# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities




WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
## Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



15

# Low Intensity Pulsed Ultrasound: A Laboratory and Clinical Promoter in Tissue Engineering

## Abstract

This chapter will focus on the current and future applications of low intensity pulsed ultrasound (LIPUS) in tissue engineering at laboratory and clinical levels. Also, this chapter will explain the molecular basis of LIPUS stimulatory effects on cell expansion, differentiation and matrix production of different tissues. In addition, this chapter will provide information about the differences between LIPUS application in the laboratory settings and in the clinical applications with emphasis on tissue engineering applications. Moreover, this chapter will discuss why variability in outcome might occur when applying LIPUS in laboratory or clinical settings. Also, we will provide some tips on how to prevent potential inconsistency in treatment results with emphasis on the technique sensitivity and how a scientist or a clinician should be aware of this sensitivity of the application technique in order to optimize the utilization of LIPUS in different settings. This chapter will set a few hypotheses and potential new applications of utilizing LIPUS in other tissue engineering applications. The outcome of this chapter will be an understanding of the advantages and limitations of using LIPUS in tissue engineering applications either at the laboratory or in clinical settings.

Tarek H. El-Bialy

## 1. Introduction

Tissue engineering utilizes cells, scaffold or housing and growth factors to maintain the specific characteristics of the tissue to be engineered. Tissue engineering efforts are faced with challenges including the availability of cells to be used for engineering specific tissue (Chu et al., 2002). Another challenge in tissue engineering is the availability of biocompatible, biodegradable scaffolds that enhance cell adhesion as well as cell proliferation and matrix production. The degradation rate of the scaffolds is optimum when it occurs in a timely pattern with matrix production and maturation of the cell seeded within the scaffold. For some tissues, like bone, cartilage, skin and nerve tissue engineering, differentiation growth factors are readily available. However the key growth factors that are important in differentiating specialized tissues, like dentine in teeth tissue engineering, for example, are still not available. This again puts another roadblock in engineering these specialized tissues. Ultrasound is a form of mechanical acoustic pressure wave at frequencies above the limit of human hearing that when transmitted through biological tissues can produce different biological effects. The most widely used applications in

medicine are operative (usually has a frequency that ranges between 2 - 8 K Hz), therapeutic (usually has a of frequency that ranges between 20 K Hz 3M Hz either in continuous or pulsed modes), and diagnostic (usually has a frequency that ranges between 1.6-12 M Hz) (Ziskin, 1987; Maylia & Nokes, 1999; Harris, 2005). The most commonly used type of therapeutic ultrasound is US that utilizes intensities between 30 mW to as high as 1-3 $W/cm^2$ and hence it may cause considerable heating in living tissues. The low intensity pulsed ultrasound (LIPUS) is widely accepted in cell and tissue repair due to the nonthermal effects of this type of ultrasound.

## 2. LIPUS utilization in cell expansion

The availability of optimized cell density (CD) is a major challenge in tissue engineering, either due to donor site morbidity (if to be harvested from another organ within the same individual) or precursor cells, sometimes also called undifferentiated mesenchymal stromal cells or (mesenchymal stem cells) (Puelacher et al., 1994; Carrier et al., 1999; Holy et al., 2000; Goldstein, 2001; Panossian et al., 2001). It has been reported that one of the important parameters impacting tissue engineering is the cell seeding density. Seeding densities more than $8 \times 10^6$ cells $cm^3$ of tissue engineering scaffold promotes extracellular matrix production, cell distribution and/or cell alignment when compared to low cell seeding densities ($<4 \times 10^6$ cells $cm^3$ of scaffold). Low cell seeding densities is reported to be associated with decreased cell proliferation and minimal or no mechanical integrity (Freed et al., 1998; Carrier et al., 1999; Li et al., 2001; Saini & Wick, 2003; Leddy et al., 2004, Hsieh-Bonassera et al., 2009).

In this context, several studies have attempted to expand mesenchymal stem cells (MSCs) before differentiating them for engineering different tissues. These reports include, but are not limited to using different growth factors and /or mechanical stresses to enhance stem cell expansion. It has been reported that chondrocytes increased in number by 100-fold within 17 days when a special growth factor cocktail was used in the culture medium (Chen, et al., 2009). Also, ultra-nanocrystalline diamond films have been shown to enhance neural stem cell expansion for tissue engineering (Wang et al., 2006).

Moreover other growth factors that have been used to enhance stem cell expansion include ascorbic acid-functionalized poly (methyl methacrylate) (AA-PMMA) (Tamama et al., 2005); epidermal growth factor receptor (EGFR) (Hsieh-Bonassera et al., 2009) ; and Flt3 ligand (FL) was used in addition to IL-11 or G-SCF (Granulocyte-Colony Stimulating factor) to stimulate the growth of rat MSCs (Jacobsen et al., 1995). These (conjugated FL) factors expanded the progenitors more than 40 times after 2 weeks incubation. On the other hand, prolactin-like protein E (PLP-E) + human thrombopoietin (TPO) showed no effect on the expansion of human MSCs (Lefebvre et al., 2001). Glucocorticoids were found to be effective in recruiting cells rather than stimulating their proliferation (Purpura et al., 2004). It was reported that Dkk-1 stimulates MSCs expansion (Horwitz, 2004). Culture flasks coated with heparin and N-(O-beta-(6-O-sulfogalactopyranosyl)-6-oxyhexyl)-3,5-bis(dodecyloxy)-benzamide have been shown to expand MSCs (Takagi, 2005). A similar study has shown that Angiopoietin-like proteins expanded HSCs 24-30 fold in ten days (Zhang et al., 2006). However, more studies are needed to evaluate the effect of these factors on MSCs behavior (especially differentiation potential) after expansion. Other attempts using pulsed electromagnetic fields (PEMF) showed that chondrocytes- and osteoblast-like cell

proliferation increased (De Mattei et al., 2001; Hartig et al., 2000). However, no studies tested the effect of PEMF on MSCs expansion. Others used LIPUS to enhance periosteal cell expansion for 5, 10, and 20 minutes for 2 and 4 days. No cell proliferation was noted (Leung et al., 2004). This may be due to the small doses of ultrasound that were used. It has been shown that ultrasound effect on tissue is evident after three weeks of application (Tsai et al., 1992). Also, LIPUS was used for 20 minutes for one day to stimulate MSCs expansion and differentiation into chondrocytes. Matrix production was enhanced; however, cell proliferation was not enhanced (Ebisawa et al., 2004). This may also be due to using LIPUS in pellet culture. MSCs are known to have cell-cell growth inhibition when they are in close proximity to each other.

On the other hand, another report using LIPUS showed that LIPUS increases matrix production and proliferation of the intervertebral disc cell culture (Iwashina et al., 2006). The effect of LIPUS was different depending on the type of cells from different origins (nucleus pulposus [NP] or from annulus fibrosus [AF]). More recently, LIPUS has been shown to enhance chondrocyte proliferation in three-dimensional collagen scaffolds (Kobayashi et al., 2009).

