

Diminished Cartilage Creep Properties and Increased Trabecular Bone Density following a Single, Sub-Fracture Impact of the Rabbit Femoral Condyle

Joseph Borrelli Jr,¹ Melissa A. Zaegel,² Mario D. Martinez,² Matthew J. Silva²

¹UT Southwestern Medical Center, 1801 Inwood Rd, WA4.312, Dallas, Texas 75390-8883, ²Washington University School of Medicine, St. Louis, Missouri

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ABSTRACT: Traumatic injury to articular cartilage can lead to post-traumatic arthritis. We used a custom pendulum device to deliver a single, near-fracture impact to the medial femoral condyles of rabbits. Impact was localized to a region ~3 mm in diameter, and impact stress averaged ~100 MPa. Animals were euthanized at 0, 1, and 6 months after impact. Cartilage mechanical properties from impacted and sham knees were evaluated by creep-indentation testing, and periarticular trabecular bone was evaluated by microCT and histomorphometry. Impact caused immediate and statistically significant loss of cartilage thickness (-40% vs. sham) and led to a greater than twofold increase in creep strain. From 0 to 6 months after impact, the ability of cartilage to recover from creep deformation became significantly impaired (percent recovery different from control at 1 and 6 months). At 1 month, there was a 33% increase in the trabecular bone volume fraction of the epiphysis beneath the site of impact compared to control, and increased bone formation was observed histologically. Taken together, these findings demonstrate that a single, high-energy impact below the fracture threshold leads to acute deleterious changes in the viscoelastic properties of articular cartilage that worsen with time, while at the same time stimulating increased bone formation beneath the impact site. © 2010 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 28:1307–1314, 2010

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Post-traumatic osteoarthritis (PTA) is often a long-term sequelae of joint injuries including ligamentous disruption and intra-articular fracture. Although the exact mechanism by which PTA develops after injury is not fully understood, there is mounting evidence that it results from a combination of metabolic changes within the articular chondrocytes, changes within the contents of the extracellular matrix, and changes in the biomechanical properties of the cartilage and underlying trabecular bone.^{1–13} These changes seem to be additive in that as the cells lose their ability to respond to injury and trabecular bone stiffens, it may predispose this already injured cartilage to greater stress and further deterioration.

To study these phenomena further we developed an *in vivo* articular cartilage injury model.^{1,3} This model allows the application of a controlled sub-fracture impact, at a clinically relevant loading rate, to the posterior aspect of the medial femoral condyle of rabbits. With this model we have shown that advanced degenerative changes in cartilage—including near complete loss of extracellular proteoglycan and decreased chondrocyte number and metabolic activity—can result from a single sub-fracture impact.² However, the effect of impact and recovery on the functional properties of cartilage, as assessed by mechanical testing, has not yet been reported in this model.

Changes to trabecular periarticular bone, a common feature of joint degeneration, have been observed in some models of cartilage impact. Increased thickness of the sub-chondral bone layer was reported 7–12 months post-impact in a rabbit model,^{4,13} and increased trabecular

bone density was observed 6 months after sub-chondral damage in a canine model.⁷ Unpublished observations of impacted samples from a recent study¹ suggested that trabecular bone density was increased in the region beneath the sub-chondral plate in the absence of sub-chondral bone fracture. This response might be related to the “bone bruise” phenomenon that is often observed by clinical magnetic resonance imaging (MRI) after knee trauma.^{9,11} Quantitative evaluation of the post-impact changes in trabecular bone is needed to more completely describe the joint response to sub-fracture trauma.

We used the rabbit knee impact model to assess the effects of impact on the mechanical properties of articular cartilage and the density of the sub-chondral bone. We hypothesized that a single high-energy impact leads to deleterious changes in the mechanical properties of the injured cartilage that worsen with time, and further that impact stimulates changes in the underlying bone.

MATERIALS AND METHODS

In Vivo Impact

The medial femoral condyles of the right knees of 67, three-month-old (3–3.5 kg) New Zealand White rabbits (Myrtle's Rabbitry, Thompson Station, TN) were subjected to a single impact using a slightly modified version of a protocol described previously.¹ The medial femoral condyle of the left knee served as a sham control and only underwent arthrotomy. Briefly, rabbits were anesthetized with an intra-muscular injection of 30 mg/kg ketamine and 6 mg/kg xylazine, and anesthesia was maintained with inhaled isoflurane (1–3%). The posterior aspect of each knee was shaved and prepped for sterile surgery. The right medial femoral condyle was exposed by arthrotomy and the leg was fixed to a polyethylene block using two 1.6 mm Kirschner wires. One trans-condylar K-wire was inserted in the femur and a second was inserted through the

Correspondence to: Joseph Borrelli, Jr (T: 214-645-3336; F: 214-645-3350; E-mail: joseph.borrelli@utsouthwestern.edu)

