Copyright © 2006 by The Journal of Bone and Joint Surgery, Incorporated

Effect of Impact on Chondrocyte Viability During Insertion of Human Osteochondral Grafts

By Boris H. Borazjani, MD, Albert C. Chen, PhD, Won C. Bae, PhD, Shantanu Patil, MD, Robert L. Sah, MD, ScD, Gary S. Firestein, MD, and William D. Bugbee, MD

Investigation performed at University of California-San Diego, La Jolla, California

Background: Osteochondral grafts, used to treat chondral and osteochondral defects, require high insertional forces that may affect the viability of chondrocytes in the graft. The objectives of this study were to (1) measure the loading impact during insertion of osteochondral grafts, (2) evaluate the effect of insertional loading on chondrocyte viability, and (3) assess this effect on chondrocyte apoptosis and activation of caspase-3.

Methods: The distal parts of twelve fresh femora from six adult human cadavers were harvested within seventy-two hours after the death of the donor. From each femur, four 15-mm-diameter cylindrical osteochondral grafts were isolated; two of these grafts (a total of twenty-four grafts in the study) were transplanted with standard impact insertion into recipient sockets in the other condyle of the ipsilateral femur. The other two grafts served as unloaded controls. Loads were measured during the insertion of ten of the twenty-four transplanted grafts. Full-thickness cartilage disks were then removed from the grafts, incubated for up to forty-eight hours, and analyzed for cell viability, TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)-positive reactivity, and caspase-3 activation, each as a function of the depth from the articular surface.

Results: The insertion of an osteochondral graft was characterized, on the average (and standard deviation), by 10 ± 4 impacts, each generating 2.4 ± 0.9 kN of load and 13.3 ± 4.9 MPa of stress for a duration of 0.57 ± 0.13 ms with a 0.62 ± 0.25 N·s impulse. Impact insertion increased cell death in the superficial 500 μm to 21% at one hour ($p < 0.001$) and 47% at forty-eight hours ($p < 0.001$) and also increased cell death in deeper layers at forty-eight hours. Some cell death was due to apoptosis, as indicated by an increase in caspase-3 activation at eight hours ($p < 0.01$) and TUNEL-positive cells at forty-eight hours ($p < 0.05$) in the superficial 500 μm of impacted cartilage.

Conclusions: Impact insertion of osteochondral grafts generates damaging loads that cause chondrocyte death, particularly in the superficial zone, mainly as a result of apoptosis mediated by the activation of caspases.

Clinical Relevance: Chondrocyte death that occurs during impact insertion of osteochondral grafts may lead to compromised function. Understanding the mechanisms and consequences of such impact loading may provide insights into potential therapeutic interventions, or lead to changes in the insertion technique, to decrease the cell injury associated with impact loading.

Osteochondral grafting is sometimes used as a method of treating focal chondral and osteochondral defects in the femoral condyles and elsewhere[1-5]. Impaction forces are used to insert the osteochondral donor tissue into the recipient site and to generate a secure fit[6]. The effect of such impaction forces on the viability of the chondrocytes that reside within the cartilage tissue of the implant has not been studied previously, either in vivo or in situ, to our knowledge.

A commentary is available with the electronic versions of this article, on our web site (www.jbjs.org) and on our quarterly CD-ROM (call our subscription department, at 781-449-9780, to order the CD-ROM).

Trauma can damage articular cartilage and its chondrocytes. Traumatic impact on joints in vivo results in chondrocyte death both in humans[7-9] and in experimental animals[10,11]. In vitro, biomechanical factors associated with mechanically induced chondrocyte death include the loading rate[12-14] and duration[15,16], contact stress[17-23], strain rate[24-26], and overall compression level[21,27]. The duration of an impact can affect the cartilage and chondrocyte response. Slowly applied loads may initially allow tissue fluid to support the load but then gradually transfer load to the matrix[28]. In contrast, suddenly applied loads may not allow time for fluid movement and thus may cause variable degrees of tissue deformation depending on

mechanical constraints[9,18]. In classic mechanics, the intensity of an impact is described by the impulse, defined as the product of load and time, with units of N·s[29]. Unlike accidental or experimental trauma, insertional loading of osteochondral grafts typically involves multiple impacts rather than a single impact. The biomechanical characteristics of insertional impacts have not been described previously, to our knowledge.

The process by which excessive mechanical loading of cartilage can lead to chondrocyte death may involve apoptosis[21,30-32]. Apoptosis occurs through an ordered sequence of cellular events, characteristically including activation of a cascade of cysteine-dependent aspartate-specific proteases (caspases)[11,33]. The regulated degradation of nuclear DNA by a caspase-activated deoxyribonuclease (CAD) is a hallmark of apoptosis[34]. In the typical sequence of cellular events during apoptosis, caspase activation is an early event[35], while DNA fragmentation is a late event that occurs in days[21,36]. Active caspase-3, an executioner caspase, facilitates the assembly of CAD into its active form, leading to the production of DNA fragments[37]. In human cartilage disks subjected to 30% compression, signs of apoptosis may appear as early as six hours after injury and the percentage of cells undergoing apoptosis may continue to increase for up to seven days after injury[21].

We hypothesized that the impact loading used to implant osteochondral grafts leads to chondrocyte death due in part to apoptosis mediated by activation of caspases. The specific aims of this study were to (1) characterize the impact load during the insertion of osteochondral grafts, (2) evaluate the effect of insertional loading on cell viability, and (3) assess this effect on apoptosis and activation of caspase-3.

Materials and Methods

Osteochondral Harvest and Grafting

All donor tissue was obtained from University of California-San Diego Lifesharing (San Diego, California). The distal parts of twelve fresh human femora were removed en bloc from six human cadavers, four male and two female, with a mean age (and standard deviation) at the time of death of 53 ± 4 years (range, forty-six to fifty-six years). The specimens were obtained under sterile conditions within seventy-two hours after the donor's death and were stored at 4°C in serum-free culture medium for up to seventy-two hours. Only normal articular cartilage (grade 1A according to the classification system of Noyes and Stabler[38]) was used in the study. A surgeon who was experienced with the osteochondral grafting procedure performed the experimental harvest and implant procedures.

A set of four cylindrical osteochondral grafts, 15 mm in diameter with 10 mm of subchondral bone (Fig. 1, *B*), was harvested from each femur with use of osteochondral allograft instruments (Arthrex, Naples, Florida) under continuous irrigation with lactated Ringer solution in order to minimize thermal damage to the cartilage. The subchondral bone was subjected to pulse lavage with lactated Ringer solution to remove marrow elements. From each set of four grafts, two grafts (one from the trochlea and one from the condyle; Fig. 1, *A*) were transplanted (designated as "loaded") to two recipient sites in the other condyle of the ipsilateral knee. The other two grafts (one from the condyle and one from the trochlea) were not implanted or impacted and were used as controls (designated as "unloaded"). In each pair of donor knees, medial grafts from one knee were transplanted to the lateral side of that knee, and lateral grafts from the other knee were grafted to the medial side of that knee. Thus, a total of twenty-four grafts from twelve femora from six cadavers were transplanted into recipient sockets.