Another precursor cell proliferation and differentiation that has been recently reported to be stimulated by LIPUS are human periodontal ligament cells that have been differentiated into cementoblasts (teeth root outer layer cells) (Inubushi et al., 2008). This is an important report on the potential use of LIPUS in dental tissue engineering. The scantiness of cementoblasts for teeth tissue engineering made it almost impossible in the past to tissue engineer teeth. However, with this recent report, it could be possible to tissue engineer teeth in the near future using LIPUS as a cell expansion and differentiation enhancer. Also, it has been reported that LIPUS enhanced cell proliferation in human fibroblasts, osteoblasts, and monocytes (Doan et al., 1999).

In a pilot study in our lab, we have shown that LIPUS enhances the expansion of bone marrow stem cells in vitro (Figure 1). This stimulatory effect is dose dependent.

IntechOpen

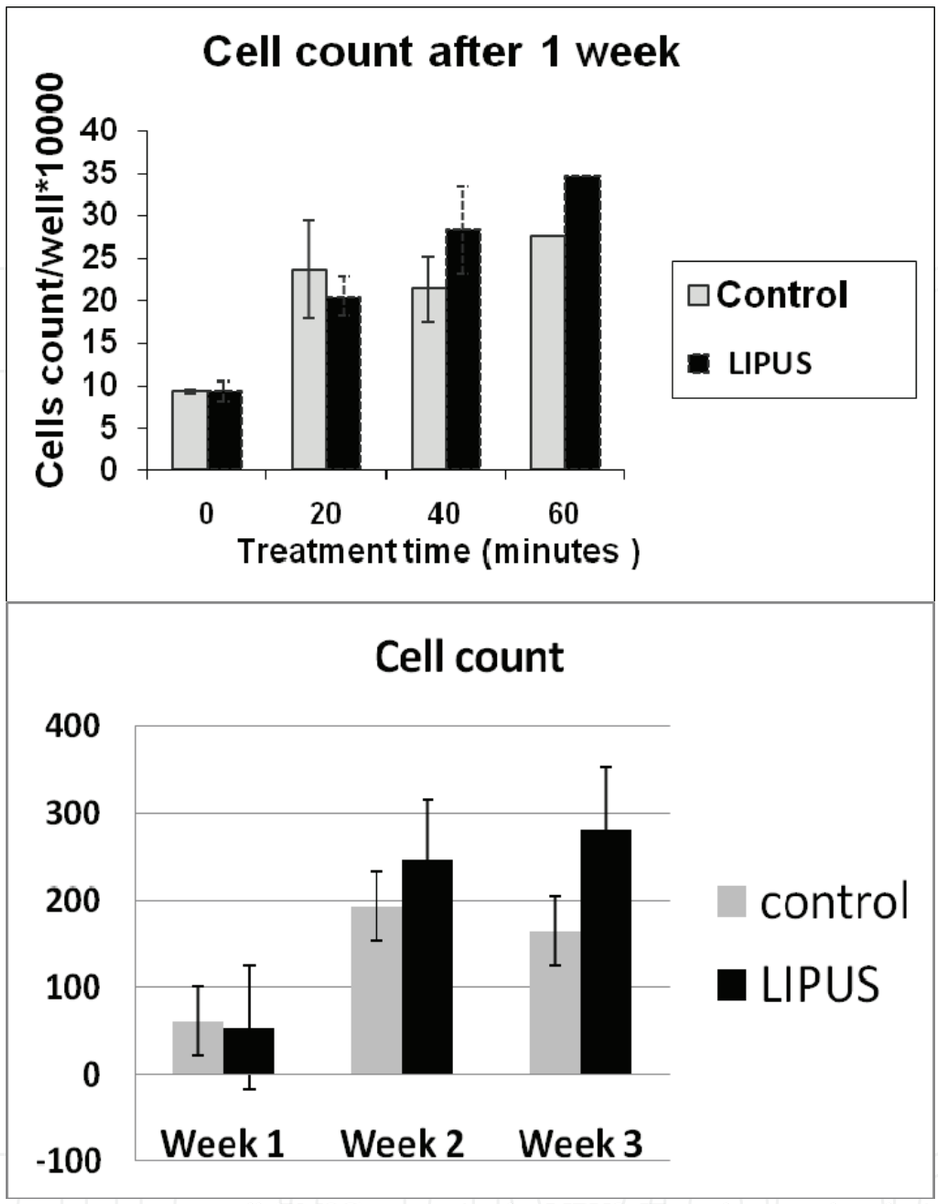



Fig. 1. Bone marrow stem cell (BMSC) expansion by LIPUS. IT can be seen that the increased LIPUS treatment time (20, 40, 60 minutes per day) or 2 and 3 weeks application led to increased BMSC expansion as measured by cell count.

## 3. Effect of LIPUS on in-vitro matrix formation for tissue engineering

Recent studies showed consistent agreement that LIPUS enhances different tissue matrix production by different cells in vitro. These cells include chondrocytes from different tissue origins (Wu et al., 1996; Doan et al., 1999; Iwabuchi et al., 2005; Mukai et al., 2005; Hsu et al., 2006; Iwashina, et al., 2006; Schumann et al., 2006a; Inubushi et al., 2008; Korstjens et al., 2008; Takeuchi et al., 2008; Tien et al., 2008; Kobayashi et al., 2009). It is to be noted that the stimulatory effect on chondrocyte matrix production has been recently shown to be dose

dependent (Schumann et al., 2006a; Tien et al., 2008). A more interesting in-vitro study showed that herniated disc resorption (HDR) treatment is accelerated by LIPUS. This effect was mediated by activation of matrix metalloproteinase-3 (MMP-3) maturation through tumor necrosis factor-alpha (TNF-alpha) and macrophage chemoattractant protein-1 (MCP-1) pathways (Iwabuchi et al; 2005). When compared to Bioreactors, LIPUS produced more matrix at 28 days in tissue engineered cartilage, while bioreactors continued matrix production until day 42 in-vitro (Hsu et al., 2006). It is to be noted that bioreactors cannot be applied clinically to engineered tissues while LIPUS can be applied. Moreover, the longer tissue culture periods could be questionable regarding possible errors or potential culture infection. The in-vitro conditioning of chondrogenic differentiated mesenchymal stem cells by LIPUS for cartilage tissue engineering in vivo has been recently tested. It has been concluded that type I and type X collagens and matrix metalloproteinase-13 are stimulated by LIPUS which indicate that LIPUS preconditioning in-vitro can be an effective treatment to upregulate chondrogenic differentiation of MSCs in vivo (Cui et al., 2007).

Studies also reported that LIPUS stimulated matrix formation and maturation by osteoblasts from different bone origins (Tsai et al., 1992; Saito et al., 2004; Sena et al., 2005; Tam et al., 2008; Suzuki et al., 2009). The foundation for the applications of LIPUS to enhance bone fracture healing in clinical situations was based on the detailed in-vitro study by Tsai et al. (Tsai et al., 1992) Since then, many in-vitro and in-vivo studies were conducted and reported on the molecular basis of LIPUS stimulation of bone cell matrix production and hence its clinical applications. The most recent studies showed that LIPUS enhances different early and late osteoblastic differentiation gene expressions. In most of these reports, alkaline phosphates were markedly increased by LIPUS in different bone cell lines. The enhanced osteogenic differentiation by LIPUS is suggested to be due to activation of early differentiation genes (c-jun, c-myc, COX-2, Egr-1, TSC-22) as well as the bone differentiation marker genes, osteonectin and osteopontin, phosphorylation (Tam et al., 2008) as well as activation of ERK1/2 and p38 MAPK pathways (Sena et al., 2005). Most importantly, LIPUS enhances bone morphogenetic proteins expression in bone cells (Suzuki et al., 2009). The importance of this report that LIPUS enhances BMPs expression is that LIPUS might be a future substitute of many BMPs-based therapies.

