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# Effect of Osteochondral Graft Insertion Forces on Chondrocyte Viability

Shantanu Patil,\* MD, William Butcher,<sup>†</sup> MD, Darryl D. D'Lima,\* MD, PhD, Nikolai Steklov,\* William D. Bugbee,<sup>†</sup> MD, and Heinz R. Hoenecke,<sup>†‡</sup> MD

From the \*Shiley Center for Orthopaedic Research & Education at Scripps Clinic and the <sup>†</sup>Division of Orthopaedic Surgery, Scripps Clinic, La Jolla, California

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**Background:** Because chondrocytes are responsible for articular cartilage matrix synthesis and maintenance, reduced chondrocyte viability could compromise graft survival, healing, and clinical outcome.

**Hypothesis:** Typical forces used in osteochondral grafting reduce the viability of the chondrocytes in the graft.

**Study Design:** Controlled laboratory study.

**Methods:** Osteochondral grafting was performed in 4 fresh-frozen cadaver knees (n = 16 per knee). Impact force was measured during extrusion of the donor graft from the harvester into the recipient site, seating the graft flush with the articular surface of the surrounding cartilage using a tamp, and recessing the graft surface below the recipient articular surface. The magnitudes of forces measured during cadaver surgery (200, 400, and 800 N) were reproduced using a drop-tower apparatus on 80 fresh osteochondral grafts harvested from knee blocks provided by tissue banks. Cell viability and glycosaminoglycan release in media were measured at 48 hours after injury.

**Results:** Forces were relatively low (range, 124-356 N) during graft extrusion from the harvester into the recipient defect or during flush seating (range, 191-418 N) of the graft. Attempts to recess the graft generated significantly greater force (range, 147-685;  $P < .01$ ). When the donor graft length was 2 mm longer than the depth of the recipient hole, the mean impact force generated was even higher (range, 240-1114 N) than the force seen in a donor graft of equal length. No reduction in viability was seen at 200-N and 400-N impacts. However, a significant decrease in chondrocyte viability was seen in the group impacted with 800 N (only 50% of cells were viable, compared with 91% in the sham group;  $P < .01$ ). Glycosaminoglycan levels in culture media did not correlate significantly with insertion force.

**Conclusion:** Typical graft insertion forces did not significantly reduce chondrocyte viability. However, increased graft length relative to the depth of the recipient hole and attempts to recess the graft generated higher forces, which reduced chondrocyte viability.

**Clinical Relevance:** Any theoretical benefits of cancellous bone compaction that may occur in grafts that are recessed or are longer than the recipient holes must be balanced against the potential reduction in chondrocyte viability.

**Keywords:** autologous osteochondral grafting; cartilage injury; chondrocyte viability; cartilage repair; osteochondral autograft transfer system (OATS); insertion forces

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Posttraumatic arthritis is one of the leading causes of secondary arthritis among the younger population.<sup>4,6,12</sup> Because the spontaneous healing potential of damaged

cartilage is minimal, untreated partial-thickness and full-thickness cartilage defects progress and can lead to post-traumatic osteoarthritis. No interventions are currently available that consistently prevent the development of this form of osteoarthritis.<sup>10</sup> The therapeutic management of young and middle-aged patients with osteochondral defects in the weightbearing regions of the knee continues to be a challenge.

Unlike procedures that target repair or regeneration, osteochondral grafting immediately fills the defect with mature articular cartilage and therefore qualifies as a cartilage lesion replacement procedure. Osteochondral grafting involves harvesting a cylindrical plug of cartilage attached to underlying subchondral bone and implanting

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<sup>‡</sup>Address correspondence to Heinz R. Hoenecke, MD, Division of Orthopaedic Surgery, Scripps Clinic, 11025 N. Torrey Pines Road, Suite 140, La Jolla, CA 92037 (e-mail: bhoeneck@san.rr.com and Blake.Judy@scrippshealth.org).

