fibronectin or RGD peptide) the integrins cluster in the cell membrane forming focal adhesions. Focal adhesion kinase (FAK) and paxillin (an intracellular protein) are key proteins in the focal adhesion complex. FAK binds directly to the integrin subunit molecules as well as binding to paxillin, thereby providing the link between the integrin and the cell's cytoskeleton.

Two groups using different cell lines have, independently, demonstrated that integrin activation occurs after treatment with LIPUS, as observed through the formation of focal adhesions on the surface of cells [46,47]. Zhou et al. [46] first reported of the increase in focal adhesion formation in response to LIPUS in human fibroblasts, whilst a separate group reported increased integrin expression in osteoblasts with 1 MHz continuous ultrasound at intensities between 62.5 and 250 mW/cm^2, and LIPUS [48,49]. With continuous ultrasound and LIPUS, integrin expression was transiently increased over a period of 24 hours, with new integrin synthesized on the surface of MC3T3-E1 and primary mouse osteoblasts [48,49] (Fig. 4). At this time the significance of new integrin synthesis is unknown, however it does give ultrasound-stimulated cells a greater capacity to attach to the surrounding matrix, which may make the cell more responsive to motion in the surrounding environment. Although integrin expression has been demonstrated with both LIPUS and continuous ultrasound, only LIPUS has been shown to activate integrin intracellular signalling, by the formation of focal adhesions in fibroblasts [46] and activated focal adhesion kinase in osteoblasts [49]. In addition ILK, integrin linked kinase, has been shown to be activated with LIPUS in chondrocytes [50]. Therefore considering Yang et al. [48] showed integrin expression, but not integrin signalling, resulted from continuous ultrasound, the low frequency 1 kHz nanomotion resulting from LIPUS application [38] may play a role in integrin signalling.

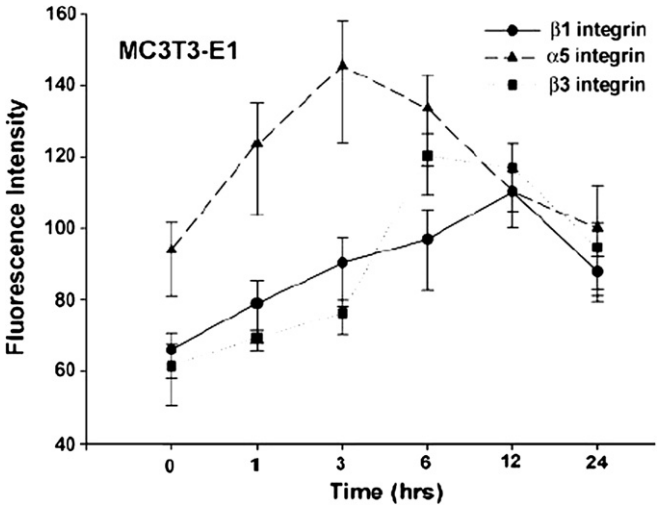

Fig. 4. Increase in the expression of integrin subunits in a 24 h period after 20 min stimulation of LIPUS (Tang et al. [49]).

The downstream events after the formation of focal adhesions are difficult to elucidate, however a role for the focal adhesion kinase (FAK) in the intracellular signalling in LIPUS stimulated cells has been determined [49]. This involves the phosphorylation of tyrosine 397 of FAK, which creates a potential binding site for SH2 domains of other signalling proteins. One of the potential signalling pathways downstream of FAK is the ERK signalling pathway. Phosphorylation of ERK results in its translocation into the nucleus and activation of gene transcription. Zhou et al. [46] showed that inhibiting MEK-1, using PD98059, prevented ultrasound-induced phosphorylation of ERK1/2 (Fig. 5a). The inhibition of ERK phosphorylation correlated with inhibition of DNA synthesis (Fig. 5b) thereby demonstrating the involvement of the ERK cascade in LIPUS induced cell proliferation. Having established ERK1/2 activation is affected by ultrasound, it was demonstrated that ERK activation is not required for focal adhesion formation and actin polymerization. However, ultrasound-induced focal adhesion formation still occurred in the presence of the MEK-1 inhibitor (Fig. 6). This same ERK 1/2 pathway was seen to be activated in mouse osteoblast cultures which could be blocked by using specific antibodies directed to α5β1 and αvβ3 integrins. The PI3 kinase pathway has also been identified to work through Akt to activate NF-κβ [49], thus demonstrating that numerous pathways are initiated via integrin activation in cells after