Another cell type that has been reported to be stimulated by LIPUS to enhance matrix production is macrophages. It has been shown that LIPUS up-regulated the phagocytosis of human primary macrophages through the activation of Src and ERKs and promoted the protein expression of CD147 and MMPs, as well as increased the level of protein tyrosine phosphorylation (Ikeda et al., 2006; Li et al., 2007a). Also, it has been reported that the effect of LIPUS on macrophages depends on cell adhesion, and relates to the integrin-MEK-ERK pathway (Li et al., 2007b). The stimulatory effect of LIPUS on the phagocytosis of macrophages may be important in tissue engineering, especially during tissue replacement and scaffold degradation for optimum integration between the engineered and original tissues. Another effect of LIPUS on macrophages is the ability of LIPUS to liberate fibroblast mitogenic factors from macrophages which are known to be a source of many important growth factors which can act as wound mediators during tissue repair. This stimulatory effect of ultrasound appeared to be mediated by producing permeability changes in these cells as well as to stimulate the cell's ability to synthesize and secrete fibroblast mitogenic factors (Young & Dyson, 1990a).

Also, LIPUS has been shown to enhance epithelial cell repair (Hill et al, 2005) as well as to accelerate tendon healing via regulation of vascular endothelial growth factor (VEGF) expression (Hill et al., 2005; Lu et al., 2008). Other reports showed that LIPUS enhances VEGF expression by fibroblasts, osteoblasts and monocytes (Doan et al., 1999). Moreover, other angiogenesis-related cytokines, IL-8 and bFGF, were significantly stimulated in osteoblasts by LIPUS (Reher et al., 1999). Earlier reports also supported the importance of LIPUS in supporting angiogenesis (Young & Dyson, 1990b; Young & Dyson, 1990c). The importance of VEGF and angiogenic factors expression by LIPUS in different tissues is very important in tissue engineering, especially bone tissue engineering. Angiogenesis is reported to be a key factor in bone maturation and bone fracture healing (Rabie et al., 2002; Rabie et al., 2001; El-Bialy et al., 2003).

Very recently, it has been reported that dental tissue matrices can be formed by LIPUS in vitro and in vivo. The first report about new dental tissue formation was reported while LIPUS was applied to osteodistracted rabbit mandibles (El-Bialy, 2003). A follow up pilot clinical study in human patients showed for the first time that human dental tissue matrices (dentine and cementum) are formed by LIPUS in 28 days (El-Bialy et al., 2004). These two studies triggered several in-vitro studies and potential tissue engineering teeth using LIPUS (Dalla-Bona et al., 2006; Scheven et al., 2007; Dalla-Bona et al., 2008; Inubushi et al., 2008; Scheven et al., 2009a; Scheven et al., 2009b). Moreover, recently, we have shown that LIPUS has an anabolic effect on human gingival fibroblasts, which in turn may help to use these cells in craniofacial tissue engineering (Mostafa et al., 2009). The preliminary conclusion of these studies may suggest that LIPUS can play an important role in dental and craniofacial tissue engineering.

Another potential application of LIPUS in enhancing tissue matrix formation for tissue engineering is via enhancing gene delivery to promote dentine, bone and other tissue engineering. Recent appreciable number of studies reported that LIPUS can provide a synergistic tool to enhance gene delivery to different cell types for tissue engineering (Inagaki et al., 2006; Kaigler et al., 2006; Kimelman et al., 2006; Nozaki et al., 2006; Tachibana et al., 2006; Saito et al., 2007; Shen et al., 2008; Sheyn et al., 2008; Kamimura & Liu, 2009; Osawa et al., 2009; Sakai et al., 2009;). The sonoporation technique, which utilizes ultrasound to facilitate drug delivery or gene transfection into cells has been accepted both in vitro and in vivo, however clinical application of this technique for tissue engineering still needs further optimization in-vitro experiments before it might be applicable clinically. The ultrasound used in sonoporation is quite different from LIPUS, however an optimization experiment for utilizing LIPUS in sonoporation might be justified.

## 4. Therapeutic ultrasound and its role in enhancing tissue engineering in vivo

It has been widely accepted that LIPUS can enhance cartilage repair based on the previously mentioned in-vitro studies. Moreover, it has been suggested that LIPUS can precondition chondrogenic derived stem cells for in-vivo cartilage tissue engineering (Schumann et al., 2006b; Cui et al., 2007). In an in-vivo pilot study, we have shown that LIPUS can enhance tissue engineered articular condyles in rabbits (El-Bialy et al., 2009). The optimum LIPUS treatment for maximum tissue engineered cartilage/or articular joints is still yet to be established.

On the other hand, there are controversial reports about the clinical efficacy of LIPUS in enhancing repair of fractured bone in human patients. While earlier reports showed that LIPUS enhances and accelerates repair of fractured bones (Heckman et al., 1994; Cook et al., 1997; Kristiansen et al., 1997; Hadjiargyrou et al., 1998; Mayr et al., 2000; Warden et al., 2000; Nolte et al., 2001; Jingushi et al., 2007; Walker et al., 2007; Griffin et al., 2008; Busse et al., 2009; El-Bialy et al., 2009), recent reports showed no difference in the healing of the LIPUS treated and nontreated fractured bones (Emami et al., 1999; Rue et al., 2004; Handolin et al., 2005; Lubbert et al., 2008).

The controversial reports about the clinical success of using LIPUS in repairing bone fractures could be due to the difference in the proximity of the fractured bone to the LIPUS transducer. It can be noted that most published reports with successful LIPUS treatment were performed on clinically accessible bones for LIPUS application, while deep bones or bones with thick muscle covering showed less success in accelerating bone fracture healing by LIPUS. A simple explanation of this is that the LIPUS power attenuates exponentially as a function of distance. In a rough estimate of LIPUS power attenuation, we have presented a simplified mathematical calculation as follows based on our experience in LIPUS attenuation of rabbit mandibles. An order-of magnitude estimate of the degree of attenuation and relative dose strengths between the direct and indirect sides can be made based on the following assumptions: 1) plane wave propagation through one side of the jaw and mandible, through the tongue and into the other side of the jaw and mandible (in reality, there will be additional loss due to scattering, especially with the presence of air regions); and 2) the pressure attenuation coefficient for teeth/mandible is α bone= 1.5 Np/cm and for the tongue is α tongue = 0.15 Np/cm at 1.5 MHz (Goss et al., 1978; Goss et al., 1980) assuming the bone thickness is 0.5 cm and the tongue thickness is 2 cm. With these assumptions the fraction of the intensity reaching the other side of the jaw would be:

$$I/I_0 = \text{Exp}\,[-2\,\alpha\,\text{bone}\,d\,\text{bone}]\,\text{Exp}\,[-2\,\alpha\,\text{tongue}\,d\,\text{tongue}] \quad (1)$$

$$= \text{Exp}\,[-2(1.5)(0.5)]\,\text{Exp}\,[-2(0.15)(2)]$$

$$= 0.122.$$

Where I is the intensity at the other side and $I_0$ is the intensity incident on the first (treated) side. This is a very rough estimate that may be taken as an upper bound; but, it predicts that 12.2% of the energy gets to the far (indirect) side relative to the near (direct) side. It has to be noted as previously mentioned that the stimulatory effect of LIPUS in enhancing tissue matrix production and bone fracture healing is dose dependent (El-Bialy et al., 2002; Schumann et al., 2006a). This could explain in part the differences in clinical outcomes between studies when considering differences in bone sizes.