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proximal tibia. (Note that the femoral K-wire for this study was placed from medial to lateral through the femoral condyles in order to accommodate the higher impact forces used in this study compared to our earlier studies.)^{1,3}

The medial femoral condyle was impacted using a custom-built pendulum device. A 3-mm diameter stainless steel impactor with a biconcave surface was aligned with the posterior aspect of the condyle. Uniform contact of the impactor with the condyle was confirmed using pressure sensitive film (Pressurex[®] Super Low Pressure, Sensor Products, Inc., Madison, NJ). In a previous validation study,¹ we assessed the uniformity of pressure over the contact region and determined that pressure varied by an average of 9% between sub-regions within the contact area. Thus, the peak stresses are not likely to be that much greater than the average stress (=force/area). The impactor was brought into contact with the surface of the condyle, and the pendulum arm was released from a fixed height, striking the impactor a single time. Impact force data were recorded at 2,000 Hz (LabVIEW 8.0, National Instruments, Austin, TX). An arthrotomy was performed on the left (sham) knee and the impactor was contacted with the medial femoral articular surface with minimal force. The joints were irrigated and closed using 3-0 coated Vicryl suture internally and 3-0 nylon suture for the skin incision. Post-operatively rabbits were allowed food and water ad libitum and free cage activity. Animals were euthanized by overdose of pentobarbital (150 mg/kg i.v.) at time 0, 1, or 6 months after surgery. All procedures were approved by Washington University's Animal Studies Committee.

Our study design had two impact levels, "low" and "high." Prior to impact we attached one of two masses, 4,350 or 4,900 g, to the pendulum arm. Preliminary studies on cadaveric rabbits indicated that the two masses produced average impact forces equal to 70% and 90%, respectively, of the force to fracture the underlying bone. We chose these high values because our prior study showed little cartilage degradation after an impact at ~45% of the fracture threshold.³ Because these impact forces were close to the average fracture threshold, we observed a number of unwanted fractures. Gross visual inspection immediately after impact revealed an impaction type fracture in the condyles of 12 rabbits, indicating sub-chondral bone fracture, while the femurs from an additional 7 rabbits had shaft fractures. These 19 animals were euthanized immediately and excluded from the study. In addition, one rabbit died in preparation for surgery and one died post-operatively. Thus, we had successful sub-fracture impacts in 46 rabbits that survived to their designated time point. The peak forces recorded at impact indicated a 7% nonsignificant difference between the two impact groups (low: 745 ± 95 N; high: 802 ± 106 N; $p = 0.088$). These values were similar to a recent study using the same model,² as were values of time-to-peak force (low: 17 ± 1 ms; high: 18 ± 2 ms; $p = 0.038$). Impact area was estimated based on the pressure film exposed prior to impact (low: 6.7 mm²; high: 7.7 mm²; $p < 0.001$). The average impact stress (force/area) was 109 MPa and did not differ between groups (low: 112 ± 25 MPa; high: 104.1 ± 11 MPa; $p = 0.21$).

Creep-Indentation Testing

Mechanical creep-indentation tests were performed on 39 pairs of right (impact) and left (sham) medial femoral condyles using a custom-built testing apparatus. At the time of death, bilateral hind limbs were amputated above the knee and stored at -20°C until testing. Prior to testing, each hind limb was thawed overnight at 4°C. The femur was dissected and the

medial condyle isolated using a diamond-wafering saw (Buehler Lake Bluff, IL). The cartilage was kept moist by constant irrigation using saline plus protease inhibitors (150 mM sodium chloride, 2.3 mM ethylenediaminetetraacetic acid, 5 mM benzamidine HCl, 10 mM *N*-ethylmaleimide, and 1 mM phenylmethylsulfonylfluoride; Sigma, St. Louis, MO).¹⁰ The bone surface of each condyle was glued to the bottom of a Petri dish and oriented so that the articular surface at the site of impact was perpendicular to vertical. The sample was submerged in saline plus protease inhibitors and allowed to equilibrate for 10 min. Testing was done at room temperature.

The creep-indentation apparatus and protocol for in situ testing were based on previous reports.^{10,14} The apparatus uses gravity to apply a constant force in the vertical direction to the articular cartilage surface via an indenter. A pulley-counterweight design is used to balance the weight of the indenter pin. Low-friction motion of the indenter assembly was accomplished using a series of air bearings (New Way Precision, Aston, PA). A 0.5-mm diameter porous, stainless steel filter (0.05 mm pore size, 50% volume fraction; Mott Corporation, Farmington, CT) was affixed to the end of the indenter shaft. Displacement was monitored using a linear variable differential transducer (100 MHR LVDT, range ±2.54 mm; Schaevitz, Hampton, VA) and recorded using LabVIEW. The indenter tip was brought into incipient contact with the cartilage surface in the center of the impacted region. A 2-g tare mass was applied, followed by a 10-min equilibration period. A 5-g test mass was then added for the creep-indentation test. The test mass was half that used in a previous study on rabbit knee cartilage¹⁴ and was chosen to produce ~20% creep strain on normal samples. Displacement data were recorded at 30 Hz during the initial 2 min and at 0.5 Hz for the remainder of the test (48 min). A 50-min creep period was chosen based on a previous study.¹⁴ The test mass was then removed, and the specimen was allowed to recover for 30 min while displacement data were collected. Final displacement values were taken to be equilibrium values. We confirmed that displacement reached 99% of final value prior to the last 5 min of the creep tests and the last 3 min of recovery.