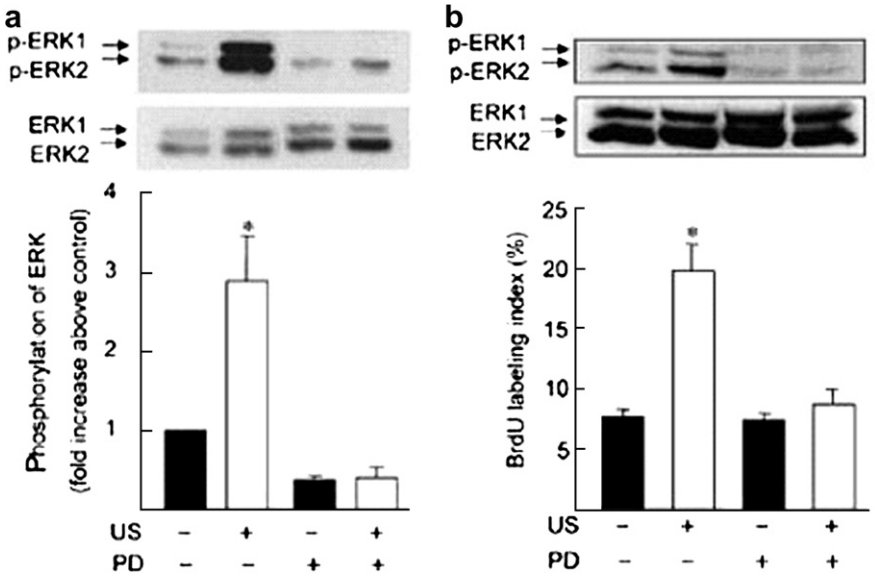

Fig. 5. Ultrasound-induced activation of ERK1/2 is regulated by MEK-1 and required for ultrasound-induced BrdU incorporation. Quiescent skin fibroblasts treated with 11 minutes of LIPUS stimulation. Phosphorylation of ERK1/2 was analysed by Western blotting with antibody to threonine and tyrosine dual phosphorylated ERK1/2. (a) Quantitation of phosphorylation presented as the fold increase in phosphorylation of ERK1/2 above control unstimulated level. (b) BrdU incorporation data represent the mean ±SE percentage of BrdU-positive nuclei in three independent wells per condition. $^{*}p < 0.05$ compared to control cells. (Zhou et al. [46]).