Another consideration that needs to be taken into account while using LIPUS for tissue repair and/or tissue engineering is that the differences in LIPUS power could be different with different applications. In another words, when using LIPUS to enhance cell proliferation and/or matrix production in vitro, lower power is needed as there is no intervening tissues between the transducer and the cells. While in animal or clinical applications, many tissues can be intervening between the point of LIPUS application and the target bone or tooth, which needs further experimentation to reach the optimum LIPUS needed to reach each target for optimum results.

It is to be noted that different cell types might respond differently to LIPUS treatment time. While stem cells are stimulated by LIPUS in a dose dependent response (Schumann et al., 2006a); the optimum LIPUS treatment for skin fibroblasts is 10 minutes per day (Zhou et al., 2004). Moreover, clinical applications using LIPUS has been accepted to be 20 minutes per day for 3-4 weeks (Heckman et al., 1994; Cook et al., 1997; Kristiansen et al., 1997; Hadjiargyrou et al., 1998; Mayr et al., 2000; Warden et al., 2000; Nolte et al., 2001; Jingushi et al., 2007; Walker et al., 2007; Griffin et al., 2008; Busse et al., 2009), in-vitro responses vary from one day to 21 days. It is to be remembered that in-vitro experiments allow direct effect of LIPUS to the cells, while in-vivo application suffers from LIPUS power attenuation as explained above. In this context, in-vitro results might not be completely applicable to in-vivo applications.

*Conclusion and future directions:*

We can conclude that there is ample literature that supports that LIPUS can be utilized to enhance cell proliferation and differentiation, matrix production with the differentiated cells, gene transfection for cell differentiation as well as tissue repair both in vitro and in vivo. The optimum LIPUS treatment time and dose is still yet to be studied. The potential applications of LIPUS in tissue engineering could be used for bone, cartilage, skin, nerve, and possibly teeth tissue engineering. A final remark is that the daily use of LIPUS for an extended period of time should not be of concern as the safety of this type of low level power ultrasound has been addressed before and proven to be safe (Mende et al., 1996; Hata et al., 1997; Blaas & Eik-Nes, 1998; Turnbull & Foster, 2002).

## 5. References

Blaas, H.G. & Eik-Nes, S.H. (1998). Advances in the imaging of the embryonic brain. *Croatian Medical Journal,* Jun;39(2):128-131, ISSN 0353-9504 (Print) 1332-8166 (Electronic).

Busse, J.W.; Kaur, J.; Mollon, B.; Bhandari, M.; Tornetta, P. 3rd; Schünemann, H.J. & Guyatt, G.H. (2009). Low intensity pulsed ultrasonography for fractures: systematic review of randomised controlled trials. *BMJ (Clinical research ed),* Feb 27;338:b351, ISSN 0959-8138 (Print) 1468-5833 (Electronic).

Carrier, R.L.; Papadaki, M.; Rupnick, M.; Schoen, F.J.; Bursac, N.; Langer, R.; Freed, L.E. & Vunjak-Novakovic, G. (1999). Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization, *Biotechnology and Bioengineering,* Sep 5; 64(5):580–589, ISSN 0006-3592 (Print) 1097-0290 (Electronic).

Chen, Y.C.; Lee, D.C.; Hsiao, C.Y.; Chung, Y.F.; Chen, H.C.; Thomas, J.P.; Pong, W.F.; Tai, N.H.; Lin, I.N. & Chiu, I.M. (2009). The effect of ultra-nanocrystalline diamond films on the proliferation and differentiation of neural stem cells. *Biomaterials,* Jul;30(20):3428-3435, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Chu, T.M.; Orton, D.G.; Hollister, S.J.; Feinberg, S.E. & Halloran, J.W. (2002). Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures. *Biomaterials,* Mar;23(5):1283-1293, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Cook, S.D.; Ryaby, J.P.; McCabe, J.; Frey, J.J.; Heckman, J.D. & Kristiansen, T.K. (1997). Acceleration of tibia and distal radius fracture healing in patients who smoke. *Clinical Orthopaedics and Related Research,* Apr;(337):198-207, ISSN 0009-921X (Print) 1528-1132 (Electronic).

Cui, J.H.; Park, S.R.; Park, K.; Choi, B.H. & Min, B.H. (2007). Preconditioning of mesenchymal stem cells with low-intensity ultrasound for cartilage formation in vivo. *Tissue Engineering,* Feb;13(2):351-360, ISSN 1076-3279 (Print) 1557-8690 (Electronic).

Dalla-Bona, D.A.; Tanaka, E.; Oka, H.; Yamano, E.; Kawai, N.; Miyauchi, M.; Takata, T. & Tanne, K. (2006). Effects of ultrasound on cementoblast metabolism in vitro: *Ultrasound in Medicine & Biology,* Jun;32(6):943-948, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Dalla-Bona, D.A.; Tanaka, E.; Inubushi, T.; Oka, H.; Ohta, A.; Okada, H.; Miyauchi, M.; Takata, T. & Tanne, K. (2008). Cementoblast response to low- and high-intensity ultrasound. *Archives of Oral Biology,* Apr;53(4): p. 318-323, ISSN 0003-9969 (Print) 1879-1506 (Electronic).

De Mattei, M.; Caruso, A.; Pezzetti, F.; Pellati, A.; Stabellini, G.; Sollazzo, V. & Traina G.C. (2001). Effects of pulsed electromagnetic fields on human articular chondrocyte proliferation. *Connective Tissue Research,* 42(4):269-279, ISSN 0300-8207 (Print) 1607-8438 (Electronic).

Doan, N.; Reher, P.; Meghji, S. & Harris, M. (1999). In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *Journal of Oral and Maxillofacial Surgery,* Apr;57(4):409-419, ISSN 0278-2391 (Print) 1531-5053 (Electronic).

Ebisawa, K.; Hata, K.; Okada, K.; Kimata, K.; Ueda, M.; Torii, S. & Watanabe, H. (2004). Ultrasound enhances transforming growth factor beta-mediated chondrocyte differentiation of human mesenchymal stem cells. *Tissue Engineering,* May-June;10(5-6):921-929, ISSN 1076-3279 (Print) 1557-8690 (Electronic).

El-Bialy, T.; Uludag, H.; Doschak, M.; Jomha, N. & Badylak, S. (2009). Functionally integrated tissue engineered mandibular condyle. Canadian Biomedical Engineering Conference, Calgary, May, 2009. (Proceeding of the Canadian Acoustics Association meeting, October, 2008).

El-Bialy, T.H.; el-Moneim Zaki, A. & Evans, C.A. (2003). Effect of ultrasound on rabbit mandibular incisor formation and eruption after mandibular osteodistraction. *American Journal of Orthodontics and Dentofacial Orthopedics,* Oct;124(4):427-434, ISSN 0889-5406 (Print) 1097-6752 (Electronic).