Cartilage Thickness Determination

Cartilage thickness was measured at the site of testing immediately after creep testing and recovery, as described.¹⁰ The porous-tipped indenter was removed from the system and replaced with a needle probe. Using a micrometer, the needle probe was driven manually through the cartilage. Force (Model 31, 1,000-g load cell; Sensotec, Columbus, OH) and displacement data were recorded at 60 Hz. The procedure was performed at five sites within the impacted area of each specimen. Each force-displacement curve had three distinct, quasi-linear regions: a slope nearly equal to zero (as the probe passes through air or saline); an intermediate slope (as the probe passes through cartilage); and a steep slope (as the probe contacts the sub-chondral bone). The cartilage thickness was defined as the displacement spanned by the second region. An average value was calculated from the five tests for each specimen. The average coefficient of variation for the five tests was 14.2% for sham and 23.2% for impact specimens.

Creep Analysis

Data from three specimens were excluded because of technical errors during testing. Thus, data from 36 pairs of specimens were used for statistical analysis. The resulting creep-indentation and recovery curves (displacement vs. time) were analyzed for total creep displacement, time to 70% creep, time

to 95% creep, recovery displacement, and percent recovery ($100 \times$ recovery displacement/creep displacement). If the percent recovery was $<100\%$, the "lost" recovery displacement was added to the thickness measurement (obtained after creep and recovery), giving a value assumed to be the cartilage thickness prior to indentation testing. This latter value was used to convert creep displacement to creep strain.

Between group comparisons (i.e., low vs. high impact; 0 month vs. 1 month vs. 6 months) were performed using analysis of variance (ANOVA; SAS v9.1.3, SAS Institute, Inc., Cary, NC). When the overall ANOVA model was significant, Tukey's *studentized range* (HSD) test was used to determine the pairwise comparisons of means that were significantly different. Due to nonnormal distributions, ANOVAs were performed using ranks of the raw data. Impact and sham values within an animal were compared using paired *t*-tests, except when required conditions were not met and Wilcoxon's test was used as a nonparametric alternative. ANOVA of impacted specimens indicated that for the majority of parameters, there were no differences between low (70%) and high (90%) impact level. Therefore, we pooled the two impact levels when comparing impact versus sham and when examining the effect of time. There were significant changes in several creep parameters from sham specimens with time (described below). To account for this, ANOVA of time effects was done on both absolute and normalized values (impacted/sham).

Trabecular Bone Analysis by MicroCT and Histomorphometry

Preliminary analysis by micro-computed tomography on several samples used for another study¹ suggested that there was a localized increase in trabecular bone density beneath the impact site. The increase was evident at 1 month with no further changes from 1 to 6 months (data not shown). Therefore, seven rabbits (high impact, 1 month) were assigned for detailed analysis of trabecular bone by microCT and dynamic histomorphometry. Rabbits were injected with calcein green (10 mg/kg i.p.) and alizarin complexone (30 mg/kg i.p.) on days 7 and 17 after impact, respectively, to label bone-forming surfaces. Postmortem, bilateral femora were fixed overnight in 4% paraformaldehyde and stored in 70% ethanol. The medial and lateral condyles of each knee were isolated using a diamond-wafering saw (Buehler), and a radiopaque marker was glued to the impacted (or sham) area of each condyle. The condyles were suspended in 1.5% agarose in a 20.5-mm diameter specimen tube and scanned using a desktop microCT system (standard resolution, 55 kV, 200 ms integration time, 145 mA; μ CT 40; Scanco Medical AG, Bassersdorf, Switzerland). For analysis, two cylindrical volumes of interest (VOIs, 1.75 mm diameter) were created within the trabecular bone of each medial condyle. A posterior VOI (150 slices thick) was positioned near the site of impact, with the edge of the VOI offset 0.8 mm from the sub-chondral plate. A distal VOI (100 slices thick) was positioned away from the site of impact, with the edge offset 0.8 mm from the distal surface of the medial condyle, 6 mm anterior to the impact site. Comparisons between the posterior and distal VOIs of the medial condyle were done from right (impact), and left (sham), knees to determine the effect of impact on trabecular bone. In addition, we analyzed a posterior VOI on the lateral condyle of both knees, which allowed us to determine the effect of the transcondylar K-wire (which was placed through the right femur only). Three-dimensional trabecular bone volume fraction (BV/TV) was computed using the manufacturer's software (threshold = 275 on a scale of 0–1,000).

