Low Intensity Pulsed Ultrasound and Articular Cartilage Repair

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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 osteoarthritis reparative and repair effects.

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

INTRODUCTION

Low intensity pulsed ultrasound (LIPUS), a non-thermal form of physical stimulation found to impact bone healing positively, is associated with possible favorable mechanical and biochemical effects on the process of endochondral ossification, as well as articular cartilage chondrocyte cell metabolism. 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 and Rothenberg et al, 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, including favorable impacts on scaffold-free cultured chondrocytes as well as gene expression in chitosan scaffolds and 3D matrices. Other studies, however, found the favorable impact to be only of temporary significance, while others found no significant effect, or emergent effects only after prolonged stimulation periods. Yet, LIPUS has been shown to impact synovial inflammation, increase vascularity in erosive cartilage, and to potentially decrease the extent of cartilage histopathology, while potentially accelerating soft tissue and bone healing processes.

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[19] 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 it 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[29], and even among human osteoarthritic chondrocytes[19], as well as those substrates derived from well-accepted animal models of osteoarthritis[17,18,29]. 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-B[29], normally required to promote chondrogenesis in these cells[20].
Investigated whether LIPUS stimulates chondrocyte proliferation

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

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.
High-density semi open culture

Methods

36 rabbits were divided into an early control, early osteoarthritis, early treatment, late control, late osteoarthritis, and late treatment groups.

Results

LIPUS has limited potential to provide an effective matrix effect.

Bovine articular chondrocytes were isolated from knee articular cartilage of 2-wk-old rabbits and treated with LIPUS for 20 min per day. FLIPUS attenuated release of type II collagen + proteoglycans + reduced chondrocyte apoptosis + improved chondrocyte proliferation, and increased type IX collagen contents and histologic and macroscopic observations were assessed. Synthesis of glycosaminoglycans was increased in LIPUS-treated chondrocytes with a collagen sponge. LIPUS applications enhanced matrix production in the high density cultures.

30 osteoarthritic and 30 normal rabbits were randomized into three groups: control, model, and treatment. LIPUS treatment attenuated cartilage degeneration, decreased the number of osteoclastic cells and increased MMP-13 expression in rats with osteoarthritis (p < 0.05). Compared with the sham treatment, LIPUS significantly reduced Mankin scores, inflammatory cells and effusion, while MMP-13 decreased.

15-wk-old male Wistar rats were divided into two experimental groups and a control group. To examine whether early intervention with LIPUS helps delay traumatically induced osteoarthritis progression in male Sprague-Dawley rats. Synthesis of glycosaminoglycans was reduced in the early treatment group than the early osteoarthritis group.

40 knee injured rabbits were divided into 4 groups, including a control group, a model group, a FGF2 group, and a FGF2 + LIPUS group. 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.

1154 3rd post surgery day and continued for 4 consecutive wks , 5 days/wk

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.

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.

<table>
<thead>
<tr>
<th>Authors</th>
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<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Takeuchi et al[24]</td>
<td>Effect of LIPUS on cell growth was examined in 3D-cultured chondrocytes with a collagen sponge</td>
<td>LIPUS promoted the proliferation of cultured chondrocytes and type IX collagen</td>
</tr>
<tr>
<td>Unaka et al[8]</td>
<td>High-density semi open culture system of rat chondrocytes</td>
<td>LIPUS applications enhanced matrix production in the high density cultures</td>
</tr>
<tr>
<td>Vaughan et al[5]</td>
<td>Bovine articular chondrocytes cultured in agarose and monolayer cultures</td>
<td>LIPUS has limited potential to provide an effective matrix effect</td>
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</table>

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.