El-Bialy, T.; El-Shamy I. & Graber, T.M. (2004). Repair of orthodontically induced root resorption by ultrasound in humans. *American Journal of Orthodontics and Dentofacial Orthopedics* Aug;126(2):186-193, ISSN 0889-5406 (Print) 1097-6752 (Electronic).

El-Bialy, T.H.; Royston, T.J.; Magin, R.L.; Evans, C.A.; Zaki, A.E. & Frizzell, L.A. (2002). The effect of pulsed ultrasound on mandibular distraction. *Annals of Biomedical Engineering,* Nov-Dec;30(10):1251-1261, ISSN 0090-6964 (Print) 1521-6047 (Electronic).

Emami, A.; Petrén-Mallmin, M. & Larsson, S. (1999). No effect of low-intensity ultrasound on healing time of intramedullary fixed tibial fractures. *Journal of OrthopaedicTrauma*, May;13(4):252-257, ISSN 0890-5339 (Print) 1531-2291 (Electronic).

Freed, L.E.; Hollander, A.P.; Martin, I.; Barry, J.R.; Langer, R. & Vunjak-Novakovic, G. (1998). Chondrogenesis in a cell–polymer–bioreactor system. *Experimental Cell Research,* Apr 10;240(1):58-65, ISSN 0014-4827 (Print) 1090-2422 (Electronic).

Goldstein, A.S. (2001). Effect of seeding osteoprogenitor cells as dense clusters on cell growth and differentiation. *Tissue Engineering,* Dec;7(6):817-27, ISSN 1076-3279 (Print) 1557-8690 (Electronic).

Goss, S.A.; Johnston, R.L. & Dunn, F. (1980). Compilation of empirical ultrasonic properties of mammalian tissues, II. *The Journal of the Acoustical Society of America,* Jul;68(1):93-108, ISSN 0001-4966 (Print) 1520-8524 (Electronic).

Goss, S.A.; Johnston, R.L. & Dunn, F. (1978). Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. *The Journal of the Acoustical Society of America,* Aug;64(2):423-457, ISSN 0001-4966 (Print) 1520-8524 (Electronic).

Griffin, X.L.; Costello, I. & Costa, M.L. (2008). The role of low intensity pulsed ultrasound therapy in the management of acute fractures: a systematic review. *The Journal of Trauma,* Dec;65(6):1446-1452, ISSN 0022-5282 (Print) 1529-8809 (Electronic).

Hadjiargyrou, M.; McLeod, K.; Ryaby, J.P. & Rubin, C. (1998). Enhancement of fracture healing by low intensity ultrasound. *Clinical Orthopaedics and Related Research,* Oct;(355 Suppl):S216-229, ISSN 0009-921X (Print) 1528-1132 (Electronic).

Handolin, L.; Kiljunen, V.; Arnala, I.; Kiuru, M.J.; Pajarinen, J.; Partio, E.K. & Rokkanen, P. (2005). No long-term effects of ultrasound therapy on bioabsorbable screw-fixed lateral malleolar fracture. *Scandinavian Journal of Surgery* 94(3):239-242, ISSN 1457-4969 (Print).

Harris, G.R. (2005). Progress in medical ultrasound exposimetry. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control,* May;52(5):717-736, ISSN 0885-3010 (Print) 1525-8955 (Electronic).

Hartig, M.; Joos, U. & Wiesmann, H.P. (2000). Capacitively coupled electric fields accelerate proliferation of osteoblast-like primary cells and increase bone extracellular matrix formation in vitro. *European Biophysics Journal,* 29(7):499-506, ISSN 0175-7571 (Print) 1432-1017 (Electronic).

Hata, T.; Aoki, S.; Manabe, A.; Hata, K. & Miyazaki, K. (1997). Three dimensional ultrasonography in the first trimester of human pregnancy. *Human Reproduction (Oxford, England),* 12(8):1800-1804, ISSN 0268-1161 (Print) 1460-2350 (Electronic).

Heckman, J.D.; Ryaby, J.P.; McCabe, J.; Frey, J.J. & Kilcoyne, R.F. (1994). Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. *The Journal of Bone and Joint Surgery. American volume,* Jan;76(1):26-34, ISSN 0021-9355 (Print) 1535-1386 (Electronic).

Hill, G.E.; Fenwick, S.; Matthews, B.J.; Chivers, R.A. & Southgate, J. (2005). The effect of low-intensity pulsed ultrasound on repair of epithelial cell monolayers in vitro. *Ultrasound in Medical & Biology,* Dec;31(12):1701-1706, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Holy, C.E.; Shoichet, M.S. & Davies, J.E. (2000). Engineering three-dimensional bone tissue in vitro using biodegradable scaffolds: investigating initial cell-seeding density and culture period. *Journal of Biomedical Materials Research,* Sep 5;51(3):376-382, ISSN 0021-9304 (Print) 1097-4636 (Electronic).

Horwitz, E.M. (2004). Dkk-1-mediated expansion of adult stem cells. *Trends in Biotechnology,* Aug;22(8):386-388, ISSN 0167-7799 (Print) 1879-3096 (Electronic).

Hsieh-Bonassera, N.D.; Wu, I.; Lin, J.K.; Schumacher, B.L.; Chen, A.C.; Masuda, K.; Bugbee, W.D. & Sah, R.L. (2009). Expansion and redifferentiation of chondrocytes from osteoarthritic cartilage: cells for human cartilage tissue engineering. *Tissue*

*engineering, Part A,* May 20 [Epub ahead of print], ISSN 1076-3279 (Print) 1557-8690 (Electronic).

Hsu, S.H.; Kuo, C.C.; Whu, S.W.; Lin, C.H. & Tsai, C.L. (2006). The effect of ultrasound stimulation versus bioreactors on neocartilage formation in tissue engineering scaffolds seeded with human chondrocytes in vitro. *Biomolecular Engineering,* Oct;23(5):259-264, ISSN 1389-0344 (Print).

Ikeda, K.; Takayama, T.; Suzuki, N.; Shimada, K.; Otsuka, K. & Ito, K. (2006). Effects of low-intensity pulsed ultrasound on the differentiation of C2C12 cells. *Life Sciences,* Oct 12;79(20):1936-1943, ISSN 0024-3205 (Print) 1879-0631 (Electronic).

Inagaki, H.; Suzuki, J.; Ogawa, M.; Taniyama, Y.; Morishita, R. & Isobe, M. (2006). Ultrasound-microbubble-mediated NF-&kappaB decoy transfection attenuates neointimal formation after arterial injury in mice. *Journal of Vascular Research,* Feb;43(1):12-18, ISSN 1018-1172 (Print)1423-0135 (Electronic).

Inubushi, T.; Tanaka, E.; Rego, E.B.; Kitagawa, M.; Kawazoe, A.; Ohta, A,; Okada, H.; Koolstra, J.H.; Miyauchi, M.; Takata, T. &, Tanne, K. (2008). Effects of ultrasound on the proliferation and differentiation of cementoblast lineage cells. *Journal of Periodontology,* Oct;79(10):1984-1990, ISSN 0022-3492 (Print).