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the graft in the cartilage lesion. This procedure has potential for the treatment of isolated cartilage defects in young, active patients.<sup>7,8</sup> Autologous osteochondral grafts have been performed for over a decade with encouraging short-term to middle-term clinical results.<sup>10</sup>

One of the concerns regarding osteochondral grafting is the forces used to insert the graft. Mechanical loading of cartilage beyond the physiologic range has been shown to result in chondrocyte death.<sup>3,11,13,17,18</sup> After an injurious compression, chondrocytes undergo apoptosis, which continues to increase up to 7 days after injury.<sup>3,14</sup> The net impact force generated during an osteochondral grafting procedure could therefore affect the viability of the chondrocytes in the osteochondral graft. Chondrocytes are responsible for matrix synthesis and maintenance. In addition, cartilage injury is accompanied by matrix damage and eventual degradation of cartilage, leading to arthritic changes in the joint.<sup>15</sup> Reduced chondrocyte viability could compromise graft survival, healing, and clinical outcomes.

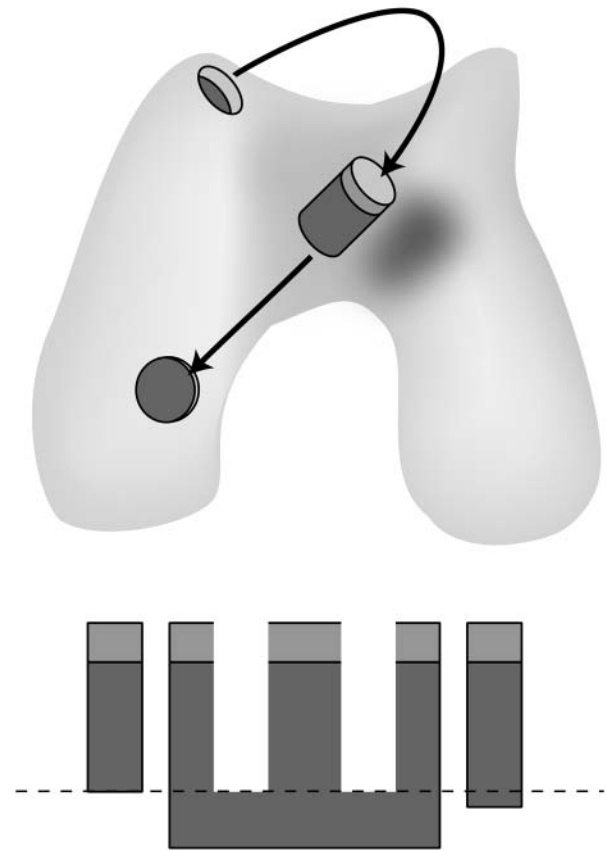
This study was conducted in 2 phases. In the first phase, we measured osteochondral graft insertion forces during cadaveric surgery. In the second phase, we monitored chondrocyte death and markers of matrix degradation after subjecting live osteochondral grafts to insertional forces. Our objective was to determine whether the forces generated during osteochondral grafting cause cell death and matrix degeneration.

## MATERIALS AND METHODS

### Measurement of Graft Insertion Forces

Osteochondral graft transplantation was carried out in 4 fresh-frozen cadaveric knees. Cylindrical osteochondral grafts 8 mm in diameter were harvested from the non-weightbearing regions of the femoral condyles and transplanted into prepared donor areas in the weightbearing regions. Three surgeons performed the technical procedure using standard manufacturer-provided equipment (ProCART, Aesculap USA Inc, Center Valley, Pennsylvania). The harvested grafts were trimmed to 2 lengths—either equal to or 2 mm longer in length than the recipient holes (nominal depth of recipient holes was ~15 mm; Figure 1). This was achieved by trimming the graft to an even length at 4 quadrants around the circumference followed by creation of a recipient hole of appropriate depth.