The grafts were inserted with use of a tamp until their cartilage surface was flush with that of the surrounding host cartilage. Of the twenty-four grafts, ten from six femora from three donors were inserted with a load-cell-instrumented tamp (surface area, 7.3 cm^2)[6] (Fig. 1, *C*) to allow load measurement during graft insertion. (Load was not measured during insertion of every graft because the instrumented tamp was not always available, and sufficient data were obtained from the ten grafts.) For each impact, the loading force was taken as the peak, the magnitude of the load impulse was calculated as the area under the force-time curve, and the duration was considered to be the interval between half-maximum loads (Fig. 1, *D*). The load history, the characteristics of each impact, the total number of impaction taps, and the tap number at which the peak impact was maximal were recorded for each inserted graft (Fig. 1, *E*). The loading force was normalized to graft area to estimate compressive stress.

Cartilage Retrieval

After insertion of the osteochondral grafts, cartilage disks were removed from each transplanted graft and from each control graft for subsequent analysis. The retrieved grafts were placed in Dulbecco modified Eagle medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 50 µg/mL gentamicin at 5% CO_2/95% O_2 at 37°C. Full-thickness cartilage was removed from the subchondral bone with a scalpel and then was cut with a dermal punch to yield six, seven, or eight 3-mm-diameter full-thickness disks from each graft.

Quantification of Cell Death

Six disks from each graft were incubated in Dulbecco modified Eagle medium, with a culture duration of one, four, or forty-eight hours (two disks each). Disks from an individual graft were distributed evenly among the time points to control for the potential regional variation in cartilage properties[39]. At each time point, disks were cut vertically in half. One half was stained with phosphate-buffered saline solution containing calcein-AM and ethidium homodimer-1 (LIVE/DEAD Viability/Cytotoxicity Kit; Molecular Probes, Eugene, Oregon) for one hour at 4°C and rinsed twice in phosphate-buffered saline solution for twenty minutes each. The samples were imaged with use of a fluorescence microscope (Eclipse TE300; Nikon, Melville, New York), an arc lamp, G-2A (for "dead" images; Nikon) or B-2A (for "live" images; Nikon) filter cubes, a Plan Fluor 4× objective lens (NA = 0.13; Nikon), and a SPOT RT camera (Diagnostic In-

struments, Sterling Heights, Michigan). Each disk was imaged at the cut vertical surface. From these images, cell death was quantified as a function of the depth from the articular surface, in bins of 100-μm thickness, to a depth of 1500 μm. To achieve this, the articular surface was localized by fitting a line to 5~10 cells at the surface, rotating the image and cells such that the articular surface was horizontally positioned, and tabulating the cells located at certain depths. Since there is a margin of cell death near a lacerated surface (as a result of isolation with a dermal punch circumferentially)[40,41], cell viability was analyzed in regions excluding the area within 100 μm of the lacerated surfaces. Live and dead cells were counted with custom image processing routines with use of MATLAB (The MathWorks, Natick, Massachusetts)[42]. Images were processed by spatial filtering (5 × 5 Laplacian of Gaussian filter, standard deviation of ~1) to accentuate regions representative of cells, filtering (2 × 2 median) to suppress noise, and thresholding to identify and localize cells. This automated method of cell counting had ~90% sensitivity and specificity for both live and dead cells as determined by manual counting of randomly cropped regions containing >100 cells for eight sample images with varying overall cell density, from the lowest to the highest (data not shown). For a given region of interest, the percentage of cell death was quantified as the number of dead cells divided by the total number of cells (live and dead).

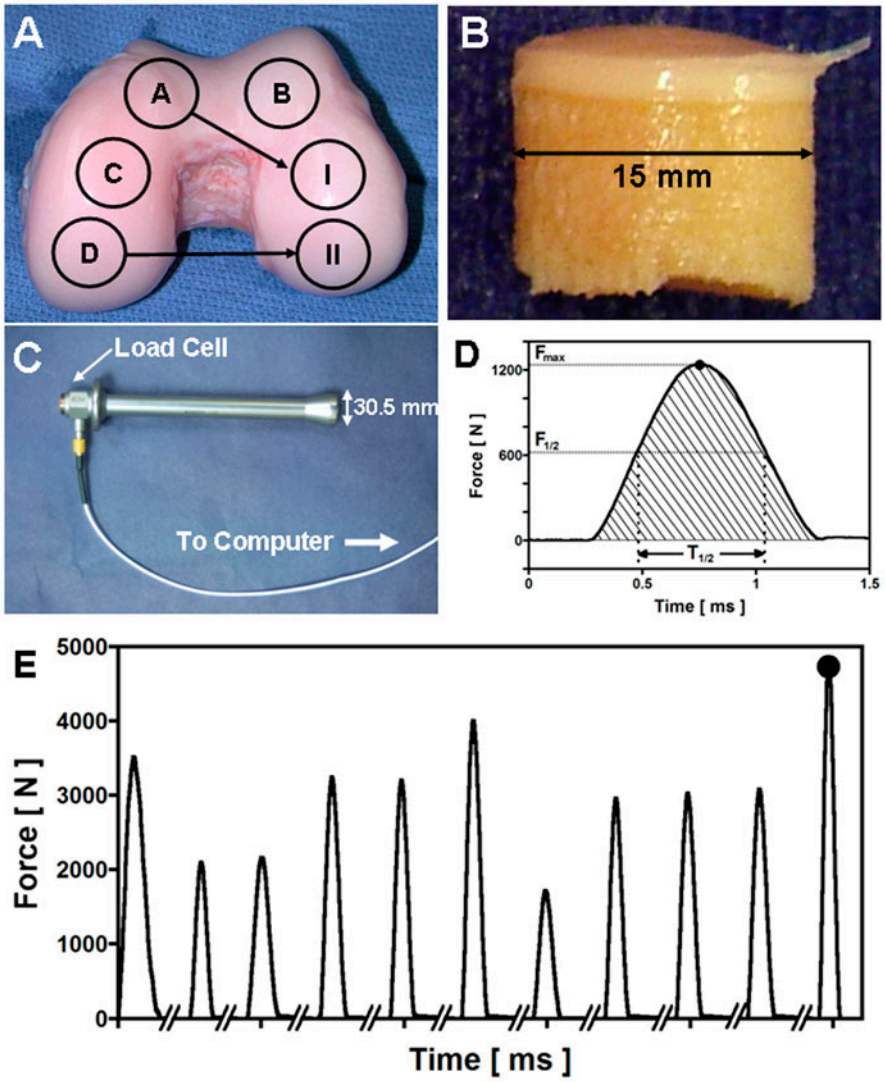


Fig. 1

A: Circles A through D represent four osteochondral grafts from human femoral condyles. Grafts A and D were transplanted into recipient sites I and II, respectively, on the other condyle of the ipsilateral knee. Grafts B and C were controls. *B:* The osteochondral graft, prepared as described in the text. *C:* The load-cell-instrumented tamp used to insert the osteochondral graft. *D:* Impact parameters measured during insertion of the osteochondral grafts. The cross-hatched area under the force-time curve is the impulse. *E:* Typical force-time tracing during insertion of an osteochondral graft, with the maximum peak force (●) indicated.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL)