Iwabuchi, S.; Ito, M.; Hata, J.; Chikanishi, T.; Azuma, Y. & Haro, H. (2005). In vitro evaluation of low-intensity pulsed ultrasound in herniated disc resorption. *Biomaterials,* Dec;26(34):7104-7114, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Iwashina, T.; Mochida, J.; Miyazaki, T.; Watanabe, T.; Iwabuchi, S.; Ando, K.; Hotta, T. & Sakai, D. (2006). Low-intensity pulsed ultrasound stimulates cell proliferation and proteoglycan production in rabbit intervertebral disc cells cultured in alginate. *Biomaterials,* Jan;27(3):354-361, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Jacobsen, S.E.; Okkenhaug, C.; Myklebust, J.; Veiby, O.P. & Lyman, S.D. (1995). The FLT3 ligand potently and directly stimulates the growth and expansion of primitive murine bone marrow progenitor cells in vitro: synergistic interactions with interleukin (IL) 11, IL-12, and other hematopoietic growth factors. *The Journal of Experimental Medicine,* Apr 1;181(4):1357-1363, ISSN 0022-1007 (Print)1540-9538 (Electronic).

Jingushi, S.; Mizuno, K.; Matsushita, T. & Itoman, M. (2007). Low-intensity pulsed ultrasound treatment for postoperative delayed union or nonunion of long bone fractures. *Journal of Orthopaedic Science,* Jan;12(1):35-41, ISSN 0949-2658 (Print) 1436-2023 (Electronic).

Kaigler, D.; Cirelli, J.A. & Giannobile, W.V. (2006). Growth factor delivery for oral and periodontal tissue engineering. *Expert Opinion on Drug Delivery*. Sep;3(5):647-662, ISSN 1742-5247 (Print)1744-7593 (Electronic).

Kamimura, K. & Liu, D. (2009). Physical approaches for nucleic acid delivery to liver. *The AAPS Journal,* Jan;10(4):589-595, ISSN 1550-7416 (Electronic).

Kimelman, N.; Pelled, G.; Gazit, G.P.Z. & Gazit, D. (2006). Applications of gene therapy and adult stem cells in bone bioengineering. *Regenerative Medicine,* Aug;1(4):549-561, ISSN 1746-0751 (Print)1746-076X (Electronic).

Kobayashi, Y.; Sakai, D.; Iwashina, T.; Iwabuchi, S. & Mochida, J. (2009). Low-intensity pulsed ultrasound stimulates cell proliferation, proteoglycan synthesis and expression of growth factor-related genes in human nucleus pulposus cell line. *European Cells & Materials,* Jun 30;17:15-22, ISSN 1473-2262 (Electronic).

Korstjens, C.M.; van der Rijt, R.H.; Albers, G.H.; Semeins, C.M. & Klein-Nulend. J. (2008). Low-intensity pulsed ultrasound affects human articular chondrocytes in vitro. *Medical & Biological Engineering & Computing,* Dec;46(12):1263-1270, ISSN 0140-0118 (Print)1741-0444 (Electronic).

Kristiansen, T.K.; Ryaby, J.P.; McCabe, J.; Frey, J.J. & Roe, L.R. (1997). Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. *The Journal of Bone and Joint Surgery. American Volume,* Jul;79(7):961-973, ISSN 0021-9355 (Print) 1535-1386 (Electronic).

Leddy, H.A.; Awad, H.A. & Guilak, F. (2004). Molecular diffusion in tissue-engineered cartilage constructs: effects of scaffold material, time, and culture conditions. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials,* Aug 15;70(2):397-406, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Lefebvre. P.; Lin, J.; Linzer, D.I. & Cohen, I. (2001). Murine prolactin-like protein E synergizes with human thrombopoietin to stimulate expansion of human megakaryocytes and their precursors. *Experimental Hematology,* Jan;29(1):51-58, ISSN 0301-472X (Print) 1873-2399 (Electronic).

Leung, K.S.; Cheung, W.H.; Zhang, C.; Lee, K.M. & Lo, H.K. (2004). Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. *Clinical Orthopaedics and Related Research,* Jan;(418):253-259, ISSN 0009-921X (Print)1528-1132 (Electronic).

Li,Y.; Ma, T.; Kniss, D.A.; Lasky, L.C. & Yang, S.T. (2001). Effects of filtration seeding on cell density, spatial distribution, and proliferation in nonwoven fibrous matrixes, *Biotechnology Progress,* Sep-Oct;17(5):935:944, ISSN 8756-7938 (Print) 1520-6033 (Electronic).

Li, Y.P.; Zhou, S.X.; Schmelz, A. & Bachem, M.G. (2007a). [Molecular mechanisms of low intensity pulsed ultrasound-mediated cellular behavior in human primary macrophages]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi/Zhongguo Bing Li Sheng Li Xue Hui,* Dec;15(6):1253-1256, ISSN 1009-2137 (Print).

Li, Y.P.; Zhou, S.X.; Andreas, S. & Max, G.B. (2007b). Effect of LIPUS on the cellular behavior of human primary macrophages. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi,* Dec;23(12):1113-1116, ISSN 1007-8738 (Print).

Lu, H.; Qin, L.; Cheung, W.; Lee, K.; Wong, W. & Leung, K. (2008). Low-intensity pulsed ultrasound accelerated bone-tendon junction healing through regulation of vascular endothelial growth factor expression and cartilage formation. *Ultrasound in Medicine & Biology,* Aug;34(8):1248-1260, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Lubbert, P.H.; van der Rijt, R.H.; Hoorntje, L.E. & van der Werken, C. (2008). Low-intensity pulsed ultrasound (LIPUS) in fresh clavicle fractures: a multi-centre double blind randomised controlled trial. *Injury*, Dec;39(12):1444-1452, ISSN 0020-1383 (Print) 1879-0267 (Electronic).

Maylia, E. & Nokes, L.D. (1999). The use of ultrasonics in orthopaedics. a review. *Technology and Health Care,* 7(1): 1–28, ISSN 0928-7329 (Print).

Mayr, E.; Rudzki, M.M.; Rudzki, M.; Borchardt, B.; Häusser, H. & Rüter, A. (2000). [Does low intensity, pulsed ultrasound speed healing of scaphoid fractures?].

*Handchirurgie, Mikrochirurgie, Plastische Chirurgie,* Mar;32(2):115-122, ISSN 0722-1819 (Print) 1439-3980 (Electronic).

Mende, U.; Zoller, J.; Dietz, A.; Wannenmacher, M.; Born, I.A. & Maier, H. (1996). [Ultrasound diagnosis in primary staging of head-neck tumors]. *Der Radiologe,* Mar;36(3):207-216, ISSN 0033-832X (Print)1432-2102 (Electronic).

Mostafa, N.Z.; Uludağ, H.; Dederich, D.N.; Doschak, M.R. & El-Bialy, T.H. (2009). Anabolic effects of low-intensity pulsed ultrasound on human gingival fibroblasts. *Archives of Oral Biology,* Aug;54(8):743-8, ISSN 0003-9969 (Print) 1879-1506 (Electronic).

Mukai, S.; Ito, H.; Nakagawa, Y.; Akiyama, H.; Miyamoto, M. & Nakamura, T. (2005). Transforming growth factor-beta1 mediates the effects of low-intensity pulsed ultrasound in chondrocytes. *Ultrasound in Medicine & Biology,* Dec;31(12):1713-1721, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Nolte, P.A.; van der Krans, A.; Patka, P.; Janssen, I.M.; Ryaby, J.P. & Albers, G.H. (2001). Low-intensity pulsed ultrasound in the treatment of nonunions. *The Journal of Trauma,* Oct;51(4):693-702; discussion 702-3, ISSN 0022-5282 (Print) 1529-8809 (Electronic).