The grafts were transplanted in 3 steps. First, the graft was extruded from the harvester into the recipient hole (extrusion) by tapping the graft through an inserter using a mallet. Extrusion from the harvester into the recipient hole typically resulted in ~2 mm of graft left protruding above the surrounding cartilage surface. Next, a mallet and 8-mm tamp were used to seat the graft such that the articular surface of the graft was flush with that of the surrounding cartilage (flush seating). Finally, the graft was tapped with a 6-mm tamp such that the articular surface of the graft was ~2 mm below the surface of the surrounding cartilage (recessing). The inserter and tamp were instrumented with a load cell (multipurpose force sensor, PCB

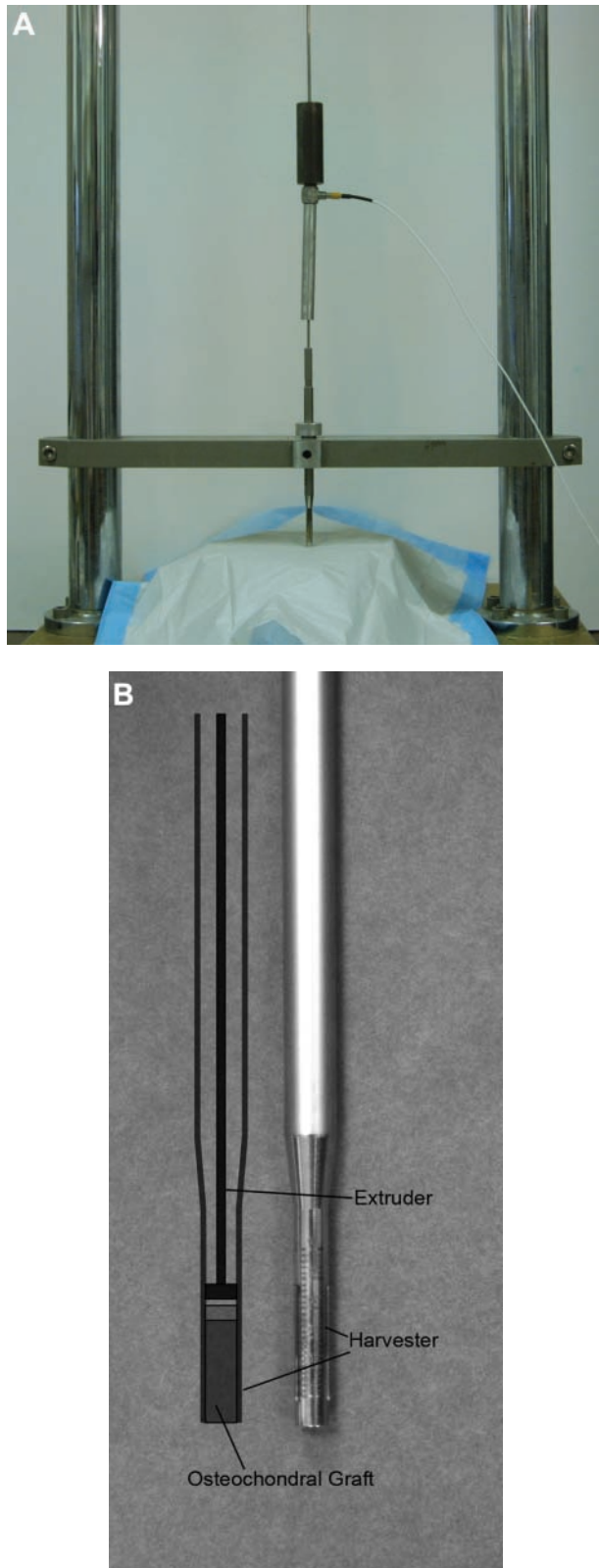


**Figure 1.** Diagram demonstrating donor graft length relative to depth of recipient hole. Left, graft length was matched to depth of recipient hole; right, graft length was 2 mm longer than depth of recipient hole.

