

Low Intensity Pulsed Ultrasound and Articular Cartilage Repair

Marks R¹

¹ Department of Health and Behavior Studies, Columbia University, Teachers College, USA

Conflict-of-interest statement: The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Marks R, Department of Health and Behavior Studies, Teachers College, Columbia University, Box 114, 525W, 120th Street, New York, NY 10027, USA.

Email: rm226@columbia.edu

Telephone: +1-212-678-3445

Fax: +1-212-678-8259

Received: July 1, 2019

Revised: July 15, 2019

Accepted: July 18 2019

Published online: August 28, 2019

ABSTRACT

Low intensity pulsed ultrasound [LIPUS], a physical modality that has demonstrated utility in bone healing contexts including non union fracture situations has been proposed to also impact articular cartilage chondrocyte cell metabolism in a favorable way. This current review focuses on two questions: a) whether LIPUS produces any clinically significant impact on chondrocyte structure or function; b) whether the impacts observed support a role for LIPUS in efforts to foster cartilage regeneration and healing applications. Drawn from the English language literature published over the last 35 years, the majority of the available basic or preclinical studies presently reviewed demonstrate LIPUS applications to consistently yield favorable cartilage chondrogenic responses, regardless of substrate, and to be worthy of further exploration in the clinical realm for promoting much sought

after osteoarthritic reparative and repair effects.

Key words: Articular Cartilage; Chondrocytes; Low Intensity Pulsed Ultrasound; Osteoarthritis; Regeneration, Repair; Treatment

© 2019 The Author(s). Published by ACT Publishing Group Ltd. All rights reserved.

Marks R. Low Intensity Pulsed Ultrasound and Articular Cartilage Repair. *International Journal of Orthopaedics* 2019; 6(4): 1151-1160 Available from: URL: <http://www.ghrnet.org/index.php/ijo/article/view/2671>

INTRODUCTION

Low intensity pulsed ultrasound (LIPUS), a non-thermal form of physical stimulation found to impact bone healing positively^[1-3], is associated with possible favorable mechanical and biochemical effects on the process of endochondral ossification, as well as articular cartilage chondrocyte cell metabolism^[2,4]. Since osteoarthritis, an incurable widespread debilitating joint disease where articular cartilage damage predominates remains an immense global health and socioeconomic issue, the use of LIPUS in fostering articular cartilage chondro-protective or reparative effects has hence been studied for some time. As recounted by Vaughan *et al*^[5] and Rothenberg *et al*^[6], in addition to the successful current clinical applications of ultrasound in various forms, several laboratory investigations conducted over several decades have shown LIPUS to produce favorable effects on cartilage matrix properties and expression^[7], including favorable impacts on scaffold-free cultured chondrocytes^[8] as well as gene expression in chitosan scaffolds^[9] and 3D matrices^[9]. Other studies, however, found the favorable impact to be only of temporary significance^[8], while others found no significant effect^[8], or emergent effects only after prolonged stimulation periods^[10]. Yet, LIPUS has been shown to impact synovial inflammation^[11], increase vascularity in erosive cartilage^[12], and to potentially decrease the extent of cartilage histopathology^[7], while potentially accelerating soft tissue and bone healing processes^[6].

At the same time, other favorable effects on muscle, and tendon, as well as inflammatory processes, imply that LIPUS applied to offset or treat osteoarthritis, the most prevalent joint disease, might be especially helpful in creating a favorable cartilage environment,

and/or for attenuating or reversing its predicted rate of deterioration in the face of limited current intervention approaches. Moreover, a biophysical modality that could be applied in the home or clinic and one that is safe and non-invasive, while positively fostering articular cartilage repair or limiting or slowing rates of its further damage, would undoubtedly be of immense value when considering the impact of the magnitude of the suffering presently encountered worldwide by osteoarthritis sufferers, as well its profound economic and social disease costs, when considering the limitations of available drugs, and resultant burgeoning surgical demands among aging populations. It might also impact the opioid epidemic to some degree which prevails in part due to the failure of medicine to relieve chronic osteoarthritic pain effectively without narcotics. Its thoughtful application may also provide a mechanism for fostering more direct drug delivery to chondrocytes, while positively affecting chondrocyte intracellular ion transport, cell signaling, and gene expression processes.

In light of the limitations of cartilage to heal, and the possible favorable use of LIPUS to induce disease modifying conformational changes within and around osteoarticular cartilage chondrocytes, this brief aimed to explore the past as well as recent data concerning the possible utility of LIPUS for fostering osteoarthritic cartilage repair, as well as for fostering seeding processes with respect to articular cartilage regeneration efforts, ideas accepted by several researchers, but not all^[13].

Key questions currently focused on in this review are a) whether LIPUS produces any uniform trend or significant impact on chondrocyte structure or function; b) whether the impacts observed support a role for LIPUS in efforts to foster clinical cartilage regeneration and healing applications in osteoarthritis related realms.

METHODS

Desired research publications were sought primarily from the **PUBMED, Science Direct, Scopus, Academic Search Complete,** and **EMBASE** data bases. Acceptable articles were those that specifically focused on the impact of LIPUS on articular cartilage, regardless of source, and only preclinical studies were examined as a basis for ascertaining a possible need for further research in this realm, and the possible direction of this. No restriction was placed on the disease model, method or substrate employed, or research design, but outcomes examined had to reflect upon some relationship between LIPUS applications and chondrocyte function or cartilage composition in either healthy or diseased or damaged tissue states. Years of specific interest were those extending from January 1 2015 - May 1, 2019 using the key words **articular cartilage** and **low intensity ultrasound [LIPUS]**, however, all publications published over the last 35 years and deemed eligible were screened, and accepted for further analysis after it was clear they fulfilled the current eligibility criteria. Background data on this topic were also reviewed, as were relevant items from other sources such as CINAHL, along with relevant citations of certain articles in an effort to ensure the inclusion of the most important and available topical data in this scoping review. Excluded were studies that did not address the key questions underpinning this review, including other biophysical modalities, studies on clinical samples, and non-English studies and English abstracts. Items published prior to 2015, and those dating from 2015 were tabulated separately, to better discern trends over time, and all were subjected to examination and narrative review, where applicable. No systematic meta-analyses were conducted given the limited number of comparable studies of

any type in this emerging field.

RESULTS

The present review, which embodied almost all published data in the English language peer reviewed data bases over the past 35 years, revealed that ultrasound, a physical modality used clinically for many years^[6] and that can be delivered in various modes, including a low frequency pulsed mode, quite consistently produces observed cellular responses that would appear favorable to articular cartilage chondrocytes examined either in cell cultures, or in the context of animal models of osteoarthritis (Tables 1-3). As outlined in PUBMED, a leading data base, with a total of 287 related papers published over the last 30 years using the terms low intensity ultrasound [LIPUS] and cartilage alone; with 95 of these publications having been listed in the past 5 years, this is an area of high ongoing interest. While very few clinical studies on this topic prevail, among the published preclinical studies, which include both outcome as well as explanatory approaches, Table 1 highlighting the most relevant background publications published prior to 2015, consistently shows LIPUS applications of differing doses to yield more favorable outcomes than not, regardless of substrate, as far as allaying osteoarthritis cartilage based disease progression in one or more potential ways. Further demonstrated is the considerable promise for LIPUS based laboratory efforts to foster cartilage repair or develop synthetic cartilage using LIPUS technology. In addition, those studies fulfilling the present review criteria and portrayed in Table 2, which are the more recent studies on this topic and that involve the use of cellular or explant substrates to identify and examine chondrocyte or stem cell responses to LIPUS applications, similarly show favorable results as a whole in the context of the topic of articular cartilage repair, along with notable plausible mechanisms of action to explain its apparent efficacy. As well, Table 3, housing those most recent LIPUS studies employing various animal models of osteoarthritis likewise provides fairly solid supportive evidence of the potential for LIPUS to favorably impact osteoarthritic chondrocytes, and cartilage structure and function, and to mediate this via several well established mechanistic pathways and cell functions known to be clinically salient as far as allaying osteoarthritis cartilage-based disease progression is concerned. Most striking is the considerable promise shown by LIPUS applications for reversing osteoarthritis pathology, laboratory efforts to foster cartilage repair, its ability to heighten other regenerative approaches synergistically, and for developing or enhancing synthetic cartilage, especially in the context of human chondrocytes, stem cells, and diseased osteoarthritis chondrocytes (Tables 1-3).