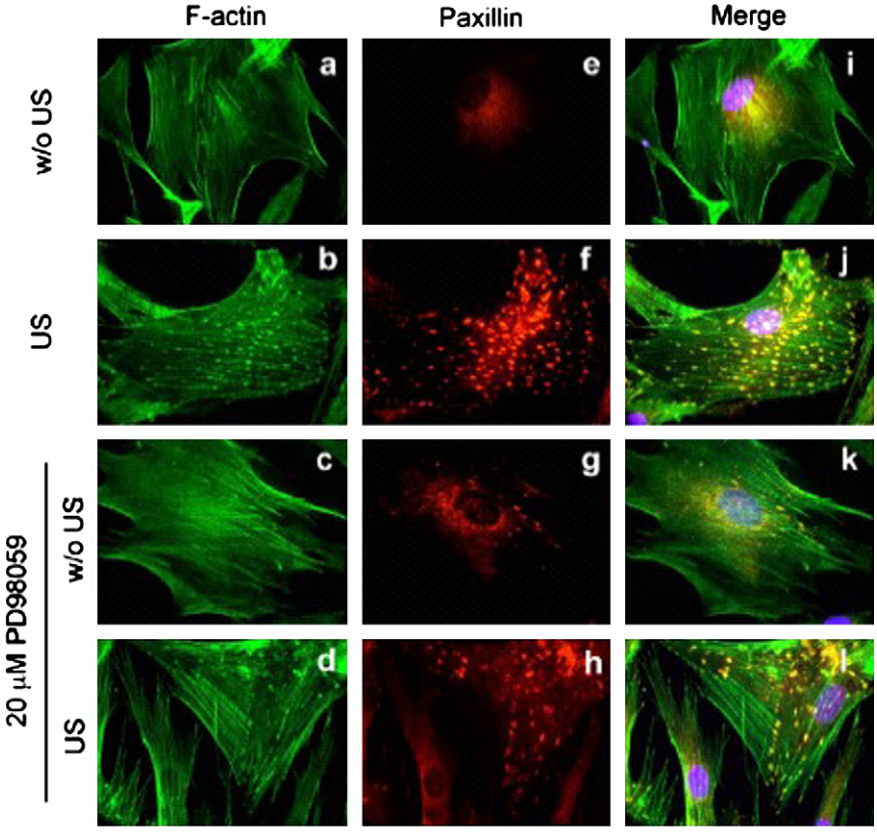

Fig. 6. Ultrasound induced focal adhesion formation occurs in the presence of PD98059. Quiescent fibroblasts grown on glass coverslips were stimulated with LIPUS for 11 min in the absence (a, b, e, f, i, j), or presence of 20 mM PD98059 (c, d, g, h, k, l). F-actin filaments and paxillin were visualized, with the merged pictures showing F-actin, paxillin, and nucleus (Zhou et al. [46]).

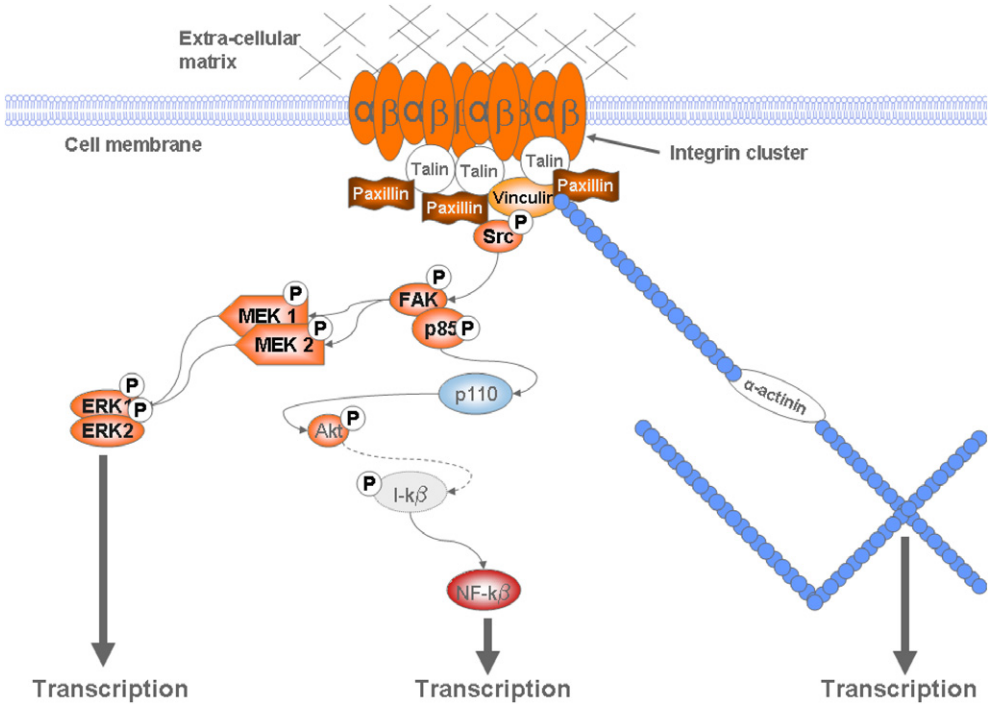

Fig. 7. Summary of intracellular pathways activated by LIPUS stimulation.

exposure to low intensity pulsed ultrasound. A summary of the pathways known to be activated by LIPUS is given in Fig. 7. The increase in the nitric oxide response to shear stresses following LIPUS exposure, as reported by McCormick et al. [44], might be explained by the enhanced reorganisation of actin cytoskeleton leading to the improvement in cytoskeletal stiffness that was observed by Zhou et al. following the increase in integrin expression [46].