The condyles were then dehydrated in ascending concentrations of ethanol and embedded in methylmethacrylate using a standard procedure for undecalcified bone. Sagittal sections were cut (using the Buehler diamond-wafering saw) in duplicate through the impact or sham regions, ground to 30–40 μ m thickness and mounted on glass slides. Slides were visualized by epifluorescence microscopy (DP-30; Olympus American Incorporated, Center Valley, PA) using a fluorescein isothiocyanate (FITC) filter for calcein and a tetramethylrhodamine isothiocyanate (TRITC) filter for alizarin. Images were captured and digitally overlaid. For analysis, a rectangular area of interest (AOIs, 1.75 mm length \times 2.50 mm width) was created within the trabecular bone of each condyle. A posterior AOI was positioned near the site of impact, with the edge of the AOI offset 0.8 mm from the sub-chondral bone for the medial condyles and 0.4 mm for the lateral condyles. A custom program (Matlab, Natick, MA) was used to quantify bone area and labeled area. Standard surface measures of bone formation were judged inappropriate due to the presence of labeled trabecular plates in the plane of the section. Due to technical difficulties with embedding and grinding, only data from three pairs of knees were suitable for analysis.

RESULTS

Displacement versus time curves from creep-indentation tests of both sham and impacted specimens exhibited the expected behavior for a viscoelastic material (Fig. 1).

Analysis of the creep and recovery curves from sham (control) specimens revealed time-dependent changes from 3 to 9 months age (0–6 months follow-up). Total creep strain in sham specimens was $\sim 40\%$ less at 6 months follow-up than at 0 or 1 months ($p < 0.05$; Fig. 2). In addition, time to 70% creep and 70% recovery was significantly less at 6 months than at 0 and 1 months ($p < 0.05$), indicating a faster rate of initial deformation. Percent recovery was approximately 90% and did not change with time ($p = 0.67$). Cartilage thickness did not change with time ($p = 0.98$). Thus, with normal skeletal

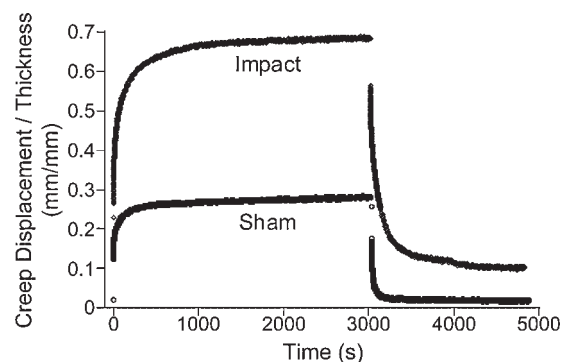


Figure 1. Displacement (normalized by cartilage thickness) history for creep indentation and recovery of a representative pair of sham and impacted (low, 1 month) specimens. The total creep strain was 68% for the impacted side versus 28% for the uninjured sham side. In addition, the time to reach 70% of total creep and 70% of recovery was greater for the impacted side. Total recovery of the impacted side was less than the sham side, even when expressed as a percent of total creep (85% vs. 94%). (For the curves shown, the creep displacement for the sham and impact specimens reached 99% of their final value 7.8 and 18.3 min prior to the end of testing, respectively. The recovery displacement values for the sham and impact specimens reached 99% of their final value 17.9 and 3.9 min prior to the end of testing.)

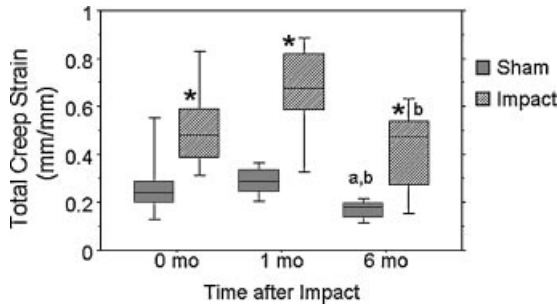


Figure 2. Total (equilibrium) strain from creep-indentation testing of rabbit femoral condyles from sham, low and high impact groups. In the sham group, creep strain was less at 6 months than 0 and 1 month, indicating changes in stiffness of articular cartilage with normal development. Low and high impact groups did not differ from each other, thus pooled impact data are shown. Impact resulted in significantly increased creep strain at all time points compared to sham ($p < 0.05$). (Box plot: the height of each box depicts the 25th–75th percentile values, while the error bars depict the 10th and 90th percentile values; the median value is depicted by the horizontal line within the box.) $p < 0.05$; *Impact different from sham; ^adifferent from 0 month; ^bdifferent from 1 month.

development from 3 to 9 months age articular cartilage had increased equilibrium stiffness but this change did not alter its ability to recover from creep deformation.