In addition to the above examples, and despite the lack of parallel clinical evidence, the past as well as the currently emerging LIPUS associated data are believed to hold considerable promise as regards cartilage repair^[41,42]. In particular, very similar LIPUS-related chondrogenic effects are seen regardless of substrate, such as mesenchymal stem cells^[20], and even among human osteoarthritic chondrocytes^[19], as well as those substrates derived from well-accepted animal models of osteoarthritis^[17,19,29]. Importantly positive metabolic outcomes have also been observed, even among osteoarthritic explants^[19] and animal models of idiopathic human osteoarthritis^[17], and when normal animal activity has been permitted^[21], and among mesenchymal stem cells, LIPUS promotes chondrogenesis even in the absence of Transforming Growth Factor- β ^[20], normally required to promote chondrogenesis in these cells^[20].

Table 1 Selected studies published prior to 2015 concerning low intensity pulsed ultrasound (LIPUS) and articular cartilage and joint related effects. Highlighted studies** are those of special note.

Authors	Substrate	Methods	Measures	Results
Bhatia et al[14]	Arthritic right radiocarpal joints of 8 cow calves induced by intraarticular injections of turpentine oil	Calves were randomly divided into groups of 4 A= control, B=US for 7 min per day for 7 days starting on day 5	Joint tissue histology	Joint tissues of group A showed severe inflammation, B showed regeneration
**Choi et al[15]	Human articular chondrocytes from osteoarthritis patients cultured in 3-dimensional alginate beads	Low intensity ultrasound (LIUS) was applied at 0, 100, 200, and 300 mW/cm(2) for 10 min per day for 2, 7, or 15 days to cells	Histological and biochemical enzymatic analyses	LIUS induced the viability of cells only at day 15, but not until day 7 after treatment
Chung et al[11]	Inflamed rat knee joint	LIPUS was applied to the knees each day for 10 days after inflammation induction	Proinflammatory factors and synovial staining patterns were assessed using immunohistology	LIPUS applications yielded a potent anti-inflammatory effect
Cook [16]	Knees of 18 dogs	2 plugs of cartilage from both knees were extracted, and one was treated with LIPUS	Articular surfaces were examined at 6wk and 12 wk grossly and histologically	LIPUS improved interface cartilage repair more effectively compared to controls
Coords et al[1]	Diabetic and non diabetic Wistar rats	Daily LIPUS was applied to fracture sites	Femoral fracture growth factor, and cartilage formation were assessed	LIPUS increased growth factor expression and cartilage formation, regardless of group diabetes status
Gurkan et al[17]	Male Hartely guinea pigs who develop osteoarthritis naturally	30mW/cm2 LIPUS was applied for 20 min per day from 3-10 months to assess its preventive as well as its remedial ability at degeneration onset and at a later stage	Joint cartilage graded according to Mankin scores, plus immunohistochemical analyses	LIPUS attenuated the process of cartilage degeneration, more so early on in the treatment schedule than later on
Hasanova et al[9]	Chitosan scaffolds	Low intensity diffuse ultrasound was applied to bovine chondrocytes embedded in Citosan matrices at constant durations but varying intensities	Chondrocyte biosynthesis and gene expression were assessed	The stimulation regimen modulated chondrocyte biosynthetic activity, and integrin mRNA expression
Huang et al[18]	Examined arthritic cartilage of rats with various severities of induced osteoarthritis	27 rats with 3 different stages (Grade I, II, III) of papain induced knee arthritis received 7 min pulse sonication treatment, 3 times/wk for 4 wk;27 rats-controls	Bone scan and histology	"Severity indexes" based on bone scan decreased after sonication
**Korstens et al[19]	Investigated whether LIPUS stimulates chondrocyte proliferation	Chondrocytes and exokants were exposed to LIPUS	Sulphate incorporation into proteoglycans by LIPUS was 1.3-fold higher in higher in degenerative than collateral monolayers as assessed biochemically, + 1.9-fold higher as assessed by autoradiography	LIPUS stimulates chondrocyte proliferation and matrix production; LIPUS might foster cartilage tissue repair in osteoarthritic patients
Lee et al[20]	Examined effect of low intensity ultrasound [LIUS] on chondrogenesis of rabbit on mesenchymal stem cells (rMSCs) in a 3-D alginate culture and on the maintenance of chondrogenic phenotypes after replating them on a monolayer culture	LIUS treatment was applied to the rMSCs	Chondrogenic markers	LIUS increased: (i) matrix formation; (ii) collagen type II, aggrecan, and Sox-9; (iii) the expression of tissue inhibitor of metalloprotease-2 (iv) the capacity to maintain the cells' chondrogenic phenotypes
Li et al. [21]	24 rabbits with knee osteoarthritis	Four contrasting groups, including 3 using LIPUS, and a control group were studied	After 4 wks articular cartilage pathology was examined histologically	LIPUS, plus LIPUS applied with nano magnets decreased Mankin scores and suppressed MMP-13 enzyme activity
Lu et al. [22]	18-wk old rabbits with partial patellectomy	LIPUS was applied to patellar bone tendon junctions	Histological analyses of vascular endothelial growth factor and chondrogenesis were conducted	Better cartilage healing took place in experimental versus control conditions
Nishikori et al[23]	Rabbit cultured chondrocytes embedded in Atelocollagen gel	LIPUS was administered 20 min per day versus sham intervention	Histology and stiffness of the composites were assessed	The active treatment appeared to improve the chondrocyte implant quality
Oyonarte et al[12]	Mandibular condyles of growing rats	LIPUS was compared to condylar effects of mesenchymal stem cells	Imaging and histological analyses were conducted	LIPUS had more impact on overall condylar development than stem cells alone

Table 1 Selected studies published prior to 2015 concerning low intensity pulsed ultrasound (LIPUS) and articular cartilage and joint related effects. Highlighted studies** are those of special note.

Takeuchi et al[24]	Effect of LIPUS on cell growth was examined in 3D-cultured chondrocytes with a collagen sponge	Ras/mitogen-activated protein kinase (MAPK) and the integrin/phosphatidylinositol 3 kinase (PI3K)/Akt pathways as well as proteins involved in chondrocyte proliferation were examined in LIPUS-treated chondrocytes	Immunohistochemical analyses and Western blotting analysis	LIPUS promoted the proliferation of cultured chondrocytes and type IX collagen
Uenaka et al[8]	High-density semi open culture system of rat chondrocytes	Cartilage constructs were exposed to LIPUS for 20 min per day	mRNA expression for cartilage matrix molecules plus collagen and proteoglycan contents and histologic and macroscopic observations were assessed	LIPUS applications enhanced matrix production in the high density cultures
Vaughan et al [5]	Bovine articular chondrocytes cultured in agarose and monolayer cultures	LIPUS [PLIUS] was applied for 20 min per day at 30 or 100 W.cm-2	Synthesis of glycosaminoglycans	LIPUS has limited potential to provide an effective matrix effect

Table 3 Summary of methods and results of key recently published in vivo animal model studies that examined post-LIPUS osteoarthritic outcomes [2015-2019]. Highlighted studies** are those of special note.