Piezolectronics Inc, Depew, New York). The total number of mallet taps and the peak forces for each tap were recorded during each of the 3 stages of graft insertion.

### Measurement of Cartilage Injury

Fresh knee blocks were obtained from tissue banks within 72 hours of death. Selection criteria were donor age between 18 and 45 years and no or mild surface fibrillation of articular cartilage. A total of 80 osteochondral grafts were obtained from human femoral condyles using the same harvesting technique and instrumentation. To account for variability among donors and location within the knee, we attempted to match the site of graft harvest. Grafts were harvested from adjacent areas at the same location and assigned to the different loading groups for each time point. An inserter was assembled on the harvested grafts (inside the harvester) and the assembly mounted at the base of a drop tower (Figure 2). This assembly simulated graft insertion forces (but without inserting the graft in a recipient site). Upon analysis of measurements made during the cadaveric surgery, we noted that the highest impacts were generated during recession of grafts that were longer than the recipient depth (5 of these impacts



**Figure 2.** A, the drop tower used to simulate surgical forces on the live harvested graft. The height of the drop weight was adjusted to generate different levels of impact. B, close-up view and diagram of graft assembly in the harvester during impact.

would average approximately 800 N). We therefore chose to simulate 5 impacts at 800 N and chose to compare the results against 5 impacts at 400 or 200 N. A weight was dropped on the load cell-inserter-graft assembly 5 times to simulate 5 mallet taps. The height was adjusted to generate nominal peak forces of 200, 400, or 800 N at impact on the load cell on the inserter to simulate the range of forces that were recorded during the various stages of graft insertion during the cadaveric experiments described in the first phase. Grafts were assigned to each peak force group (200, 400, or 800 N;  $n = 20$  per group). Peak impact forces of 200, 400, and 800 N on a graft with a diameter of 8 mm would generate average peak stresses of 4, 8, and, 16 MPa, respectively. In addition, a fourth control group ( $n = 20$ ) was harvested and assembled on the impact tower but not subjected to any impact. After the prescribed impact, the full-thickness chondral portion was sharply excised from subchondral bone using a scalpel and cultured in Dulbecco's Modified Eagle's Medium supplemented with 0.1% serum. Chondrocyte viability was measured at 48 and 120 hours after impact. These time points were based on pilot studies that did not find significant changes in viability after 5 days post injury. Thin sections of the cartilage explant were obtained perpendicular to the articular surface; these were incubated in calcein AM to stain live cells. Dead cells were counterstained with propidium iodide. Digital photographs of each section were recorded with a fluorescent microscope at  $\times 10$ . Three locations were analyzed: 1 field of view each including the superficial, middle, and deep zones. The top 10% of the cartilage thickness was defined as the superficial zone; the remaining 90% was equally divided to represent the middle and deep zones (Figure 3, column A). Cell viability was recorded as the percentage of calcein-positive cells relative to the total number of calcein and propidium iodide-positive cells. To reduce the effect of surgical harvesting on the cell viability, we excluded the cut margins of the graft from the cell viability analysis. In pilot studies, cell viability immediately after harvest was mean  $93\% \pm 4\%$ . To quantify matrix damage after impact, glycosaminoglycans released in the media during culture were measured by the dimethyl methylene blue assay.<sup>5</sup>