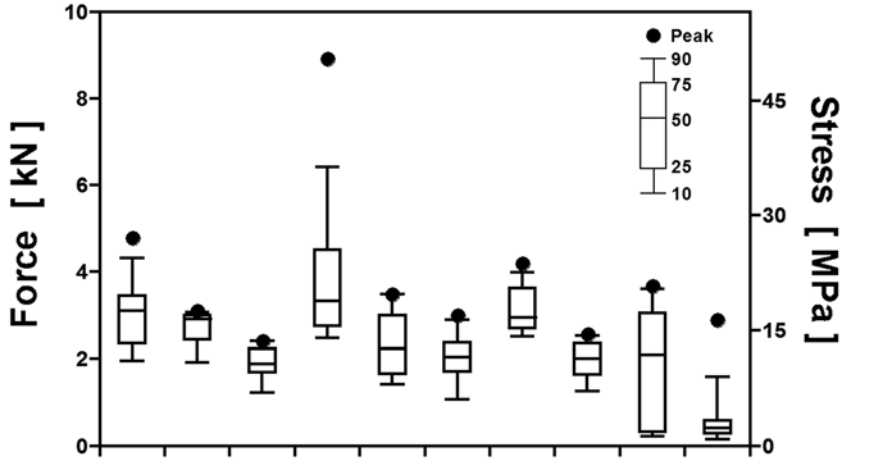
Apoptosis was assessed forty-eight hours after graft insertion. For this analysis, one cartilage disk from each of five inserted grafts and five control grafts (from five donors) was incubated for forty-eight hours. That time point was selected because the number of apoptotic cells increases with time, especially from six to ninety-six hours after application of load[21,36]. After incubation, cartilage disks were placed in 10% buffered formalin for twenty-four hours, embedded in paraffin, and cut into 3-μm-thick sections. In situ detection of apoptosis was performed with use of an ApoAlert DNA Fragmentation Assay Kit (Clontech, Mountain View, California). Fluorescein-dUTP labels DNA strand breaks and allows direct detection of DNA fragmentation by emitting a bright green signal when viewed with fluorescence microscopy. Apoptosis was quantified as a function of the depth from the articular surface by counting the number of TUNEL-positive cells and total cells in three sequential bins of 500-μm thickness over a depth of 1500 μm in three to six sections for each sample and determining the percentage of TUNEL-positive cells.

Immunohistochemical Analysis for Active Caspase-3

Caspase-3 activation was assessed eight hours after insertion of the graft. For this analysis, one cartilage disk from each of four inserted grafts and four control grafts (from four donors) was incubated for eight hours. That time point was chosen because the number of activated caspase-3 cells increases with time, from four to twenty-four hours after application of load[35,43,44]. Cartilage samples were fixed, embedded in paraffin, and cut into 3-μm sections, as described above. The presence of active caspase-3 was determined by immunohistochemical analysis with the VECTASTAIN avidin-biotin-peroxidase complex (ABC) kit (Vector Laboratories, Burlingame, California). In accordance with the manufacturer's instructions, rabbit polycolonal anti-caspase-3 antibody (1:66 dilution; R&D Systems, Minneapolis, Minnesota) was used as the primary antibody at 4°C overnight. A biotinylated goat anti-rabbit antibody (R&D Systems) was used as the secondary antibody. The reaction was then visualized by diaminobenzidine (DAB) (Sigma, St. Louis, Missouri), resulting in a brown color. Caspase-3 activation was quantified by the number of DAB-positive cells and expressed as the percentage of total cells. It was quantified as a function of the depth from the articular surface in three sequential bins of 500-μm thickness over a depth of 1500 μm in three to six sections for each sample, as described above for TUNEL staining.

Statistical Analysis

Pilot data from preliminary experiments demonstrated a 10% standard deviation for nonviable cells in the superficial layer of cartilage. Thus, to detect a 20% difference at a significance of $\alpha = 0.05$ and a power $(1 - \beta)$ of 0.80, six donors were needed.



		1	2	3	4	5	6	7	8	9	10	mean ± SD
Core												
Impacts		11	9	5	11	5	7	20	8	9	10	10 ± 4
Impact of Peak Force	#start	11	6	5	10	5	7	19	4	7	5	8 ± 5
	#end	1	4	1	2	1	1	2	5	3	6	3 ± 2
	%	100	67	100	91	100	100	95	50	78	50	83 ± 21

Fig. 2

Variation in the impact force to which each osteochondral graft was subjected. The tenth, twenty-fifth, fiftieth, seventy-fifth, and ninetieth percentiles of impact force are shown for each graft, as is the peak force (●). The vertical axis on the right shows the equivalent stress, calculated as the force normalized to the surface area of the articular surface of the graft. The table below lists the number of taps used to seat each individual graft within the recipient socket. The table also indicates the number of the tap at which the peak force occurred, relative to the starting or ending tap and as a percentage of the total number of taps.

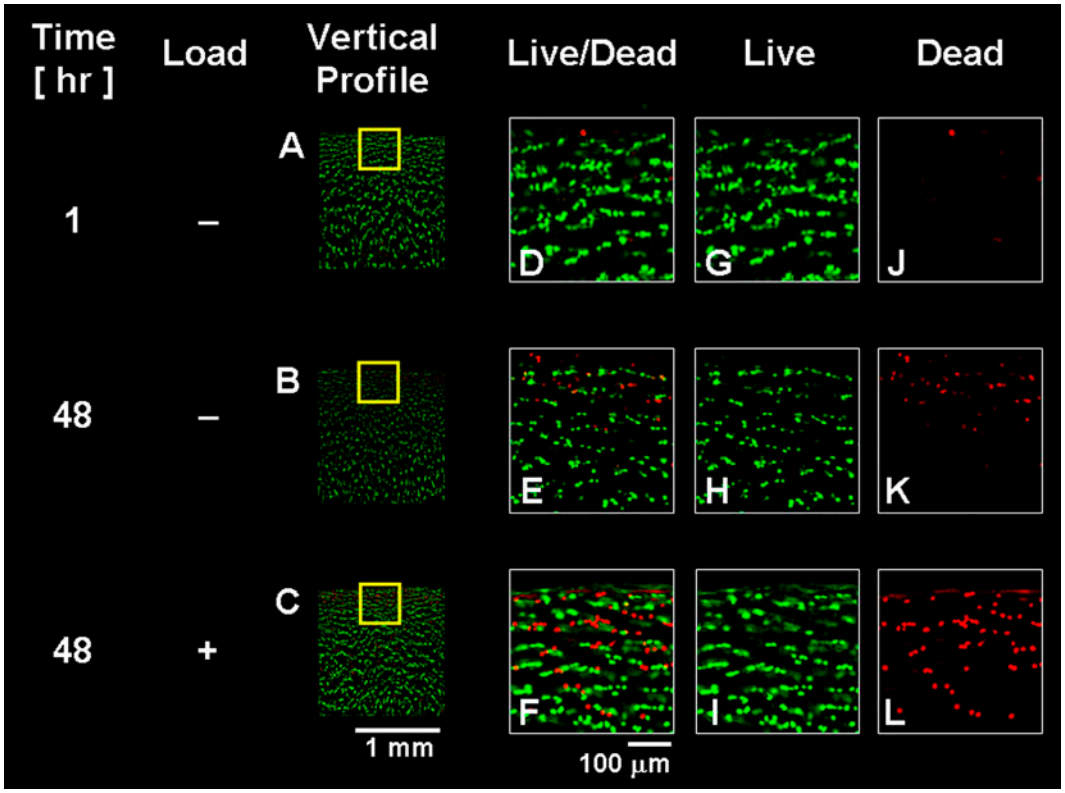


Fig. 3

Effect of impact load on cell death of osteochondral grafts. *A, B,* and *C:* Localization of live (green) and dead (red) chondrocytes in the vertical section of articular cartilage with (+) or without (–) load at one hour and forty-eight hours. Higher-magnification images of live and dead (*D, E,* and *F*), live (*G, H, and I*), and dead (*J, K,* and *L*) chondrocytes are shown.