Authors	Methods	Results
**Fujita et al[31]	15-wk-old male Wistar rats were divided into two experimental groups and a control group	LIPUS treatment attenuated cartilage degeneration, decreased the number of osteoclastic cells and restored the expression of aggrecan after an initial decrease induced by mechanical overloading
**He et al[32]	Temporomandibular [TMJ] osteoarthritis [OA] was induced by trauma, At 8 wks, experimental group began LIPUS treatments for 4 wks (5 days/wk)	The TMJ-OA model was successfully established, and LIPUS attenuated cartilage retrogression determined histologically
**Hsieh et al[33]	To examine whether early intervention with LIPUS helps delay traumatically induced osteoarthritis progression in male Sprague-Dawley rats, LIPUS doses of 1.0 MHz, 0.1 W/cm2, were applied on the 3rd post surgery day and continued for 4 consecutive wks , 5 days/wk	Compared with the sham treatment, LIPUS significantly reduced Mankin scores, inflammatory cells and matrix metalloproteinase 13 expression in rats with osteoarthritis (p < 0.05)
**Jia et al[34]	30 osteoarthritis and 30 normal rabbits were randomized into three groups	FLIPUS attenuated release of type II collagen + proteoglycans + reduced chondrocyte apoptosis + effusion
Ji et al[35]	Effect of LIPUS on expression of tissue inhibitor of metalloproteinase-2 (TIMP-2) articular cartilage cells of rabbits with knee osteoarthritis (OA) was examined	Compared with the normal control group, expression of TIMP-2 in OA model group was increased, while MMP-13 decreased
Kanaguchi et al[36]	Temporomandibular joints of 24 adult rats divided into 4 groups: control and mono-iodoacetate groups, were injected with contrast media and mono-iodoacetate, respectively, at 12 wks and observed up to 20 wks; and LIPUS and mono-iodoacetate + LIPUS groups, injected with contrast media and mono-iodoacetate, respectively, at 12 wks with LIPUS performed from 16-20 wks	At 20(OA) wks, the mono-iodoacetate +LIPUS showed higher bone density
Li et al[7]	30 5-month-old female Sprague-Dawley rats were randomly assigned to six groups	Micro-computed tomography indicated that the thickness and sulfated glycosaminoglycan content of cartilage decreased, but the thickness of the subchondral cortical bone plate and the formation of subchondral bone increased in the osteoarthritic group under normal joint use conditions
Pan et al[37]	Chondrocytes were isolated from knee articular cartilage of 2-wk-old rabbits and treated with LIPUS	LIPUS plus 0.1 mg/mL CCO solution promoted chondrocyte proliferation and type II collagen and TGF-β1 expression synergistically in vitro (P < 0.05), but also promoted proliferation in the absence of CCO
Tang et al[38]	40 knee injured rabbits were divided into 4 groups, including a control group, a model group, a fibroblast growth factor [FGF2] group and a FGF2 + LIPUS	In the FGF2 group and FGF2 + LIPUS groups, tissues of knee joint gradually repaired
Xia et al[4]	36 rabbits were divided into an early control, early osteoarthritis, early treatment, late control, late osteoarthritis, and late treatment groups	Results showed less severe cartilage damage in the early treatment group than the early osteoarthritis group, but no significant difference in cartilage damage or Mankin score between the late treatment and late osteoarthritis groups
Zahoor et al[39]	Intra-articular fractures of the medial tibial plateau of 30 rats were surgically created and LIPUS was applied to the operated joints either for the first 2 wk (LIPUS1-2 group) or in weeks 4 and 5 after intra-articular fracture (LIPUS4-5 group)	Among the altered gait parameters, maximal and average paw print areas in LIPUS1-2 + 4-5 groups, but not LIPUS 0 group, had either reached baseline or was recovered
Zhou et al[40]	Divided 18 adult rabbits into a sham operated, an operated untreated group, and an operation with LIPUS therapy delivered daily on day 4 after cartilage surgery	Compared to the operation alone, the severity of cartilage injury was decreased in the LIPUS group.

Table 2 Summary methods and results key recently published in vitro explants/culture studies [2015-2019]. **=those of special note.

Authors	Methods	Results
**Cheng et al [25]	Normal and osteoarthritis chondrocytes were exposed to LIPUS, and mRNA and protein expression of cartilage, metalloproteinases and integrin-FAK-PI3K/Akt signal pathway-related genes were examined using standardized procedures	Compared with levels in normal chondrocytes, expression levels, the ECM-related genes were significantly lower in osteoarthritis chondrocytes and those of metalloproteinase-related genes were significantly higher
Nishida et al[26]	Human chondrocytic cell line (HCS)-2/8, rat primary epiphyseal and articular cartilage cells, and CCN2-deficient chondrocytes that impaired chondrocyte differentiation, were treated with LIPUS for 20 min at 3.0 MHz frequency and 60 mW/cm ² power	Gene expression of the chondrocyte differentiation markers and CCN2 production were increased in the cultured chondrocytes treated with LIPUS. In addition, Ca ²⁺ influx and phosphorylation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)1/2 were increased by LIPUS treatment, and the stability of TRPV4 and BKCa channel mRNAs was decreased by siRNA against CCN2
Sekino et al[3]	Investigated effects optimal LIPUS intensity on cartilage matrix regeneration in mouse cells	LIPUS induced collagen and aggrecan synthesis and decreased MMP13 expression
**Tan et al[27]	Cultured explants of porcine cartilage and human osteoarthritic cartilage was subjected to daily LIPUS or PEMF	Non treated explants showed signs of atrophy at 1 week, but not in treated explants where cell clusters were observed
Ting et al[28]	Examined the effect of LIPUS on scaffold-free dedifferentiated bovine articular cartilage chondrocytes	Those tissues exposed to TGF-B3 produced increases in total collagen and glycosaminoglycan along with cartilage-specific gene expression
**Uddin et al[29]	Examined the specific impact of LIPUS on human cartilage cells and explants in the presence and absence of IL-1B using Western blot analysis in an effort to specifically study the cellular pathway associated with IL-1B activity	LIPUS stimulation applied using a unit with a 10cm ² transducer to cells and explants cultured on 35mm plates for a 20min period per day as approved for bone healing stimulations, increased proteoglycan content as well as inhibiting IL-1B induced loss of proteoglycans
Yamaguchi et al[30]	An osteochondral defect was created on both femur grooves of Wistar rats. Four wks later, bone m mesenchymal stromal cells (MSCs) were injected into the right knee joint	MSC injection improved the cartilage repair score, and LIPUS irradiation improved BV/TV

These positive LIPUS induced effects include, but are not limited to, its ability to significantly attenuate the impact of destructive chondrolytic enzymes known to destroy cartilage matrix in injured joints^[46], in a dose dependent manner in the context of cultured chondrocytes and articular cartilage explants^[47], whose effect is greater in early rather than late osteoarthritis^[17]. On the other hand, LIPUS not only attenuates the production of an array of damaging catabolic responses associated with the natural history of osteoarthritis^[27], but its thoughtful application is shown to concurrently induce favorable anabolic chondrocyte responses, as shown by associated changes in the extracellular matrix protein content and the parallel upregulation of chondrogenic genes^[32]. Sekino *et al*^[3] too observed that an optimally developed LIPUS dosage effectively improves collagen and aggrecan synthesis and remodeling when applied to mouse chondroprogenitor cell line ATDC5 for 20 min per day, findings largely consistent with those reported by Min *et al*^[44] and DU *et al*^[45].

Other potentially relevant observed benefits of LIPUS applications to chondrocyte explants, and osteoarthritis cartilage models include its ability to favorably modulate Transforming Growth Factor-B3 levels that can help regulate the reconstruction of injured cartilage^[28,37], along with the induction of favorable subchondral bone alterations^[21], plus the induction of CCN2, a protein involved in cartilage repair^[26]. Ji *et al*^[35] who sought to examine whether the expression of serum TIMP-2, significantly decreased in osteoarthritis, would be upregulated by LIPUS, showed this in fact did occur.