Impacted specimens also exhibited characteristic creep behavior but had markedly greater creep strain and less percent recovery than sham controls (Fig. 1). Total creep strain was more than twofold greater in impacted specimens than sham controls at each time point ($p < 0.05$; Fig. 2). This change was associated with a decrease of ~40% in cartilage thickness in impacted specimens versus control ($p < 0.05$; Table 1). Percent recovery of impacted specimens was not different from shams at 0 month but was significantly less than shams at 1 and 6 months, indicating progressive degeneration following impact (Fig. 3). In addition, time to 70% creep was significantly greater in impacted specimens than shams at 6 months, while time to 70% recovery was significantly greater at both 1 and 6 months.

There were several significant changes in the creep properties of the impacted specimens with time. Total creep strain decreased from 1 to 6 months (Fig. 2; $p = 0.003$), suggesting partial recovery of equilibrium stiffness (although still different from normal). To account for normal age-related changes during the 6-month follow-up interval, we also expressed the properties of the impacted specimens as a fraction of their paired sham values and analyzed these normalized data. Time to 70% recovery was greater at 6 months than at 0 months ($p = 0.03$), and time to 70% creep was greater at 1 and 6 months than 0 month, although this difference did not reach significance ($p = 0.09$). Thus, there was some evidence of improvement in the creep properties of impacted specimens with time (decreased creep) but also some evidence of deterioration (longer time to creep and recover, and diminished recovery after 1 month).

Impact significantly increased the amount of trabecular bone in the medial femoral condyle directly beneath

Table 1. Creep-Indentation Properties of Articular Cartilage at the Posterior Aspect of the Medial Femoral Condyle of NZW Rabbits at Three Time Points after Sham, Low, or High Mass Impact

Parameter	Sham			Low			High		
	0 Month (n = 10)	1 Month (n = 12)	6 Months (n = 15)	0 Month (n = 6)	1 Month (n = 6)	6 Months (n = 8)	0 Month (n = 4)	1 Month (n = 6)	6 Months (n = 7)
Cartilage thickness (mm)	0.334 ± 0.102	0.344 ± 0.108	0.347 ± 0.091	0.199^c ± 0.052	0.207^c ± 0.089	0.218^c ± 0.162	0.158^c ± 0.101	0.137^c ± 0.066	0.176^c ± 0.082
Creep strain (mm/mm)	0.281 ± 0.162	0.289 ± 0.061	0.169 ^{ab} ± 0.040	0.507^c ± 0.244	0.719^{ec} ± 0.094	0.399^{bc} ± 0.198	0.554^c ± 0.075	0.606^c ± 0.273	0.436^c ± 0.163
Percent recovery (%)	85.0 ± 12.4	90.3 ± 6.3	87.5 ± 9.9	83.7 ± 9.9	74.3^c ± 19.1	70.9^c ± 19.3	78.3 ± 24.7	75.3^c ± 18.2	68.8^c ± 19.5
T _{70% creep} (s)	29.8 ± 12.8	25.1 ± 11.1	10.6 ^{ab} ± 8.2	27.3 ± 9.2	52.5 ± 30.6	20.5^c ± 14.5	21.7 ± 11.2	32.4 ± 27.3	40.6^c ± 34.9
T _{70% recovery} (s)	16.5 ± 10.1	17.2 ± 11.4	6.8 ^{ab} ± 2.7	23.4 ± 18.4	117.4^{ac} ± 63.6	90.8^c ± 113.5	30.6 ± 27.6	48.4^{cd} ± 31.9	67.3^c ± 80.8

^aDifferent from 0 month ($p < 0.05$).

^bDifferent from 1 month ($p < 0.05$).

^cImpact different from sham (low and high levels are pooled for this comparison).

^dDifferent from low impact group ($p < 0.05$).

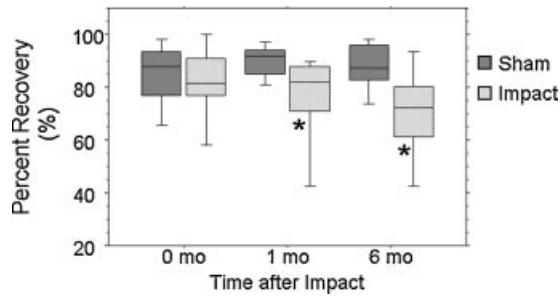


Figure 3. Total (equilibrium) recovery normalized by total (equilibrium) creep displacement from indentation testing of rabbit femoral condyles from sham, low, and high impact groups. Low and high impact groups did not differ from each other, thus pooled impact data are shown. Sham specimens recovered 85–90% of creep deformation. Recovery of low and high impact specimens did not differ from sham specimens at 0 month but was significantly less than sham at 1 and 6 months ($p < 0.05$ by ANOVA), suggesting a deterioration of viscoelastic properties with time after impact. (Box plot: the height of each box depicts the 25th–75th percentile values, while the error bars depict the 10th and 90th percentile values; the median value is depicted by the horizontal line within the box.) *Impact different from sham; $p < 0.05$.