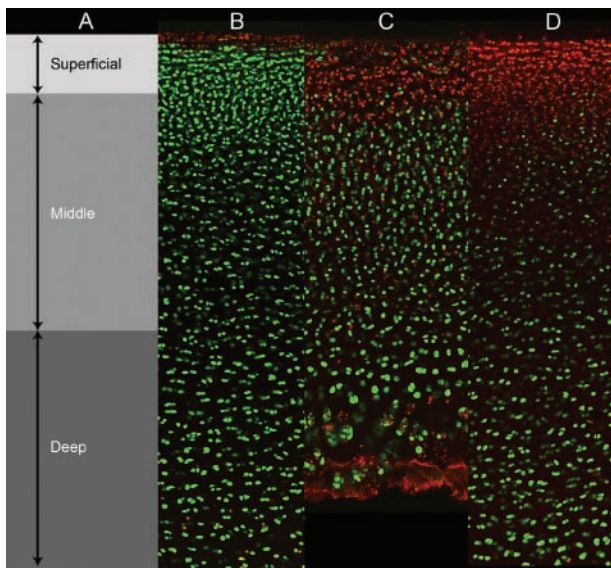
### Statistical Analysis

Multifactor analysis of variance with the Tukey post hoc test was used to detect statistically significant differences in cell viability and glycosaminoglycan loss between groups of grafts impacted at different forces. We determined that a sample size of 10 per group could detect a mean difference in viability of 20% or glycosaminoglycan loss of 30% or more with a power  $>80\%$  at  $\alpha = .05$ .

## RESULTS

### Measurement of Graft Insertion Forces

There was a surgeon-to-surgeon variability in the number of taps (Table 1) and in the magnitude of forces. One surgeon tended to use more taps with lower forces. However, the differences in mean forces were not statistically significant.



**Figure 3.** A, Diagram showing the definition of superficial (top 10%), middle (40%), and deep zones (40%). Representative photomicrographs showing cell viability of B, control (sham impact) group specimen (viability = 92%); C, 400-N impact specimen (viability = 67%); and D, 800-N impact specimen (viability = 53%).

**TABLE 1**

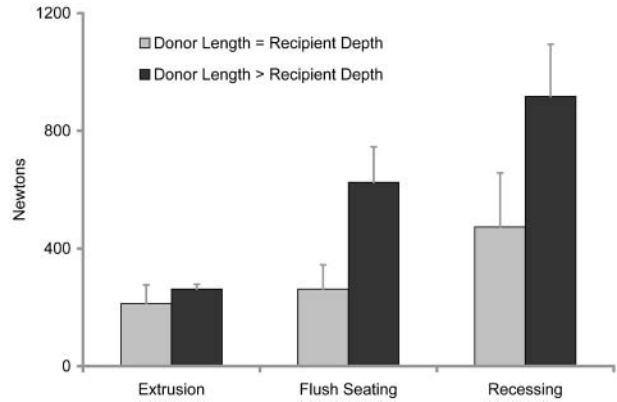
Number of Mallet Impacts (Mean ± Standard Deviation) Recorded During Grafting

Grafting Stage	Extrusion	Flush Seating	Recessing
	44 ± 20	12 ± 5	11 ± 4

Forces were relatively low (range, 124-356 N) during graft extrusion from the harvester into the recipient defect or during flush seating (range, 191-418 N) of the graft (Figure 4). Attempts to recess the graft generated significantly greater force, (range, 147-685 N;  $P < .01$ ). When the donor graft length was 2 mm longer than the depth of the recipient hole, the mean impact force generated was even higher (absolute range, 240-1114 N; median relative increase of 172 N) than the force seen in a donor graft of equal length (Figure 4). To simulate the mallet taps with better reproducibility, we used a drop-tower apparatus. A comparison between forces recorded during the osteochondral grafting and those measured using the drop-tower apparatus is shown in Figure 5.

**Measurement of Cartilage Injury**

Mean chondrocyte viability was 91% in sham impact (control) grafts (Figure 3). A significant decrease in chondrocyte viability was seen at 800 N, with only 50% of cells remaining viable at 120 hours ( $P < .01$ ). The reduction in cell viability under impact loads of 200 N (to 80%) and 400 N (to 67%) was not statistically significant. However, the group means were linearly correlated strongly with impact



