

# Improved Cartilage Repair After Treatment With Low-Intensity Pulsed Ultrasound

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Low-intensity pulsed ultrasound accelerates bone healing via upregulation of cartilage formation and maturation phases of endochondral bone formation. The current authors evaluated the effect of ultrasound therapy on the repair of full-thickness osteochondral defects. Bilateral, 3.2 mm diameter by 5.0 mm deep osteochondral defects were created in the patellar groove of 106 adult male New Zealand rabbits. The defects were treated with daily low-intensity pulsed ultrasound therapy on the right knee. The left knee was not treated. In Part I, the effect of ultrasound therapy was evaluated at 4, 8, 12, 24, and 52 weeks after surgery. In Part II, the effect of the length of treatment (5, 10, or 40 minutes of daily ultrasound therapy) compared with standard 20 minute therapy was evaluated. The repair cartilage was evaluated and graded on a standard scale for the gross and histologic appearance. Ultrasound treatment significantly improved the morphologic features and histologic characteristics of the repair cartilage compared with nontreated controls. Earlier, better

repair with less degenerative changes at later times was observed in defects treated with ultrasound. Doubling the treatment time to 40 minutes daily significantly increased the histologic quality of the repair cartilage. In the current animal model, daily low-intensity pulsed ultrasound had a significant positive effect on the healing of osteochondral defects.

## List of Abbreviations Used

BMP	bone morphogenetic protein
EDTA	ethylenediaminetetraacetic acid
GAG	glycosaminoglycan
MRNA	messenger ribonucleic acid
OP-1	osteogenic protein-1
TGF- $\beta$	transforming growth factor-beta

As many as 650,000 knee surgeries are done annually to treat articular cartilage damage, including 200,000 with focal chondral defects. Osteochondral defects may occur in any age group but occur most frequently in young active patients.<sup>2</sup> Injury commonly is located in the distal femur or patella and arises from patellar dislocations, twisting injuries, trauma, or persistent osteochondritis dissecans. With time, the injury results in degeneration of the

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joint surfaces, particularly if the defect is left untreated.<sup>18</sup>

Cartilage is an avascular musculoskeletal tissue with a relatively low capacity for self-regeneration or repair. In the absence of available stem cells, there is little hope for cartilage repair after injury and the tissue progresses along a degenerative pathway that destroys the biochemical and biomechanical properties. Various treatments for osteochondral lesions have failed to achieve universal success. In an attempt to bring stem cells into the area of injury, subchondral bone drilling or the microfracture technique has been advocated.<sup>2,18,19</sup> The fibrocartilage repair tissue formed has a greater stiffness, permeability and Type I collagen content, and a significantly reduced GAG content compared with hyaline cartilage. The inferior load bearing capacity of the resulting fibrocartilage can result in degeneration of the repair with time necessitating additional reconstructive procedures.

Mosaicplasty or the transplantation of autologous osteochondral plugs from nonweight-bearing portions of the knee to a defect site has had some success.<sup>3,14,22</sup> The technique is limited by availability of donor cartilage. In addition, this is an invasive, technically demanding procedure with the potential of donor site pain among other reported complications.<sup>22</sup> Other concerns with the procedure include the necessity of the donor plugs to conform to the surface geometry of the weightbearing surface and the long-term disposition of the interface between the plugs themselves.

Autologous chondrocyte transplantation has shown early success for the treatment of chondral defects.<sup>5,6,8</sup> The technique consists of harvesting a small piece of the marginal articular cartilage from the femoral condyle. Retrieved cells are multiplied in explant culture and later implanted at the defect under a periosteal flap. Although the early results with this technique are promising, the need for multiple surgeries, cost, and the long-term disposition of the repair tissue may limit the use of this technique.<sup>21</sup>

The use of growth factors including those from the BMP family have been hypothesized

as a potential method to stimulate cell proliferation and expression of cartilage phenotype for chondral defect repair.<sup>9,13,29,30</sup> Initial animals studies in which BMP-2 and BMP-7 (OP-1) were implanted have shown some promise.<sup>9,13,30</sup> However, these materials are not yet clinically available and the extrapolation of animal results to use in human clinical application is difficult.

All of these interventions involve a surgical procedure. Low-intensity pulsed ultrasound has been shown to promote the endochondral portion of fracture healing and may influence the healing of osteochondral defects without surgical intervention.<sup>23,24,32,34</sup> Ultrasound has been shown to accelerate fracture healing when applied for 20 minutes daily in the non-thermal, low energy level of 30 mW/cm<sup>2</sup> range (Sonic Accelerated Fracture Healing System [SAFHS], Exogen Inc, Piscataway, NJ).<sup>10,15,16</sup> In a prospective, randomized, double blind and placebo controlled study of closed or Grade I open tibial fractures, Heckman et al<sup>15</sup> reported an approximate 38% decrease in healing time with the use of daily ultrasound therapy. In a similar study, Kristiansen et al<sup>16</sup> found acceleration of each stage of fracture healing in distal radius fractures and a 38% decrease in overall healing time with the use of ultrasound.

Animal fracture models and *in vitro* studies of chondrocytes have been used to elucidate the role ultrasound plays in advancing endochondral ossification.<sup>23,34</sup> Yang et al<sup>34</sup> reported that when ultrasound was applied to a rat femur fracture model, there was an increase in the expression of genes encoding cartilage production and therefore the acceleration of fracture healing was a result of the earlier synthesis of extracellular matrix proteins in cartilage resulting in altered chondrocyte maturation and earlier endochondral bone formation. In an *in vitro* study using rat chondrocytes, ultrasound stimulation resulted in a significant increase in aggrecan mRNA levels and proteoglycan synthesis although cell proliferation and alpha Type II procollagen mRNA were not affected.<sup>23</sup> These findings suggest the ultrasound has a direct stimulatory effect on cartilage forma-

tion, maturation, and extracellular matrix production.