Mechanisms of action and possible pathways of influence implicated in the aforementioned processes include the integrin/focal adhesion kinase/mitogen-activated protein kinase signaling pathway. According to Jang *et al*^[48], LIPUS enhances the migration of chondrogenic progenitor cells towards injured cartilage sites, which could delay or prevent the onset of post-traumatic osteoarthritis. LIPUS may further augment cartilage cell proliferation and extracellular matrix production by regulating the TGF-BRII and Smad2 cellular pathways^[37], as well as by alleviating osteoarthritis-

induced prostaglandin E2 and nitric oxide in the synovial fluid of affected joints, in addition to reducing chondrocyte apoptosis^[34], and by increasing the number of nests containing 4-6 chondrocytes by 3.9 fold in degenerative explants^[19].

In sum, regardless of year of study, model examined, study methods and aims, LIPUS exposure, and varied application approaches, favorable morphological, histological, functional, and intracellular signaling outcomes have been observed more often than not in the published preclinical literature when LIPUS is applied in some way to various forms of osteoarthritic cartilage and/or chondrocytes. In addition, when LIPUS is combined with fibroblast growth factor, or integrated within microbubbles, or applied after mesenchymal stromal cell injection to treat osteochondral defects^[30], the synthesis and secretion of collagen by chondrocytes, as well as concurrent cartilage repair appears to be enhanced^[48,49].

Unsurprisingly, several independent researchers who conducted well-controlled studies have concluded LIPUS applications may hold immense promise in the clinical sphere for preventing, slowing, or reversing osteoarthritis pathology^[21,25,31,36,38,50], and hence further efforts to examine the hypothesized relationships depicted in Figure 1 and mechanisms of action implied in the literature would appear to be of considerable value. As well, establishing which LIPUS parameters are most likely to yield optimally favorable chondrocyte gene expression influences as implied by Miller *et al*^[51,52], and which have the best potential for promoting the production of functional chondrocytes in laboratory based cartilage scaffolds as outlined by Guo *et al*^[53], should be explored further.

DISCUSSION AND CONCLUSION

Ultrasound, widely used in medicine as both a diagnostic and therapeutic tool, and a form of acoustic radiation that can be manipulated experimentally to produce a variety of favorable anabolic and beneficial biological effects in various tissues^[55], such as bone^[26,56], has been hypothesized to exert a favorable effect on joint

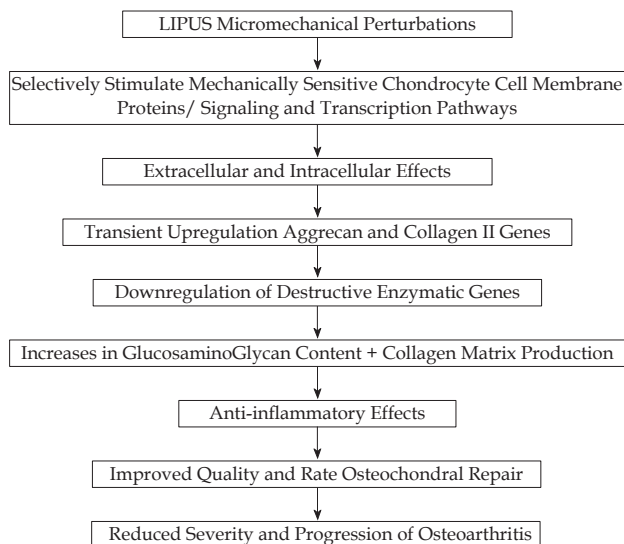


Figure 1 Schematic representation of the impact of LIPUS on chondrocyte functions that maybe useful for treating osteoarthritis symptoms, and for regenerative cartilage repair efforts as adapted from [5, 11, 16, 19, 21, 25, 26, 29, 46, 47, 50, 54, 63, 64, 66-70]

tissues such as cartilage, implicated in osteoarthritis^[56]. In particular, a variant of ultrasound delivered at low intensity in pulsed form known as LIPUS, a non-thermal wave form of mechanical stimulation has specifically been studied for some time to ascertain its potentially beneficial effects on the repair of injured or diseased articular cartilage chondrocytes, known to be a very limited process, but one possibly requiring some form of mechanical stimulation^[48]. As well, almost 15 years ago, Lee *et al*^[20] concluded carefully calibrated and delivered LIPUS applications had the potential to serve as an efficient and cost-effective method for inducing chondrogenic differentiation of mesenchymal stromal cells *in vitro* for purposes of fostering cartilage tissue engineering attempts.

In this respect, and despite some evidence to the contrary^[5,13], and possible publication bias, along with limited external validity to the clinic, Gurkan *et al*^[17] and many others, such as Korstjens *et al*^[19], have observed that LIPUS, can be applied effectively in various *in vitro* contexts to attenuate, if not totally reverse, osteoarthritis cartilage degeneration. Moreover, these effects are apparently causal as demonstrated in several well-designed controlled studies conducted using a variety of osteoarthritic animal models^[16,63].

These LIPUS induced effects are attributed in part to its ability to stimulate chondrocyte migration, proliferation, and differentiation, as well as to down-regulate chondrocytic ERK1/2 and p38 extracellular signaling kinases, known to be highly activated during inflammation^[63]. LIPUS induced changes also include related modulations in type II collagen and proteoglycan and matrix metalloproteinase-13 levels^[33,63]. As such, it appears LIPUS is potentially able to delay degeneration in early osteoarthritis^[17], and may prove of especially high value as far as the development of efficacious osteoarthritis disease modifying interventions go, when compared to presently available treatment options that commonly provide only symptomatic relief.

LIPUS may also improve overall chondral histology^[6], while preventing or delaying excess cartilage degradation^[29,33], and in accord with early work^[6] may specifically reduce the expression not only of matrix metalloproteinase-13^[29,33], but interleukin-1B^[29], an additional catabolic degradative overexpressed enzyme found in osteoarthritis. On the other hand, in addition to clearly heightening type II collagen production in early stage osteoarthritis^[4], LIPUS

applications help to reduce synovial inflammation^[33], increase chondrocyte numbers^[36], and enhance other strategies designed to promote anabolic cartilage chondrocyte repair processes^[37].

Indeed related research published over the past 35 years in various laboratories is striking in its agreement of the hypothesis that certain dosages and applications of LIPUS are likely to exert favorable clinically important biophysical influences on both normal chondrocytes, as well as those from osteoarthritic animal and human joint sources. Moreover, notwithstanding the limitations of *in vitro* and *ex vivo* studies, and their potentially limited generalizability to the clinical sphere, a sizeable number of the presently reviewed studies found positive outcomes that could auger well for bioengineering efforts, as well for attenuating retrogression of cartilage. Showing favorable results among differing models of osteoarthritis, diverse chondrocyte cell cultures, and when applying diverse research approaches as far as cartilage repair goes strengthens confidence in the findings. Moreover, most studies applied some laudable and rigorous strategies and efforts to ensure that the models of osteoarthritic damage and LIPUS interactions were valid and not confounded by changes in environmental conditions, among other factors. The assumption that instruments and methods used to assess LIPUS outcome were carefully chosen, calibrated, valid, and reliable, along with the fact that ultrasound can propagate in the human knee^[56], tends to heighten the credibility and possible clinical relevance of the cumulative body of preclinical findings. Parallel research in advance of clinical trials, also provides further support for a variety of plausible biophysical mechanisms of action to explain LIPUS outcomes, that are highly consistent with known mechanotransducer effects on anabolic as well as catabolic chondrocyte pathways^[57-60], and potential osteoarthritis related therapeutic genes^[32].