the impact site at 1 month. MicroCT analysis indicated that trabecular bone volume fraction (BV/TV) beneath the site of impact was greater compared to the same site in the sham knee, as well as other sites in the impacted knee ($p < 0.01$; Fig. 4). Similar increases were observed for trabecular thickness and number (data not shown). The increase in trabecular bone volume was localized to the area beneath the impact site and thus was not attributed to the effects of the transcondylar K-wire or to any limb effects. For example, BV/TV in the distal region of the impacted medial femoral condyle (away from the

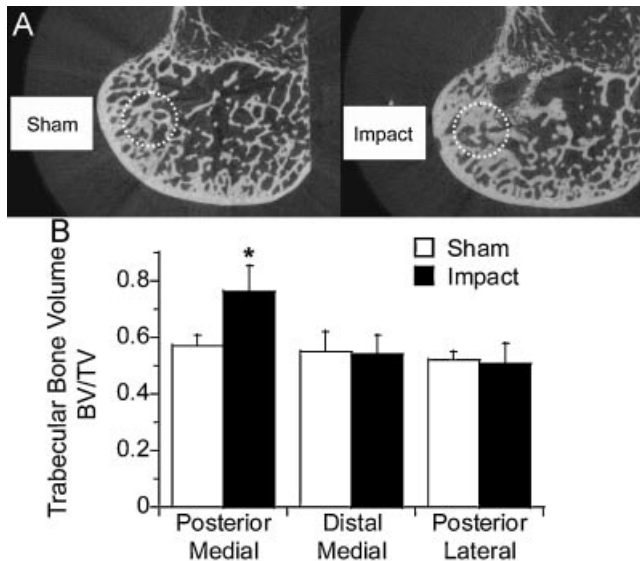


Figure 4. (A) MicroCT images of the medial femoral condyles of representative sham (left) and impact (right) knees illustrating the posterior site of impact and the region of analysis (dashed circle). The impact specimen appears to have more trabecular bone beneath the impact site. (B) Mean (\pm SD) bone volume fraction (BV/TV) of trabecular bone in the region of interest. Near the site of impact (posterior part of the medial condyle), BV/TV was 33% greater in impact side versus sham side ($p = 0.005$). Away from the site of impact, BV/TV did not differ between impact and sham sides.

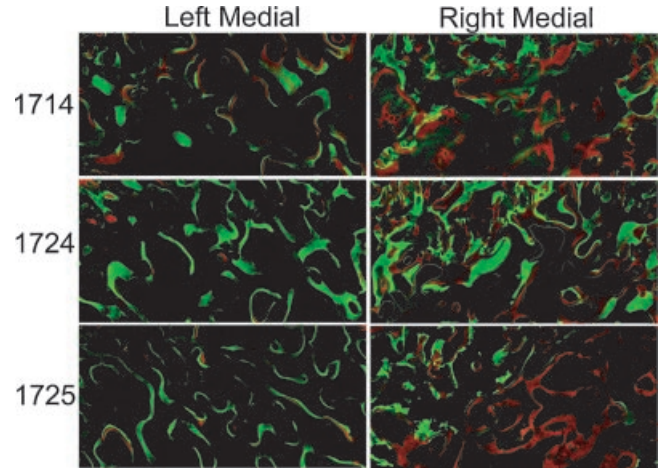


Figure 5. Fluorescent photomicrographs of trabecular bone from the posterior aspect of the medial femoral condyle of sham (left) and impacted (right) knees from three rabbits. Calcein green and alizarin complexone (red) were administered on days 7 and 17 after impact, respectively, to label newly mineralizing surfaces. Greater labeled area is evident in impacted samples, indicating an increase in bone formation after impact. Impacted specimens had a more plate-like microstructure.

impact site) was not different from same region of the sham knee ($p = 0.75$), nor were there any differences between the lateral femoral condyles of the impacted versus sham knees ($p = 0.61$).

Consistent with the microCT findings, histomorphometric analysis indicated increased trabecular bone formation in the region beneath the impact site (Fig. 5). Absolute labeled area (labeled with either calcein or alizarin) was 77% greater in impacted condyles compared to contralateral sham condyles, and labeled area per bone area was 38% greater (Table 2).

DISCUSSION

We used an in vivo model of articular cartilage injury to investigate the effects of near-fracture impact on the in situ mechanical properties of articular cartilage and the density of the underlying trabecular bone. We found that a single, high-force impact produces acute loss of cartilage thickness and a reduction in cartilage equilibrium stiffness (i.e., increased creep strain) but no immediate change in the ability of cartilage to recover from creep deformation. From 0 to 6 months after impact, cartilage thickness remains diminished, stiffness remains compromised, and the ability of cartilage to recover from creep deformation becomes significantly impaired. Within 1 month after impact, there was a significant increase in trabecular bone volume beneath the impact site due to increased bone formation. Taken together, these findings support our hypothesis that a single high-energy impact leads to acute deleterious changes in the mechanical properties of the injured cartilage that worsen with time, while also stimulating changes in the underlying bone.