The effects of loading and of depth from the articular surface on cell viability were assessed with repeated-measures two-way analysis of variance and post hoc testing. The effects of loading and of depth from the articular surface on apoptosis and caspase-3 activation were assessed with repeated-measures analysis of variance and post hoc testing. Statistical analyses were performed with Systat 10.2 (Point Richmond, California). Data are expressed as the mean and standard deviation.

Results

Biomechanics of Graft Insertion

The mean loading force of all ninety-five impacts was 2.5 ± 1.3 kN (52% coefficient of variation), corresponding to a stress of 14.0 ± 7.3 MPa. The impact duration averaged 0.56 ± 0.18 ms, and the impulse averaged 0.63 ± 0.30 N·s. The means and coefficients of variation were similar when the analysis was performed in terms of grafts (ten grafts from three donors; Fig. 2) instead of impacts: the loading force averaged 2.4 ± 0.9 kN (37% coeffvcient of variation), the corresponding stress averaged 13.3 ± 4.9 MPa, the impact duration averaged 0.57 ± 0.13 ms, and the impulse averaged 0.62 ± 0.25 N·s. On the average, the maximum impact applied to each osteochondral graft was associated with a loading force of 3.9 ± 1.9 kN (49% coefficient of variation; Fig. 2), a stress of 22.1 ± 10.8

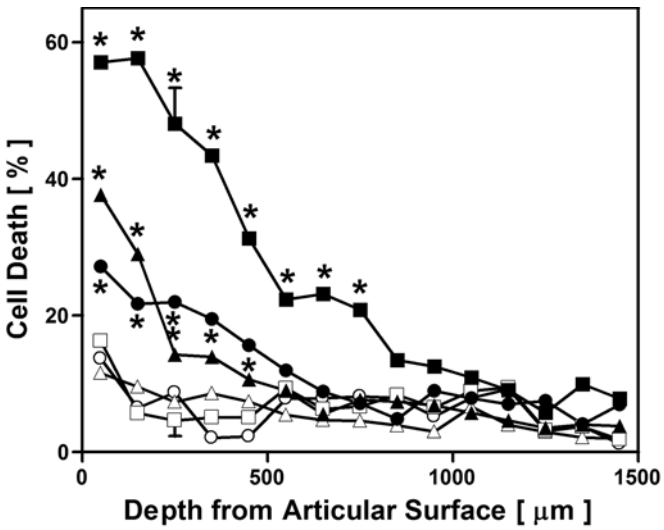


Fig. 4

Time and depth-associated distribution of chondrocyte death in unloaded (○, △, □) and loaded (●, ▲, ■) osteochondral grafts at one hour (circles), four hours (triangles), and forty-eight hours (squares) after graft insertion. The error bars indicate the standard deviation, and the asterisks indicate a significant difference when compared with the time-matched unloaded samples ($p < 0.05$).

MPa, an impact duration of 0.48 ± 0.12 ms, and an impulse of 0.82 ± 0.20 N·s. An average of 10 ± 4 impacts were required to insert an osteochondral graft into the recipient socket, with the maximum impact being 8 ± 5 taps from the start (first impact), corresponding to 83% ± 21% of the total number of impacts, or 3 ± 2 taps from the end (last impact).

Analysis of Chondrocyte Death After Load

Loading diminished cell viability, especially near the articular surface (Figs. 3 and 4). The average cell death in the unloaded samples did not differ significantly ($p = 0.46$) among the three time points after graft insertion (6% ± 2%, 6% ± 2%, and 7% ± 3% at one, four, and forty-eight hours, respectively). However, at each time point, the application of load increased cell death ($p < 0.001$) compared with that in the unloaded samples. One hour after insertional loading, cell death was significantly increased ($p < 0.001$) in the superficial 500 μm, with an average of 21% ± 4% cells dead compared with 9% ± 2% in

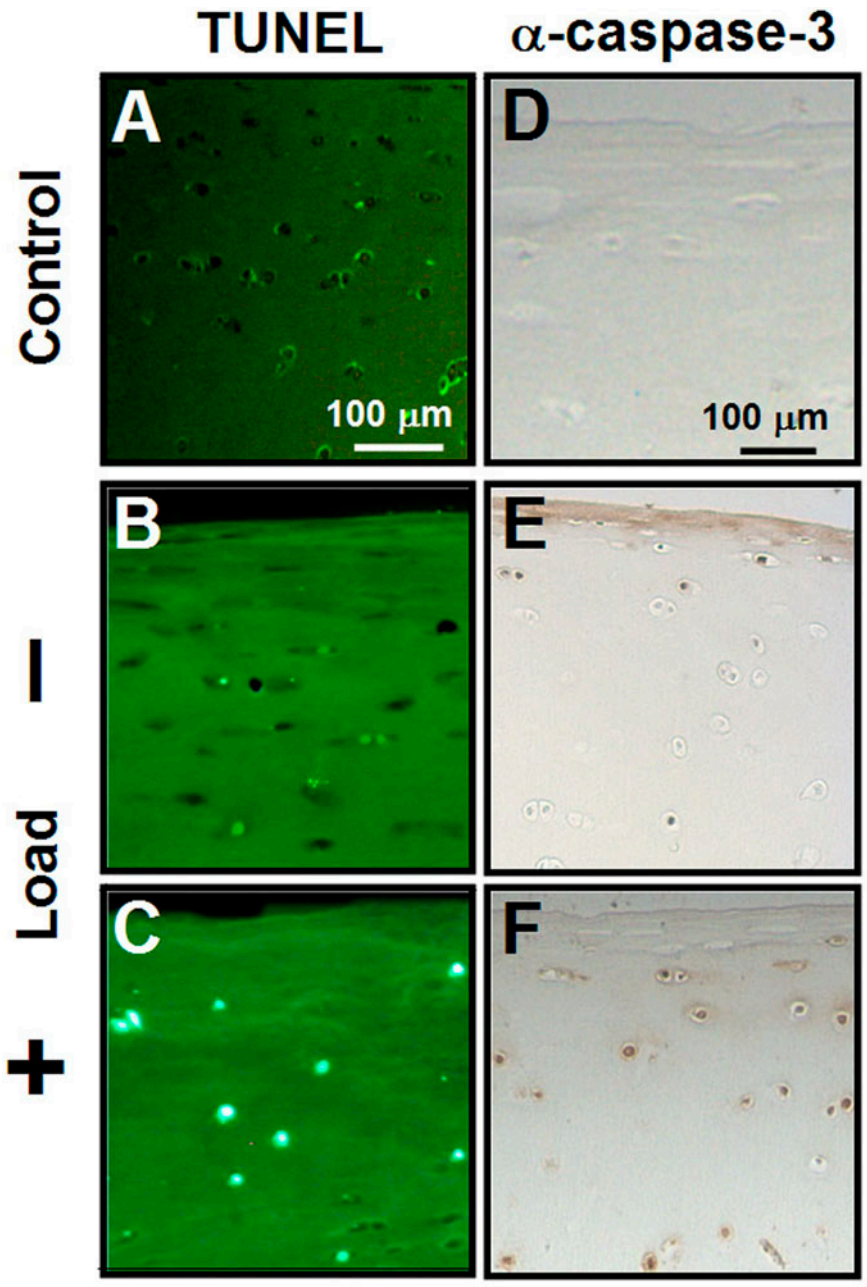


Fig. 5

Effect of impact load on apoptosis and caspase-3 activation in osteochondral grafts. *A, B,* and *C:* Localization of TUNEL-positive (green) chondrocytes in articular cartilage with (+) or without (–) load and in negative controls at forty-eight hours after graft insertion. *D, E,* and *F:* Localization of active-caspase-3-positive (brown) chondrocytes in articular cartilage with (+) or without (–) load and in negative controls at eight hours.