As mentioned previously, these mechanisms of action include, but are not limited to, an apparent reduction in matrix metalloproteinases-13 immunopositive cells^[36], downregulation of interleukin 1 [IL 1], a potent degradative enzyme responsible for considerable inflammatory damage in osteoarthritis that is not readily attenuated by standard pharmacologic interventions^[61], and the activation of the circadian Per-2 gene^[32]. Another is related to its potential for stimulating chondrocyte proliferation along with heightened matrix production^[19,29]. Moreover, LIPUS appears to stimulate chondrocyte proteoglycan synthesis^[62], while down regulating potent catabolic cartilage enzymes^[3,29,33,47], which can otherwise degrade one or more essential functional and structural components of the chondrocyte extracellular matrix^[63], concurrently.

As such, if the observed ability of LIPUS to upregulate cartilage specific gene expression mechanisms that are potentially therapeutic^[32], such as tissue inhibitor of metalloproteinase-2 (TIMP-2)^[35], along with its concomitant influence on repressing catabolic enzyme actions and production, such as matrix metalloproteinase (MMP-13)^[35] are clinically transferable, one could anticipate a resounding impact as far as heightening our ability to ameliorate the extent and magnitude of osteoarthritis disablement. However, because the chondrocytic mechanotransducing processes initiated or forged by LIPUS may be time sensitive, research to examine an array of timing parameters and/or possible time frames for optimally modulating favorable LIPUS associated outcomes and avoiding unfavorable ones is strongly recommended. As well, examining the relative impact of other LIPUS stimulation parameters as this affects the sensitivity of the chondrocyte membrane and its receptors in both health and disease may help to shed new light on LIPUS and its potential for fostering desirable repair of cartilage defects, especially

osteoarticular cartilage reparative and protective processes.

In addition, efforts to carefully examine the impact of LIPUS on multiple chondrocyte cell membrane signaling pathways, and target genes, especially those not previously examined, is strongly encouraged for further examining treatment mechanisms, as is research to examine the utility of matching the frequency of the driving force of LIPUS to that of the chondrocyte system's natural frequency of vibration as outlined by Miller *et al*^[51,52], and Louw *et al*^[68].

In the interim, while the literature cited above is not without limitations, or necessarily inclusive of all research, it is concluded that a variety of favorable adaptive chondrocyte responses are likely to be observed following several modes of applying LIPUS in experimental settings that do not occur in control samples^[21,69]. Moreover, these favorable responses along with analogous research to tentatively explain LIPUS mechanisms of action parallel those that emerge or are implicated in the context of chondrocyte cell biology and the nature of the osteoarthritis disease process.

In light of the formidable current and emergent global challenges in managing disabling osteoarthritis in aging populations, along with its limited reversibility, and treatment options, especially those that can foster articular cartilage repair, findings that LIPUS appears to favorably influence anabolic:catabolic cartilage metabolic factor ratios, and can do this consistently in accord with established chondrocyte membrane mechanotransduction and transport mechanisms through variously defined dose parameters, is surely noteworthy, and should not be ignored. To the contrary, it appears that consistent with knowledge that manipulation of the cartilage physical microenvironment by biophysical cues^[64], plus analogous views presented here and others^[66-70], dedicated efforts to extend the findings of LIPUS applications in the context of the laboratory to the clinic is likely to prove highly beneficial to clinicians and others seeking a feasible tool for cartilage tissue repair, as proposed by Korstiens *et al*^[19], as well as many current or future osteoarthritis sufferers, and should focus on discerning and replicating both its independent effects, as well as its potential utility as an adjunctive or additive therapeutic strategy^[21,37,38,71,72], or both, as far as favorably influencing osteoarthritic articular cartilage reparative, regenerative, and reconstructive processes.

REFERENCES

1. Coords M, Breitbart E, Paglia D, Kappy N, Gandhi A, Cottrell J, Cedeno N, Pounder N, O'Connor JP, Lin SS. The effects of low-intensity pulsed ultrasound upon diabetic fracture healing. *J Orthop Res* 2011; **29**(2): 181-188. [DOI: 10.1002/jor.21223]; Epub 2010 Sep 30. [PMID: 20886648].
2. Massari L, Benazzo F, Falez F, Perugia D, Pietrogrande L, Setti S, Osti R, Vaienti E, Ruosi C, Cadossi R. Biophysical stimulation of bone and cartilage: state of the art and future perspectives. *Int Orthop* 2019; **43**(3): 539-551. [DOI: 10.1007/s00264-018-4274-3]. Epub 2019 Jan 15. [PMID: 30645684]; [PMCID: PMC6399199].
3. Sekino J, Nagao M, Kato S, Sakai M, Abe K, Nakayama E, Sato M, Nagashima Y, Hino H, Tanabe N, Kawato T, Maeno M, Suzuki N, Ueda K. Low-intensity pulsed ultrasound induces cartilage matrix synthesis and reduced MMP13 expression in chondrocytes. *Biochem Biophys Res Commun* 2018; **506**(1): 290-297. [DOI: 10.1016/j.bbrc.2018.10.063]. Epub 2018 Oct 19. [PMID: 30348521].
4. Xia P, Shen S, Lin Q, Cheng K, Ren S, Gao M, Li X. Low intensity ultrasound treatment at an early osteoarthritis stage protects rabbit cartilage from damage via the integrin/focal adhesion kinase/mitogen-activated protein kinase signaling pathway. *J Ultrasound Med* 2015; **34**(11): 1991-1999. [DOI: 10.7863/ultra.14.10016]. Epub 2015 Sep 22. [PMID: 26396170].
5. Vaughan NM, Grainger J, Bader DL, Knight MM. The potential of pulsed low intensity ultrasound to stimulate chondrocytes matrix synthesis in agarose and monolayer cultures. *Med Biol Eng Comput* 2010; **48**(12): 1215-1222. [DOI: 10.1007/s11517-010-0681-3]. Epub 2010 Oct 12. [PMID: 20938751]; [PMCID: PMC2993893].
6. Rothenberg JB, Jayaram P, Naqvi U, Gober J, Malanga GA. The role of Low-Intensity Pulsed Ultrasound on cartilage healing in knee osteoarthritis: a review. *PM R* 2017; **9**(12): 1268-1277. [DOI: 10.1016/j.pmrj.2017.05.008]. Epub 2017 Jun 9.
7. Li X, Sun Y, Zhou Z, Zhang D, Jiao J, Hu M, Hassan CR, Qin YX. Mitigation of articular cartilage degeneration and subchondral bone sclerosis in osteoarthritis progression using low-intensity ultrasound stimulation. *Ultrasound Med Biol* 2019; **45**(1): 148-159. [DOI: 10.1016/j.ultrasmedbio.2018.08.022]. Epub 2018 Oct 12. [PMID: 30322672]; [PMCID: PMC6289639].
8. Uenaka K, Imai S, Ando K, Matsusue Y. Relation of low-intensity pulsed ultrasound to the cell density of scaffold-free cartilage in a high-density static semi-open culture system. *J Orthop Sci*. 2010; **15**(6): 816-824. [DOI: 10.1007/s00776-010-1544-3]. Epub 2010 Nov 30. [PMID: 21116901].
9. Hasanova GI, Noriega SE, Mamedov TG, Guha Thakurta S, Turner JA, Subramanian A. The effect of ultrasound stimulation on the gene and protein expression of chondrocytes seeded in chitosan scaffolds. *J Tissue Eng Regen Med* 2011; **5**(10): 815-822. [DOI: 10.1002/term.384]. Epub 2011 Jan 17. [PMID: 22002925].
10. Loyola-Sánchez A, Richardson J, Beattie KA, Otero-Fuentes C, Adachi JD, MacIntyre NJ. Effect of low-intensity pulsed ultrasound on the cartilage repair in people with mild to moderate knee osteoarthritis: a double-blinded, randomized, placebo-controlled pilot study. *Arch Phys Med Rehabil* 2012; **93**(1): 35-42. [DOI: 10.1016/j.apmr.2011.07.196]; [PMID: 22200383].
11. Chung JI, Barua S, Choi BH, Min BH, Han HC, Baik EJ. Anti-inflammatory effect of low intensity ultrasound (LIUS) on complete Freund's adjuvant-induced arthritis synovium. *Osteoarthritis Cartilage* 2012 Apr; **20**(4): 314-322. [DOI: 10.1016/j.joca.2012.01.005]; [PMID: 22289897].
12. Oyonarte R, Becerra D, Díaz-Zúñiga J, Rojas V, Carrion F. Morphological effects of mesenchymal stem cells and pulsed ultrasound on condylar growth in rats: a pilot study. *Aust Orthod J* 2013; **29**(1): 3-12. [PMID: 23785932].
13. Yang SW, Kuo CL, Chang SJ, Chen PC, Lin YT, Manousakas I, Kuo SM. Does low-intensity pulsed ultrasound treatment repair articular cartilage injury? A rabbit model study. *BMC Musculoskelet Disord* 2014 Feb 10; **15**:36. [DOI: 10.1186/1471-2474-15-36]. [PMID: 24507771]; [PMCID: PMC3923237].
14. Bhatia R, Sobti VK, Roy KS. Gross and histopathological observations on the effects of therapeutic ultrasound in experimental acute chemical arthritis in calves. *Zentralbl Veterinarmed A* 1992; **39**(3): 168-173. [PMID: 1621463].
15. Choi BH, Woo JI, Min BH, Park SR. Low-intensity ultrasound stimulates the viability and matrix gene expression of human articular chondrocytes in alginate bead culture. *J Biomed Mater Res A* 2006; **79**(4): 858-864. [PMID: 16886219].
16. Cook SD, Salkeld SL, Patron LP, Doughty ES, Jones DG. The effect of low-intensity pulsed ultrasound on autologous osteochondral plugs in a canine model. *Am J Sports Med* 2008; **36**(9): 1733-1741. [DOI: 10.1177/0363546508316766]; Epub 2008 May 15. [PMID: 18483198].
17. Gurkan I, Ranganathan A, Yang X, Horton WE Jr, Todman M, Huckle J, Pleshko N, Spencer RG. Modification of osteoarthritis in the guinea pig with pulsed low-intensity ultrasound treatment. *Osteoarthritis Cartilage* 2010; **18**(5): 724-733. [DOI: 10.1016/j.joca.2010.01.006]. Epub 2010 Feb 6. [PMID: 20175971];