Previous studies have documented loss of cartilage mechanical properties with time after sub-fracture impact, although using lower stress levels than we

Table 2. Histomorphometric Parameters from Trabecular Bone of the Posterior Femoral Condyle ($n = 3$)

Condyle	Labeled Area (mm ²)	Bone Area (mm ²)	Labeled Area/Bone Area	Bone Area/Tissue Area
Right medial (impact)	1.31 ± 0.38	4.47 ± 0.06	0.29 ± 0.09	0.73 ± 0.01
Left medial (sham)	0.74 ± 0.17	3.51 ± 0.37	0.21 ± 0.05	0.57 ± 0.06

Mean ± SD values for each sample were averaged from two sections; because of few samples, statistical comparisons are not presented.

applied. Natoli et al.¹⁵ impacted bovine osteochondral explants at 3 and 9 MPa and reported that reductions in cartilage stiffness were greater at 4 weeks after impact than at 1 day, indicating progressive deterioration of tissue integrity. Haut et al.⁵ developed an in vivo model of sub-fracture impact to the rabbit patella, producing damage of the retro-patellar cartilage at impact stresses of ~20 MPa. Using this model, cartilage stiffness was not altered acutely (time-zero) but was significantly reduced at 3–6 months after impact.^{4,12,13} Stiffness loss persisted up to 36 months without either worsening or improving and was accompanied by increased permeability.⁴ In the current study we used a higher energy impact (~20 J) than used in these previous studies, generating peak stresses of ~100 MPa. We chose this high-stress loading condition because in a prior study using the same model we found that impact stresses of up to 55 MPa did not lead to progressive cartilage degradation.³ The impact we used in the current study produced acute damage leading to increased creep strain (i.e., decreased equilibrium stiffness), in contrast to the studies described above that applied lower stresses. With time in our study, we found that the ability of cartilage to recover from creep deformation was diminished, consistent with the progressive deterioration that was noted by others.^{4,12,15}

The progressive deterioration of articular cartilage after impact is consistent with previous reports of compromised cell viability, matrix composition, and biosynthetic activity.^{6,12,15,16} Jeffrey et al.¹⁶ studied the metabolic response of chondrocytes to a single impact in articular cartilage biopsies. These experiments reported a loss of both extracellular proteoglycans and proteins, as well as decreased substrate production immediately following a single impact to cartilage explants. Newberry et al.¹² reported progressive thinning of impacted cartilage in rabbit knees as well as histological evidence of early osteoarthritis. Huser and Davies⁶ demonstrated that a single high-energy impact can cause cartilage surface damage, proteoglycan loss, and chondrocyte death in vitro. These changes were observed at 48 h and increased with time. Milentijevic et al.⁸ used an open joint impact model and reported that cell death at the site of impact occurred for peak stress >20 MPa and matrix damage occurred for peak stress >30 MPa. Similarly, in a recent study, we showed a profound alteration in chondrocyte metabolism including decreased type II procollagen mRNA expression and decreased BMP-2 protein following a single impact of the same magnitude as used in the current study.² The loss of biosynthetic activity with time indicates that the articular cartilage loses its ability to self-repair after a high-stress impact.

Thus, it is not surprising that in the current study mechanical behavior did not improve substantially with time and showed some signs of deterioration.

Impact caused a local increase in trabecular bone density (BV/TV) directly sub-adjacent to the impact site within 1 month. MicroCT indicated that trabecular bone volume was greater in the impact-adjacent area compared to similar sites in the sham knee, and to nonadjacent sites in the impacted medial femoral condyle. By analyzing bone from the lateral femoral condyle (nonimpacted) we were able to confirm that the increase in trabecular bone volume was not attributed to the effect of the transcondylar K-wire. The microCT findings were supported by dynamic histomorphometry analysis that indicated increased trabecular bone formation. Others have noted increased thickness of the sub-chondral bone layer following in vivo impact, although these changes were not observed until 7–12 months post-impact.^{4,13} Gradual changes in sub-chondral bone may reflect an interaction between cartilage stiffness and the stiffness of its bony bed.¹⁷ By contrast, the relatively rapid increase in trabecular bone density that we observed is more likely to be a direct response to impact. “Bone bruises” are often observed by clinical MRI in the trabecular bone adjacent to sites of impact in the knee joint and appear to represent acute hemorrhage or edema.^{9,11} We propose that the increased trabecular density we observed 1 month after impact is a consequence of the bone bruise rather than changes in the overlying cartilage. We do not believe that the bony changes we observed resulted from trabecular fracture. Samples that had obvious punch defects were excluded, and in preliminary studies we examined several samples using high-resolution contact radiographs and found no evidence of fracture. Further, microCT imaging of numerous samples did not reveal any sub-chondral bone or trabecular bone fractures. Thus, although we cannot rule out subtle bone damage, we believe that the responses we observed were not the result of fracture.

Analysis of the creep-indentation properties of articular cartilage from the sham knees of young rabbits revealed important developmental changes. As these animals matured from 3 to 9 months age, articular cartilage thickness did not change but the cartilage was stiffer and reached creep equilibrium in less time. These findings are consistent with data from bovine articular cartilage that showed an increase in cartilage stiffness during development in association with increased collagen content.¹⁸ Interestingly, after 6–8 months of age, there appear to be no further changes in the mechanical properties of articular cartilage in the normal rabbit

knee,⁴ consistent with the age when skeletal growth is complete in the rabbit.¹⁹

There are several limitations to our study. First, our intent was to examine two levels of loading, at 70 and 90% of the fracture threshold. We selected masses of 4,350 g (low) and 4,900 g (high) to achieve the desired forces based on preliminary studies. Nevertheless, forces recorded at impact were only different by 7% between groups (rather than the desired 20%) and this difference was not significant ($p = 0.08$). As a result, there were no detectable differences in any outcome measures between the low and high groups. Both groups represented a severe, near-fracture impact. A second limitation is that we did not estimate intrinsic material properties of cartilage (e.g., modulus, permeability) because of the large strains generated in our creep tests. Linear biphasic theory has been used to estimate material properties from creep-indentation tests of cartilage,^{10,14,15} but this theory assumes infinitesimal strains¹⁰ and thus was inappropriate for our study. Our testing protocol applied a 5-g test mass, which resulted in ~20% creep strain in control specimens (as intended). However, average values of creep strain in impacted specimens were >40%, much larger than we anticipated. Clearly, use of a lighter test mass would be required to achieve equilibrium strain values in a range acceptable for small-strain analysis. In addition, even if strains were reduced, the significant differences in cartilage thickness between samples would make estimation of material properties across groups problematic. Despite the lack of material property estimates, the main effects of impact were readily observed by analysis of the properties derived directly from the time–displacement data. And because testing conditions were fixed, comparisons between groups are valid for evaluation of relative behaviors. A third limitation is that our thickness measurements (performed immediately after completion of mechanical testing) sometimes yielded values that were less than the creep displacement. This is because not all the creep displacement was recovered, that is, there was “lost” displacement after 30 min of recovery. To account for this, we added this “lost” displacement to the measured thickness, which may have introduced some error in our thickness values. Lastly, our histomorphometric analysis is limited by few samples. Technical difficulties with embedding led to artifactual damage during grinding and the resulting sections from four pairs of knees were of too poor quality to allow quantitative analysis. Nonetheless, the available data were consistent with microCT findings and support the logical inference that an increase in bone density in the first month after impact is due to increased bone formation.

The cartilage impact model we used has both strengths and limitations. Among the strengths, it is an *in vivo* model that allows application of a highly controlled impact at a loading rate similar to that predicted for a fall from a standing height onto an outstretched hand.¹ In addition, it produces an isolated cartilage injury without other joint injury or bone

fracture. Thus, it allows examination of the effects of cartilage injury without other confounding factors. Although our model does require an arthrotomy, our cumulative experience is that the arthrotomy does not lead to joint inflammation and that the sham knees do not differ from normals. On the other hand, the isolated injury that our model creates does not replicate the multifactorial injuries seen with joint trauma. Our goal is not to simulate a complex knee injury that may involve ligament rupture and/or bone fracture but to isolate one component of such an injury, that is, the direct trauma to the cartilage surface.

Our findings have clinical significance to the problem of traumatic cartilage injury. When combined with our previous report on changes in proteoglycan content and chondrocyte biosynthetic activity,² the current study demonstrates that profound cartilage damage can be produced by an impact that does not cause bone fracture. Following such a severe impact there is little or no evidence of cartilage self-repair. Interestingly, in the 6-month duration of this study, we did not observe expansion of the damaged area to adjacent cartilage or evidence of full-blown joint degeneration, suggesting that the joint as a whole is relatively tolerant of localized injury. However, with sufficient time joint degeneration may occur, especially in the setting where local bone density is increased leading to increased contact stress.

In summary, changes in the properties of articular cartilage and of the underlying bone were initiated from a single, high-energy impact in the absence of fracture. Acute loss of cartilage thickness and stiffness was demonstrated following impact. These negative changes persisted over 6 months and were accompanied by a loss of the ability of cartilage to recover from creep deformation. There was no evidence of self-repair, consistent with the lack of biosynthetic activity and loss of proteoglycan content reported previously.² In addition, we observed an increase in trabecular bone density subjacent to the impact site, perhaps the result of a bone bruise. Taken together, loss of cartilage structural integrity and increased stiffness of the supporting bone is likely to contribute to further deterioration of the articular cartilage. We conclude that a single high-energy impact can lead to cartilage degeneration and bony changes consistent with post-traumatic osteoarthritis.

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