In Vitro Effects of Bupivacaine on the Viability and Mechanics of Native and Engineered Cartilage Grafts

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Background: Although the toxic effects of bupivacaine on chondrocyte monolayer culture have been well described, its cellular and mechanical effects on native and engineered articular cartilage remain unclear. For the repair of articular cartilage defects, fresh autologous and allogenic cartilage grafts are commonly used, and engineered cell-based therapies are emerging. The outcome of grafting therapies aimed at repairing damaged cartilage relies largely on maintaining proper viability and mechanical suitability of the donor tissues.

Purpose: To investigate the in vitro effects of single bupivacaine exposure on the viability and mechanics of 2 cartilage graft types: native articular cartilage and engineered neocartilage.

Study Design: Controlled laboratory study.

Methods: Articular cartilage explants were harvested from the bovine stifle femoral condyles, and neocartilage constructs were engineered from bovine stifle chondrocytes using the self-assembling process, a scaffold-free approach to engineer cartilage tissue. Both explants and neocartilage were exposed to chondrogenic medium containing a clinically applicable bolus of 0.5%, 0.25%, or 0% (control) bupivacaine for 1 hour, followed by fresh medium wash and exchange. Cell viability and matrix content (collagen and glycosaminoglycan) were assessed at $t = 24$ hours after treatment, and compressive mechanical properties were assessed with creep indentation testing at $t = 5$ to 6 days after treatment.

Results: Single bupivacaine exposure was chondrotoxic in both explants and neocartilage, with 0.5% bupivacaine causing a significant decrease in chondrocyte viability compared with the control condition ($55.0\% \pm 13.4\%$ vs $71.9\% \pm 13.5\%$; $P < .001$). Bupivacaine had no significant effect on matrix content for either tissue type. There was significant weakening of the mechanical properties in the neocartilage when treated with 0.5% bupivacaine compared with control, with decreased aggregate modulus (415.8 ± 155.1 vs 660.3 ± 145.8 kPa; $P = .003$), decreased shear modulus (143.2 ± 14.0 vs 266.5 ± 89.2 kPa; $P = .002$), and increased permeability (14.7 ± 8.1 vs $6.6 \pm 1.7 \times 10^{-15}$ m^4/Ns; $P = .009$). Bupivacaine exposure did not have a significant effect on the mechanical properties of native cartilage explants.

Conclusion: Single bupivacaine exposure resulted in significant chondrotoxicity in native explants and neocartilage and significant weakening of mechanical properties of neocartilage. The presence of abundant extracellular matrix does not appear to confer any additional resistance to the toxic effects of bupivacaine.

Clinical Relevance: Clinicians should be judicious regarding the use of intra-articular bupivacaine in the setting of articular cartilage repair.

Keywords: knee; articular cartilage; pain management; joint resurfacing

Local anesthetics are commonly used as intra-articular injections to reduce pain caused by acute injury, degenerative disease, or surgery.[13,39] Multiple studies have shown the deleterious effects of these anesthetics on chondrocyte viability, with chondrotoxic effects occurring in a time-

and dose-dependent manner.[9,10,23,24,35] Historically, continuous-infusion intra-articular pain pumps have been associated with frank chondrolysis.[19] Subsequent investigations have reported that significant chondrocyte death can still occur after a short exposure period, raising concern about the potential detrimental effects of single-injection local anesthetics.[10]

Despite concerning evidence demonstrating the chondrotoxicity of local anesthetics, there remains widespread clinical use of these agents for the relief of symptoms because of the lack of obvious detrimental effects in the

in vivo setting.[22,37] In both normal and anterior cruciate ligament–transected knees of rats, weekly injections of 0.5% bupivacaine for 5 consecutive weeks did not induce any changes in chondrocyte viability compared with saline injection.[22] However, unlike humans, rodents can exhibit spontaneous articular cartilage repair because of lifelong open growth plates and thinner cartilage.[27,41] Another reason for the skepticism regarding the chondrotoxicity of anesthetics is that the majority of studies examining the toxic effects have been performed on monolayer culture, which does not adequately simulate in vivo conditions of chondrocytes embedded within the hyaline extracellular matrix (ECM). Some studies have suggested that an intact ECM may confer a protective effect against the toxicity of these agents.[9,22,38] Even if chondrocyte viability diminishes, changes to the ECM may not occur, and therefore the mechanical functionality of articular cartilage may remain intact. To date, the effects of local anesthetics on the ECM and mechanical characteristics of articular cartilage have not been well studied.