Nozaki, T.; Ogawa, R.; Watanabe, A.; Nishio, R.; Fuse, H. & Kondo, T. (2006). [Ultrasound-mediated gene transfection: problems to be solved and future possibilities]. *Journal of Medical Ultrasonics.* Nov;33(3):135-142, ISSN 1346-4523 (Print) 1613-2254 (Electronic).

Osawa, K.; Okubo, Y.; Nakao, K.; Koyama, N. & Bessho, K. (2009). Osteoinduction by microbubble-enhanced transcutaneous sonoporation of human bone morphogenetic protein-2. *The Journal of Gene Medicine,* Jul;11(7):633-641, ISSN 1099-498X (Print) 1521-2254 (Electronic).

Panossian, A.; Ashiku, S.; Kirchhoff, C.H.; Randolph, M.A. & Yaremchuk, M.J. (2001). Effects of cell concentration and growth period on articular and ear chondrocyte transplants for tissue engineering. *Plastic and Reconstructive Surgery,* Aug;108:392-402, ISSN 0032-1052 (Print) 1529-4242 (Electronic).

Puelacher, W.C.; Kim, S.W.; Vacanti, J.P.; Schloo, B.; Mooney, D. & Vacanti, C.A. (1994). Tissue-engineered growth of cartilage: the effect of varying the concentration of chondrocytes seeded onto synthetic polymer matrices. *International Journal of Oral and Maxillofacial Surgery,* Feb;23(1):49-53, ISSN 0901-5027 (Print) 1399-0020 (Electronic).

Purpura, K,A,; Aubin, J.E. & Zandstra, P.W. (2004). Sustained in vitro expansion of bone progenitors is cell density dependent. *Stem Cells*. 2004;22(1):39-50, ISSN 0250-6793.

Rabie, A.B.; Shum, L. & Chayanupatkul, A. (2002). VEGF and bone formation in the glenoid fossa during forward mandibular positioning. *American Journal of Orthodontics and Dentofacial Orthopedics,* Aug;122(2):202–209, ISSN 0889-5406 (Print) 1097-6752 (Electronic).

Rabie, A.B.; Zhao, Z.; Shen. G.; Hagg, E.U. & Robinson, W. (2001). Osteogenesis in the glenoid fossa in response to mandibular advancement. *American Journal of Orthodontics and Dentofacial Orthopedics,* Apr;119(4):390–400, ISSN 0889-5406 (Print) 1097-6752 (Electronic).

Reher, P.; Doan, N.; Bradnock, B.; Meghji, S. & Harris, M. (1999). Effect of ultrasound on the production of IL-8, basic FGF and VEGF. *Cytokine,* Jun;11(6):416-423, ISSN 1043-4666 (Print) 1096-0023 (Electronic).

Rue, J.P.; Armstrong, D.W. 3rd; Frassica, F.J.; Deafenbaugh, M. & Wilckens, J.H. (2004). The effect of pulsed ultrasound in the treatment of tibial stress fractures. *Orthopedics*. Nov;27(11):1192-1195, ISSN 0147-7447 (Print) 1938-2367 (Electronic).

Saini, S., & Wick, T.M. (2003). Concentric cylinder bioreactor for production of tissue engineered cartilage: effect of seeding density and hydrodynamic loading on construct development. *Biotechnology Progress,* Mar-Apr;19(2):510-521, ISSN 8756-7938 (Print)1520-6033 (Electronic).

Saito, M.; Soshi, S.; Tanaka, T. & Fujii, K. (2004). Intensity-related differences in collagen post-translational modification in MC3T3-E1 osteoblasts after exposure to low- and high-intensity pulsed ultrasound. *Bone*. Sep;35(3):644-655, ISSN 8756-3282 (Print) 1873-2763 (Electronic).

Saito, M.; Mazda, O.; Takahashi, K.A.; Arai Y.; Kishida, T.; Shin-Ya, M.; Inoue, A.; Tonomura, H.; Sakao, K.; Morihara, T.; Imanishi, J.; Kawata, M. & Kubo, T. (2007). Sonoporation mediated transduction of pDNA/siRNA into joint synovium in vivo. *Journal of Orthopaedic Research,* Nov;25(10):1308-1316, ISSN 0736-0266 (Print) 1554-527X (Electronic).

Sakai, T.; Kawaguchi, M. & Kosuge, Y. (2009). siRNA-mediated gene silencing in the salivary gland using in vivo microbubble-enhanced sonoporation. *Oral Diseases,* Jun 10 (Epub ahead of print), ISSN 1354-523X (Print) 1601-0825 (Electronic).

Scheven, B.A.; Man, J.; Millard, J.L.; Cooper, P.R.; Lea, S.C.; Walmsley, A.D. & Smith, A.J. (2009a). VEGF and odontoblast-like cells: stimulation by low frequency ultrasound. *Archives of Oral Biology,* Feb;54(2):185-191, ISSN 0003-9969 (Print) 1879-1506 (Electronic).

Scheven, B.A.; Millard, J.L.; Cooper, P.R.; Lea, S.C.; Walmsley, A.D. & Smith, A.J. (2007). Short-term in vitro effects of low frequency ultrasound on odontoblast-like cells. *Ultrasound in Medicine & Biology,* Sep;33(9):1475-1482, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Scheven, B.A.; Shelton, R.M.; Cooper, P.R.; Walmsley, A.D. & Smith, A.J. (2009b). Therapeutic ultrasound for dental tissue repair. *Medical Hypotheses*, Oct;73(4):591-593, ISSN 0306-9877 (Print) 1532-2777 (Electronic).

Schumann, D.; Kujat, R.; Zellner, J.; Angele, M.K.; Nerlich, M.; Mayr, E. & Angele, P. (2006a). Treatment of human mesenchymal stem cells with pulsed low intensity ultrasound enhances the chondrogenic phenotype in vitro. *Biorheology*. 43(3-4):431-43, ISSN 0006-355X (Print) 1878-5034 (Electronic).

Schumann, D.; Kujat, R.; Nerlich, M. & Angele, P. (2006b). Mechanobiological conditioning of stem cells for cartilage tissue engineering. *Bio-medical Materials and Engineering,* 16(4 Suppl):S37-52, ISSN 0959-2989 (Print) 1878-3619 (Electronic).

Sena, K.; Leven, R.M.; Mazhar, K.; Sumner, D.R. & Virdi, A.S. (2005). Early gene response to low-intensity pulsed ultrasound in rat osteoblastic cells. *Ultrasound in Medicine & Biology*, May;31(5):703-708, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Shen, Z.P.; Brayman, A.A.; Chen, L. & Miao, C.H. (2008). Ultrasound with microbubbles enhances gene expression of plasmid DNA in the liver via intraportal delivery. *Gene Therapy,* Aug;15(16):1147-1155, ISSN 0969-7128 (Print) 1476-5462 (Electronic).

Sheyn, D.; Kimelman-Bleich, N.; Pelled, G.; Zilberman, Y.; Gazit, D. & Gazit, Z. (2008). Ultrasound-based nonviral gene delivery induces bone formation in vivo. *Gene Therapy*. Febr;15(4):257-266, ISSN 0969-7128 (Print) 1476-5462 (Electronic).