The purpose of the current study was to examine the effect of daily low-intensity pulsed ultrasound therapy on the repair of full-thickness osteochondral defects in a rabbit model. The effect of length of ultrasound treatment and duration of daily ultrasound treatment was investigated using gross and histologic analyses.

## MATERIALS AND METHODS

### Experimental Design

One hundred six adult male New Zealand White rabbits were used. All animals received bilateral, full-thickness osteochondral defects 3.2 mm in diameter and 5 mm deep in the central region of the patellar groove of the femur. The investigation was conducted in two parts. Part I used 70 animals to examine the effect of daily ultrasound therapy using the clinically available ultrasound device and 20 minute daily treatment time. The right knee in these animals was treated with 20 minutes of daily low-intensity pulsed ultrasound 6 days weekly using the commercially available ultrasound signal. The left knee was not treated. Groups of 12 animals each were sacrificed at 4, 8, and 12 weeks postoperative. Eighteen animals were sacrificed at 24 weeks postoperative. Three groups of six animals each were sacrificed after receiving 12, 18, or 24 weeks of daily ultrasound therapy. Eight animals received daily ultrasound therapy for 24 weeks postoperative and were sacrificed at 52 weeks postoperative, and a final group of eight animals received daily ultrasound therapy for 52 weeks and were sacrificed at 52 weeks postoperative.

Part II of the study used 36 animals and investigated the effect of either shortening or lengthening the daily treatment time. The right knee of all animals was treated for 20 minutes daily with the ultrasound device and in groups of 12 animals each the left knee was treated with either 5, 10, or 40 minutes of ultrasound therapy. All animals in Part II of the study were sacrificed at 4 weeks postoperative.

### Animal and Surgical Model

Adult male New Zealand White rabbits weighing approximately 4 kg at acquisition were used. All animals were at least 12 months of age. Specific attention was given to selecting animals of uniform

size to limit variability in loading of the osteochondral defects.

Using standard aseptic techniques, surgery was done with the animals under isoflurane gas anesthesia. The animals were monitored by electrocardiogram and heart rate monitors. Both hind limbs were prepared and draped in sterile fashion. Through a median parapatellar incision, the connective tissue securing the patella was released partially to dislocate the patella and expose the medial femoral condyle and patellar groove. Using a drill bit, a 3.2 mm diameter by 5 mm deep osteochondral defect in the patellar sulcus of the femur was created by hand. After irrigation with saline, the joint was closed in layers. Routine anteroposterior (AP) radiographs were taken after surgery to ensure proper defect location.

Butorphanol tartrate (Fort Dodge Animal Health, Fort Dodge, IA; 0.2 mg/kg body weight) was administered subcutaneously as required for pain. Animals were administered intramuscular antibiotics for 4 days after surgery. Animals were kept in recovery cages postoperatively until fully conscious and weightbearing, after which they were transferred to standard cages and allowed unrestricted motion.

### Ultrasound Device and Treatment

The standard treatment consisted of 20 minutes of daily ultrasound therapy with the above described ultrasound device. The ultrasound device delivers a low level acoustic pressure wave signal with an intensity of 30 mW/cm<sup>2</sup> at a frequency of 1.5 MHz and burst width of 200  $\mu$ s (equivalent to the intensity used for diagnostic ultrasound) to the skin for 20 minutes daily. The ultrasound therapy was administered 6 days weekly beginning on postoperative Day 3. In Part II of the study, ultrasound therapy also was investigated at 5, 10, and 40 minutes of application daily. Animals were sedated by intramuscular injection of ketamine and xylazine (83 mg/mL Ketaset and 17 mg/mL Rompun, Fort Dodge Animal Health) at the dosage of 0.3 mg/kg body weight to administer the therapy. This dosage is approximately 1/2 of the anesthetic dosage intended to provide sedation only. The ultrasound transducer was placed on the distal femur at the lateral condyle with ample ultrasound gel. The sites were shaved periodically to ensure contact between the transducer, coupling gel, and the skin. Each ultrasound device was tested before use with a signal detector to ensure that the units were emitting the appropriate signal.

## Evaluations

Animals were sacrificed by an intravenous barbiturate overdose (Beuthanasia-D Veterinary Euthanasia Solution, Schering-Plough Animal Health, Kenilworth, NJ; 0.45 mL/kg body weight). Right and left distal femurs were harvested en bloc, carefully labeled, and kept in cool physiologic saline until gross grading and microphotography was completed.

Each harvested knee with a defect was graded for gross appearance according to Moran et al<sup>20</sup> independently by two observers blinded to the treatment group. This analysis apportions points based on the formation of intraarticular adhesions, restoration of articular surface, erosion, and appearance of the cartilage. Eight points is the best possible grade (Table 1).

The individual specimens then were fixed by immersion in 4% paraformaldehyde solution. After fixation, the specimens were decalcified slowly in EDTA. The defect area was bisected across the diameter of the defect. The resulting halves and surrounding tissue were embedded in paraffin and sectioned across the defect site. At least two sections, 5 to 7  $\mu\text{m}$  thick, from three different levels were cut from each block. Level 1 was closest to the defect center. Level 3 was closest to the defect perimeter and Level 2 was centered between Levels 1 and 3.