The purpose of this study was to evaluate the in vitro effects of bupivacaine, one of the most commonly used anesthetics, on 2 types of mature, ECM-dense cartilage grafts: native articular cartilage explants and engineered neocartilage. Native articular cartilage explants are representative of fresh autografts and allografts, which are frequently used clinically to treat articular cartilage damage in patients. Bovine articular cartilage has historically been used to study the effects of medication exposure in vitro.[10,23,28] It has also been used as a model to study osteoarthritis pathogenesis.[30,32,34] There are extensive data on the histology and mechanical properties of the tissue.[30,34] Moreover, the large joint size allows for sufficient explant material to perform statistically powered in vitro studies. Cell-based engineered cartilaginous tissues are emerging as therapeutic alternatives. The scaffold-free, self-assembling process produces robust neocartilage with abundant ECM.[21] This biomimetic neocartilage has histologic, biochemical, and mechanical properties (tensile and compressive stiffnesses of 6.12 MPa and 326 kPa, respectively)[15] similar to those of native articular cartilage (10.1 MPa and 472 kPa, respectively)[3,7] and is a promising novel strategy being investigated for cartilage repair.[21,31,40] To examine the effects of bupivacaine, native articular cartilage and engineered neocartilage underwent single exposure to bupivacaine, followed by quantification of chondrocyte viability, biochemical content, and mechanical properties. It was hypothesized that single bupivacaine exposure would cause chondrotoxicity and decreased mechanical properties in both native explants and neocartilage.

METHODS

Explant Harvest

Full-thickness cartilage punches, 3 mm in diameter, were harvested from the femoral condyles of 3 juvenile bovine stifle joints (Research 87) less than 36 hours after slaughter using an aseptic technique. All cadaveric specimens were grossly normal without any abnormalities of the articular cartilage. Punches were rinsed in Dulbecco's modified Eagle medium (DMEM; Gibco) with high glucose/GlutaMAX and with 1% (vol/vol) penicillin/streptomycin/fungizone (PSF; BD Biosciences). Punches were trimmed to 1- to 2-mm thickness, preserving the articular surface, superficial zone, and part of the middle zone. This was done to have the native cartilage be comparable with the engineered neocartilage in terms of thickness, zones, and surface area exposed to the treatments. Explants were maintained in chondrogenic medium (DMEM with high glucose/GlutaMAX containing 1% PSF, 1% [vol/vol] Insulin-Transferrin-Selenium-Plus (ITS+) premix, 1% [vol/vol] nonessential amino acids, 100 nM of dexamethasone, 40 µg/mL of L-proline, 50 µg/mL of ascorbate-2-phosphate, and 100 µg/mL of sodium pyruvate; all from Sigma) and 3% (vol/vol) fetal bovine serum (Atlanta Biologicals) until bupivacaine exposure later that same day.

Neocartilage Engineering

Articular cartilage was harvested from the femoral condyles and trochlea of juvenile bovine stifle joints (n = 3; Research 87) within 36 hours of slaughter. The cartilage was minced into 1- to 2-mm^3 pieces and digested in 0.2% (wt/vol) type 2 collagenase (Worthington) in DMEM with 1% PSF and 3% fetal bovine serum for 18 hours using an orbital shaker at 37°C. After digestion, chondrocytes were filtered through 70-µm cell strainers, resuspended in DMEM, and frozen in cryovials in liquid nitrogen until use. After thawing, primary (P0) chondrocytes were resuspended in chondrogenic medium, and neocartilage constructs were formed using the self-assembling process as previously described.[21] Briefly, nonadherent self-assembly wells, 3 mm in diameter, were made from 2% (wt/vol) agarose and saturated with chondrogenic medium for 2 days before construct seeding. Neocartilage constructs were seeded at a density of 1.62 × 10^6 P0 cells per construct in 50 µL of chondrogenic medium into each 3 mm–diameter agarose well. Constructs were maintained at 37°C and 10% CO_2. On day 7, constructs were unconfined from the self-assembly wells and transferred to a 24-well plate. The medium was changed daily until

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construct unconfinement, after which the medium was exchanged every other day for the remaining duration of the 28-day culture. Constructs were exposed to bupivacaine on day 28 of culture.