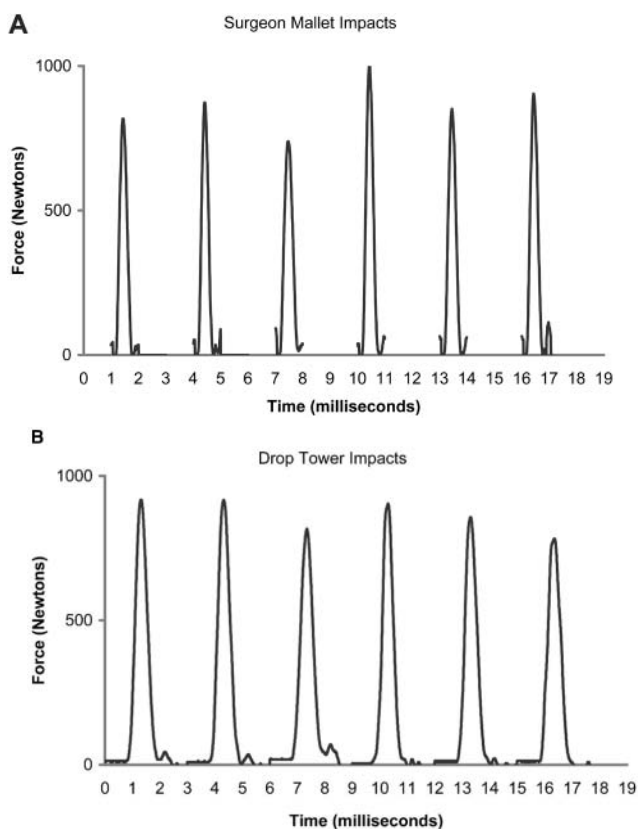
**Figure 4.** Mean forces (± standard deviation) were measured during the 3 stages of graft insertion: extruding the graft from the harvester into the recipient hole (extrusion), seating the graft with a tamp such that the articular surface of the graft was flush with that of the surrounding cartilage (flush seating), and recessing the graft such that the articular surface of the graft was ~2 mm below the surface of the surrounding cartilage (recessing). The highest force was generated while attempting to recess the graft. Forces were also recorded when the graft length was 2 mm longer than the recipient hole. Significantly higher forces were recorded in the flush seating and recessing stages of graft insertion.

load ( $R^2 = .89, P = .03$  at 48 hours;  $R^2 = .98, P < .001$  at 120 hours). The mean percentage of dead cells in the superficial zone was consistently greater than that in the middle or deeper zones and was statistically significant for the 800-N impact group (Figure 6;  $P = .01$ ). The rate of release of glycosaminoglycans decreased significantly with longer time in culture (Figure 7;  $P < .01$ ). Pairwise comparisons did not yield a statistically significant difference between groups ( $P = .56$ ).

**DISCUSSION**

The recommendations for graft insertion vary from flush seating to recessed seating inside the recipient holes. The rationale for seating the graft flush is to reconstruct the articular surface at the defect. On the other hand, recessing the graft can protect it from high contact forces in the immediate postoperative period,<sup>2</sup> and cartilage remodeling can correct small magnitudes of recession.<sup>9</sup> In this study, substantial force was generated during graft insertion in the osteochondral graft insertion procedure. Initial insertion of the graft by extrusion from the harvester did not require much force, but tamping the graft surface to seat it flush with the surrounding articular surface required higher force. Recessing the graft required the highest force. These results suggest that recession should be avoided or, if deemed necessary, the recipient hole should be lengthened to avoid high insertion force.