**TABLE 1. Gross Grading Scheme Used in the Evaluation of the Osteochondral Defects**

Intraarticular adhesions	Grades
None	2
Minimal with fine loose fibrous tissue	1
Major with dense fibrous tissue	0
Restoration of articular surface	
Complete	2
Partial	1
None	0
Erosion of cartilage	
None	2
Defect site or site border	1
Defect site and adjacent normal cartilage	0
Appearance of cartilage	
Translucent	2
Opaque	1
Discolored or irregular	0
Total score	8 possible points

One section from each level was stained with either Goldner's trichrome or safranin-o and fast green stains to indicate GAG content in the matrix.

Histologic sections were graded according to Caplan et al,<sup>7</sup> which apportions points based on the nature of the repair cartilage, structural characteristics, and cellular changes (Table 2). Six sections from each specimen were evaluated independently by two observers blinded to treatment group. The observer determined the histologic grade for each specimen after examining all of the sections from that specimen. Examination of intraobserver error showed that the grades from each observer could be averaged together and a composite grade for each specimen could be determined.

**TABLE 2. Histologic Grading Scale Used in the Evaluation of Sections From Each Experimental Defect**

Cell morphologic features	Grades
Normal	4
Mostly hyaline cartilage	3
Mixed hyaline and fibrocartilage	2
Mostly fibrocartilage	1
Some fibrocartilage, most nonchondrocytic cells	0
Reconstruction of subchondral bone	
Normal	3
Reduced subchondral bone reconstruction	2
Minimal subchondral bone reconstruction	1
No subchondral bone reconstruction	0
Matrix staining	
Normal	4
Slightly reduced	3
Reduced	2
Significantly reduced	1
No staining	0
Filling of defect (cartilage layer)	
100%	2
50% or 150% (overfill or underfill)	1
0%	0
Surface regularity	
Regular, smooth	1
Irregular	0
Bonding	
Both interfaces bonded	2
One interface or partial bonding	1
Neither interface bonded	0
Total	16 possible points

### Statistical Analysis

By pairing a nontreated control defect site with an experimental treatment site within each animal reduces the effect of individual variations in animal healing and allows for matched paired comparisons. One way and two way analysis of variance (ANOVA) was used to screen for any effects of animal to animal variance and intraobserver variance. Once shown that neither variable significantly influenced the means, data were pooled. One way and two way ANOVA for the evaluation period, treatment effect, and length of treatment were used to determine significance of gross and histologic data. Subsequent post hoc analysis consisted of matched paired Student t tests to determine the influence of ultrasound treatment within each evaluation period. When multiple t test comparisons were made, the Bonferroni correction was used. For all statistical tests, significance was defined as  $p \leq 0.05$  (95% confidence interval).

### RESULTS

The animals tolerated the surgical and ultrasound treatment procedures well. In Part I of the study, one animal from the 52 week group died 19 days before the scheduled sacrifice date; however, statistical analysis showed that the results from this animal could be pooled with others in the 52-week group. One animal was eliminated from the gross and histologic analysis in the 24-week group because of poor surgical

placement of a defect. Two animals were eliminated from the 4-week histologic evaluation because of infections. One specimen from the 8-week histologic analysis was eliminated because of improper histologic sectioning. In Part II of the study, one animal in the 5-minute treatment group died at 17 days postoperative of complications related to the administration of ketamine. This animal was not replaced. Two animals in the 10-minute treatment group also were excluded from the analysis. One animal had a wound infection at sacrifice. The other animal was eliminated because of improper surgical placement of the defect.

### Part 1: The Effect of Daily Ultrasound Treatment

Ultrasound treated defects had significantly improved gross appearance grades at 4, 8, and 12 weeks and significantly improved histologic grades at 4, 12, 24, and 52 weeks postoperative compared with nontreated controls (Tables 3, 4). Two-way ANOVA for the effect of time and experimental treatment showed that the postoperative healing period did not significantly influence the mean gross or histologic grade ( $p = 0.2968$  and  $p = 0.1315$ , respectively). The experimental ultrasound treatment significantly affected the mean gross and histologic grade (both  $p < 0.0001$ ). Two-way ANOVA found no effect caused by

**TABLE 3. (Part I) Gross Grading Results at Each Evaluation Period, Pair-Wise Within Animal t Test**

Treatment Groups	Ultrasound	Control	p Value
4 weeks	6.6 ± 1.0 (12)	5.0 ± 1.5 (12)	0.0044
8 weeks	7.1 ± 1.1 (12)	5.9 ± 1.4 (12)	0.0333
12 weeks	6.6 ± 1.1 (12)	5.7 ± 1.1 (12)	0.0492
24 weeks (12 weeks of treatment)	6.8 ± 1.2 (6)	6.8 ± 0.5 (6)	NS
24 weeks (18 weeks of treatment)	6.3 ± 1.0 (5)	6.1 ± 0.7 (5)	NS
24 weeks (24 weeks of treatment)	7.0 ± 0.5 (6)	6.3 ± 0.8 (6)	NS
24 weeks pooled	6.7 ± 0.9 (17)	6.4 ± 0.7 (17)	NS
52 weeks (24 weeks of treatment)	6.8 ± 1.0 (8)	6.3 ± 1.4 (8)	NS
52 weeks (52 weeks of treatment)	6.7 ± 1.7 (8)	6.1 ± 1.5 (8)	NS
52 weeks pooled	6.7 ± 1.4 (16)	6.2 ± 1.4 (16)	NS
All groups pooled	6.7 ± 1.1 (69)	5.9 ± 1.3 (69)	0.0001

\*NS =  $p > 0.05$ ; (mean ± standard deviation [sample size]).

**TABLE 4. (Part I) Histologic Grading Results at Each Evaluation Period, Pair-Wise Within Animal t Test**

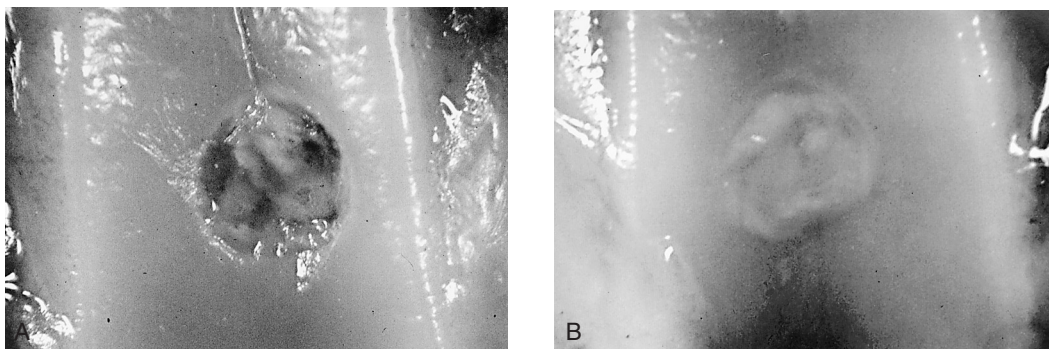
Treatment Groups	Ultrasound	Control	p Value
4 weeks	9.2 ± 2.8 (10)	6.8 ± 2.4 (10)	0.0129
8 weeks	9.4 ± 2.9 (11)	9.0 ± 2.8 (11)	NS
12 weeks	9.9 ± 1.9 (12)	8.1 ± 2.2 (12)	0.0009
24 weeks (12 weeks of treatment)	10.0 ± 3.3 (6)	7.0 ± 3.0 (6)	0.0131
24 weeks (18 weeks of treatment)	9.2 ± 1.8 (5)	7.8 ± 2.6 (5)	NS
24 weeks (24 weeks of treatment)	9.7 ± 2.4 (6)	7.7 ± 2.7 (6)	0.0152
24 weeks pooled	9.6 ± 2.6 (17)	7.5 ± 2.7 (17)	0.0002
52 weeks (24 weeks of treatment)	9.1 ± 1.0 (8)	7.3 ± 2.1 (8)	0.0068
52 weeks (52 weeks of treatment)	8.8 ± 3.5 (8)	7.6 ± 3.7 (8)	NS
52 weeks pooled	8.9 ± 2.4 (16)	7.4 ± 2.9 (16)	0.0100
All groups pooled	9.4 ± 2.5 (66)	7.7 ± 2.7 (66)	p < 0.0001

\*NS = p > 0.05; (mean ± standard deviation [sample size]).

increasing the number of ultrasound treatments (12, 18, or 24 weeks of ultrasound therapy evaluated at 24 weeks, and 24 or 52 weeks of ultrasound therapy evaluated at 52 weeks) on the mean gross and histologic grade (p = 0.2386 and p = 0.8550, respectively). However, the ultrasound treatment significantly improved the mean gross and histologic grades (both p < 0.0001).

Grossly, at 4, 8, and 12 weeks the ultrasound treated defects had significantly greater grades compared with the contralateral nontreated control defects (Table 3). Figure 1 is an

example of the gross appearance of a pair of osteochondral defects from an animal evaluated at 4 weeks. From 8 to 12 weeks, there was little improvement in the gross appearance of the defects regardless of treatment. The mean gross grades for ultrasound and nontreated control defects improved at 24 weeks over the 12 week mean gross grades but the difference was not statistically significant. From 24 to 52 weeks, the ultrasound treated and nontreated control mean gross grades decreased slightly but again this did not represent a statistically significant effect. It was difficult to detect ev-



**Fig 1A–B.** (A) The gross appearance of a nontreated control defect at 4 weeks postoperative. The defect is not yet covered with cartilage. The repair tissue is disorganized and easily differentiated from the host cartilage. (B) The gross appearance of the contralateral osteochondral defect treated with 4 weeks of daily ultrasound. The defect margins are difficult to discern from the host cartilage. The repair cartilage has a smooth homogenous appearance.

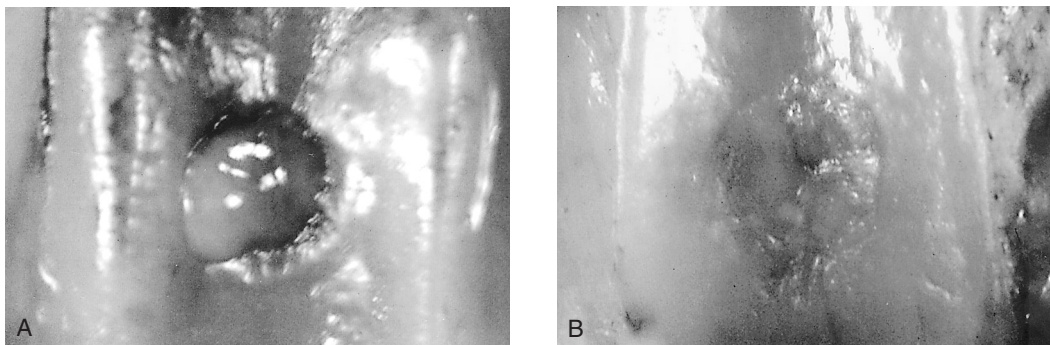
idence of degeneration grossly in the long-term evaluation because differences were subtle and better elucidated from the histologic observations.

Increasing the number of ultrasound treatments did not significantly affect the mean gross grades. Osteochondral defects treated with ultrasound throughout the 24-week evaluation had increased mean gross grades compared with defects treated with 12 and 18 weeks of therapy evaluated at 24 weeks. However, the difference in means was not statistically significant. Figure 2 is an example of the 24-week postoperative appearance of a pair of osteochondral defects from an animal treated with 12 weeks of ultrasound therapy. Similarly, there was no significant improvement in the gross appearance of defects treated for 52 weeks of ultrasound compared with defects treated with 24 weeks of ultrasound and evaluated at 52 weeks.

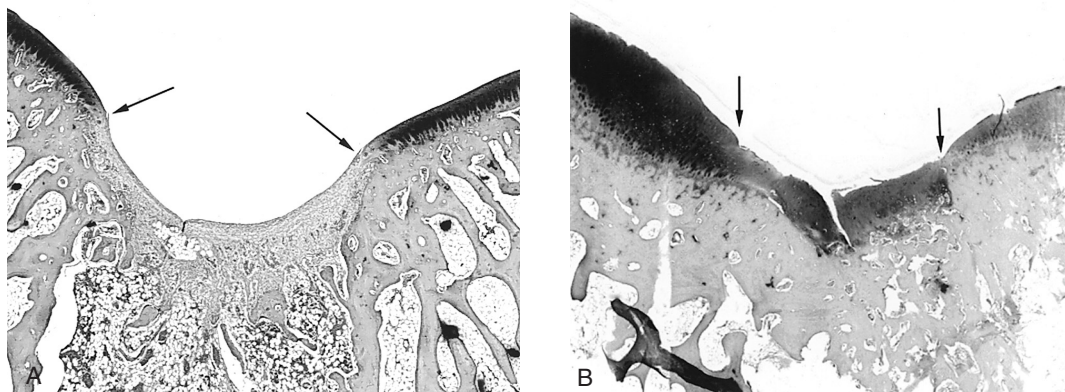
At 4 and 12 weeks postoperative, the ultrasound-treated defects had significantly greater histologic grades compared with contralateral nontreated control defects (Table 4, Fig 3). At 8 weeks, the ultrasound-treated defects had a slightly greater mean histologic grade compared with controls; however, the difference was not statistically significant. The mean his-

tologic grades for ultrasound-treated and nontreated defects at the 24 and 52 week evaluations did not change significantly from the 12 week grades. Similar to the gross results, increasing the postoperative ultrasound treatment duration from 12 or 18 weeks to 24 weeks or from 24 to 52 weeks also did not significantly affect the mean histologic grades. Figure 4 is a microphotograph of an osteochondral defect evaluated at 24 weeks after receiving 12 weeks of ultrasound therapy and the contralateral nontreated control defect. Statistical analysis showed that, when pooled, the mean total histologic grade for all defects treated with ultrasound evaluated at 24 weeks (12, 18, and 24 weeks of daily treatment) was significantly greater than the mean grade for contralateral control defects (Table 4). Similarly, the pooled histologic grade for the ultrasound treated defects evaluated at 52 weeks was significantly greater than the mean histologic grade for nontreated controls (Fig 5).

Analysis of the subscores of the histologic grading scale (cell morphologic features, reconstruction of subchondral bone, matrix staining, filling of the cartilage defect, surface regularity, and bonding) was done to elucidate the dynamics of the cartilage repair and deterioration process. Specifically, the effect of ul-



**Fig 2A–B.** (A) The 24-week gross appearance of a nontreated control defect. Although the defect is covered with repair tissue, the repair has a swollen appearance and is not integrated with the host cartilage. A large pannus of opaque tissue is present. (B) The 24-week gross appearance of the contralateral osteochondral defect treated with 12 weeks of daily ultrasound. The repair cartilage is translucent and well integrated with the host cartilage. There is little evidence of degenerative changes and the articular surface is relatively smooth.



**Fig 3A–B.** (A) A photomicrograph of a nontreated control defect at 4 weeks postoperative. The defect margins are noted by arrows (Original magnification,  $\times 5$ ). A thin layer of subchondral bone has been regenerated and marrow is present in the defect. The repair layer overlying the early subchondral bone is made up of undifferentiated cells and coarse horizontally oriented fibrous tissue. There is no proteoglycan staining within the repair using safranin-o staining. (B) A photomicrograph of a histologic section of the contralateral osteochondral defect treated with 4 weeks of daily ultrasound (Original magnification,  $\times 5$ ). The defect margins are noted by arrows. The subchondral bone layer is nearly restored to its normal height. The repair cartilage is not yet fully organized but has a hyaline-like appearance and intense safranin-o staining. The repair cartilage is bonded to the host cartilage.

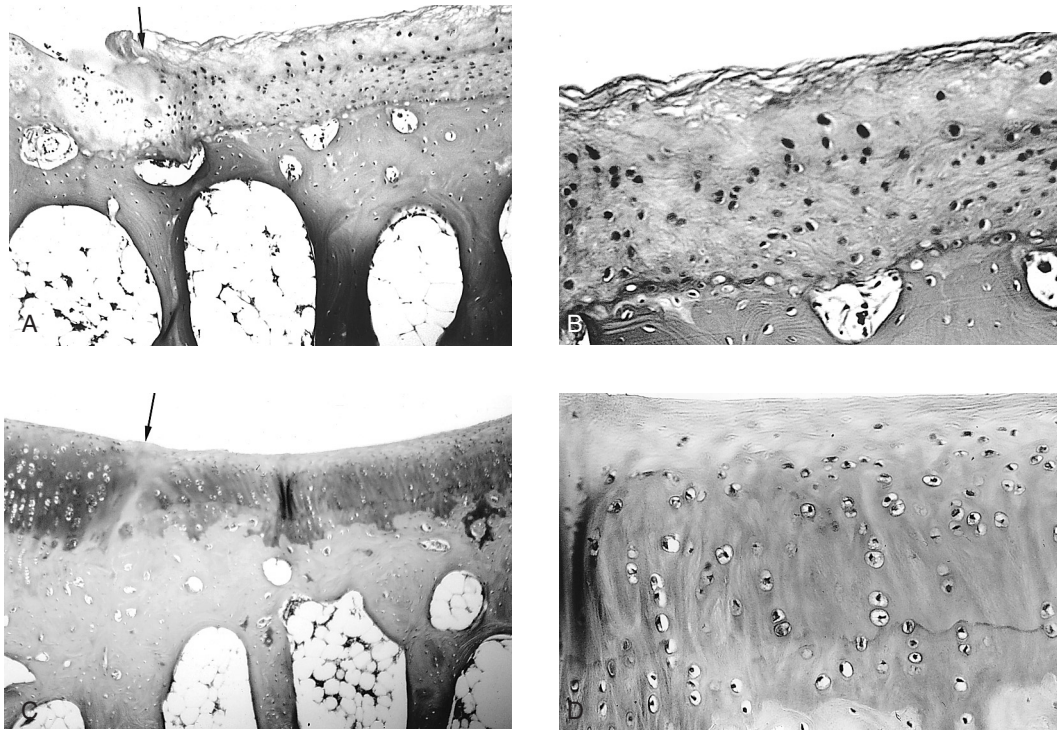
trasound treatment was examined in the markers for cartilage regeneration (cell morphologic features, matrix staining, cartilage filling) or in the reduction of markers for cartilage degeneration (matrix staining, surface regularity, and bonding).

Initially, it was hypothesized that the improved histologic grades in the ultrasound-treated defects was a reflection of earlier restoration of subchondral bone and subsequent early reformation of the new cartilage layer. Time and the experimental treatment had a significant effect on the subchondral bone histologic subscore ( $p = 0.00001$  and  $0.0120$ , respectively). Although the mean subchondral bone grade was greater at all evaluations in ultrasound defects, matched paired  $t$  tests at each evaluation period of the mean subchondral bone grades were not statistically significant.

Ultrasound treatment significantly improved the surface regularity of the repair cartilage compared with nontreated controls at 24 and 52 weeks postoperative ( $p = 0.0085$  and  $p = 0.0183$ , respectively). The surface regu-

larity did not decrease significantly with time in defects treated with ultrasound. However, for nontreated control defects, the mean surface regularity grade decreased significantly from 4 weeks to 12, 24, and 52 weeks ( $p = 0.05, 0.05, \text{ and } 0.01$ , respectively). Bonding of the repair cartilage with the intact host cartilage also was improved significantly with ultrasound treatment compared with nontreated controls at 4 and 52 weeks ( $p = 0.0499$  and  $p = 0.0001$ , respectively). At 52 weeks, the mean grade for bonding in nontreated controls decreased significantly from 8 and 12 week values ( $p = 0.01$  and  $0.05$ , respectively). In contrast, the mean bonding grade at 24 and 52 weeks for ultrasound-treated defects was not significantly different from earlier periods. The ultrasound-treated defects also had more anatomic filling of the cartilage defect layer compared with controls at the 24 week evaluation ( $p = 0.0438$ ). Intensity of the extracellular matrix staining for proteoglycans was improved significantly in the ultrasound-treated defects compared with nontreated controls at



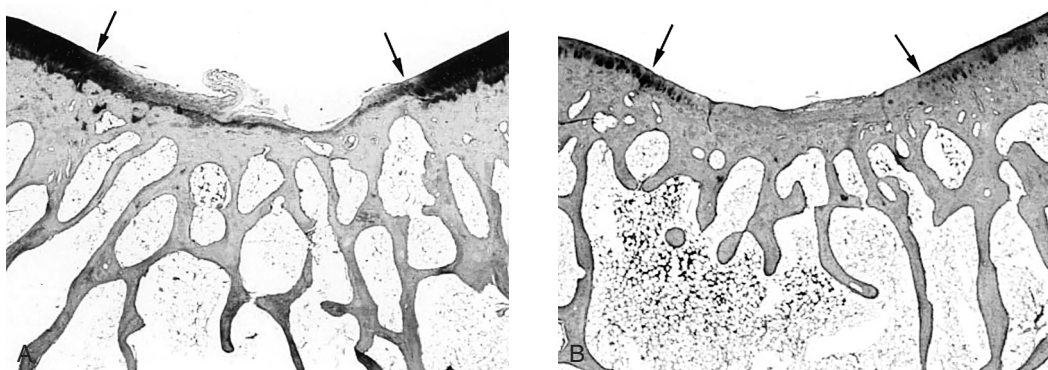


**Fig 4A–B.** (A) The 24-week postoperative histologic appearance of a nontreated control osteochondral defect near the interface of the host cartilage (arrow, gross appearance shown in Figure 2A). Chondrocyte clusters have formed at the interface and safranin-o intensity is decreased markedly. Cysts also have formed (Stain, safranin; original magnification,  $\times 32$ ). (B) A high power ( $\times 80$ ) view of the repair tissue in the center of the same defect showing an absence of safranin-o staining, irregular parallel arrangement of the collagen fibers, and the absence of a tidemark region into the subchondral bone. (C) The 24-week postoperative histologic appearance of the contralateral defect treated with 12 weeks of ultrasound therapy (Original magnification,  $\times 32$ ; gross appearance shown in Figure 2B). The newly regenerated cartilage layer is slightly thinner than the host cartilage (arrow at the defect margin). However, both are stained with safranin-o in a normal pattern and intensity. (D) A high power ( $\times 80$ ) view of the repair tissue in the same defect showing the columnar arrangement of the chondrocytes, intense safranin-o staining, a normal tidemark and calcified cartilage zone. Collagen fibers appear to have an orderly arrangement perpendicular to the surface of the subchondral bone.

12 and 24 weeks ( $p = 0.0014$  and  $p = 0.0040$ ). The matrix staining subscore mean decreased significantly with time for the ultrasound and nontreated control defects ( $p = 0.0451$  and  $0.0159$ , respectively). However, a significant reduction in the matrix staining grade did not occur until 52 weeks postoperative from the 12 week results in the ultrasound treated defects ( $p = 0.05$ ), whereas matrix staining decreased significantly from 8 to 24 weeks in the nontreated control defects ( $p = 0.05$ ).

## Part II: Evaluation of the Length of Daily Ultrasound Treatment

Differences in the paired mean gross and histologic grades for the 5 and 10 minutes of ultrasound therapy compared with the standard 20-minute ultrasound treatment were not statistically significant (Tables 5, 6). However, when gross grading results were pooled, treatment with 20 and 40 minutes of ultrasound significantly improved the mean gross grade over nontreated controls ( $p = 0.05$  and  $0.01$ ,



**Fig 5A–B.** (A) A photomicrograph of a histologic section from a nontreated control osteochondral defect at 52 weeks postoperative. There is an elevation of the subchondral bone layer in the area of the defect (arrows at the defect margins). The thin cartilage layer is fibrous and fibrocartilaginous tissue. Safranin-o staining is minimal and severe delamination of the repair from the subchondral bone has occurred (Original magnification,  $\times 5$ ). (B) A photomicrograph of the contralateral osteochondral defect treated with 52 weeks of daily ultrasound. The subchondral bone layer is fully restored to its normal level. The repair cartilage layer is well bonded to the host cartilage and there are few degenerative changes at the interface. However, there is little safranin-o stain present in the repair which appears to be a combination of cartilage and fibrocartilage (Original magnification,  $\times 5$ ).

**TABLE 5. (Part II) Gross Grading Results Comparing Ultrasound Treatment Duration to the Standard 20 Minute Treatment, Pair-Wise Within Animal Comparisons**

Treatment Groups	Standard 20 Minute Treatment (contralateral defect)	p Value
5 minutes 5.7 $\pm$ 1.0 (11)	6.7 $\pm$ 0.9 (11)	NS
10 minutes 6.2 $\pm$ 1.5 (10)	6.2 $\pm$ 1.2 (10)	NS
40 minutes 6.5 $\pm$ 0.8 (12)	6.0 $\pm$ 1.2 (12)	NS

\*NS =  $p > 0.05$ ; [mean  $\pm$  standard deviation (sample size)].

**TABLE 6. (Part II) Histologic Grading Results Comparing Ultrasound Treatment Duration to the Standard 20 Minute Treatment, Pair-Wise Within Animal Comparisons**

Treatment Groups	Standard 20 Minute Treatment (contralateral defect)	p Value
5 minutes 7.2 $\pm$ 2.6 (11)	8.2 $\pm$ 2.5 (11)	NS
10 minutes 7.8 $\pm$ 2.9 (10)	7.8 $\pm$ 2.8 (10)	NS
40 minutes 8.4 $\pm$ 2.5 (12)	6.4 $\pm$ 2.1 (12)	0.0195

\*NS:  $p > 0.05$ ; [mean  $\pm$  standard deviation (sample size)]

respectively). The 40-minute ultrasound treatment resulted in the highest mean histologic grade obtained at 4 weeks postoperative. Doubling the ultrasound treatment time from 20 to 40 minutes daily significantly improved the histologic appearance ( $p = 0.0195$ ) compared with the standard 20 minute treatment time (Table 6). However, the difference in the mean histologic grades for the 5- and 10-minute treatment lengths compared with the standard treatment time was not statistically significant.

## DISCUSSION

The concept of using biophysical stimulation at a cellular level to modulate tissue formation and repair is not new. Pulsed electromagnetic fields, used in the promotion of bone nonunion healing, may influence the formation of cartilage and extracellular matrix during endochondral bone formation.<sup>1</sup> Pulsing direct current with a resultant electric field across tissue also has been shown to enhance bone and cartilage formation.<sup>4,17</sup>

Low-intensity pulsed ultrasound is a form of mechanical energy transmitted into the body as high frequency acoustic pressure waves producing micromechanical stresses in tissues.<sup>11,12</sup> Ultrasound is thought to promote bone formation in a manner comparable with that first postulated by Wolff's Law.<sup>33</sup> Bone's physiologic response to mechanical force includes the formation, deposition, and resorption of bone. As a result, it not surprising that on a cellular level, low-intensity ultrasound has been shown to increase <sup>45</sup>Ca uptake in cartilage and bone cell cultures, and plays a role in the modulation of TGF- $\beta$  synthesis and parathyroid hormone response in mesenchymal and osteoblast cells.<sup>25-28</sup> In chondrocytes, ultrasound upregulates the expression of extracellular matrix proteins and collagen phenotypes, precursors in endochondral bone formation.<sup>23</sup>

It has been shown that daily therapy with low-intensity pulsed ultrasound is safe and beneficial in the treatment of fractures.<sup>15,16</sup> In rabbits and humans, ultrasound accelerates the early events of endochondral bone healing.<sup>10,11,16,24,32,34</sup> In the

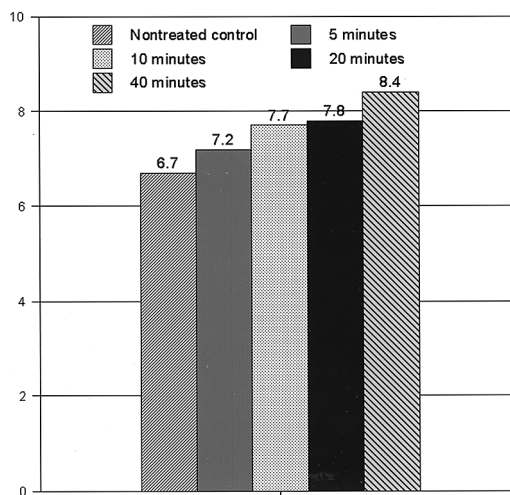
animal model used in the current study, daily ultrasound therapy had its greatest effect early, before 24 weeks of healing. The gross appearance of osteochondral defects treated with ultrasound therapy was most improved over controls at 4, 8, and 12 weeks. Histologically, the improvement also was significant at early evaluations. However, at 24 and 52 weeks postoperative as the histologic appearance of untreated controls began to degenerate, less degeneration occurred in the ultrasound-treated defects. The early regeneration and improved quality of the repair cartilage in the ultrasound-treated defects may help prevent or at least delay the degenerative process.

Analysis of the histologic subscores showed that ultrasound treatment improved markers for degeneration of the repair tissue particularly at evaluations after 12 weeks. Surface regularity was improved with ultrasound treatment compared with nontreated control defects. In ultrasound-treated defects, bonding of the repair cartilage to the host cartilage also was maintained with time and had significantly greater bonding grades compared with controls at 4 and 52 weeks. Surface regularity and bonding decreased significantly with time in nontreated control defects. The presence of metachromatic staining of the extracellular matrix is an indicator of hyaline cartilage, whereas the absence of staining is indicative of degeneration via proteoglycan loss and change in the biochemical composition of the tissue. Significantly increased matrix staining and a delay in the reduction of matrix staining with time was observed in ultrasound-treated defects compared with controls.

In the current rabbit model, 12 weeks of ultrasound treatment seemed to be adequate to see the sustained benefit of daily therapy. When evaluated at 24 weeks, there was no significant improvement in the quality of defect healing when ultrasound was applied beyond the first 12 weeks postoperative, with 18 and 24 weeks of therapy having equivalent mean gross and histologic grades compared with 12 weeks of treatment. Similarly, there was no significant increase in mean gross and histologic grades when defects were evaluated at

52 weeks after receiving 52 weeks of ultrasound therapy compared with those treated with just 24 weeks of therapy. With time, there was no significant change in the ultrasound group results from 24 to 52 weeks, indicating that perhaps the crucial effects of ultrasound occur before 24 weeks of healing. The effect of the number of ultrasound treatments required to see a sustained benefit in this animal model also seems to depend on the application of therapy within the first 12 weeks of healing.

Part II of the study was a first attempt at optimizing the daily ultrasound treatment time to determine whether shorter (or longer) therapy time is required to observe the benefits of the treatment. Decreasing the number of treatments and the length of each treatment is desirable in developing a clinical ultrasound therapy regime that ensures patient compliance. Increasing the treatment time from the standard 20 minutes to 40 minutes resulted in a statistically significant histologic improvement. Overall, there was a trend toward improved cartilage repair with increasing daily ultrasound treatment time (Fig 6). These findings are in agreement with observations in models of bone healing after treat-



**Fig 6.** Graphic summary of the mean histologic grades comparing 0, 5, 10, 20, and 40 minutes of ultrasound treatment at 4 weeks postoperative. A trend in increasing mean grade was observed with increasing ultrasound therapy treatment time.

ment with ultrasound. In a rabbit fibular osteotomy model, Tsai et al<sup>31</sup> compared the rate of bone formation after treatment with daily low intensity ultrasound for either 5, 15, or 25 minutes. They reported that at 4 weeks after surgery the amount of bone formed increased significantly with the length of the ultrasound treatment, where 25 minutes of therapy generated the most bone formation. The results of the current study indicate that the effects of treatment with low-intensity pulsed ultrasound may be dose dependent. In this experimental model, optimal histologic results were observed with a 40-minute daily treatment. The results also indicated a minimum 12-week duration. This would be a reasonable treatment protocol; however, trials in humans will best determine the optimum therapy balancing issues of patient compliance and clinical outcomes.

The exact biologic mechanism is not yet fully understood, although it generally is accepted that low-intensity pulsed ultrasound influences the cartilage formation and maturation phases of fracture healing.<sup>23,34</sup> The current study has shown that ultrasound therapy can positively influence the healing of articular defects, particularly those involving the subchondral bone, and may prove to be an adjunct to procedures such as mosaicplasty and subchondral drilling. Ultrasound may impart accelerated and improved quality of the repair, slowing the possible degenerative process associated with these defects. These improvements may lead to a faster postoperative recovery period and greater functional scores in intermediate and long-term followup.

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