- [PMCID: PMC2873836].
18. Huang MH, Ding HJ, Chai CY, Huang YF, Yang RC. Effects of sonication on articular cartilage in experimental osteoarthritis. *J Rheumatol* 1997; **24**(10): 1978-1984. [PMID: 9330942].
 19. 19. Korstjens CM, van der Rijt RH, Albers GH, Semeins CM, Klein-Nulend J. Low-intensity pulsed ultrasound affects human articular chondrocytes in vitro. *Med Biol Eng Comput* 2008; **46**(12): 1263-1270. [DOI: 10.1007/s11517-008-0409-9]; Epub 2008 Oct 14. [PMID: 18853213].
 20. Lee HJ, Choi BH, Min BH, Son YS, Park SR. Low-intensity ultrasound stimulation enhances chondrogenic differentiation in alginate culture of mesenchymal stem cells. *Artif Organs* 2006; **30**(9): 707-715. [PMID: 16934100].
 21. Li X, Lin Q, Wang D, Dai Y, Cheng K, Yu J, Lin A, Gao M, Chen A. The effects of low-intensity pulsed ultrasound and nanomagnet applications on the expressions of MMP-13 and MAPKs in rabbit knee osteoarthritis. *J Nanosci Nanotechnol* 2013; **13**(1): 722-727. [PMID: 23646806].
 22. Lu H, Qin L, Cheung W, Lee K, Wong W, Leung K. Low-intensity pulsed ultrasound accelerated bone-tendon junction healing through regulation of vascular endothelial growth factor expression and cartilage formation. *Ultrasound Med Biol* 2008; **34**(8): 1248-1260. [DOI: 10.1016/j.ultrasmedbio.2008.01.009]; Epub 2008 Apr 18. [PMID: 18378382].
 23. Nishikori T, Ochi M, Uchio Y, Maniwa S, Kataoka H, Kawasaki K, Katsube K, Kuriwaka M. Effects of low-intensity pulsed ultrasound on proliferation and chondroitin sulfate synthesis of cultured chondrocytes embedded in Atelocollagen gel. *J Biomed Mater Res* 2002; **59**(2): 201-6. [PMID: 11745554].
 24. 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. 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 Res Ther* 2008; **10**(4): R77. [DOI: 10.1186/ar2451. Epub 2008 Jul 11. [PMID: 18616830]; [PMCID: PMC2575623].
 25. Cheng K, Xia P, Lin Q, Shen S, Gao M, Ren S, Li X. Effects of low-intensity pulsed ultrasound on integrin-FAK-PI3K/Akt mechanochemical transduction in rabbit osteoarthritis chondrocytes. *Ultrasound Med Biol* 2014; **40**(7): 1609-1618. [DOI: 10.1016/j.ultrasmedbio.2014.03.002]. Epub 2014 Apr 16.
 26. Nishida T, Kubota S, Aoyama E, Yamanaka N, Lyons KM, Takigawa M. Low-intensity pulsed ultrasound (LIPUS) treatment of cultured chondrocytes stimulates production of CCN family protein 2 (CCN2), a protein involved in the regeneration of articular cartilage: mechanism underlying this stimulation. *Osteoarthritis Cartilage* 2017; **25**(5): 759-769. [DOI: 10.1016/j.joca.2016.10.003]. Epub 2016 Oct 8. [PMID: 27729291].
 27. Tan L, Ren Y, van Kooten TG, Grijpma DW, Kuijjer R. Low-intensity pulsed ultrasound (LIPUS) and pulsed electromagnetic field (PEMF) treatments affect degeneration of cultured articular cartilage explants. *Int Orthop* 2015; **39**(3):549-557. [DOI: 10.1007/s00264-014-2542-4]. Epub 2014 Sep 30. [PMID: 25267432].
 28. Ting SY, Montagne K, Nishimura Y, Ushida T, Furukawa KS. Modulation of the effect of transforming growth factor- β 3 by low-intensity pulsed ultrasound on scaffold-free dedifferentiated articular bovine chondrocyte tissues. *Tissue Eng Part C Methods* 2015; **21**(10): 1005-1014. [DOI: 10.1089/ten.TEC.2014.0428]. Epub 2015 Jun 11. [PMID: 25915185].
 29. Uddin SM, Richbrough B, Ding Y, Hettinghouse A, Komatsu DE, Qin YX, Liu CJ. Chondro-protective effects of low intensity pulsed ultrasound. *Osteoarthritis Cartilage* 2016; **24**(11): 1989-1998. [DOI: 10.1016/j.joca.2016.06.014]; Epub 2016 Jun 27. [PMID: 27364595; [PMCID: PMC5071131].
 30. Yamaguchi S, Aoyama T, Ito A, *et al.* Effect of low-intensity pulsed ultrasound after mesenchymal stromal cell injection to treat osteochondral defects: an in vivo study. *Ultrasound Med Biol* 2016; **42**(12): 2903-2913. [DOI:10.1016/j.ultrasmedbio.2016.07.021]
 31. Fujita M, Sato-Shigeta M, Mori H, Iwasa A, Kawai N, Hassan AH, Tanaka E. Protective effects of low-intensity pulsed ultrasound on mandibular condylar cartilage exposed to mechanical overloading. *Ultrasound Med Biol* 2019; **45**(4): 944-953. [DOI: 10.1016/j.ultrasmedbio.2018.12.006]; Epub 2019 Feb 4. [PMID: 30732913].
 32. He D, An Y, Li Y, wang J, Wu G, Chen L, Zhu G. RNA sequencing reveals target genes of temporomandibular joint osteoarthritis in rats after the treatment of low-intensity pulsed ultrasound. *Gene* 2018; **672**: 126-136. [DOI:10.1016/j.gene.2018.06.002].
 33. 33. Hsieh YL, Chen HY, Yang CC. Early intervention with therapeutic low-intensity pulsed ultrasound in halting the progression of post-traumatic osteoarthritis in a rat model. *Ultrasound Med Biol* 2018; **44**(12): 2637-2645. [DOI: 10.1016/j.ultrasmedbio.2018.08.007]; Epub 2018 Sep 24. [PMID: 30262135].
 34. Jia L, Wang Y, Chen J, Chen W. Efficacy of focused low-intensity pulsed ultrasound therapy for the management of knee osteoarthritis: a randomized, double blind, placebo-controlled trial. *Sci Rep* 2016 Oct 17; **6**: 35453. [DOI: 10.1038/srep35453]; [PMID: 27748432]; [PMCID: PMC5066246].
 35. Ji JB, Li XF, Liu L, Wang GZ, Yan XF. Effect of low intensity pulsed ultrasound on expression of TIMP-2 in serum and expression of mmp-13 in articular cartilage of rabbits with knee osteoarthritis. *Asian Pac J Trop Med* 2015; **8**(12): 1043-1048. [DOI: 10.1016/j.apjtm.2015.11.003]; Epub 2015 Nov 14.
 36. Kanaguchi Arita A, Yonemitsu I, Ikeda Y, Miyazaki M, Ono T. Low-intensity pulsed ultrasound stimulation for mandibular condyle osteoarthritis lesions in rats. *Oral Dis* 2018; **24**(4): 600-610. [DOI: 10.1111/odi.12798]; Epub 2017 Nov 24. [PMID: 29069539].
 37. Pan YL, Ma Y, Guo Y, Tu J, Guo GP, Ma SM, Zheng SY, Tu PC, Yu BB, Huang GC. Effects of Clematis chinensis Osbeck mediated by low-intensity pulsed ultrasound on transforming growth factor- β /Smad signaling in rabbit articular chondrocytes. *J Med Ultrason* (2001). 2019; **46**(2): 177-186. [DOI: 10.1007/s10396-018-0920-z]; Epub 2019 Jan 18. [PMID: 30659392].
 38. Tang ZF, Li HY. Effects of fibroblast growth factors 2 and low intensity pulsed ultrasound on the repair of knee articular cartilage in rabbits. *Eur Rev Med Pharmacol Sci* 2018; **22**(8): 2447-2453. [DOI: 10.26355/eurrev_201804_14838]; [PMID: 29762847].
 39. Zahoor T, Mitchell R, Bhasin P, *et al.* Effect of low-intensity pulsed ultrasound on joint injury and post-traumatic osteoarthritis: an animal study. *Ultrasound Med Biol* 2018; **44**(1): 234-242. [DOI:10.1016/j.ultrasmedbio.2017.09.014]
 40. Zhou HY, Li Q, Wang JX, Xie YJ, Wang SQ, Lei L, Gao YQ, Huang MM, Hu Y, Xu FY, Zhang C. Low-intensity pulsed ultrasound repair in mandibular condylar cartilage injury rabbit model. *Arch Oral Biol* 2019; **104**: 60-66. [DOI: 10.1016/j.archoralbio.2019.05.018]; [PMID: 31174095].
 41. Zhou XY, Zhang XX, Yu GY, Zhang ZC, Wang F, Yang YL, Li M, Wei XZ. Effects of Low-intensity pulsed ultrasound on knee osteoarthritis: a meta-analysis of randomized clinical trials. *Biomed Res Int* 2018 Jul 15; 2018: 7469197. [DOI: 10.1155/2018/7469197]; [PMID: 30105243]; [PMCID: PMC6076961].
 42. Devrimsel G, Metin Y, Serdaroglu Beyazal M. Short-term effects of neuromuscular electrical stimulation and ultrasound therapies on muscle architecture and functional capacity in knee osteoarthritis: a randomized study. *Clin Rehabil* 2019; **33**(3): 418-427. [DOI: 10.1177/0269215518817807]; Epub 2018 Dec 4. [PMID: 30514113].
 43. Wang X, Lin Q, Zhang T, Wang X, Cheng K, Gao M, Xia P, Li