Suzuki, A.; Takayama, T.; Suzuki, N.; Kojima, T.; Ota, N.; Asano, S. & Ito, K. (2009). Daily low-intensity pulsed ultrasound stimulates production of bone morphogenetic protein in ROS 17/2.8 cells. *Journal of Oral Science,* Mar;51(1):29-36, ISSN 1343-4934 (Print) 1880-4926 (Electronic).

Tachibana, K.; Irie, Y. & Ogawa, K. (2006). [Emerging technologies in therapeutic ultrasound]. *Journal of Medical Ultrasonics,* Feb;33(6):631-639, ISSN 1346-4523 (Print) 1613-2254 (Electronic).

Takagi, M. (2005). Cell processing engineering for ex-vivo expansion of hematopoietic cells. *Journal of Bioscience and Bioengineering,* Mar;99(3):189-196, ISSN 1389-1723 (Print) 1347-4421 (Electronic).

Takeuchi, R.; Ryo, A.; Komitsu, N.; Mikuni-Takagaki, Y.; Fukui, A.; Takagi, Y.; Shiraishi, T.; Morishita, S.; Yamazaki, Y.; Kumagai, K.; Aoki, I. & Saito, T. (2008). Low-intensity pulsed ultrasound activates the phosphatidylinositol 3 kinase/Akt pathway and stimulates the growth of chondrocytes in three-dimensional cultures: a basic science study. *Arthritis Research & Therapy,* 10(4):R77, ISSN 1478-6354 (Print) 1478-6362 (Electronic).

Tam, K.F.; Cheung, W.H.; Lee, K.M.; Qin, L. & Leung, K.S. (2008). Osteogenic effects of low-intensity pulsed ultrasound, extracorporeal shockwaves and their combination - an in vitro comparative study on human periosteal cells. *Ultrasound in Medicine & Biology*, Dec;34(12):1957-1965, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Tamama, K.; Fan, V.H.; Griffith, L.G.; Blair, H.C. & Wells, A. (2005). Epidermal growth factor as candidate for ex vivo expansion of bone marrow-derived mesenchymal stem cells. *Stem Cells,* Mar;24(3):686-695, ISSN 0250-6793.

Tien, Y.C.; Lin, S.D.; Chen, C.H.; Lu, C.C.; Su, S.J. & Chih, T.T. (2008). Effects of pulsed low-intensity ultrasound on human child chondrocytes. *Ultrasound in Medicine & Biology*, Jul;34(7):1174-1181, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Tsai, C.L.; Chang, W.H. & Liu, T.K. (1992). Preliminary studies of duration and intensity of ultrasonic treatments on fracture repair. *The Chinese Journal Of Physiology,* 35(1):21-26, ISSN 0304-4920 (Print). Erratum in: *The Chinese journal of physiology,* 35:168, 1992.

Turnbull, D.H. & Foster, F.S. (2002). In vivo ultrasound biomicroscopy in developmental biology. *Trends in Biotechnology,* 20(8):S29-33, ISSN 0167-7799 (Print) 1879-3096 (Electronic).

Walker, N.A.; Denegar, C.R. & Preische, J. (2007). Low-intensity pulsed ultrasound and pulsed electromagnetic field in the treatment of tibial fractures: a systematic review. *Journal of Athletic Training,* Oct-Dec;42(4):530-535, ISSN 1062-6050 (Print) 1938-162X (Electronic).

Wang, Y.; Singh, A.; Xu, P.; Pindrus, M.A.; Blasioli, D.J. & Kaplan, D.L. (2006). Expansion and osteogenic differentiation of bone marrow-derived mesenchymal stem cells on a vitamin C functionalized polymer. *Biomaterials,* Jun;27(17):3265-3273, ISSN 0142-9612 (Print) 1878-5905 (Electronic).

Warden, S.J.; Bennell, K.L.; McMeeken, J.M. & Wark, J.D. (2000). Acceleration of fresh fracture repair using the sonic accelerated fracture healing system (SAFHS): a

review. *Calcified Tissue International,* Feb;66(2):157-163, ISSN 0171-967X (Print) 1432-0827 (Electronic).

Wu, C.C.; Lewallen, D.G.; Bolander, M.E.; Bronk, M.E.; Kinnick, R. & Greenlead, J.F. (1996). Exposure to low-intensity ultrasound stimulates aggrecan gene expression by cultured chondrocytes. *Transactions - Orthopaedic Research Society,* 1996;21:622.

Young, S.R. & Dyson, M. (1990a). Macrophage responsiveness to therapeutic ultrasound. *Ultrasound in Medicine & Biology,* 16(8):809-816, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Young, S.R. & Dyson, M. (1990b). Effect of therapeutic ultrasound on the healing of full-thickness excised skin lesions. *Ultrasonics,* May;28(3):175-180, ISSN 0041-624X (Print) 1874-9968 (Electronic).

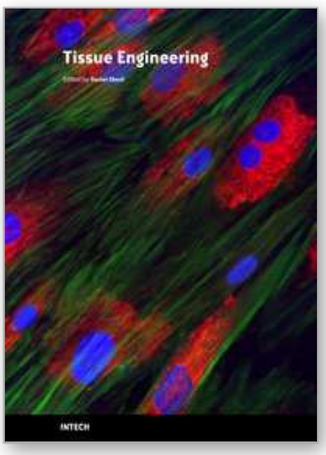
Young, S.R.; & Dyson, M. (1990c). The effect of therapeutic ultrasound on angiogenesis. *Ultrasound in Medicine & Biology,* 16(3):261-269, ISSN 0301-5629 (Print) 1879-291X (Electronic).

Zhang, C.C.; Kaba, M.; Ge, G.; Xie, K.; Tong, W.; Hug, C. & Lodish, H.F. (2006). Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. *Nature Medicine,* Feb;12(2):240-245, ISSN 1078-8956 (Print) 1546-170X (Electronic).

Zhou, S.; Schmetz, A.; Seufferlein, T.; Li, Y.; Zhao, J. & Bachem, M.G. (2004). Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts. *The* Journal of Biological Chemistry, Dec 24;279(52):54463-9, ISSN 0021-9258 (Print) 1083-351X (Electronic).

Ziskin, M.C. (1987). Applications of ultrasound in medicine: comparison with other modalities, Ultrasound: medical applications, biological effects, and hazard potential, Repacholi, M.H., Grandolfo, M., Rindi, A., (Eds.), 49-59, Plenum Press, ISBN 0306424118, New York, NY.

IntechOpen




**Tissue Engineering**

Edited by Daniel Eberli

ISBN 978-953-307-079-7
Hard cover, 524 pages
**Publisher** InTech
**Published online** 01, March, 2010
**Published in print edition** March, 2010

The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tarek H. El-Bialy (2010). Low Intensity Pulsed Ultrasound: A Laboratory and Clinical Promoter in Tissue Engineering Abstract, Tissue Engineering, Daniel Eberli (Ed.), ISBN: 978-953-307-079-7, InTech, Available from: http://www.intechopen.com/books/tissue-engineering/low-intensity-pulsed-ultrasound-a-laboratory-and-clinical-promoter-in-tissue-engineering-abstract

INTECH
open science | open minds

**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2010 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen