Effect of Mechanical Mincing on Minimally Manipulated Articular Cartilage for Surgical Transplantation

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Background: Point-of-care treatment options for medium to large symptomatic articular cartilage defects are limited. Minced cartilage implantation is an encouraging single-stage option, providing fresh viable autologous tissue with minimal morbidity and cost.

Purpose: To determine the histological properties of mechanically minced versus minimally manipulated articular cartilage.

Study Design: Controlled laboratory study.

Methods: Remnant articular cartilage was collected from fresh femoral condylar allografts. Cartilage samples were divided into 4 groups: cartilage explants with or without fibrin glue and mechanically minced cartilage with or without fibrin glue. Samples were cultured for 42 days. Chondrocyte viability was assessed using live/dead assay. Cellular migration and outgrowth were monitored using bright-field microscopy. Extracellular matrix deposition was assessed via histological staining. Proteoglycan content and synthesis were assessed using dimethylmethylene blue assay and radiolabeled 35S-sulfate, respectively. Type II collagen (COL2A1) gene expression was analyzed via polymerase chain reaction.

Results: The mean viability of minced cartilage particles (34% ± 14%) was not significantly reduced compared with baseline (46% ± 13%) on day 0 ($P = .90$). After culture, no significant difference in the percentage of live cells was appreciated between mechanically minced (58% ± 23%) and explant (73% ± 14%) cartilage in the presence of fibrin glue ($P = .52$). The addition of fibrin glue did not significantly affect the viability of cartilage samples. The qualitative assessment revealed comparable cellular migration and outgrowth between groups. Proteoglycan synthesis was not significantly different between groups. Histological analysis findings were positive for COL2A1 in all groups, and matrix formation was appreciated in all groups. COL2A1 expression in minced cartilage (1.72 ± 1.88) was significantly higher than in explant cartilage (0.15 ± 0.07) in the presence of fibrin glue ($P = .01$).

Conclusion: Mechanically minced articular cartilage remained viable after 42 days of culture in vitro and was comparable with cartilage explants with regard to cellular migration, outgrowth, and extracellular matrix synthesis.

Clinical Relevance: Mechanically minced articular cartilage is an encouraging intervention for the treatment of symptomatic cartilage defects. Further translational work is warranted to determine the viability of minced cartilage implantation as a single-stage therapeutic intervention in vivo.

Keywords: knee; cartilage; cartilage restoration

Articular cartilage defects in the knee are relatively common and possess limited inherent capacity for self-repair.[40] When left untreated, chondral defects progress in size and depth, leading to premature osteoarthritic changes with resultant pain and dysfunction.[3,25,42] Because of the avascular nature of articular cartilage and the limited ability of chondrocytes to migrate and divide, chondral healing is limited, resulting in mechanically inferior fibrocartilage.[2,8,26] As a result, cartilage restoration therapies involving the restoration of chondral defects with tissue closely approximating the biological, histochemical, and mechanical properties of native hyaline cartilage are utilized to improve clinical outcomes while providing long-term durability. Traditional single-stage operative management of smaller cartilage defects includes microfracture, osteochondral autograft transplantation, and osteochondral allograft (OCA) transplantation. However,

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microfracture produces varied clinical outcomes secondary to the unsatisfactory mechanical durability of resultant fibrocartilage repair tissue.[11,29,41,42,48] Osteochondral autograft transplantation is resource intensive, technically challenging, and associated with donor-site morbidity, while OCA transplantation outcomes have been complicated by increased failure rates requiring a reoperation or revision arthroplasty.[5-7,13,19,23,32,49]

Recent advances in tissue engineering have led to cellular-based therapies, such as autologous chondrocyte implantation, with the goal of replacing larger ($\geq$4 cm^2) cartilage defects with tissue comparable with native hyaline cartilage.[20-22] However, despite encouraging histological and clinical outcomes, autologous chondrocyte implantation is a 2-stage procedure associated with substantial costs.[12,26,27,30,43]

As such, single-stage cellular-based procedures, such as the utilization of mechanically minced autogenous articular cartilage, have emerged as a low-cost viable alternative to current treatment options. Through mechanical stimulation, minced cartilage has been shown to facilitate chondrocyte extracellular matrix production, capable of integrating into chondral defects and maturing to hyaline-like cartilage.[10,17,39] Clinical studies utilizing mechanically minced cartilage consisting of autograft and juvenile allograft particulated cartilage have demonstrated encouraging results.[10,18,37,47] Meanwhile, multiple investigations have reported the satisfactory fixation of minced cartilage particulates within the defect area using fibrin glue.[1,4,31]

The purpose of this study was to determine the differences and similarities between minimally manipulated versus mechanically minced articular cartilage harvested from fresh femoral condylar allografts. We hypothesized that minced articular cartilage fragments cultured in vitro would demonstrate continued cell viability, migration, and production of extracellular matrix components comparable with minimally manipulated articular cartilage.

METHODS

Human Tissue Acquisition

Local research ethics board approval was obtained before the initiation of the study. Remnant cartilage samples were obtained from 6 fresh human femoral condylar allografts after osteochondral plugs were removed from the condyles and implanted into patients undergoing OCA transplantation (Figure 1). Before implantation, all 6 donor condyles were preserved in nutrient-rich antibiotic media and stored at a temperature range of 1°C-10°C. After the surgical procedure, approximately half of the residual cartilage was harvested from the donor condyle and mechanically minced using a 4.0-mm arthroscopic shaver (Torpedo; Arthrex). Cartilage was captured using an in-line suction receptacle (GraftNet; Arthrex). Remnant cartilage directly adjacent to the harvest area was mechanically procured using 4-mm punch biopsy to obtain minimally manipulated cartilage (explant) to serve as control tissue. All cartilage tissue was collected from areas of the condyle that were grossly normal based on a visual inspection. Both the explant (control group) and the mechanically minced (test group) cartilage samples were transported in Dulbecco's modified Eagle medium/nutrient mixture F-12 containing 20% serum. Samples were divided into 4 subgroups: minimally manipulated (explant) cartilage (four 4-mm specimens per well) cultured with and without fibrin glue (Evicel [human]; Ethicon) and mechanically minced cartilage cultured with and without fibrin glue. The fibrin glue utilized in this study was prepared from a 2-mL sterile solution of Biological Active Component 2 (55-85 mg/mL fibrinogen) and a 2-mL sterile solution of human thrombin (800-1200 IU/mL thrombin). Aliquots of minced cartilage were then carefully placed into the designated wells of a 24-well culture plate. Samples cultured in fibrin glue were added to wells after 3 drops of the prepared glue were added to the designated well base. Each sample was then gently pressed into the well until the glue completely covered the sample. Because of the adherent and frail characteristics of mechanically minced cartilage, an accurate wet weight could not be reliably measured. As such, estimates of the volume that the glue needed to cover the designated samples and the volume of minced cartilage that would provide enough cells for the assays were used.

Culture Media and Conditions

Specimens were cultured in a solution containing Dulbecco's modified Eagle medium, Ham's F-12, fetal bovine serum, penicillin/streptomycin/amphotericin B (composed of 10,000 U/mL of penicillin, 10,000 μg/mL of streptomycin, and 25 μg/mL of amphotericin B), and gentamicin (100 μg/mL). All culture components were purchased from Invitrogen. Day 0 was defined as the day that tissue was collected from allografts after surgery. Mechanically minced and explant cartilage were cultured for 42 days, during which time the medium was changed every other day. Cultures were maintained at 37°C in a humidified atmosphere

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Figure 1. Femoral condylar allograft after osteochondral allograft removal.

of 5% CO_2. Cellular migration was monitored every 48 hours using bright-field microscopy.

Cell Viability

Cell survival was measured using calcein acetoxymethyl and ethidium homodimer–1 (EthD-1; Molecular Probes).[51] Live cells emit green fluorescence after metabolizing calcein via a ubiquitous intracellular esterase, while EthD-1 enters cells when plasma membrane integrity is compromised after cell death, staining nuclear DNA red. Cell viability was recorded as the number of live cells divided by the number of total cells. Images were acquired on an Eclipse TE2000-S microscope (Nikon) using MetaMorph Software (Version 6.1r6; Molecular Devices). Cell counting was performed using ImageJ software (National Institutes of Health). Each image was enhanced to maximize image quality, and automated cell counting parameters were set based on the size of the smallest and largest cells visualized. Each color image was then converted to a binary image, and stained cells meeting threshold requirements were automatically counted. The ratio of dead to live cells was then calculated.

Histological Analysis

On day 0 and day 42, tissue samples were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, sectioned at 6 μm using a microtome, and stained with 7safranin O and hematoxylin and eosin to assess the proteoglycan (PG) content.[46] The same Eclipse E800 microscope (Nikon) with imaging software was used to analyze the integrity of the cartilage surface and matrix, detecting abnormalities in cellularity and depletion of safranin O staining, reflective of PG distribution.

PG Synthesis

On day 0 and day 42, cultured tissue was pulse labeled with 10 uCi/mL of 35S-sulfate for 4 hours, after which mechanically minced and explant cartilage samples were collected and stored at −20°C until papain digestion and filtration assay. 35S-sulfate PGs in the media and papain-digested explants were quantified by liquid scintillation counting after rapid filtration assay as previously described.[38] The concentration of 35S-sulfate PGs synthesized was normalized to the cellular DNA content measured via DNA assay using Hoechst 33258 (Catalog #09460; Polysciences).[28] PG synthesis was normalized to micrograms of DNA utilizing radioactive 35S-sulfate (PerkinElmer), Alcian blue 8GX (Acros Organics), and Hoechst 33258.

PG Content

The cultured media were collected and stored at −20°C for 42 days. Media samples from each subgroup were pooled weekly. Media were not digested. To assay the PG content in culture media, we used dimethylmethylene blue assay (Catalog #03610; Polysciences).[14]

Immunohistochemistry

Serial sections were then used for immunohistochemistry. Before primary antibody staining, slides were incubated at 60°C in a water bath in a 10-mM sodium citrate buffer (pH 6.0) for 20 minutes to unmask the antigen. Sections were incubated overnight at 4°C with primary antibody: anti–type II collagen (anti–COL2A1) (20 μg/mL) (ab34712; Abcam). A horseradish peroxidase micropolymer secondary antibody was used from the horseradish peroxidase/3,3′-diaminobenzidine Detection IHC Detection Kit (ab236469; Abcam) for 15 minutes, followed by a diaminobenzidine chromogen for no more than 10 minutes as a visual control for staining. The slides were washed, dehydrated, and mounted. The evaluation and documentation of results were performed using the same Eclipse E800 microscope (Nikon) with NIS-Elements imaging software (Nikon).

Reverse Transcription Polymerase Chain Reaction

On day 0 and day 42, cartilage tissue was transferred to an RNAse-free tube (Eppendorf), flash frozen in liquid nitrogen, and stored at −80°C until RNA isolation. Total RNA was extracted using the Trizol reagent (Invitrogen) as previously described.[15] Reverse transcription for cDNA was performed per the kit's specifications (#95048-100; Quanta Biosciences) to measure COL2A1 gene expression (Table 1). Prime polymerase chain reaction (PCR) custom plates were used for reverse transcription PCR (#10025216; Bio-Rad Laboratories).

Genes With Amplicon Context Sequence

Thermal cycling with SYBR green was performed using the CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories). Cycle details are included in Appendix Figure A1 (available in the online version of this article).

TABLE 1
Genetic Context Sequence for Polymerase Chain Reaction

Gene Name	Gene Symbol	Reference Sequence Accession No.
Collagen, type II, alpha 1	COL2A1	NC_000012.11, NG_008072.1, NT_029419.12
Gene Symbol	Amplicon Context Sequence	
COL2A1	CAGCTATGGAGATGACAATCTGGCTCCCAACACTGCCAACGTCCAGATGACCTTC CTACGCCTGCTGTCCACGGAAGGCTCCCAGAACATCACCTACCACTG	

Livak and Schmittgen's[34] method was used to analyze the relative changes in gene expression from real-time quantitative PCR experiments. The housekeeping gene used for normalization was glyceraldehyde 3-phosphate dehydrogenase.

Statistical Analysis

Data are reported as the mean ± standard deviation of 6 biological replicates (donor samples). Minimally manipulated (explant) cartilage (control group) with or without fibrin glue and mechanically minced cartilage with or without fibrin glue were compared using 1- and 2-way analyses of variance with Tukey post hoc testing for multiple comparisons, after confirming the normality of the data using the D'Agostino-Pearson test. Statistical significance was set at $P < .05$. All statistical analyses were performed using Stata software (Version 16.1; StataCorp).

RESULTS

The mean age of the 6 cadaveric donors was 22.5 ± 5.7 years (range, 14-28 years) and consisted of 3 male and 3 female specimens. Cartilage harvest was performed, on average, at 23.3 ± 2.1 days (range, 21-26 days) after donor death. No cartilage was harvested beyond the 28-day expiration date as set by the cartilage allograft supplier.

Effect of Mechanical Mincing on Chondrocyte Viability

The mean viability of minced cartilage particles on day 0 (34% ± 14%) was not significantly reduced compared with baseline (46% ± 13%; $P = .90$) (Figure 2A and Appendix Table A1, available online). After 42 days of culture, the mean viability of minced cartilage particles with and without fibrin glue was 58% ± 23% and 36% ± 27%, respectively, compared with baseline (46% ± 13%) ($P = .90$ and $P = .64$, respectively). After 42 days of culture, the mean percentages of live cells in the presence of fibrin glue in the mechanically minced group and explant group were 58% ± 23% and 73% ± 14%, respectively ($P = .52$). The mean percentages of live cells in the absence of fibrin glue in the mechanically minced group and explant group were 36% ± 27% and 46% ± 12%, respectively ($P = .78$). The mean percentage of live cells by day 42 in the mechanically minced and explant groups was not affected by the addition of fibrin glue ($P = .15$ and $P = .056$, respectively). After 42 days of culture, the mean area of cell death in the presence of fibrin glue in the mechanically minced group and explant group was 35,953.23 ± 21,537.58 and 11,011.50 ± 12,770.39, respectively ($P = .90$). The mean area of cell death in the absence of fibrin glue in the mechanically minced group and explant group was 56,498.97 ± 33,672.97 and 58,054.29 ± 27,995.23, respectively ($P = .90$) (Figure 2B and Appendix Table A2, available online). Representative images of live/dead staining can be seen in Figure 2C.

Outgrowth Assessment

Cellular migration and matrix outgrowth were present after 7 days of culture and plateaued by 42 days in both the mechanically minced and explant groups. Bright-field images showed limited chondrocyte outgrowth by day 7 (Figure 3, A-D), increasing by day 21 (Figure 3, E-H), with confluence by day 42 (Figure 3, I-L) in both mechanically minced and explant cartilage.

PG Synthesis

On day 0, the mean levels of PG synthesis were comparable between the mechanically minced (639.33 ± 239.19) and explant (767.16 ± 343.57) groups ($P = .90$). On day 42, no significant difference in the level of PG synthesis in the presence of fibrin glue was appreciated between the mechanically minced (1819.83 ± 952.61) and explant (2432.00 ± 1551.48) groups ($P = .74$). PG synthesis in the absence of fibrin glue in the mechanically minced group (1209.17 ± 378.12) was not significantly different compared with that in the explant group (1143.67 ± 418.55) on day 42 ($P = .90$). PG synthesis was significantly greater in explant tissue with fibrin glue by day 42 compared with that in explant tissue at baseline ($P = .012$). All other within-group and between-group comparisons were not significantly different (Figure 4 and Appendix Table A3, available online).

PG Content

When the PG content was evaluated, there were no significant differences between the mechanically minced and explant groups on day 42, and no differences with regard

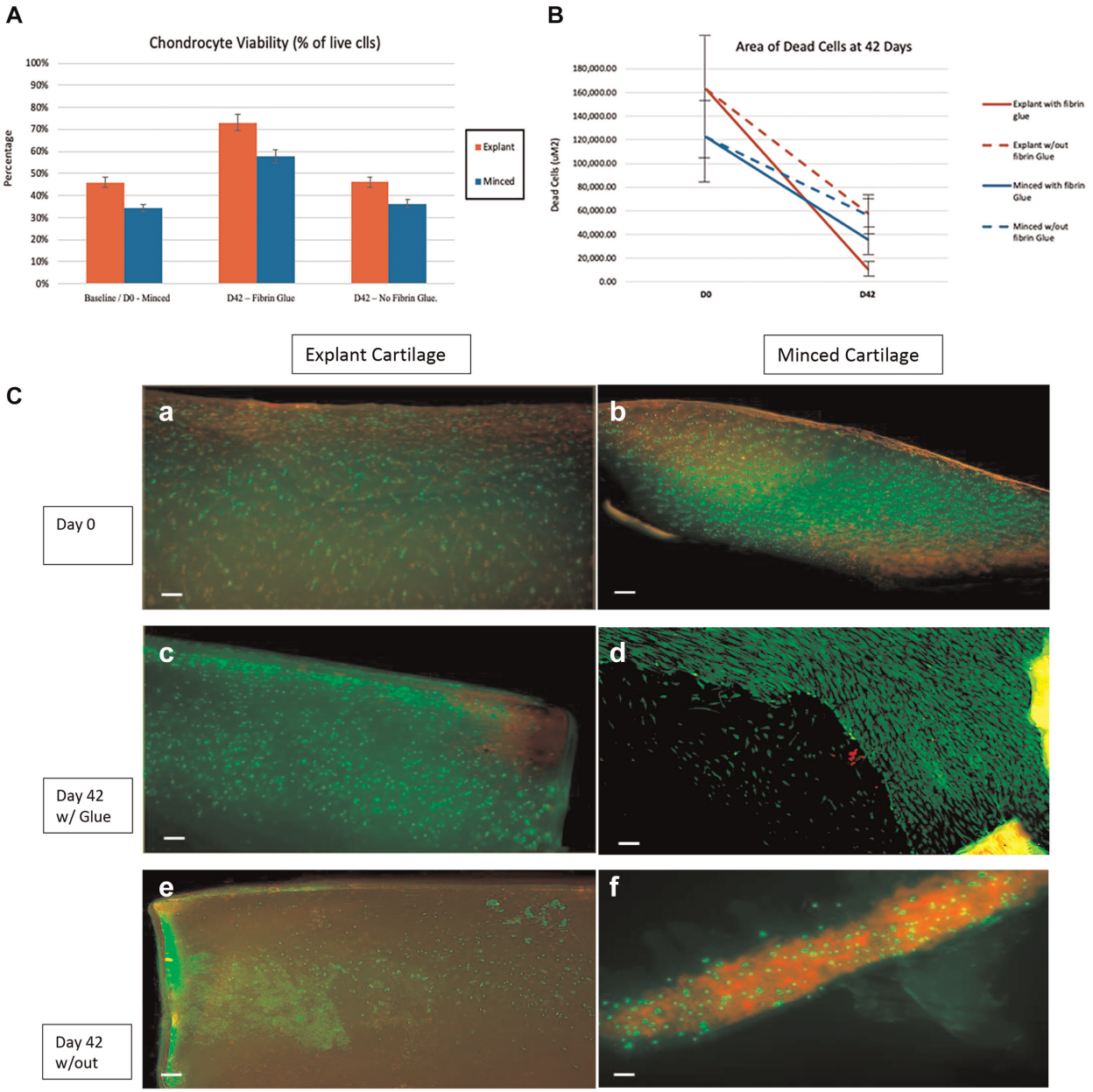

Figure 2. Effect of mincing on chondrocyte viability. (A) Comparison with baseline of chondrocyte viability for explant and minced cartilage on day 0 (D0) and day 42 (D42). (B) Area of cell death on day 0 and day 42. (C) Live/dead staining of minced and explant cartilage with and without fibrin glue. Representative images: explant cartilage on day 0 (*a*), minced cartilage on day 0 (*b*), explant cartilage with glue on day 42 (*c*), minced cartilage with glue on day 42 (*d*), explant cartilage without glue on day 42 (*e*), and minced cartilage without glue on day 42 (*f*). Scale bars: 100 μm.

to the presence or absence of fibrin glue (Figure 5 and Appendix Table A4, available online).

Immunohistochemistry and Histological Analysis

Both the explant and mechanically minced groups exhibited evidence of extracellular matrix growth after 42 days. On day 0, chondrocytes in the mechanically minced group with or without fibrin glue were positive for COL2A1, with greater degrees of matrix staining positive for COL2A1 in the presence of fibrin glue. The matrix newly formed around cells outside the tissue was also found to be positively stained for safranin O (Figure 6). Safranin O staining was positive in both the mechanically minced and the explant groups, suggesting matrix diffusion, although the mechanically minced group displayed less staining (Figure 7).

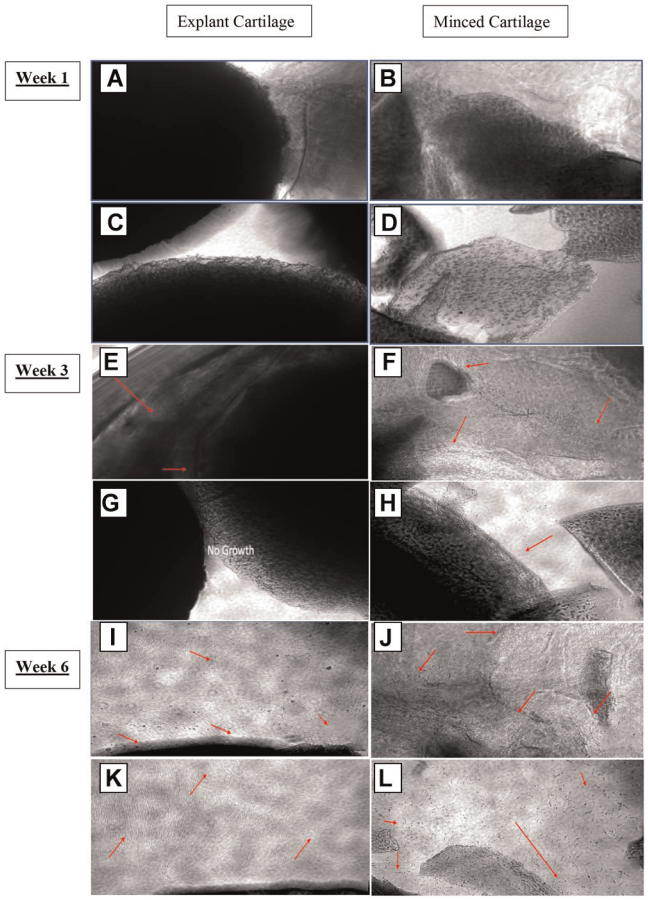

Figure 3. Bright-field images with areas of migration and growth: (A) explant cartilage with glue on day 7, (B) minced cartilage with glue on day 7, (C) explant cartilage without glue on day 7, (D) minced cartilage without glue on day 7, (E) explant cartilage with glue on day 21, (F) minced cartilage with glue on day 21, (G) explant cartilage without glue on day 21, (H) minced cartilage without glue on day 21, (I) explant cartilage with glue on day 42, (J) minced cartilage with glue on day 42, (K) explant cartilage without glue on day 42, and (L) minced cartilage without glue on day 42. Arrows indicate areas of new growth and cellular migration.

COL2A1 Gene Expression

On day 0, the mean levels of COL2A1 expression were comparable between the mechanically minced and explant groups (P = .89). After 42 days of culture, COL2A1 expression was significantly greater in the presence of fibrin glue in the mechanically minced group (1.72 ± 1.88) versus explant group (0.15 ± 0.07) (P = .01). In the absence of fibrin glue, COL2A1 expression between the mechanically minced (0.82 ± 0.66) and explant (0.53 ± 0.62) groups was not significantly different on day 42 (P = .89). All other within-group and between-group comparisons were not significantly different (Figure 8 and Appendix Table A5, available online).

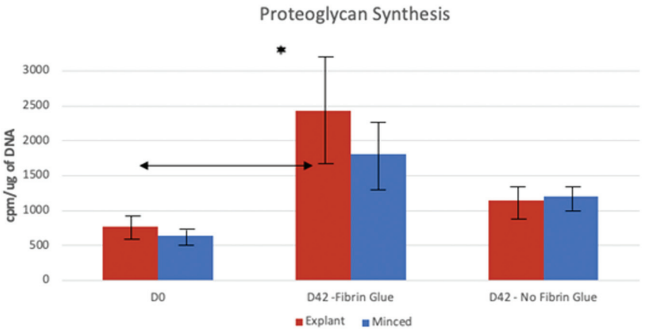

Figure 4. Proteoglycan synthesis values (cpm/ug of DNA) by day in culture. Cultured tissue was pulse labeled with 10 uCi/mL of 35S-sulfate. Significant difference between explant tissue on day 0 (D0) and explant tissue with glue on day 42 (D42) (*$P < .05$).

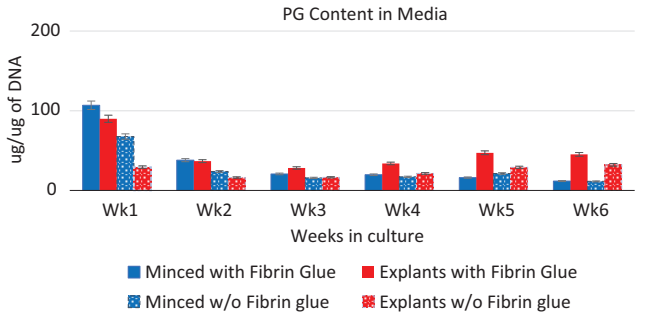

Figure 5. Proteoglycan content values (ug/ug of DNA) by week in culture. After 42 days of culture, media were pooled weekly and assayed.

DISCUSSION

The main findings from the present study are that mechanically minced articular cartilage remained viable after 42 days of in vitro culture, while the effect of mechanically mincing cartilage did not significantly damage cell viability compared with minimally manipulated cartilage. Moreover, cellular migration, outgrowth, PG synthesis and content, and COL2A1 expression in mechanically minced cartilage were comparable with those in minimally manipulated cartilage tissue. With the exception of COL2A1 expression, the addition of fibrin glue to culture media had no significant effect on the observed physiological parameters.

Mechanically mincing cartilage using an arthroscopic 4.0-mm bone-cutting shaver blade was not found to have a detrimental effect on cellular viability after 42 days of in vitro culture. Mechanical mincing using a sharp instrument has been shown to limit cell death during the preparation of cartilage fragments with minimal trauma compared with blunt trauma. Currently utilized techniques of cartilage mincing include the use of scalpel blades, custom mincing devices, and shavers.[47] Using bovine cartilage, Redman et al[45] demonstrated that blunt trauma created using

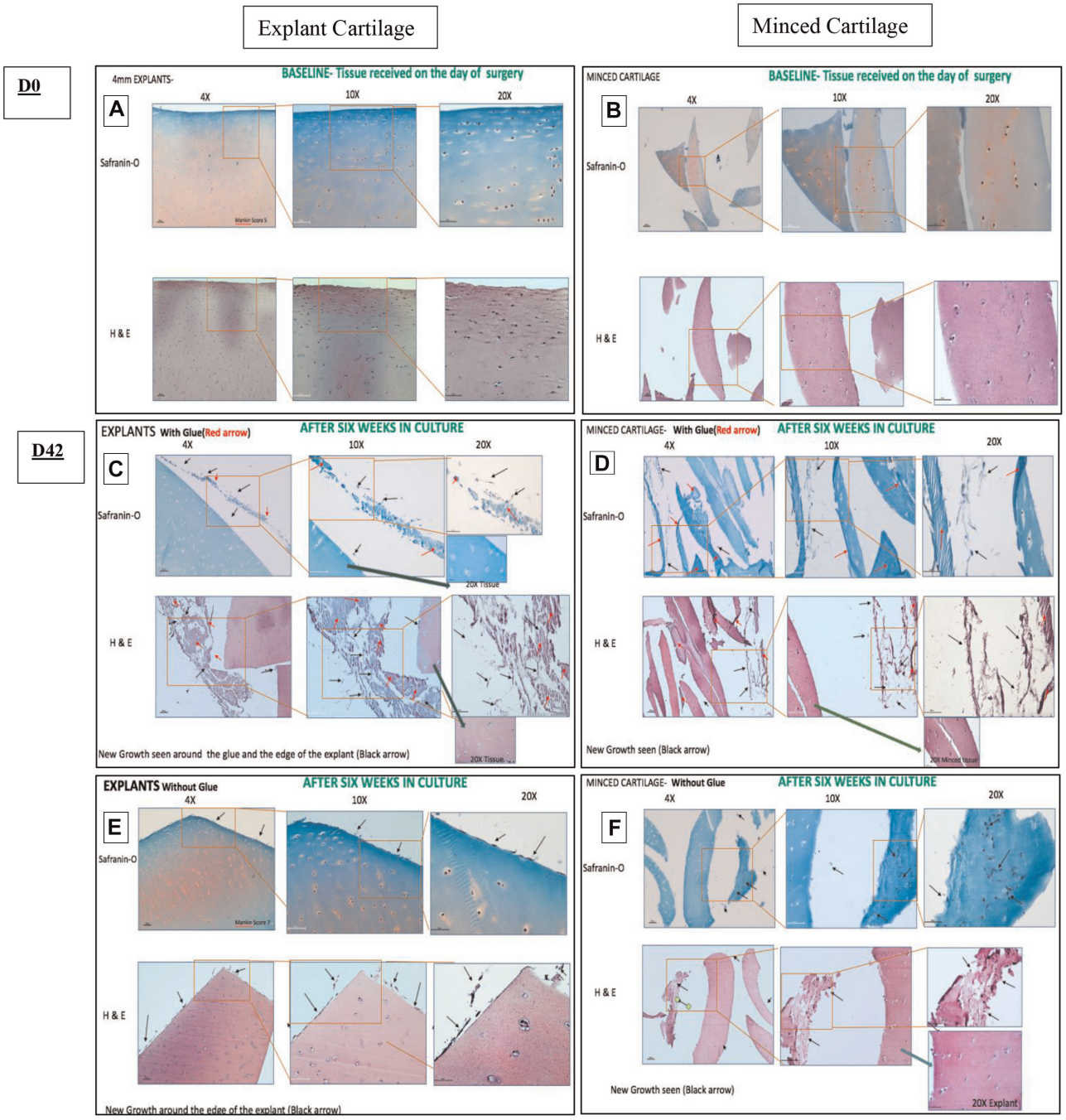

Figure 6. Extracellular matrix deposition by chondrocytes derived from minced and explant tissue in fibrin glue after 42 days of culture. Representative images of safranin O staining (n = 6) and hematoxylin and eosin (H & E) staining (n = 6): (A) explant cartilage on day 0 (D0), (B) minced cartilage on day 0, (C) explant cartilage with glue on day 42 (D42), (D) minced cartilage with glue on day 42, (E) explant cartilage without glue on day 42, and (F) minced cartilage without glue on day 42. Black arrows indicate areas of new growth, and red arrows indicate areas of fibrin glue. Scale bars: 100 μm for 4× and 10× images and 50 μm for 20× images.

a trephine resulted in a 100-μm zone of necrosis, which was not appreciated when preparation was performed sharply using a scalpel. Tew et al[50] described cellular and extracellular matrix cartilage responses to blunt trauma in a bovine cartilage injury model, reporting cell death in combination with necrosis and apoptosis after explants were traumatized centrally using a 1.22-mm trephine. Conversely, utilizing cartilage harvested from the femoral condyles of 12 human knees undergoing knee arthroplasty, Levinson et al[31] observed no differences in outgrowth potential, differentiation behavior, or cellular viability when comparing cartilage cut using a custom mincing device to hand mincing versus

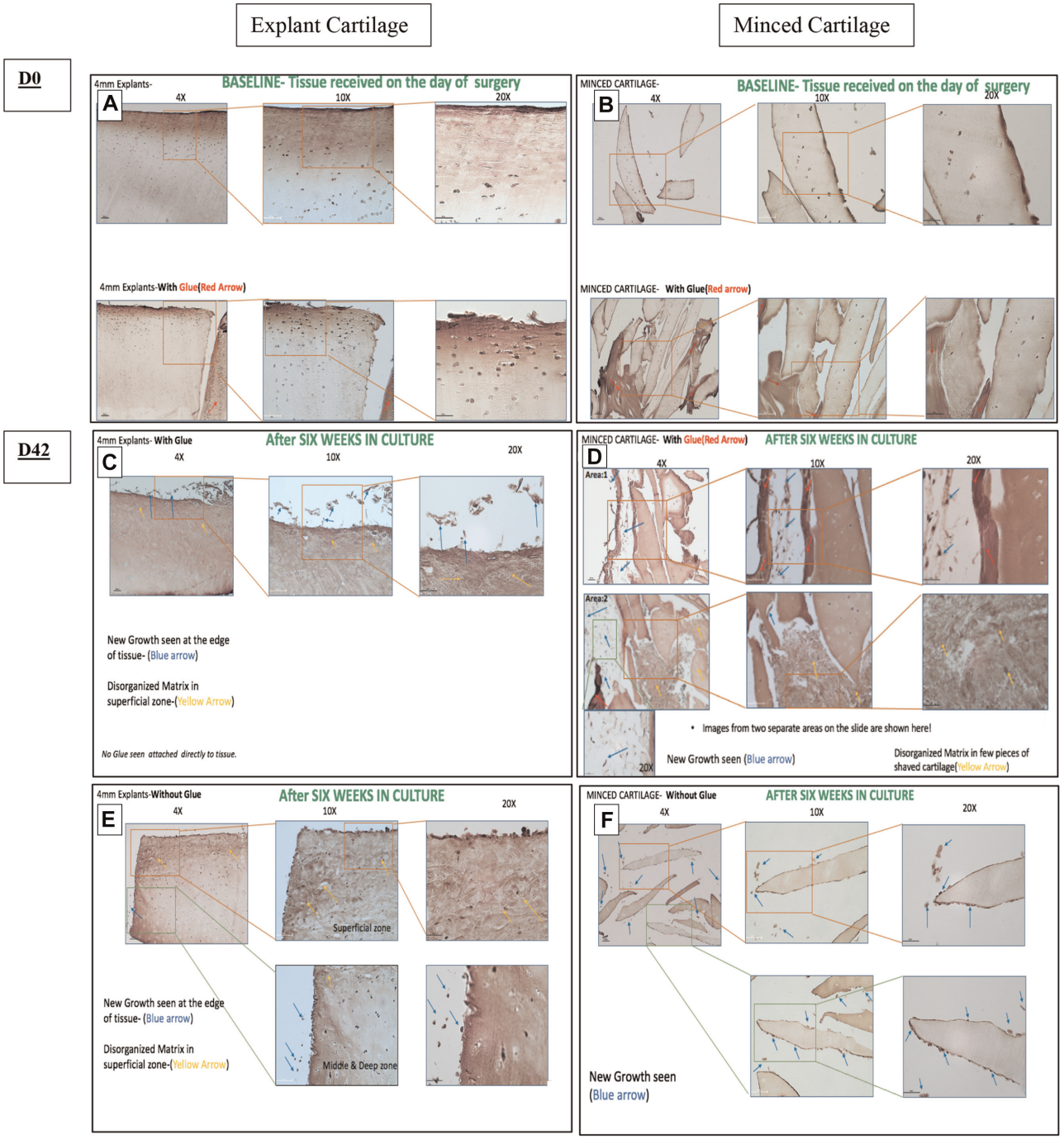

Figure 7. Positive type II collagen staining in explant and minced cartilage. Both groups showed new growth after 42 days of culture. Representative images of type II collagen immunostaining (n = 6): (A) explant cartilage on day 0 (D0), (B) minced cartilage on day 0, (C) explant cartilage with glue on day 42 (D42), (D) minced cartilage with glue on day 42, (E) explant cartilage without glue on day 42, and (F) minced cartilage without glue on day 42. Blue arrows indicate areas of new growth, yellow areas indicate disorganized matrix, and red arrows indicate areas of fibrin glue. Scale bars: 100 μm for 4× and 10× images and 50 μm for 20× images.

no mincing at 7 and 28 days. However, the authors noted mechanical mincing to be faster and to result in significantly smaller homogeneous cartilage particles. Similarly, Bonasia et al[9] reported on minced cartilage fragments harvested from the femoral head of patients undergoing hip replacement, categorized into 1 of 4 groups based on the harvested cartilage size: (1) cubes 8 mm in diameter, (2) cubes 2 mm in diameter, (3) cubes 1 mm in diameter,

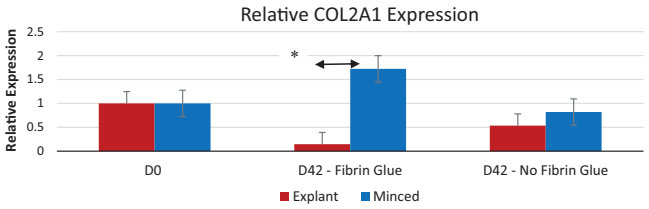

Figure 8. Polymerase chain reaction analysis for the relative expression of type II collagen (COL2A1). Significant difference between explant and minced cartilage with glue on day 42 (D42) (*$P < .05$). D0, day 0.

and (4) "cartilage paste" (<0.3 mm). At 6 weeks after harvest, the authors found that the most fragmented group, cartilage paste (<0.3 mm), demonstrated significantly enhanced extracellular matrix production based on the PG:DNA ratio compared with 8-mm ($P = .001$) and 1-mm ($P = .02$) cartilage cubes. The histological analysis score for the cartilage paste (<0.3 mm) group was significantly better compared with that for 8-mm ($P = .02$), 2-mm ($P = .04$), and 1-mm ($P = .03$) cartilage cubes. Future studies are warranted to better understand the ideal method of chondral mincing, including the use of arthroscopic instrumentation, to optimize the preservation of cell viability and chondral properties essential to defect healing while prioritizing ease of use, efficiency, and cost-effectiveness.

In this in vitro study, minced cartilage exhibited chondrocyte outgrowth and extracellular matrix deposition in which cell growth, confluence, and COL2A1 staining were observed after culture. These results are consistent with the study by Papalia et al[44] in which the authors reported that minced cartilage exhibited matrix diffusion with histological and immunohistochemical staining positive for COL2A1 and matrix components, comparable with hyaline-like cartilage.[36] Lind and Larsen[33] similarly reported on the capacity for chondral restoration when comparing outcomes after the treatment of femoral condyle defects (6 mm in diameter) with chondrocytes in a collagen membrane scaffold versus minced cartilage placed under a collagen membrane scaffold in a goat model. Using 8 adult goats, the authors reported no difference in O'Driscoll and Pineda histology scores, tissue filling, or repair tissue stiffness at 4 months. Meanwhile, Marmotti et al[35] evaluated chondrocyte outgrowth from minced cartilage fragments on a scaffold versus a scaffold alone for the treatment of osteochondral defects (7 mm in diameter; 3.5 mm in depth) in a goat model. At 2 months, a matrix positive for COL2A1 was observed in the scaffolds, while morphological and histological analyses showed the development of hyaline-like repair tissue, superior to that of defects treated with scaffolds alone. While in vitro and animal-based studies have demonstrated encouraging outcomes in regenerating chondral tissue with hyaline-like histological properties, further in vivo animal and mechanical investigations examining the viability and durability of mechanically minced cartilage are necessary before clinical application.

The addition of fibrin glue to in vitro culture media was not found to have a significant effect on cell viability or outgrowth. Nevertheless, previous investigations have reported the improved fixation of minced cartilage when utilizing fibrin glue. Levinson et al[31] analyzed chondrocyte viability and migration on a fibrin or collagen gel using cartilage samples separated into unminced, hand-minced, or device-minced groups, harvested from 12 patients undergoing knee arthroplasty. The authors reported that fibrin glue effectively acted as an encapsulating biomaterial, promoting cell migration with comparable levels of cellular outgrowth and viability with the more technically challenging collagen membrane. Meanwhile, when using a Göttingen minipig model, Christensen et al[17] demonstrated that full-thickness chondral defects (6 mm in diameter and depth) in the medial and lateral trochlear facets treated with autologous cartilage chips (minced using a scalpel) embedded in fibrin glue led to higher International Cartilage Regeneration & Joint Preservation Society scores for morphological tissue characteristics compared with marrow stimulation alone at 6 months postoperatively. Further investigations evaluating fixation techniques for a single-step autologous cartilage repair procedure with biomaterials such as fibrin glue and collagen membranes are necessary to better understand their effectiveness in providing structural integrity for minced cartilage fragments in the clinical setting.

This study is not without limitations. The cartilage utilized in this investigation was excess tissue collected at the time of transplantation from a fresh OCA, which is ideally harvested from healthy donors within 24 hours of death.[24] As such, the mean age of donors used for cartilage sourcing in this investigation was 22.5 ± 5.7 years, with a range of 14 to 28 years, which may limit the generalizability of the study findings to cartilage harvested from older populations. As the raw amount of surgically removed cartilage varied drastically among the donor population because of uncontrollable influences (surface area of the condyle, availability of postsurgical cartilage, differences in processing by multiple personnel, etc), this may potentially confound our data, as no threshold volume of cartilage harvest necessary for appropriate analyses was identified. In addition, because of the inherent adherent characteristics of mechanically minced cartilage, the exact wet weight, number of particles, and respective particle sizes could not be measured. Additionally, the mechanically minced cartilage fragments were not easily grasped using forceps, and their placement into a weighing vessel likely would have led to a resultant loss of tissue secondary to mechanical loading. As such, an estimate of the volume of shaved cartilage was made. The assessment of chondrocyte viability, which was calculated as the percentage of live cells with respect to the total number of cells (live plus dead cells), was limited, as we were unable to examine the depth of cell death or the death of cartilage by zones because of the limitations related to microscopy and the software utilized. Also, the effect, if any, that heat generated from the arthroscopic shaver could have had on chondrocyte viability was not accounted for, potentially confounding the direct effect of mincing articular cartilage on cellular viability. Furthermore, after cartilage was minced using an arthroscopic shaver, the layers and zones of cartilage

were not visible, prohibiting a quantitative histological assessment utilizing the Mankin scoring system. Moreover, we did not perform type I collagen staining, which would assist with understanding the appearance of elongated migrated cells in live/dead staining assay, along with the detection of fibrocartilaginous tissue. Finally, an in vivo component was not included. Additionally, our review of the literature identified limited cases reporting clinical outcomes after the use of minced chondrocyte implantation for the repair of articular cartilage.[16,17,35]

CONCLUSION

Mechanically minced articular cartilage remained viable after 42 days of culture in vitro and was comparable with cartilage explants with regard to cellular migration, outgrowth, and extracellular matrix synthesis. Further translational work is warranted to determine the viability of minced cartilage as a single-stage therapeutic intervention for surgical implantation in vivo.

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