A number of groups [29,30,35,51] have shown that one of the main genes to be activated in cells stimulated by LIPUS is cyclooxygenase 2 (COX-2), the rate limiting enzyme in the production of prostaglandin E2 (PGE2). Sena et al. [29] and Warden et al. [51] showed transient increases in COX-2 whereas Tang et al. [49] and Naruse et al. [30] showed secretion of PGE2 into the cell medium over 24 hours, following a single 20 minutes treatment with LIPUS. Tang et al. [49] have taken this experimentation further and demonstrated, again by using blocking monoclonal antibodies to α5β1 and αvβ3 integrins, that this enhancement of COX-2 production with LIPUS can be blocked. This directly implicates integrins as the mechanoreceptors converting the mechanical ultrasound stimulus to a biochemical response in the cell. Also the pathways initiated by LIPUS through the integrin activation, PI3 kinase, ERK and NF-κβ, are directly related to the production of COX-2, which was demonstrated by using both biochemical pathway blocking agents and dominant negative mutants transfected into cells.

It has been established that one of the key molecules in fracture repair is COX-2 and that with in vivo models the application of NSAIDs and specific COX-2 inhibitors impairs the healing of fractures (Simon et al. [52]). One of the processes in fracture repair is the mineralisation of the soft callus in the process known as endochondral ossification. The acceleration of this process will stabilize the fracture more rapidly and hence lead to accelerated healing. A number of groups [25,26] have shown that LIPUS enhances the mineralisation of cultures in vitro, which can be related to the mineralisation of the soft callus in fracture repair. Tang et al. [49] also demonstrated enhanced mineralization, and confirmed that this affect was directly related to both COX-2 and the signalling pathways initiated by LIPUS. By using a compound that blocks the production of PGE2 the enhanced mineralisation, stimulated by LIPUS, was blocked. Also the use of dominant negative mutants for FAK, PI3 kinase and Akt also blocked the LIPUS enhanced mineralisation. This was the first set of data implicating the activation of integrins in osteoblasts by LIPUS and the downstream signalling pathways of ERK, PI3 kinase and Akt resulting in COX-2 gene expression, which can then be traced to enhanced mineralization and fracture repair. As discussed earlier, numerous genes, proteins and growth factors important to fracture healing are enhanced by LIPUS, but as yet only COX-2 has been linked to a transduction pathway.

These results appear to correlate to observations seen with the in vivo models of fracture repair. Both Azuma et al. [18] and Wang et al. [21] demonstrated that using a closed rat femoral fracture model, LIPUS appeared to accelerate the process of endochondral ossification leading to a more complete mineralised callus compared to control fractures analysed at the same time points. Using the same model of fracture repair it appears that chondrocytes in the

fractures of LIPUS stimulated animals also produce greater amounts of COX-2 when compared to the control untreated group [53].

The conclusions from these observations are that the effects of LIPUS are detected by cells using integrin mechanoreceptors on the surface of cells. Numerous pathways are then initiated within the cell to activate the transcription of COX-2, which leads to an increase in PGE2. This enhanced COX-2 production with LIPUS treatment can be directly related to the process of mineralisation in osteoblast cultures in vitro and enhanced endochondral ossification in vivo.

3. Conclusion

The ability of LIPUS to accelerate fresh fracture healing has been shown in a number of peer reviewed clinical studies and through the regulatory bodies.

The clinical effects are supported by in vitro and in vivo studies, demonstrating numerous cell types and biological processes respond to this mechanical stimulus. One would expect multiple signaling pathways to be activated, with the evidence that LIPUS has a positive impact throughout the fracture healing process, and this is confirmed. In particular, the elucidation of the COX-2 PGE2 pathway clearly links to the enhanced endochondral ossification process and mineralization observed clinically.

References

[1] J.W. Busse, M. Bhandari, Therapeutic ultrasound and fracture healing, a survey of beliefs and practices, Arch. Phys. Med. 85 (2004) 1653–1656.

[2] K.N. Malizos, M.E. Hantes, V. Protopappas, A. Papachristos, Low-intensity pulsed ultrasound for bone healing: An overview. Injury, Int. J. Care Injured 37 (S1) (2006) 56–62.

[3] J.D. Heckman, J.P. Ryaby, J. McCabe, J.J. Frey, R.F. Kilcoyne, Acceleration of Tibial fracture-healing by non-invasive low-intensity pulsed ultrasound, J. Bone Joint Surg. 76A (1) (1994) 26–34.