the same area of the unloaded sample (Fig. 4). By forty-eight hours after insertion, cell death not only had increased more than twofold in the uppermost 500 μm (47% ± 11% compared with 7% ± 5% in the unloaded samples), but it also had extended deeper into the tissue, to ~1000 μm ($p < 0.001$), where cell death averaged 38% ± 16% compared with 7% ± 4% in the unloaded samples (Fig. 4).

Quantification of Apoptosis by TUNEL Assay

Impaction loading also induced apoptosis in a depth-varying manner. At forty-eight hours, the baseline percentage of cells detected as apoptotic in the unloaded samples (five donors) averaged 9% ± 2%, while the loaded samples showed a significantly higher level ($p < 0.05$) of apoptosis (26% ± 7%) (Fig. 5, *A, B,* and *C*). Application of load increased cell apoptosis in the uppermost 1500 μm ($p < 0.001$) when compared with the apoptosis in that region in the unloaded samples. Most of these TUNEL-positive cells were found in the top 500 μm, where apoptosis was increased threefold (from 5% ± 2% in the unloaded samples to 16% ± 7% in the loaded samples, Fig. 6, *A*). The trend continued through the depth of the cartilage but with lower levels of positively-stained cells (2% ± 1% in the unloaded samples compared with 7% ± 1% in the loaded samples in the next 500-μm area and 1% ± 0.5% in the unloaded samples compared with 3 ± 1% in the loaded samples in the area that was even 500 μm deeper).

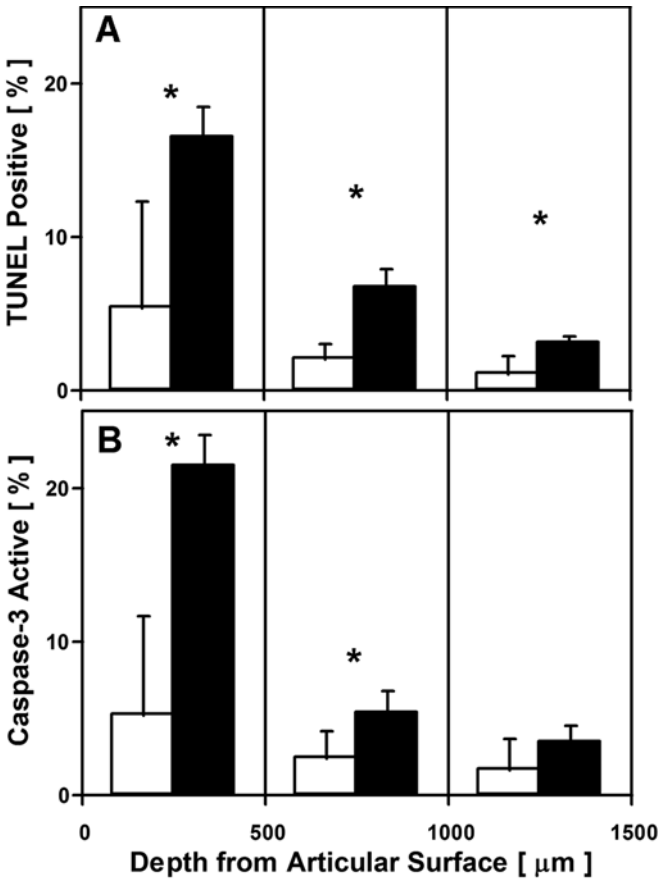


Fig. 6

Depth-associated distribution of TUNEL-positive and active-caspase-3-positive chondrocytes in unloaded (white bars) and loaded (black bars) osteochondral grafts. The data are given as the mean and standard deviation. The asterisks indicate a significant difference compared with depth-matched unloaded samples ($p < 0.01$). *A:* TUNEL-positive cell distribution in unloaded and loaded samples at forty-eight hours. *B:* Active-caspase-3-positive cell distribution in unloaded and loaded samples at eight hours.

Evaluation of Caspase-3 Activation After Load

Immunohistochemical analysis of the cartilage disks (from four donors) at eight hours (Fig. 5, *D, E,* and *F*) showed active caspase-3 in 9% ± 3% of the cells in the unloaded samples, whereas the loaded samples showed significantly higher caspase-3 activation (31% ± 7% of the cells) ($p < 0.01$). When the activated-caspase-3-positive cells were quantified as a function of depth (Fig. 6, *B*), the application of load was found to increase caspase-3 activation in the uppermost 500 μm as well as the next 500-μm layer ($p < 0.001$) when compared with the activation in the same region of the unloaded samples.

Discussion

Osteochondral grafting has been used with varying degrees of success for restoration of articular cartilage. Current clinical techniques utilizing devices that impact the graft during insertion can lead to chondrocyte death, which may diminish the performance of the graft. In the present study, we sought to characterize the mechanical parameters and chondrocyte responses to the current clinical technique of insertion. The impacts (average, ten impacts) needed to insert 15-mm-diameter grafts resulted in an average force of 2.4 kN and an area-averaged compressive stress of 13.3 MPa; the average duration of the impacts was 0.57 ms, with an average impulse of 0.62 N·s. Cell death as a result of impact insertion was localized near the impacted articular surface, extending from the top 500 μm at one hour after impact to a depth of 1000 μm by forty-eight hours (Fig. 4). An increase in caspase-3 activation at eight hours and TUNEL-positive cells at forty-eight hours, both of which are indicative of an apoptotic pathway, also demonstrated a loss of viable cells that was accentuated near the articular surface (Figs. 5 and 6).

In this study, we employed techniques used in surgery. Previous in vitro studies of impact loading have been performed on cartilage explants[13-16,18,26,45], intact animal knees[10,46,47], or osteochondral grafts from animals[12,48]. In the single other study of impact loading of human osteochondral grafts, of which we are aware, the investigators used osteoarthritic knees[40] in contrast to fresh normal human tissue (from donors just over the age limit of forty-five years for osteochondral allografting). Also, the osteochondral grafts were prepared to a size (15 mm in diameter) and shape (circular) typical of those used in human osteochondral allografting, and they were inserted into a recipient socket by a surgeon who was experienced with the procedure.