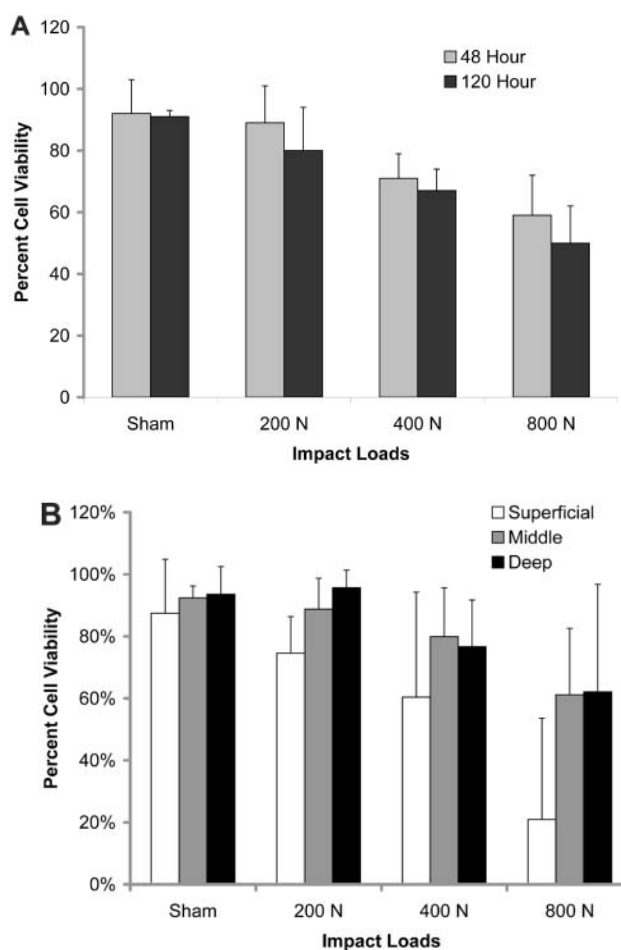
Some instrument manufacturers recommend harvesting a graft longer in length than the depth of the recipient hole (*Osteochondral Autograft Transfer System Surgical Technique*



**Figure 5.** Forces measured during the cadaveric surgery were similar to those generated using the drop tower. A, forces recorded during graft recession (5 representative peaks). B, forces recorded while impacting the explants in the 800-N group. The x-axis represents time in milliseconds but the data between impacts have been truncated to fit the taps in each graph.

*Manual*, Arthrex Inc, Naples, Florida, 2000). This technique tends to compact the cancellous bone in the graft, potentially improving the biomechanical stability. However, the results of the cadaver surgery indicate that higher forces were required for the final seating of these longer grafts. In general, compaction of the cancellous bone, which occurs when recessing a graft of matching length or seating a longer graft, was associated with higher forces. Therefore, any potential benefit of graft compaction should be weighed against the potential for reduced cell viability.

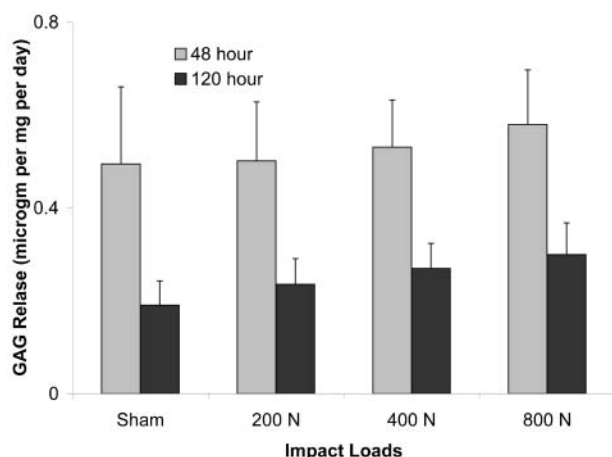
Significant chondrocyte death has been reported 24 hours after insertion of osteochondral grafts into defects created in sheep knees.<sup>16</sup> In that study, forces during insertion were not measured. Our finding that force up to 400 N did not significantly reduce chondrocyte viability is encouraging, which is in contrast to previous reports of chondrocyte death in full-thickness cartilage explants that were mechanically injured at a lower force.<sup>3,11,14,18</sup> In osteochondral grafts, the attached subchondral bone may protect the cartilage, thus preserving chondrocyte viability to some extent. In addition, in the harvester, the cartilage is typically in radially confined compression during the process of inserting the graft. Most in vitro models of chondrocyte death injured the cartilage under radially unconfined compression.<sup>3,14</sup> During the