[4] T.K. Kristiansen, J.P. Ryaby, J. McCabe, J.J. Frey, L.R. Roe, Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound, J. Bone Joint Surg. 79-A (7) (1997) 961–973.

[5] A. Emami, M. Petren-Mallmin, S. Larsson, No effect of low-intensity ultrasound on healing time of intramedullary fixed tibial fractures, J. Orthop. Trauma 13 (4) (1999) 252–257.

[6] K.S. Leung, W.S. Lee, H.F. Tsui, P.P.L. Liu, W.H. Cheung, Complex tibial fracture outcomes following treatment with low-intensity pulsed ultrasound, Ultrasound Med. Biol. 30 (3) (2004) 389–395.

[7] E. Mayr, M.M. Rudzki, B. Borchardt, H. Haüsser, A. Rüter, Does pulsed low intensity ultrasound accelerate healing of scaphoid fractures? Handchir. Mikrochir. Plast. Chir. 32 (2000) 115–122.

[8] P.A. Nolte, A. van der Krans, P. Patka, I.M.C. Janssen, J.P. Ryaby, G.H.R. Albers, Low-intensity pulsed ultrasound in the treatment of nonunions, J. Trauma 51 (4) (2001) 693–703.

[9] E. Mayr, C. Möckl, A. Lenich, M. Ecker, A. Rüter, Is low intensity ultrasound effective in treating disorders of fracture healing? Unfallchirurg 105 (2002) 108–115.

[10] D. Gebauer, E. Mayr, E. Orthner, J.P. Ryaby, Low-intensity pulsed ultrasound: effects on nonunions, Ultrasound Med. Biol. 31 (10) (2005) 1391–1402.

[11] N. Tsumaki, M. Kakiuchi, J. Sasaki, T. Ochi, H. Yoshikawa, Low-intensity pulsed ultrasound accelerates maturation of callus in patients treated with opening-wedge high tibial osteotomy by hemicallotasis, J. Bone Joint Surg. 86-A (11) (2004) 2399–2405.

[12] H. El-Mowafi, M. Mohsen, The effect of low-intensity pulsed ultrasound on callus maturation in tibial distraction osteogenesis, Int. Orthop. 29 (2005) 121–124.

[13] S.M. Gold, R. Wasserman, Preliminary results of tibial bone transports with pulsed low intensity ultrasound (Exogen™), J. Orthop. Trauma 19 (2005) 10–16.

[14] P.P. Antich, J.E. Dowdey, R.C. Murry Jr., US Patent 5,038,787 1991.

[15] P.P. Antich, S. Mehta, Ultrasound critical-angle reflectometry (UCR): a new modality for functional elastometric imaging, Phys. Med. Biol. 42 (1997) 1763–1777.

[16] S. Mehta, P.P. Antich, Measurement of shear-wave velocity by ultrasound critical angle reflectometry (UCR), Ultrasound Med. Biol. 23 (7) (1997) 1123–1126.

[17] Teijin Ltd., Personal Communication.

[18] Y. Azuma, M. Ito, Y. Harada, H. Takagi, T. Ohta, S. Jingushi, Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus, J. Bone Miner. Res. 16 (4) (2001) 671–680.

[19] N.M. Rawool, B.B. Goldberg, F. Forsberg, A.A. Winder, E. Hume, Power Doppler assessment of vascular changes during fracture treatment with low-intensity ultrasound, J. Ultrasound Med. 22 (2) (2003) 145–153.

[20] A.A. Pilla, M.A. Mont, P.R. Nasser, S.A. Khan, M. Figueiredo, J.J. Kaufman, R.S. Siffert, Non-invasive low-intensity pulsed ultrasound accelerates bone healing in the rabbit, J. Orthop. Trauma 4 (3) (1990) 246–253.

[21] S. Wang, D. Lewallen, M. Bolander, E. Chao, D. Ilstrup, J. Greenleaf, Low intensity ultrasound treatment increases strength in a rat femoral fracture model, J. Orthop. Res. 12 (1) (1994) 40–47.

[22] K.H. Yang, J. Parvizi, S.J. Wang, D.G. Lewallen, R.R. Kinnick, J.F. Greenleaf, M.E. Bolander, Exposure to low-intensity ultrasound increases aggrecan gene expression in a rat femur fracture model, J. Orthop. Res. 14 (5) (1996) 802–809.