<table>
<thead>
<tr>
<th>Authors</th>
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<tbody>
<tr>
<td>Fujita et al[31]</td>
<td>15-wk-old male Wistar rats were divided into two experimental groups and a control group</td>
<td>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</td>
</tr>
<tr>
<td>He et al[32]</td>
<td>Temporomandibular (TM) osteoarthritis [OA] was induced by trauma. At 8 wks, experimental group began LIPUS treatments for 4 wks (5 days/wk)</td>
<td>The TM-J OA model was successfully established, and LIPUS attenuated cartilage retrogression determined histologically</td>
</tr>
<tr>
<td>Hsieh et al[33]</td>
<td>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</td>
<td>Compared with the sham treatment, LIPUS significantly reduced Mankin scores, inflammatory cells and matrix metallopeptidase 13 expression in rats with osteoarthritis (p &lt; 0.05)</td>
</tr>
<tr>
<td>Jia et al[34]</td>
<td>30 osteoarthritic and 30 normal rabbits were randomized into three groups</td>
<td>FLIPUS attenuated release of type II collagen + proteoglycans + reduced chondrocyte apoptosis + effusion</td>
</tr>
<tr>
<td>Li et al[35]</td>
<td>Effect of LIPUS on expression of tissue inhibitor of metalloproteinase-2 (TIMP-2) articular cartilage cells of rabbits with knee osteoarthritis (OA) was examined</td>
<td>Compared with the normal control group, expression of TIMP-2 in OA model group was increased, while MMP-13 decreased</td>
</tr>
<tr>
<td>Kanaguchi et al[36]</td>
<td>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</td>
<td>At 20(OA) wks, the mono-iodoacetate + LIPUS showed higher bone density</td>
</tr>
<tr>
<td>Li et al[7]</td>
<td>30 5-month-old female Sprague-Dawley rats were randomly assigned to six groups</td>
<td>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</td>
</tr>
<tr>
<td>Pan et al[37]</td>
<td>Chondrocytes were isolated from knee articular cartilage of 2-wk-old rabbits and treated with LIPUS</td>
<td>LIPUS plus 0.1 mg/mL CCO solution promoted chondrocyte proliferation and type II collagen and TGF-β1 expression synergistically in vitro (P &lt; 0.05), but also promoted proliferation in the absence of CCO</td>
</tr>
<tr>
<td>Tang et al[38]</td>
<td>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 group</td>
<td>In the FGF2 group and FGF2 + LIPUS groups, tissues of knee joint gradually repaired</td>
</tr>
<tr>
<td>Xia et al[4]</td>
<td>30 rabbits were divided into an early control, early osteoarthritis, early treatment, late control, late osteoarthritis, and late treatment groups</td>
<td>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</td>
</tr>
<tr>
<td>Zhaoor et al[39]</td>
<td>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 (LIPUSI-2 group) or in weeks 4 and 5 after intra-articular fracture (LIPUSI-5 group)</td>
<td>Among the altered gait parameters, maximal and average paw print areas in LIPUSI-2 + 4-5 groups, but not LIPUS 0 group, had either reached baseline or was recovered</td>
</tr>
<tr>
<td>Zhou et al[40]</td>
<td>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</td>
<td>Compared to the operation alone, the severity of cartilage injury was decreased in the LIPUS group.</td>
</tr>
</tbody>
</table>
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\[^{[30]}\], in a dose dependent manner in the context of cultured chondrocytes and articular cartilage explants\[^{[27]}\], 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\[^{[4]}\] and DU et al\[^{[49]}\].

Other potentially relevant observed benefits of LIPUS applications to chondrocyte explants, and osteoarthritic cartilage models include its ability to favorably modulate Transforming Growth Factor-B3 levels that can help regulate the reconstruction of injured cartilage\[^{[26,57]}\], along with the induction of favorable subchondral bone alterations\[^{[29]}\], plus the induction of CCN2, a protein involved in cartilage repair\[^{[20]}\]. Ji et al\[^{[30]}\] 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\[^{[40]}\], 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\[^{[5}^\text{a}\] , as well as by alleviating osteoarthritisc- 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\[^{[38]}\], the synthesis and secretion of collagen by chondrocytes, as well as concurrent cartilage repair appears to be enhanced\[^{[46]}\].

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\[^{[21,23,31,36,38,59]}\], 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\[^{[52]}\], 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\[^{[22]}\], such as bone\[^{[28]}\], has been hypothesized to exert a favorable effect on joint
tissues such as cartilage, implicated in osteoarthritis. 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. As well, almost 15 years ago, Lee et al 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, and possible publication bias, along with limited external validity to the clinic, Gurkan et al and many others, such as Korstjens et al, 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 osteoarthritis animal models.

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. LIPUS induced changes also include related modulations in type II collagen and proteoglycan and matrix metalloproteinase-13 levels. As such, it appears LIPUS is potentially able to delay degeneration in early osteoarthritis 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, while preventing or delaying excess cartilage degradation, and in accord with early work may specifically reduce the expression not only of matrix metalloproteinase-13, but interleukin-1β, 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, LIPUS applications help to reduce synovial inflammation, increase chondrocyte numbers, and enhance other strategies designed to promote anabolic cartilage chondrocyte repair processes.

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 osteoarthritis 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, 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 cartilage pathways, and potential osteoarthritis related therapeutic genes.

As mentioned previously, these mechanisms of action include, but are not limited to, an apparent reduction in matrix metalloproteinases-13 immunopositive cells, 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, and the activation of the circadian Per-2 gene. Another is related to its potential for stimulating chondrocyte proliferation along with heightened matrix production. Moreover, LIPUS appears to stimulate chondrocyte proteoglycan synthesis, while down regulating potent catabolic cartilage enzymes, which can otherwise degrade one or more essential functional and structural components of the cartilage extracellular matrix, concurrently.

As such, if the observed ability of LIPUS to upregulate cartilage specific gene expression mechanisms that are potentially therapeutic, such as tissue inhibitor of metalloproteinase-2 (TIPPM-2) and neutrophil elastase, along with its concomitant influence on repressing catabolic enzyme actions and production, such as matrix metalloproteinase (MMP-13) 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

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, 47, 50, 54, 63, 64, 66-70].

- LIPUS Micromechanical Perturbations
- Selectively Stimulate Mechanically Sensitive Chondrocyte Cell Membrane Proteins/ Signaling and Transcription Pathways
- Extracellular and Intracellular Effects
- Transient Upregulation Aggrecan and Collagen II Genes
- Downregulation of Destructive Enzymatic Genes
- Increases in Glucosaminoglycan Content + Collagen Matrix Production
- Anti-inflammatory Effects
- Improved Quality and Rate Osteochondral Repair
- Reduced Severity and Progression of Osteoarthritis
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.[3,52] and Louët et al.[80].

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,[44, plus analogous views presented here and others,[46,70] 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.[83], 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


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