The variability in the impact mechanical parameters (e.g., a 52% coefficient of variation of impact force) could be

due to a number of factors. Surgeon control of the impact may be one factor. Other sources of variation could be differences in the bone quality of the graft recipient, the tightness of the fit of the grafts, and the relationship of the socket depth to the graft length, which could affect the seating level. The peak impact occurred near the completion of insertion (Fig. 2), suggesting that peak impact occurs when the base of the graft is seated into the recipient socket. The standard manual preparation of the graft base and recipient socket can result in variations of ~0.5 to 1 mm. Thus, an important part of the surgical technique is to trim the bone to the appropriate length, which can play a key role in the successful outcome of allograft transplantation and long-term cell viability.

Several biomechanical features of impact loading may lead to cell death. The peak stress determined in this study (13 ± 5 MPa) was in the range (14 to 20 MPa) that has been reported to induce chondrocyte death[18] (Fig. 2). The high rate at which the load (or stress) was applied may have contributed to the distribution of cell death, as high stress rates produce chondrocyte death localized to the superficial layer[45] and low stress rates produce a more diffuse distribution of cell death[13,14,26]. The stress rates that were applied were comparable with the range of 35 to 1250 MPa/s reported in the literature[14,17,46]. The depth of cell death from the articular surface has been observed previously to increase with peak stress and to decrease with an increasing stress rate[14]. Alternatively, it may be that strain and/or the strain rate are the key components leading to apoptosis[21,49-51].

Probably just as important as the load magnitude and rate is the duration over which the load is applied, or the impulse. In this study, the impulse was generally constant, with amplitudes of ~0.6 N·s. Telemetry measurements obtained from instrumented knee prostheses have shown axial forces ranging from 1500 to 2400 N (~2.8 to 3.6 × body weight) during typical levels of activity, such as walking, stair-climbing, and light jogging[52-54]. This finding suggests that high loads are probably present in the knee itself but are transmitted and dissipated by various tissues in the knee, such as the meniscus[55-57], to help create an environment that protects against cell injury during activity.

The overall density of chondrocytes in cartilage is affected by the balance between cell death and cell proliferation. Cell death induced by impact may be due to both apoptosis and necrosis. Necrosis does not require activation of specific intracellular signaling cascades, and, after the application of mechanical impact to cartilage[15,16,58], necrosis has been observed as an earlier event than apoptosis, occurring as soon as twenty to 120 minutes after impact[15]. In the present study, apoptosis was evaluated eight and forty-eight hours after graft insertion. The percentages of cells shown to be apoptotic by TUNEL (26%) and caspase-3 activation (31%) assays (Figs. 3 and 4) were similar, but they were lower than the percentage of dead cells determined by viability assay (47%). This could have been due to compression causing cell death by necrosis and the fact that the evaluation times were past the period when cell death by necrosis occurred. In addition, insertional loading could also increase the chondrocyte number by causing proliferation, which would counteract the injurious effect of the impact. Furthermore, although the grafts were prepared from en bloc knee specimens in a manner typical of the clinical procedure, it is unknown whether a fresh osteochondral allograft inserted in vivo may be protected by exposure of the injured chondrocytes to synovial fluid or the blood supply in the subchondral bone.

The chondrocytes responded differently to load according to their depth from the articular surface. In other studies, cell death was often localized at the superficial surface as a result of the high compaction and loss of fluid[14,45]. In the present study, chondrocytes were similarly affected by load, as evident in the vertical profiles. However, chondrocytes in the deep layer of cartilage appeared to be protected from damage. Although the cartilage was not evaluated below a depth of 1500 μm (the overall thickness of human cartilage in the femoral condyle ranges from 1.5 to 3 mm[59-62]), the deeper layers appear to be protected by the subchondral bone, possibly by preventing the cartilage from radially expanding at the cartilage-bone interface and thus constraining the tissue to allow the fluid to pressurize, sustain load, and diminish impact-induced damage and apoptosis[20]. In the present study, application of load did not grossly affect cell viability (i.e., increase death) in the deep profiles. The effect of the donor site on cell death was not assessed. Nonetheless, in the control tissues, the majority of the chondrocytes were viable, suggesting methods to protect the chondrocytes and their functions at the surface of the tissue may be beneficial.

Apoptosis is known to occur after an ordered sequence of cellular events. Abnormal biomechanical loading can trigger apoptosis by activation of caspases that lead to characteristic changes in DNA and other cellular constituents. The triggers of apoptosis induced by mechanical stimulation are not known, but several candidates can be proposed, including nitric oxide donors[63], expression of the Fas receptor[64], and stress-induced rearrangement of the cytoskeleton[65]. Mechanical loading could disrupt cell-matrix interactions or lead to cytoskeletal reorganization by means of three major pathways (death receptors, mitochondria, and endoplasmic reticulum), all of which are pathways in the sequential activation of caspases that converge at caspase-3[66].

Pharmacologic or other therapeutic interventions may assist in protecting grafts during insertion and may improve cell viability. Caspases can be sensitive to pharmacologic intervention[11,67,68]. Injection of the caspase inhibitor Z-VAD.fmk into a rabbit knee joint for more than seven days after an acute injury reduced cell apoptosis by 20% to 30% in one study[68]. This suggests that pharmacologic treatments may be possible and that these agents may be used to inhibit apoptosis at various points in the pathway. The profile of cell death in our study is consistent with previous trends in the literature[36], in which cell death due to impact loading has been reported to have been located primarily at the superficial region of cartilage. We found that, with time, the region of cell death extended into the middle zone, a finding consistent with other reports of delayed cell death[36]. In addition, this profile quantified how cell death increased not only with time, but also with the depth from the articular surface. A pharmacologic or technical intervention to protect re-

gions from cell death may be useful during graft procedures.

Maintaining chondrocyte viability is a fundamental principle of osteochondral grafting. The results of this study suggest that modification of graft insertion techniques may be warranted to protect graft viability. Indeed, this study has led to a change in our clinical practice in that we now minimize impact loading of grafts during insertion. Furthermore, understanding the results of cell death with impact loading may lead to therapeutic interventions that protect the graft. This may have implications not only for osteochondral grafting techniques, but also for therapies for chondral impact injuries. It is unknown what effect impact loading and subsequent chondrocyte death have on the function or longevity of the graft in vivo; thus additional studies are warranted. ■

NOTE: The authors thank Dr. Sanna Rosengren for statistical assistance, Dr. Darryl D. D'Lima for valuable discussion, and the Lifesharing Program of the University of California-San Diego for providing human donor tissue.

Boris H. Borazjani, MD
Albert C. Chen, PhD
Won C. Bae, PhD
Robert L. Sah, MD, ScD
Gary S. Firestein, MD
Department of Orthopaedic Surgery (B.H.B.), MC 0942, Department of Bioengineering (A.C.C., W.C.B., and R.L.S.), MC 0412, and Department of Rheumatology, Allergy and Immunology (G.S.F.), MC 0656, University of California-San Diego, 9500 Gilman Drive, La Jolla, CA 92093

Shantanu Patil, MD
Scripps Center for Orthopaedic Research and Education, 11025 North Torrey Pines Road, Suite 140, La Jolla, CA 92037

William D. Bugbee, MD
Department of Orthopaedic Surgery, University of California-San Diego, La Jolla Clinic, 4150 Regents Park Row, #300, La Jolla, CA 92037. E-mail address : wbugbee@ucsd.edu

In support of their research for or preparation of this manuscript, one or more of the authors received grants or outside funding from University of California-San Diego Academic Senate, National Institutes of Health, National Science Foundation, and Arthritis Foundation. None of the authors received payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity. No commercial entity paid or directed, or agreed to pay or direct, any benefits to any research fund, foundation, educational institution, or other charitable or nonprofit organization with which the authors are affiliated or associated.

doi:10.2106/JBJS.E.00992

References

1. Bobic V. Arthroscopic osteochondral autograft transplantation in anterior cruciate ligament reconstruction: a preliminary clinical study. Knee Surg Sports Traumatol Arthrosc. 1996;3:262-4.