Bupivacaine Exposure

Both native explants and neocartilage constructs were exposed to chondrogenic medium supplemented with 0.5% (wt/vol) bupivacaine, 0.25% (wt/vol) bupivacaine, or 0% bupivacaine (control) (n = 6 explants and n = 6 neocartilage constructs per group based on power analysis) for 1 hour at 37°C at 50 rpm using an orbital shaker. Bupivacaine doses were calculated based on a standard clinical 10-mL bolus injection of medication into an adult human knee and accounting for a dilution from 6.7 mL of synovial joint fluid.[20] For example, a 10-mL bolus of 0.5% bupivacaine injected into a knee joint with 6.7 mL of synovial fluid yields a bupivacaine concentration of 3 mg/mL within the joint. Using this adjusted concentration, each group of native explants or constructs was placed in a well of a 12-well plate and incubated with 1.2 mL of appropriate bupivacaine-chondrogenic medium dilution to allow for complete submersion of the tissues. After bupivacaine exposure, explants and constructs were washed 3 times with chondrogenic medium. Explants and constructs were then maintained in individual wells in a 24-well plate in chondrogenic medium at 37°C and 10% CO_2 until testing.

Viability Assessment

Twenty-four hours after bupivacaine exposure, 1 mm–thick, vertical cross sections were taken and incubated in 80 μL of chondrogenic medium plus 80 μL of LIVE/DEAD reagent (calcein acetoxymethyl, ethidium homodimer-1; Thermo Fisher) for 30 minutes. Sections were viewed via fluorescence microscopy using the Texas red and green fluorescent protein filters at 4× and 10× magnification. Images were analyzed using ImageJ (National Institutes of Health). Three regions of interest measuring 150 μm × 150 μm were taken from random, nonoverlapping areas at least 100 μm below the surface. A macro was created using the auto local threshold, watershed, and analyze particles functions to count live and dead cells to calculate viability. An average was taken from these 3 regions of interest to yield 1 measurement of viability per explant or construct.

Histological Evaluation

Twenty-four hours after exposure, samples from explants and constructs were fixed in 10% neutral buffered formalin and embedded in paraffin. Cross sections, 4 μm thick, were stained using hematoxylin and eosin to assess cellular morphology, picrosirius red to assess total collagen distribution, and safranin O to assess glycosaminoglycan (GAG) distribution.

Quantitative Biochemistry

Twenty-four hours after exposure, samples from explants and constructs were weighed to measure their wet weights.

Samples were then lyophilized and weighed again to measure their dry weights. The water content of the tissues was determined from sample weights before and after lyophilization. Lyophilized tissue samples were digested in 125 μg/mL of papain in phosphate buffer at 60°C for 18 hours. Sulfated GAG content was measured using the Blyscan dimethyl methylene blue assay kit (Biocolor Ltd). Collagen content was quantified by a modified colorimetric chloramine-T hydroxyproline assay using a Sircol collagen assay standard (Biocolor Ltd).[11] DNA content was measured using a PicoGreen cell proliferation assay (Quant-iT PicoGreen dsDNA assay kit; Thermo Fisher). Collagen and GAG content were normalized to wet weight, dry weight, and DNA.

Creep Indentation Testing

A compression indentation apparatus[1] was used to assess the creep of explants and neocartilage on day 5 or 6 after exposure. Sample thickness was determined before testing using ImageJ software. As previously described,[21] a 0.55 mm–diameter, flat-ended, porous indenter tip was applied to the samples under a 2.5- to 3.5-g or 0.5- to 4.5-g load, and specimens were allowed to creep until reaching equilibrium, resulting in 5% to 18% or 8% to 15% strain for explants and constructs, respectively. A semianalytical, seminumerical, linear biphasic model and finite element analysis were used to obtain the aggregate modulus (H_A), shear modulus, and permeability (k) from the experimental data.[2]

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8. Sample size (n = 6 per group) was determined based on previous study data[10] using viability as the primary outcome with alpha set at .05 and a minimum power of 80%. For the mechanical data, all samples that experienced strains below 8% were excluded (n = 3) to ensure conformity with the assumptions of the analytical model used. Statistical outliers were identified via the ROUT method in GraphPad Prism 8 in all data sets and removed before statistical analyses were performed. A 2-way analysis of variance with Tukey post hoc tests was used to determine differences caused by bupivacaine dose within tissue type for all quantitative data. All data are presented as means ± SDs.

RESULTS

Chondrocyte Viability

Overall, neocartilage had decreased chondrocyte viability compared with native explants (Figure 1). Histologically, chondrocyte death was localized at the tissue periphery in both groups (Figure 1A). Specimens exposed to bupivacaine exhibited reduced viability compared with controls, irrespective of tissue type. Among all specimens (both explants and neocartilage), lower chondrocyte viability was observed in the 0.5% bupivacaine-treated specimens (55.0% ± 13.4%; P < .001) and 0.25% bupivacaine-treated
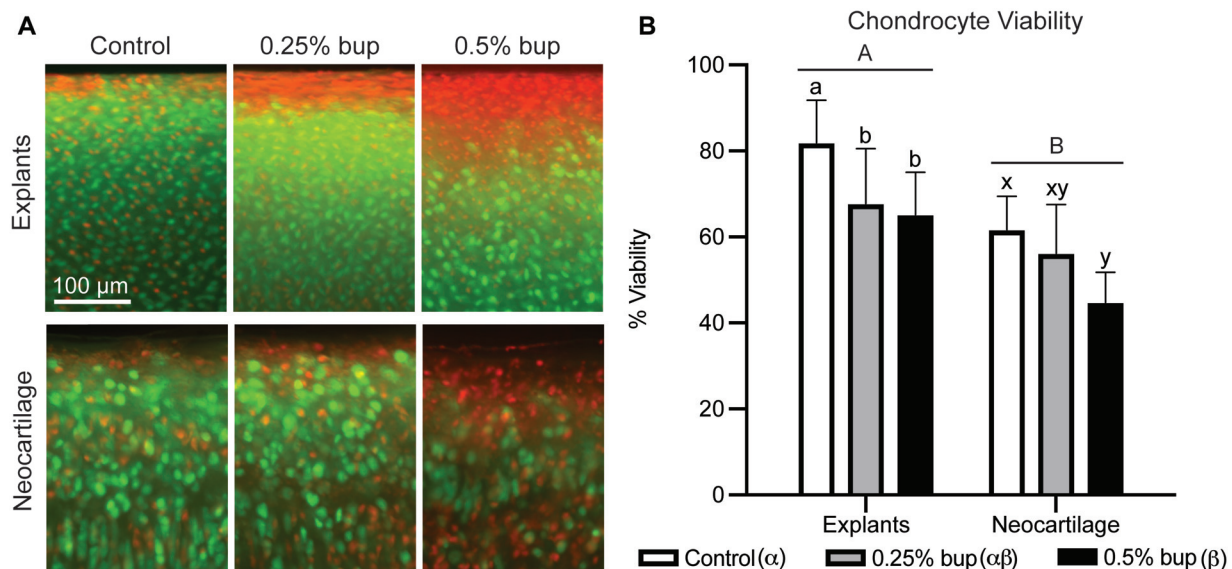
Figure 1. Chondrocyte viability in native explants and neocartilage constructs after bupivacaine (bup) exposure. (A) Top surface of cartilage tissue (10 ×). (B) A 2-way analysis of variance with Tukey post hoc tests was used to determine significant factors (tissue type and treatment), as well as differences caused by bupivacaine dose within tissue type. Tissue type and bupivacaine exposure were significant sources of variation in chondrocyte viability. Statistical significance ($P < .05$) is indicated by groups marked with different letters (eg, among tissue type: A, B; or within tissue types among bupivacaine-treated specimens: a, b or x, y).

specimens (62.0% ± 13.0%; $P = .054$) compared with controls (71.9% ± 13.5%). Differences in mean chondrocyte viability between 0.5% and 0.25% bupivacaine-treated specimens were not significant. Both bupivacaine dose ($P = .001$) and tissue type ($P < .001$) were significant factors affecting chondrocyte viability, with no significant interaction between factors (Figure 1B). Among the native explant groups, chondrocyte viability was significantly decreased in 0.5% bupivacaine-treated specimens (65.2% ± 9.9%; $P = .018$) and 0.25% bupivacaine-treated specimens (67.8% ± 12.8%; $P = .049$) compared with in the controls (82.0% ± 9.8%). Among the neocartilage groups, chondrocyte viability was significantly decreased in 0.5% bupivacaine-treated specimens compared with in the controls (44.8% ± 7.0% vs 61.7% ± 7.7%; $P = .016$).

Histology

Cell morphology in both the native explants and neocartilage constructs did not appear to be affected by bupivacaine treatment (Figure 2). This was evident by the presence of many cells within lacunae, with elongated cells in the superficial zone and a columnar arrangement of cells in the deep zone. Collagen distribution within the native explants and neocartilage constructs also appeared unaffected by bupivacaine exposure (Figure 2). Among the native explants, collagen staining was more intense in the superficial and deep zones of all treatment groups. Among neocartilage constructs, collagen was homogeneously distributed throughout the tissue except for intense staining localized at the periphery in all treatment groups. In contrast, GAG distribution did appear to be affected by

bupivacaine exposure (Figure 2). Among the native explants, GAG staining was less intense in the superficial and middle zones in the groups that received 0.25% and 0.5% bupivacaine treatment. Among the neocartilage constructs, GAG staining was less intense throughout the tissue treated with 0.25% and 0.5% bupivacaine.

Quantitative Biochemistry

Tissue type, but not bupivacaine dose, was a significant factor affecting tissue biochemical properties. Collagen/wet weight, collagen/dry weight, collagen/DNA, and GAG/DNA were greater in the explant groups compared with the neocartilage groups ($P < .001$), while hydration, GAG/wet weight, and GAG/dry weight were greater in the neocartilage groups compared with the explant groups ($P < .001$) (Table 1). Bupivacaine treatment did not have a significant effect on the biochemical content of both explants and neocartilage (Figure 3).

Compressive Mechanical Properties

Both bupivacaine dose ($P = .021$) and tissue type ($P < .001$) were significant factors affecting aggregate modulus, with no significant interaction between factors (Figure 4A). Among the self-assembled constructs, the mean aggregate modulus for 0.5% bupivacaine-treated specimens (415.8 ± 155.1 kPa) was significantly decreased compared with 0.25% bupivacaine-treated specimens (618.5 ± 103.1 kPa; $P = .012$) and controls (660.3 ± 145.8 kPa; $P = .003$). The bupivacaine dose did not have a significant effect on aggregate modulus among native explants.
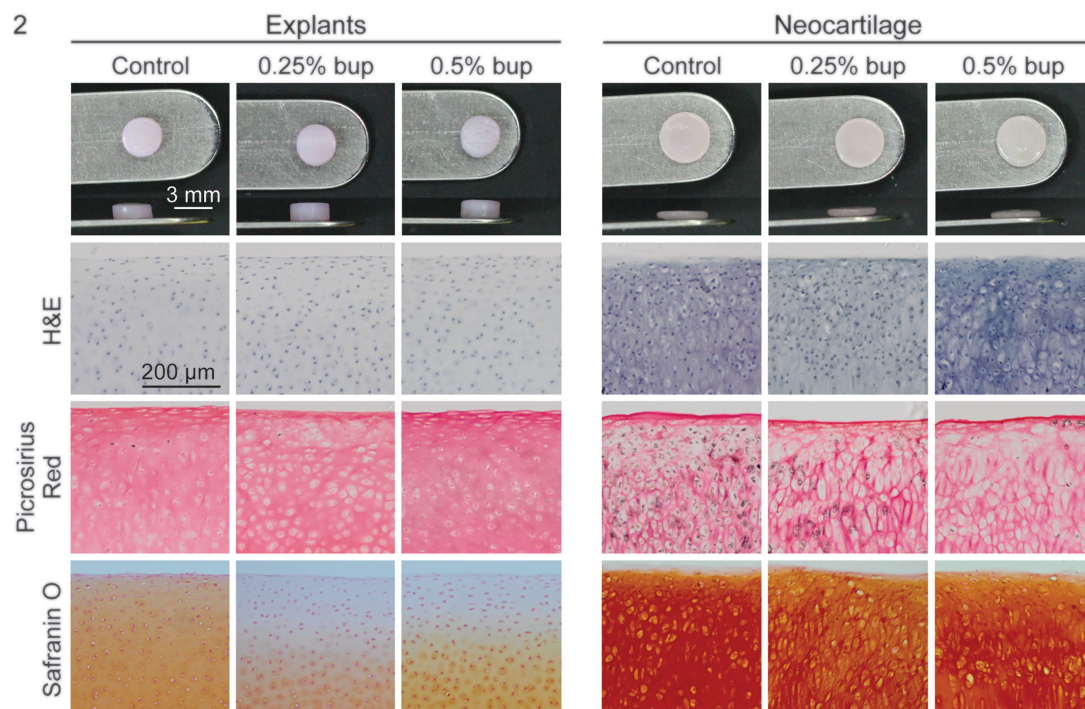
Figure 2. Gross and microscopic histology of native explants and neocartilage constructs after exposure to bupivacaine (bup). H&E, hematoxylin and eosin.

TABLE 1
Biochemical Composition of Extracellular Matrix[a]

Bupivacaine Treatment	Hydration, %	Col/ww, %	Col/dw, %	Col/DNA, µg/µg	GAG/ww, %	GAG/dw, %	GAG/DNA, µg/µg
Explants							
Control	78.3 ± 2.7	13.8 ± 3.5	62.8 ± 9.1	343.9 ± 102.6	5.0 ± 0.6	23.0 ± 3.1	125.0 ± 33.7
0.25%	80.2 ± 1.3	12.2 ± 2.5	61.2 ± 9.5	294.4 ± 81.0	4.9 ± 0.7	24.5 ± 3.5	117.2 ± 25.6
0.5%	80.6 ± 2.5	12.3 ± 2.4	63.3 ± 7.1	359.6 ± 110.9	4.3 ± 0.9	22.3 ± 3.1	128.6 ± 45.9
Neocartilage							
Control	82.2 ± 0.7	2.0 ± 0.2	11.4 ± 0.9	22.7 ± 1.2	7.5 ± 1.0	42.3 ± 5.2	84.5 ± 11.9
0.25%	81.9 ± 0.4	2.1 ± 0.2	11.7 ± 0.8	18.3 ± 2.0	8.3 ± 0.5	46.1 ± 2.2	72.4 ± 7.3
0.5%	82.6 ± 1.2	1.9 ± 0.4	10.7 ± 2.4	16.6 ± 4.8	8.0 ± 0.5	46.0 ± 2.3	71.1 ± 13.1

[a]Data are presented as mean ± SD. Biochemical composition was significantly different ($P < .001$) between explants and neocartilage for all column measures. Col, collagen; dw, dry weight; GAG, glycosaminoglycan; ww, wet weight.

Both bupivacaine dose ($P = .015$) and tissue type ($P = .011$) were significant factors affecting shear modulus, with no significant interaction between factors (Figure 4B). Among the self-assembled constructs, the mean shear modulus for 0.5% bupivacaine-treated specimens (143.2 ± 14.0 kPa) was significantly decreased compared with 0.25% bupivacaine-treated specimens (237.7 ± 48.6 kPa; $P = .018$) and controls (266.5 ± 89.2 kPa; $P = .002$). The bupivacaine dose did not have a significant effect on shear modulus among native explants.

Finally, both bupivacaine dose ($P = .021$) and tissue type ($P = .011$) were significant factors affecting permeability, with a significant interaction between factors ($P = .021$) (Figure 4C). Among the self-assembled constructs, the mean permeability for 0.5% bupivacaine-treated specimens

(14.7 ± 8.1 × 10^{-15} m⁴/Ns) was significantly increased compared with 0.25% bupivacaine-treated specimens (4.4 ± 1.8 × 10^{-15} m⁴/Ns; $P = .001$) and controls (6.6 ± 1.7 × 10^{-15} m⁴/Ns; $P = .009$). The bupivacaine dose did not have a significant effect on permeability among native explants.

DISCUSSION

In this study, a single exposure of native articular cartilage explants and neocartilage constructs to clinically relevant doses of bupivacaine resulted in significant chondrotoxicity and changes in tissue mechanics. The findings of this study suggest that the existence of abundant ECM within native cartilage and neocartilage tissue does not confer any
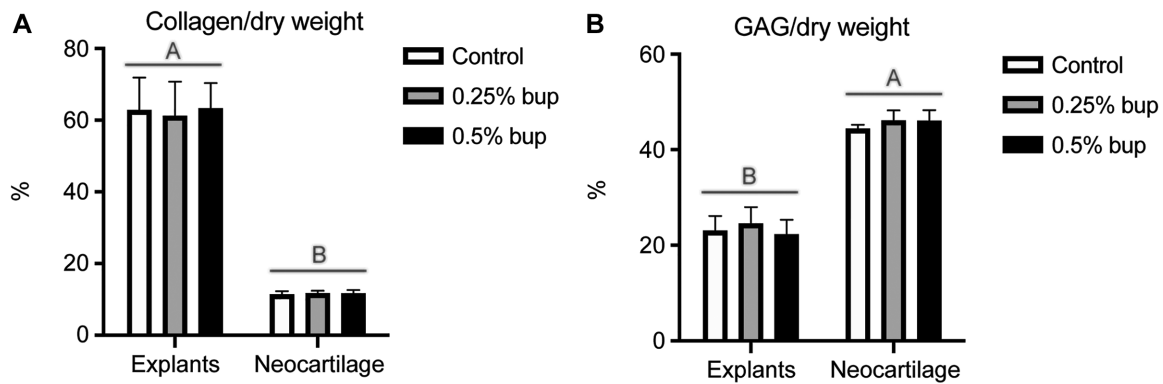
Figure 3. Biochemical composition of the extracellular matrix. Tissue type was a significant source of variation in (A) collagen per dry weight and (B) glycosaminoglycan (GAG) per dry weight. bup, bupivacaine. Statistical significance ($P < .05$) is indicated by groups marked with different letters.
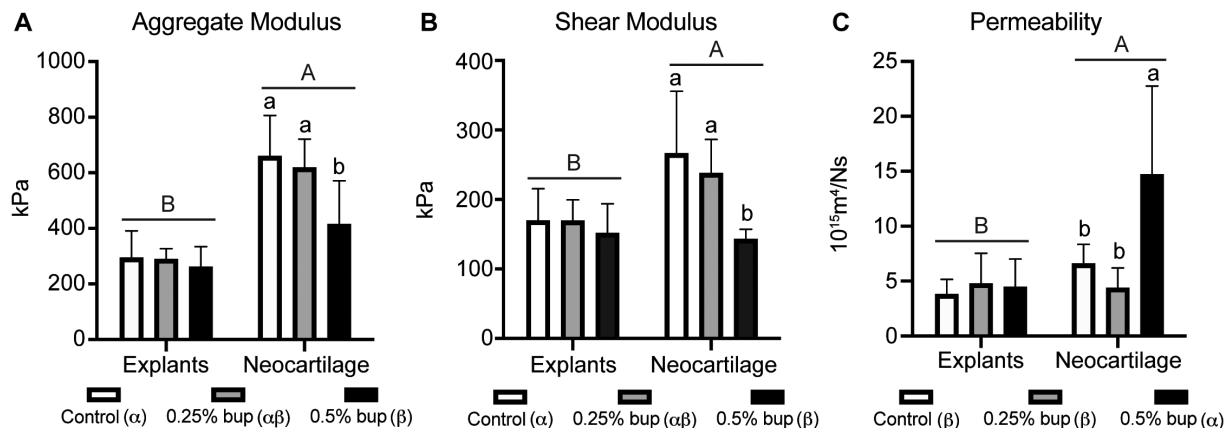

Figure 4. Compressive mechanical properties of native explants and neocartilage. Tissue type and bupivacaine (bup) exposure were significant sources of variation in the (A) aggregate modulus and (B) shear modulus. (C) For permeability, there was a significant interaction between tissue type and bupivacaine exposure. Both factors were also significant sources of variation. Statistical significance ($P < .05$) is indicated by groups marked with different letters (eg, among tissue type: A, B; or within tissue types among bupivacaine-treated specimens: a, b).

additional resistance to the chondrotoxic effects of bupivacaine. However, the effect of bupivacaine on tissue mechanics may be less deleterious for native cartilage tissue than for tissue-engineered neocartilage.

The chondrotoxic effect of bupivacaine is well known and has been the subject of study by a number of groups.[9,10,23,24,35,36] This effect is dose-dependent, with higher concentrations of bupivacaine exhibiting more chondrotoxicity.[4,5,9,12,18,36] Similar dose-dependent effects were observed in both cartilage tissue types in this study. Although the majority of previous studies have been performed using monolayer cell culture, recent studies examining the effects of bupivacaine on intact tissue explants have found similar chondrotoxicity rates.[12,16,33,36] Sherman et al[36] found that 1-hour exposures of canine cartilage explants to 0.25% and 0.125% bupivacaine had chondrotoxic effects with corresponding decreases in metabolic activity. In an in vivo rat study, Chu et al[8] demonstrated that a single intra-articular injection of 0.5% bupivacaine resulted in a reduction in chondrocyte density after 6 months. The

outcomes of the present study support the growing body of literature that demonstrates that bupivacaine is chondrotoxic to cartilage, despite the presence of intact ECM.

The primary function of articular cartilage is to provide low-friction articulation and absorption of joint loads. Thus, it is critical that cartilage mechanics not be affected by the administration of anesthetics. Although the cellular toxicity of local anesthetics has been well established, whether the mechanical properties of the articular cartilage are similarly affected is unknown and not well studied to date. This study found that the aggregate modulus and shear modulus of neocartilage constructs were significantly diminished 5 to 6 days after a single bupivacaine exposure, indicating a loss of load-bearing and energy dissipation capabilities of the tissue. While the biochemical composition of both native explants and engineered neocartilage constructs remained unchanged with bupivacaine treatment, ECM components not measured in this study, such as pyridinoline collagen crosslinks, may have been diminished, resulting in the observed changes to the neocartilage compressive properties.

It was also shown that 0.5% bupivacaine exposure resulted in an increase of neocartilage permeability compared with 0.25% bupivacaine or the control condition. Furthermore, this study found that neocartilage was more susceptible to an increase in permeability from high-dose bupivacaine exposure compared with native explants. This increased permeability may contribute to the decrease in compressive mechanical properties observed among the neocartilage constructs, but not among native explants, as a result of fluid exudation. Importantly, human cartilage is more permeable than is bovine cartilage and therefore may be susceptible to more bupivacaine-induced changes than those demonstrated by this study, warranting further investigation.[3]

Although this study did not find any significant changes in the compressive mechanical properties of native explants after bupivacaine exposure, a single measurement at 5 to 6 days after exposure may not have allowed sufficient time for alterations in the matrix and tissue mechanics to develop. It is postulated that substantial chondrocyte death would initiate a cascade of decreased matrix production and, consequently, loss of mechanical functionality over time. Therefore, longitudinal assessment of cartilage mechanics over a longer period of time after a single bupivacaine exposure is warranted.

The findings of this study demonstrate the unintended, deleterious effects of bupivacaine, which is commonly administered intra-articularly during surgery to reduce postoperative pain in the setting of articular cartilage repair. Cell-based approaches to cartilage repair, such as matrix-induced autologous chondrocyte implantation (MACI), are becoming commonly used, with many additional products in clinical development.[25] The neocartilage constructs used in this study are fabricated using the cell-based, scaffold-free, self-assembling process that generates scaffold-free neocartilage with abundant ECM and compressive, tensile, and low-friction properties similar to those of native articular cartilage.[14,15,21,26,29,40] This neocartilage has been investigated for cartilage repair in the knee and other diarthrodial joints. For example, implantation of engineered neocartilage into the temporomandibular joint disk demonstrated outstanding healing and prevention of osteoarthritis compared with empty defect controls in preclinical animal models.[40]

This study showed that neocartilage was more susceptible to bupivacaine-induced reduction in mechanical properties. Compared with self-assembled neocartilage tested in this study, MACI and other cell-based implants contain less mature matrix and may be more permeable.[17] Therefore, these implants may be even more susceptible to the chondrotoxic effects of bupivacaine and the corresponding deterioration of mechanical integrity. Additionally, most cartilage repair implants also lack a robust lamina splendens, which serves as a protective barrier to intra-articular elements.[9] Cartilage changes associated with osteoarthritis, such as fibrillation and increased hydration, may similarly make the tissue more susceptible to bupivacaine toxicity. Greater anesthetic-induced in vitro chondrotoxicity has been noted in osteoarthritic cartilage compared with intact cartilage,[5,24] and further weakening in the biomechanical

properties of osteoarthritic cartilage may occur from bupivacaine exposure.

While this study presents compelling data demonstrating the negative effects of bupivacaine on chondrocyte viability and cartilage mechanics, there are several notable limitations. First, biochemical measurement was performed once at 24 hours after bupivacaine exposure (concurrently with viability measurement) and was not performed at the same time as creep indentation testing, precluding association of biochemical and mechanical results. Second, this in vitro study may not have accurately represented in vivo conditions regarding bupivacaine exposure time, dosage, and clearance from the joint. The half-life of bupivacaine is longer than was the exposure time used in this study. Additionally, the steady concentration of medication used in this study does not reflect the pharmacokinetics of the medication in the body.[6,12] During surgery, blood and residual arthroscopic fluid may further dilute the anesthetic bolus. Third, chondrocytes used to generate neocartilage were isolated from the femoral condyles and the trochlea, whereas the explants were only harvested from the femoral condyles. It has been shown that neocartilage generated from cells derived from different topographical areas within the bovine patellofemoral joint has different functional properties.[32] Fourth, native explants in this study only consisted of the superficial and middle zones and did not contain the deep zone, and bupivacaine sensitivity may differ among each zone of cartilage.[38] Moreover, trimming the native explants to exclude the deep zone, calcified cartilage, and subchondral bone layer created a nonphysiological interface and increased surface area that may have allowed for increased penetration of bupivacaine upon exposure. Future studies should be done to (1) examine if bupivacaine exposure affects native explant and neocartilage biochemical or mechanical properties after longer-term culture and (2) determine if articular cartilage protected by a lamina splendens within an intact joint is affected by bupivacaine exposure in a similar manner to that observed in the present study. Despite these limitations, this study reports the effects of bupivacaine on both native and engineered cartilage tissues and encourages further, in-depth analyses of alterations in cartilage matrix composition and mechanical properties resulting from local anesthetic exposure.

CONCLUSION

A single bupivacaine exposure resulted in dose-dependent chondrotoxicity to native explants and neocartilage, both ECM-dense tissues. Additionally, a single bupivacaine exposure significantly increased the permeability and weakened the compressive mechanical properties of neocartilage. The existence of abundant ECM did not appear to confer any additional resistance to the chondrotoxic effects of bupivacaine. Furthermore, tissue permeability appeared to influence the susceptibility of the tissue to dose-dependent decreases in mechanical properties. This study demonstrated unexpected, deleterious effects of bupivacaine and highlights additional investigations that should be undertaken to provide clinicians with the

knowledge to judiciously use intra-articular bupivacaine in the setting of articular cartilage repair.

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