[23] P.A. Nolte, J. Klein-Nulend, G.H.R. Albers, R.K. Marti, C.M. Semeins, S.W. Goei, E.H. Burger, Low-intensity ultrasound stimulates endochondral ossification in vitro, J. Orthop. Res. 19 (2001) 301–307.

[24] C.M. Korstjens, P.A. Nolte, E.H. Burger, G.H. Albers, C.M. Semeins, I.H. Aartman, S.W. Goei, J. Klein-Nulend, Stimulation of bone cell differentiation by low-intensity ultrasound – a histomorphometric in vitro study, J. Orthop. Res. 22 (3) (2004) 495–500.

[25] K.S. Leung, W.H. Cheung, C. Zhang, K.M. Lee, H.K. Lo, Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells, Clin. Orthop. Rel. Res. 418 (2004) 253–259.

[26] J.M. Unsworth, J. Kaneez, S. Harris, J. Ridgway, S.A. Fenwick, D. Chenery, A.J. Harrison, Pulsed low intensity ultrasound enhances mineralisation in pre-osteoblast cells, Ultrasound Med. Biol. 33 (9) (2007) 1468–1474.

[27] K. Ebisawa, K. Hata, K. Okada, K. Kimata, M. Ueda, S. Torii, H. Watanabe, Ultrasound enhances transforming growth factor beta-mediated chondrocyte differentiation of human mesenchymal stem cells, Tissue Eng. 10 (5–6) (2004) 921–929.

[28] J.N. Ridgway, S. Kaneez, J.M. Unsworth, A.J. Harrison, Osteogenic gene expression in response to pulsed low intensity ultrasound, Orthop. Res. Soc. Trans. (2005).

[29] K. Sena, R.M. Leven, K. Mazhar, D.R. Sumner, A.S. Virdi, Early gene response to low-intensity pulsed ultrasound in rat osteoblastic cells, Ultrasound Med. Biol. 31 (2005) 703–708.

[30] K. Naruse, A. Miyauchi, M. Itoman, Y. Mikuni-Takagaki, Distinct anabolic response of osteoblast to low-intensity pulsed ultrasound, J. Bone Min Res. 18 (2) (2003) 360–369.

[31] J. Parvizi, C.-C. Wu, D.G. Lewallen, J.F. Greenleaf, M.E. Bolander, Low-intensity ultrasound stimulates proteoglycan synthesis in rat chondrocytes by increasing aggrecan gene expression, J. Orthop. Res. 17 (4) (1999) 488–494.

[32] F.S. Wang, Y.R. Kuo, C.J. Wang, K.D. Yang, P.R. Chang, Y.T. Huang, H.C. Huang, Y.C. Sun, Y.J. Yang, Y.J. Chen, Nitric oxide mediates ultrasound-induced hypoxia-inducible factor-1 alpha activation and vascular endothelial growth factor-A expression in human osteoblasts, Bone 35 (1) (2004) 114–123.

[33] Y.J. Chen, C.J. Wang, K.D. Yang, P.R. Chang, H.C. Huang, Y.T. Huang, Y.C. Sun, F.S. Wang, Pertussis toxin-sensitive Galphai protein and ERK-dependent pathways mediate ultrasound promotion of osteogenic transcription in human osteoblasts, FEBS Lett. 554 (1–2) (2003) 154–158.

[34] T. Kokubu, N. Matsui, H. Fujioka, M. Tsunoda, K. Mizuno, Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in mouse osteoblasts, Biochem. Biophys. Res. Commun. 256 (2) (1999) 284–287.

[35] P. Reher, M. Harris, M. Whiteman, H.K. Hai, S. Meghji, Ultrasound stimulates nitric oxide and prostaglandin E_2 production by human osteoblasts, Bone 31 (1) (2002) 236–241.

[36] J. Harle, V. Salih, J.C. Knowles, F. Mayia, I. Olsen, Effects of therapeutic ultrasound on osteoblast gene expression, J. Mater. Sci. Mater Med. 12 (10–12) (2001) 1001–1004.

[37] L. Claes, B. Willie, The enhancement of bone regeneration by ultrasound, Prog. Biophys. Mol. Biol. 93 (1–3) (2007) 384–398.