**Figure 6.** Osteochondral grafts were subjected to 5 impacts of 200, 400, or 800 N. Cell viability was measured using calcein staining. A, cell viability after 48 and 120 hours in culture was significantly lower in the 800-N impact group than in the sham group. B, the percentage of dead cells in the superficial zone was consistently greater than that in the middle or deeper zones.

cadaver surgery, initial graft insertion (extrusion from the harvester) and tamping did not usually require a force >400 N. This result suggests that the typical force applied during a routine osteochondral grafting procedure may not have any immediately deleterious effects.

A previous study reported reduced chondrocyte viability in 15-mm diameter osteochondral explants after manual impaction (mean stresses of 13 MPa) simulating an allograft transplant procedure.<sup>1</sup> The objective of the present study was to reproduce typical loads generated during the various steps of an autologous osteochondral transplant procedure and to determine whether the magnitude of the impact loads correlated with chondrocyte viability. Differences between studies included differences in the sizes of the osteochondral explants (15-mm vs 8-mm diameter), peak stresses (13 MPa vs 4, 8, and 16 MPa), and number of impacts (10 vs 5). Nevertheless, percentage cell death reported was similar, indicating that the threshold stress value for inducing cell death lies between 8 and 13



**Figure 7.** The rate of glycosaminoglycan (GAG) release decreased significantly with time in culture, indicating the more acute GAG loss after tissue harvesting. However, differences between impact load groups were not statistically significant.

MPa. For the explants used in the present study, the impact force threshold would lie between 400 and 650 N.

Glycosaminoglycan release did not appear to correlate with the force applied. Glycosaminoglycan release is often used as a measure of cartilage injury and matrix degradation. These results suggest that matrix degradation is not increased in the immediate postoperative period by the impact force typically used in osteochondral grafting. Cartilage was removed from the bone after simulated impact for culturing to remove any biologic effect of the presence of subchondral bone in culture. However, the surgical trauma of separating the cartilage from the bone could have led to increased glycosaminoglycan release, which may have overshadowed any smaller differences in glycosaminoglycan release between groups. In addition, the longer-term effect of reduced chondrocyte viability, which may result in further matrix degradation, was not tested in the present study.

This study measured forces in fresh-frozen cadaveric specimens in the laboratory and not during actual surgery. Osteochondral grafting forces measured during live surgery may be different than those measured in a cadaveric setting. We measured the length of the graft at 4 quadrants around the circumference and created the corresponding recipient hole. However, part of the variation in forces measured could be explained by some surgical variation in graft length and depth of recession. These data are representative of a single surgical manufacturer's instrument design and may not apply to other instrument designs or surgical techniques. For the viability experiments, we chose not to insert into a recipient site because controlling the force of each impact, keeping the femur sterile during the process of inserting multiple grafts from the same knee, and extracting the inserted grafts for postimpact experiments was technically challenging. Although the drop tower-generated impacts were similar in magnitude to surgeon-generated impacts, the longer

duration of the former could have affected cell viability. The number of mallet impacts (5) simulated by the drop tower was chosen based on data collected during the cadaver surgery. A higher number of mallet impacts may have further reduced chondrocyte viability. Finally, the study only measured chondrocyte viability and glycosaminoglycan release up to 120 hours after simulated impact. The effect of grafting forces on longer-term viability or matrix degradation was not measured.

## CONCLUSION

Autologous osteochondral transplantation is a frequently recommended therapeutic option for full-thickness osteochondral defects. This study reveals that typical insertion forces do not significantly damage the transplanted tissue in the immediate postoperative method. However, increase in graft length relative to the depth of the recipient hole and attempts to recess the graft generated higher forces and reduced chondrocyte viability and are therefore not recommended.

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