2. Garrett JC. Osteochondral allografts. Instr Course Lect. 1993;42:355-8.

3. Matsusue Y, Yamamuro T, Hama H. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy. 1993;9:318-21.

4. Gross AE, Silverstein EA, Falk J, Falk R, Langer F. The allotransplantation of partial joints in the treatment of osteoarthritis of the knee. Clin Orthop Relat Res. 1975;108:7-14.

5. McDermott AG, Langer F, Pritzker KP, Gross AE. Fresh small-fragment osteochondral allografts. Long-term follow-up study on first 100 cases. Clin Orthop Relat Res. 1985;197:96-102.

6. Butcher W, Patil S, Steklov N, Tucker BS, Bugbee W, D'Lima DD, Hoenecke HR Jr. Chondrocyte viability after osteochondral graft transplantation. Presented as a poster exhibit at the Annual Meeting of the American Academy of Orthopaedic Surgeons; 2004 Mar 10-14; San Francisco, CA.

7. Murray MM, Zurakowski D, Vrahas MS. The death of articular chondrocytes after intra-articular fracture in humans. J Trauma. 2004;56:128-31.

8. Colwell CW Jr, D'Lima DD, Hoenecke HR, Fronek J, Pulido P, Morris BA, Chung C, Resnick D, Lotz M. In vivo changes after mechanical injury. Clin Orthop Relat Res. 2001;391 Suppl:S116-23.

9. Kim HT, Lo MY, Pillarisetty R. Chondrocyte apoptosis following intraarticular fracture in humans. Osteoarthritis Cartilage. 2002;10:747-9.

10. Borrelli J Jr, Tinsley K, Ricci WM, Burns M, Karl IE, Hotchkiss R. Induction of chondrocyte apoptosis following impact load. J Orthop Trauma. 2003;17:635-41.

11. D'Lima DD, Hashimoto S, Chen PC, Colwell CW Jr, Lotz MK. Impact of mechanical trauma on matrix and cells. Clin Orthop Relat Res. 2001;391 Suppl:S90-9.

12. Krueger JA, Thisse P, Ewers BJ, Dvoracek-Driksna D, Orth MW, Haut RC. The extent and distribution of cell death and matrix damage in impacted chondral explants varies with the presence of underlying bone. J Biomech Eng. 2003;125:114-9.

13. Ewers BJ, Dvoracek-Driksna D, Orth MW, Haut RC. The extent of matrix damage and chondrocyte death in mechanically traumatized articular cartilage explants depends on rate of loading. J Orthop Res. 2001;19:779-84.

14. Milentijevic D, Torzilli PA. Influence of stress rate on water loss, matrix deformation and chondrocyte viability in impacted articular cartilage. J Biomech. 2005;38:493-502.

15. Chen CT, Burton-Wurster N, Borden C, Hueffer K, Bloom SE, Lust G. Chondrocyte necrosis and apoptosis in impact damaged articular cartilage. J Orthop Res. 2001;19:703-11.

16. Lucchinetti E, Adams CS, Horton WE Jr, Torzilli PA. Cartilage viability after repetitive loading: a preliminary report. Osteoarthritis Cartilage. 2002;10:71-81.

17. Repo RU, Finlay JB. Survival of articular cartilage after controlled impact. J Bone Joint Surg Am. 1977;59:1068-76.

18. Torzilli PA, Grigiene R, Borrelli J Jr, Helfet DL. Effect of impact load on articular cartilage: cell metabolism and viability, and matrix water content. J Biomech Eng. 1999;121:433-41.

19. Jeffrey JE, Gregory DW, Aspden RM. Matrix damage and chondrocyte viability following a single impact load on articular cartilage. Arch Biochem Biophys. 1995;322:87-96.

20. Milentijevic D, Helfet DL, Torzilli PA. Influence of stress magnitude on water loss and chondrocyte viability in impacted articular cartilage. J Biomech Eng. 2003;125:594-601.

21. D'Lima DD, Hashimoto S, Chen PC, Colwell CW Jr, Lotz MK. Human chondrocyte apoptosis in response to mechanical injury. Osteoarthritis Cartilage. 2001;9:712-9.

22. Lewis JL, Deloria LB, Oyen-Tiesma M, Thompson RC Jr, Ericson M, Oegema TR Jr. Cell death after cartilage impact occurs around matrix cracks. J Orthop Res. 2003;21:881-7.

23. Duda GN, Eilers M, Loh L, Hoffman JE, Kaab M, Schaser K. Chondrocyte death precedes structural damage in blunt impact trauma. Clin Orthop Relat Res. 2001;393:302-9.

24. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ. Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop Res. 2001;19:1140-6.

25. Morel V, Quinn TM. Cartilage injury by ramp compression near the gel diffusion rate. J Orthop Res. 2004;22:145-51.

26. Quinn TM, Allen RG, Schalet BJ, Perumbuli P, Hunziker EB. Matrix and cell injury due to sub-impact loading of adult bovine articular cartilage explants: effects of strain rate and peak stress. J Orthop Res. 2001;19:242-9.

27. Patwari P, Gaschen V, James IE, Berger E, Blake SM, Lark MW, Grodzinsky AJ, Hunziker EB. Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthritis Cartilage. 2004;12:245-52.

28. Mow V, Rosenwasser M. Articular cartilage: biomechanics. In: Woo SLY, Buckwalter JA, editors. Injury and repair of the musculoskeletal soft tissues. Park Ridge, IL: American Academy of Orthopaedic Surgeons; 1988. p. 427-63.

29. Panjabi MM, White AA 3rd. Biomechanics in the musculoskeletal system. Philadelphia: Churchill Livingstone; 2001.

30. Loening AM, James IE, Levenston ME, Badger AM, Frank EH, Kurz B, Nuttall ME, Hung HH, Blake SM, Grodzinsky AJ, Lark MW. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys. 2000;381:205-12.

31. Notzli H, Clark J. Deformation of loaded articular cartilage prepared for scanning electron microscopy with rapid freezing and freeze-substitution fixation. J Orthop Res. 1997;15:76-86.

32. Brown TD, Anderson DD, Nepola JV, Singerman RJ, Pedersen DR, Brand RA. Contact stress aberrations following imprecise reduction of simple tibial plateau fractures. J Orthop Res. 1988;6:851-62.

33. Lee D, Long SA, Adams JL, Chan G, Vaidya KS, Francis TA, Kikly K, Winkler JD, Sung CM, Debouck C, Richardson S, Levy MA, DeWolf WE Jr, Keller PM, Tomaszek T, Head MS, Ryan MD, Haltiwanger RC, Liang PH, Janson CA, McDevitt PJ, Johanson K, Concha NO, Chan W, Abdel-Meguid SS, Badger AM, Lark MW, Nadeau DP, Suva LJ, Gowen M, Nuttall ME. Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality. J Biol Chem. 2000;275:16007-14.

34. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature. 1998;391:43-50. Erratum in: Nature. 1998;393:6.

35. Nuttall ME, Nadeau DP, Fisher PW, Wang F, Keller PM, DeWolf WE Jr, Goldring MB, Badger AM, Lee D, Levy MA, Gowen M, Lark MW. Inhibition of caspase-3-like activity prevents apoptosis while retaining functionality of human chondrocytes in vitro. J Orthop Res. 2000;18:356-63.

36. Costouros JG, Dang AC, Kim HT. Comparison of chondrocyte apoptosis in vivo and in vitro following acute osteochondral injury. J Orthop Res. 2004;22:678-83.

37. Sakahira H, Enari M, Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature. 1998;391:20-1.

38. Noyes FR, Stabler CL. A system for grading articular cartilage lesions at arthroscopy. Am J Sports Med. 1989;17:505-13.

39. Gilmore RS, Palfrey AJ. Chondrocyte distribution in the articular cartilage of human femoral condyles. J Anat. 1988;157:23-31.

40. Huntley JS, Bush PG, McBirnie JM, Simpson AH, Hall AC. Chondrocyte death associated with human femoral osteochondral harvest as performed for mosaicplasty. J Bone Joint Surg Am. 2005;87:351-60.

41. Hunziker EB, Quinn TM. Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge. J Bone Joint Surg Am. 2003;85 Suppl 2:85-92.

42. Gonzalez RC, Woods RE, Eddins SL. Digital image processing using MATLAB. Upper Saddle River, NJ: Pearson Prentice Hall; 2004.

43. Kuhn K, Lotz M. Regulation of CD95 (Fas/APO-1)-induced apoptosis in human chondrocytes. Arthritis Rheum. 2001;44:1644-53.

44. Islam N, Haqqi TM, Jepsen KJ, Kraay M, Welter JF, Goldberg VM, Malemud CJ. Hydrostatic pressure induces apoptosis in human chondrocytes from osteoarthritic cartilage through up-regulation of tumor necrosis factor-alpha, inducible nitric oxide synthase, p53, c-myc, and bax-alpha, and suppression of bcl-2. J Cell Biochem. 2002;87:266-78.

45. Bush PG, Hodkinson PD, Hamilton GL, Hall AC. Viability and volume of in situ bovine articular chondrocytes-changes following a single impact and effects of medium osmolarity. Osteoarthritis Cartilage. 2005;13:54-65.

46. Borrelli J Jr, Torzilli PA, Grigiene R, Helfet DL. Effect of impact load on articular cartilage: development of an intra-articular fracture model. J Orthop Trauma. 1997;11:319-26.

47. Haut RC. Contact pressures in the patellofemoral joint during impact loading on the human flexed knee. J Orthop Res. 1989;7:272-80.

48. Whiteside RA, Jakob RP, Wyss UP, Mainil-Varlet P. Impact loading of articular cartilage during transplantation of osteochondral autograft. J Bone Joint Surg Br. 2005;87:1285-91. Erratum in: J Bone Joint Surg Br. 2005;87:1583.

49. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW Jr. In vitro and in vivo models of cartilage injury. J Bone Joint Surg Am. 2001;83(Suppl 2):22-4.

50. Quinn TM, Grodzinsky AJ, Hunziker EB, Sandy JD. Effects of injurious compression on matrix turnover around individual cells in calf articular cartilage explants. J Orthop Res. 1998;16:490-9.

51. Patwari P, Fay J, Cook MN, Badger AM, Kerin AJ, Lark MW, Grodzinsky AJ. In vitro models for investigation of the effects of acute mechanical injury on cartilage. Clin Orthop Relat Res. 2001;391 Suppl:S61-71.

52. Taylor SJ, Walker PS, Perry JS, Cannon SR, Woledge R. The forces in the distal femur and the knee during walking and other activities measured by telemetry. J Arthroplasty. 1998;13:428-37.

53. Taylor SJ, Walker PS. Forces and moments telemetered from two distal femoral replacements during various activities. J Biomech. 2001;34:839-48.

54. D'Lima DD, Patil S, Steklov N, Slamin JE, Colwell CW Jr. In vivo knee forces after total knee arthroplasty. Clin Orthop Relat Res. 2005;440:45-9.

55. Mow VC, Ratcliffe A, Chern KY, Kelly MA. Structure and function relationships of the menisci of the knee. In: Mow VC, Arnoczky SP, Jackson, DW, editors. Knee meniscus: basic and clinical foundations. New York: Raven Press; 1992. p 37-57.

56. Ahmed AM, Burke DL. In-vitro measurement of static pressure distribution in synovial joints—part I: tibial surface of the knee. J Biomech Eng. 1983;105:216-25.

57. Walker PS, Erkman MJ. The role of the menisci in force transmission across the knee. Clin Orthop Relat Res. 1975;109:184-92.

58. Loening A, Levenston M, James I, Nuttall M, Hung H, Gowen M, Grodzinsky A, Lark M. Injurious compression of bovine articular cartilage induces apoptosis before detectable mechanical damage. Trans Orthop Res Soc. 1999;24:42.

59. Quinn TM, Hunziker EB, Hauselmann HJ. Variation of cell and matrix morphologies in articular cartilage among locations in the adult human knee. Osteoarthritis Cartilage. 2005;13:672-8.

60. Eckstein F, Faber S, Muhlbauer R, Hohe J, Englmeier KH, Reiser M, Putz R. Functional adaptation of human joints to mechanical stimuli. Osteoarthritis Cartilage. 2002;10:44-50.

61. Franz T, Hasler EM, Hagg R, Weiler C, Jakob RP, Mainil-Varlet P. In situ compressive stiffness, biochemical composition, and structural integrity of articular cartilage of the human knee joint. Osteoarthritis Cartilage. 2001;9:582-92.

62. Shepherd DE, Seedhom BB. Thickness of human articular cartilage in joints of the lower limb. Ann Rheum Dis. 1999;58:27-34.

63. Lee DA, Frean SP, Lees P, Bader DL. Dynamic mechanical compression influences nitric oxide production by articular chondrocytes seeded in agarose. Biochem Biophys Res Commun. 1998;251:580-5.

64. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed myocyte cell death. J Clin Invest. 1995;96:2247-59.

65. DeMeester SL, Cobb JP, Hotchkiss RS, Osborne DF, Karl IE, Tinsley KW, Buchman TG. Stress-induced fractal rearrangement of the endothelial cell cytoskeleton causes apoptosis. Surgery. 1998;124:362-71.

66. Kuhn K, D'Lima DD, Hashimoto S, Lotz M. Cell death in cartilage. Osteoarthritis Cartilage. 2004;12:1-16.

67. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW Jr. Prevention of chondrocyte apoptosis. J Bone Joint Surg Am. 2001;83(Suppl 2):25-6.

68. Costouros JG, Dang AC, Kim HT. Inhibition of chondrocyte apoptosis in vivo following acute osteochondral injury. Osteoarthritis Cartilage. 2003;11:756-9.