[38] Greenleaf JF, Kinnick RR, Bronk JT, Bolander ME. Ultrasound induced tissue motion during fracture treatment. Poster Presentation AIUM annual meeting 2003, Montreal, Canada.

[39] J. Kenwright, A.E. Goodship, Controlled mechanical stimulation in the treatment of tibial fractures, Clin. Orthop. Rel. Res. 241 (1989) 36–47.

[40] J. Kenwright, J.B. Richardson, J.L. Cunningham, S.H. White, A.E. Goodship, M.A. Adams, P.A. Magnussen, J.H. Newman, Axial movement and tibial fractures, J. Bone Joint Surg. 73-B (4) (1991) 654–659.

[41] J. Kenwright, T. Gardner, Mechanical influences on tibial fracture healing, Clin. Orthop. Rel. Res. 355S (1998) S179–S190.

[42] J. Wolff, The Law of Bone Remodelling (Das Gesetz der Transformation der Knochen, Hirschwald), Springer, Berlin, 1892.

[43] K.S. Leung, W.S. Lee, W.H. Cheung, L. Qin, Lack of efficacy of low-intensity pulsed ultrasound on prevention of postmenopausal bone loss evaluated at the distal radius in older Chinese women, Clin. Orthop. Relat. Res. 427 (2004) 234–240.

[44] S.M. McCormick, V. Saini, Y. Yazicioglu, Z.N. Demou, T.J. Royston, Interdependence of pulsed ultrasound and shear stress effects on cell morphology and gene expression, Ann. Biomed. Eng. 34 (3) (2006) 436–445.

[45] J.T. Ferraro, M. Daneshmand, R. Bizios, V. Rizzo, Depletion of plasma membrane cholesterol dampens hydrostatic pressure and shear stress-induced mechanotransduction pathways in osteoblast cultures, Am. J. Physiol. Cell Physiol. 286 (4) (2004) C831–C839.

[46] S. Zhou, A. Schmelz, T. Seufferlein, Y. Li, J. Zhao, M.G. Bachem, Molecular mechanisms of low intensity pulsed ultrasound on human skin fibroblasts, J. Biol. Chem. 279 (52) (2004) 54463–54469.

[47] M. Bolander, Mayo Clinic, MN, USA, Personal Communication 2004.

[48] R.S. Yang, W.L. Lin, Y.Z. Chen, C.H. Tang, T.H. Huang, B.Y. Lu, W.M. Fu, Regulation by ultrasound treatment on the integrin expression and differentiation of osteoblasts, Bone 36 (2005) 276–283.

[49] C.H. Tang, R.S. Yang, T.H. Huang, D.Y. Lu, W.J. Chuang, T.F. Huang, W.M. Fu, Ultrasound stimulates cyclooxygenase-2 expression and increase bone formation through integrin, focal adhesion kinase, phosphatidylinositol 3-kinase, and Akt pathway in osteoblasts, Mol. Pharm. 69 (6) (2006) 2047–2057.

[50] H.C. Hsu, Y.C. Fong, C.S. Chang, C.J. Hsu, S.F. Hsu, J.G. Lin, W.M. Fu, R.S. Yang, C.H. Tang, Ultrasound induces cyclooxygenase-2 expression through integrin, integrin-linked kinase, Akt, NF-κB and p300 pathway in human chondrocytes, Cellular Signal. 19 (2007) 2317–2328.

[51] S.J. Warden, J.M. Favaloro, K.L. Bennell, J.M. McMeeken, K.W. Ng, J.D. Zajac, J.D. Wark, Low-intensity pulsed ultrasound stimulates a bone-forming response in UMR-106 cells, Biochem. Biophys. Res. Commun. 286 (3) (2001) 443–450.

[52] A.M. Simon, M.B. Manigrasso, J.P. O'Connor, Cyclo-oxygenase 2 function is essential for bone fracture healing, JBMR 17 (6) (2002) 963–976.

[53] T.A. Freeman, V. Antoci, M. Rozycka, C.S. Adams, I.M. Shapiro, J. Parvizi, Low intensity ultrasound affects MMP-13, Osteopontin and COX-2 protein expression: an in vivo study. ORS Annual Meeting, Chicago, 2006.