- X. Low-intensity pulsed ultrasound promotes chondrogenesis of mesenchymal stem cells via regulation of autophagy. *Stem Cell Res Ther* 2019; **10**(1): 41. [DOI: 10.1186/s13287-019-1142-z. [PMID: 30670079]; [PMCID: PMC6343259].
44. Min BH, Woo JI, Cho HS, Choi BH, Park SJ, Choi MJ, Park SR. Effects of low-intensity ultrasound (LIUS) stimulation on human cartilage explants. *Scand J Rheumatol* 2006; **35**(4): 305-11. [PMID: 16882596].
 45. DU D, Chen S, Yi G, Wang P, Tang Y, Zheng L, Chen J. Low-intensity pulsed ultrasound promotes extracellular matrix synthesis of human osteoarthritis chondrocytes. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2016; **32**(11): 1536-1540. Chinese. [PMID: 27774951].
 46. Iwabuchi Y, Tanimoto K, Tanne Y, Inubushi T, Kamiya T, Kunimatsu R, Hirose N, Mitsuyoshi T, Su S, Tanaka E, Tanne K. Effects of low-intensity pulsed ultrasound on the expression of cyclooxygenase-2 in mandibular condylar chondrocytes. *J Oral Facial Pain Headache* 2014; **28**(3): 261-268. [DOI: 10.11607/ofph.1156].
 47. Ito A, Aoyama T, Yamaguchi S, Zhang X, Akiyama H, Kuroki H. Low-intensity pulsed ultrasound inhibits messenger RNA expression of matrix metalloproteinase-13 induced by interleukin-1 β in chondrocytes in an intensity-dependent manner. *Ultrasound Med Biol* 2012; **38**(10): 1726-1733. [DOI: 10.1016/j.ultrasmedbio.2012.06.005. Epub 2012 Aug 21].
 48. Jang KW, Ding, Seol D, Lim TH, Buckwalter JA, Martin JA. Low-intensity pulsed ultrasound promotes chondrogenic progenitor cell migration via focal adhesion kinase pathway. *Ultrasound Med Biol* 2014; **40**(6): 1177-1186. [DOI: 10.1016/j.ultrasmedbio.2013.12.007]; Epub 2014 Mar 4.
 49. Aliabouzar M, Zhang LG, Sarkar K. Lipid coated microbubbles and low intensity pulsed ultrasound enhance chondrogenesis of human mesenchymal stem cells in 3D printed scaffolds. *Sci Rep* 2016; **6**: 37728. [DOI: 10.1038/srep37728]; [PMID: 27883051]; [PMCID: PMC5121887].
 50. Cook SD, Salkeld SL, Popich-Patron LS, Ryaby JP, Jones DG, Barrack RL. Improved cartilage repair after treatment with low-intensity pulsed ultrasound. *Clin Orthop Relat Res* 2001; **(391 Suppl)**: S231-243. [PMID: 11603707].
 51. Miller AD, Chama A, Louw TM, Subramanian A, Viljoen HJ. Frequency sensitive mechanism in low-intensity ultrasound enhanced bioeffects. *PLoS One* 2017; **12**(8): e0181717. [DOI: 10.1371/journal.pone.0181717. [PMID: 28763448]; [PMCID: PMC5538718].
 52. Miller AD, Subramanian A, Viljoen HJ. Theoretically proposed optimal frequency for ultrasound induced cartilage restoration. *Theor Biol Med Model* 2017; **14**(1): 21. [DOI: 10.1186/s12976-017-0067-4]; [PMID: 29132387]; [PMCID: PMC5684760].
 53. Guo G, Lu L, Ji H, Ma Y, Dong R, Tu J, Guo X, Qiu Y, Wu J, Zhang D. Low intensity pulse ultrasound stimulate chondrocytes growth in a 3-D alginate scaffold through improved porosity and permeability. *Ultrasonics* 2015; **58**: 43-52. [DOI: 10.1016/j.ultras.2014.12.001]; Epub 2014 Dec 16. [PMID: 25543661].
 54. Vaca-González JJ, Guevara JM, Moncayo MA, Castro-Abril H, Hata Y, Garzón-Alvarado DA. Biophysical stimuli: a review of electrical and mechanical stimulation in hyaline cartilage. *cartilage* 2019; **10**(2): 157-172. [DOI: 10.1177/1947603517730637]; Epub 2017 Sep 21. [PMID: 28933195]; [PMCID: PMC6425540].
 55. Dalecki D. Mechanical bioeffects of ultrasound. *Annu Rev Biomed Eng* 2004; **6**: 229-48. [PMID: 15255769].
 56. White D, Evans JA, Truscott JG, Chivers RA. Can ultrasound propagate in the joint space of a human knee? *Ultrasound Med Biol* 2007; **33**(7): 1104-1111. [Epub 2007 Apr 18. [PMID: 17445964].
 57. Xia P, Ren S, Lin Q, Cheng K, Shen S, Gao M, Li X. Low-intensity pulsed ultrasound affects chondrocyte extracellular matrix production via an integrin-mediated p38 MAPK signaling pathway. *Ultrasound Med Biol* 2015; **41**(6): 1690-700. [DOI: 10.1016/j.ultrasmedbio.2015.01.014]; Epub 2015 Feb 27. [PMID: 25736607].
 58. Xia P, Ren S, Lin Q, Cheng K, Xia P, Lin Q, Shen S, Gao M, Ren S, Li X. Effects of low-intensity pulsed ultrasound on integrin-FAK-PI3K/Akt mechanochemical transduction in rabbit osteoarthritis chondrocytes. *Ultrasound Med Biol* 2014; **40**(7): 1609-2618. [DOI: 10.1016/j.ultrasmedbio.2014.03.002]; Epub 2014 Apr 16. [PMID: 24742749].
 59. Crossman J, Alzaheer N, Abdallah MN, Tamimi F, Flood P, Alhadainy H, El-Bialy T. Low intensity pulsed ultrasound increases mandibular height and Col-II and VEGF expression in arthritic mice. *Arch Oral Biol* 2019; **104**: 112-118. [DOI: 10.1016/j.archoralbio.2019.05.032]; [PMID: 31177013].
 60. Jia L, Chen J, Wang Y, Zhang Y, Chen W. Focused low-intensity pulsed ultrasound affects extracellular matrix degradation via decreasing chondrocyte apoptosis and inflammatory mediators in a surgically induced osteoarthritic rabbit model. *Ultrasound Med Biol* 2016; **42**(1): 208-219. [DOI: 10.1016/j.ultrasmedbio.2015.08.010]; Epub 2015 Sep 26. [PMID: 26403700].
 61. Namazi H. Effect of low-intensity pulsed ultrasound on the cartilage repair in people with mild to moderate knee osteoarthritis: a novel molecular mechanism. *Arch Phys Med Rehabil* 2012 Oct; **93**(10): 1882; author reply 1882. [DOI: 10.1016/j.apmr.2012.01.028]; [PMID: 22989718].
 62. Parvizi J, Wu CC, Lewallen DG, Greenleaf JF, Bolander ME. Low-intensity ultrasound stimulates proteoglycan synthesis in rat chondrocytes by increasing aggrecan gene expression. *J Orthop Res* 1999; **17**(4): 488-494. [PMID: 10459753].
 63. Li X, Li J, Cheng K, Lin Q, Wang D, Zhang H, An H, Gao M, Chen A. Effect of low-intensity pulsed ultrasound on MMP-13 and MAPKs signaling pathway in rabbit knee osteoarthritis. *Cell Biochem Biophys* 2011; **61**(2): 427-434. [DOI: 10.1007/s12013-011-9206-4]; [PMID: 21567132].
 64. Huang X, Das R, Patel A, Nguyen TD. Physical stimulations for bone and cartilage regeneration. *Regen Eng Transl Med* 2018; **4**(4): 216-237. [DOI: 10.1007/s40883-018-0064-0]; Epub 2018 Jun 25. [PMID: 30740512]; [PMCID: PMC6366645].
 65. Nieminen HJ, Ylitalo T, Suuronen JP, Rahunen K, Salmi A, Saarakkala S, Serimaa R, Hægström E. Delivering agents locally into articular cartilage by intense mhz ultrasound. *Ultrasound Med Biol* 2015; **41**(8): 2259-2265. [DOI: 10.1016/j.ultrasmedbio.2015.03.025]; Epub 2015 Apr 25. [PMID: 25922135]; [PMCID: PMC4587538].
 66. Subramanian A, Budhiraja G, Sahu N. Chondrocyte primary cilium is mechanosensitive and responds to low-intensity-ultrasound by altering its length and orientation. *Int J Biochem Cell Biol* 2017; **91**(Pt A): 60-64. [DOI: 10.1016/j.biocel.2017.08.018]; Epub 2017 Sep 10. [PMID: 28870737].
 67. Naito K, Watari T, Muta T, et al. Low-intensity pulsed ultrasound (LIPUS) increases the articular cartilage type II collagen in a rat osteoarthritis model. *J Orthopaedic Res* 2010; **28**(3): 361-369. [DOI:10.1002/jor.20995].
 68. Louw TM, Budhiraja G, Viljoen HJ, Subramanian A. Mechanotransduction of ultrasound is frequency dependent below the cavitation threshold. *Ultrasound Med Biol* 2013; **39**(7): 1303-1319. [DOI: 10.1016/j.ultrasmedbio.2013.01.015]. Epub 2013 Apr 3. [PMID: 23562015]; [PMCID: PMC4183372].
 69. Jia XL, Chen WZ, Zhou K, Wang ZB. Effects of low-intensity pulsed ultrasound in repairing injured articular cartilage. *Chin J Traumatol* 2005; **8**(3): 175-178. [PMID: 15896276].
 70. Xu SY, Zhang LM, Yao XM, Zhou GQ, Li X, He BJ, Chen XG. [Effects and mechanism of low-intensity pulsed ultrasound on extracellular matrix in rabbit knee osteoarthritis]. *Zhongguo Gu Shang* 2014; **27**(9): 766-771. Chinese. [PMID: 25571661].
 71. Park SR, Park SH, Jang KW, Cho HS, Cui JH, An HJ, Choi

Marks R. Low Intensity Pulsed Ultrasound and Articular Cartilage Repair

MJ, Chung SI, Min BH. The effect of sonication on simulated osteoarthritis. Part II: alleviation of osteoarthritis pathogenesis by 1 MHz ultrasound with simultaneous hyaluronate injection. *Ultrasound Med Biol* 2005; **31**(11): 1559-1566. [PMID: 16286033].

72. Yuan LJ, Niu CC, Lin SS, Yang CY, Chan YS, Chen WJ, Ueng SW. Effects of low-intensity pulsed ultrasound and hyperbaric oxygen on human osteoarthritic chondrocytes. *J Orthop Surg Res* 2014; **9**: 5. [DOI: 10.1186/1749-799X-9-5]; [PMCID: PMC3922963]; [PMID: 24499626].