

# Impaction Affects Cell Viability in Osteochondral Tissues During Transplantation

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**ABSTRACT:** Symptomatic full-thickness defects of articular cartilage are increasingly treated with osteochondral allografts. The present study focused on the viability of cells in cartilage that had been impact loaded by the instruments used in preparation of the cartilage for transplantation. Osteochondral plugs were removed and reimplanted using a plastic tamp device fitted with a load cell. Plugs were examined at time 0 or after 48 hours or 7 days of tissue culture. During insertion, the force was  $25 \pm 6$  N and increased with time to a peak of  $307 \pm 84$  N. On average, 18 taps were necessary for the insertion of each plug, and the applied total impulse ranged from 5.7 to 17.8 N. Peak force and total impulse were highly correlated ( $R^2=0.76$ ,  $P<.001$ ). Typically, a loading cycle lasted <10 milliseconds with peak loading rates up to  $133 \pm 25$  kN/s for

each individual plug. The loading rate was dependent on the peak force, ie, the higher the applied load, the higher the rate. Cell death was 60% in the upper zone for all groups at all time points and lower (20%) for the middle and deep zones. Cell death appeared to be higher in all zones of the impacted group. Investigating the whole plug at time 0, cell viability was significantly lower for the impacted plugs compared to control (dead:  $P<.02$ , living:  $P=.05$ ). After 48 hours, as well as after 7 days, mean cell viability remained affected. These data suggest that the range of loading used in the manipulation of cartilage tissue in transplantation must be carefully considered if cell preservation is to be maintained.

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## INTRODUCTION

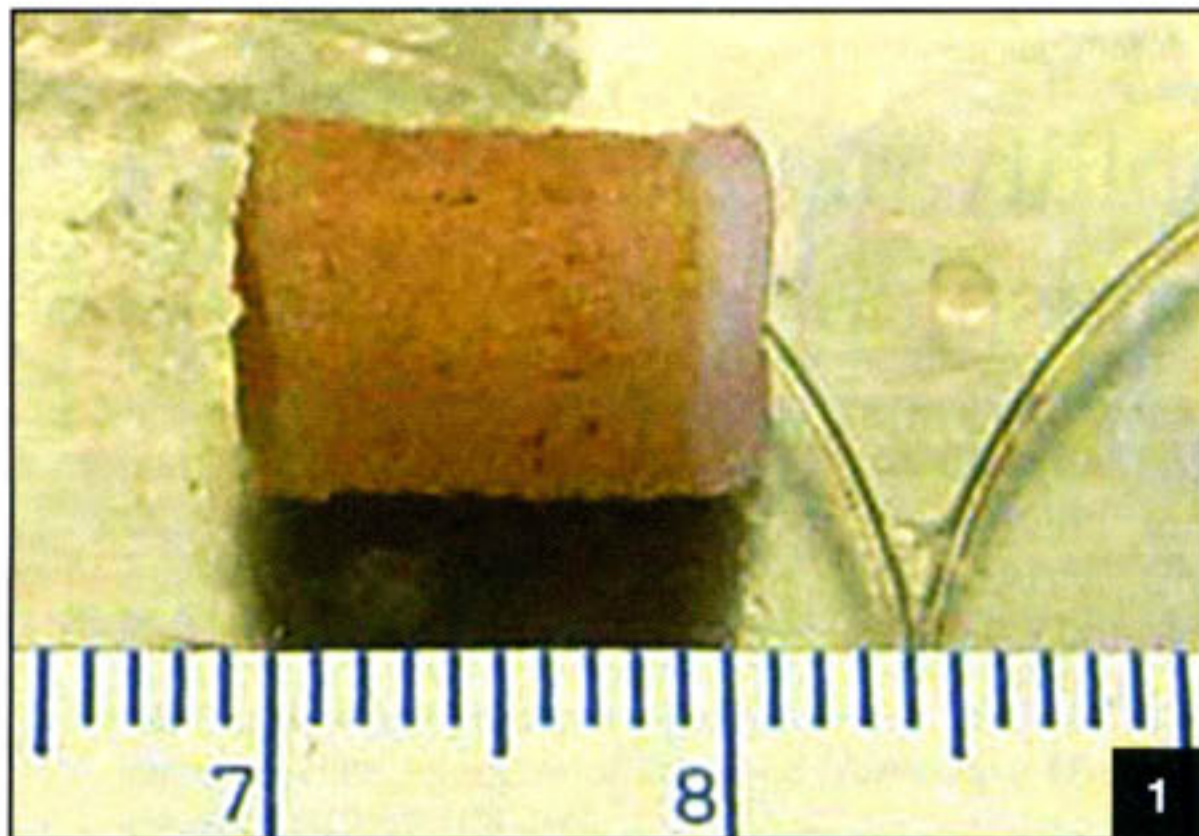
Experience with fresh osteochondral allografting extends over two decades with Gross et al<sup>8</sup> and Finkelstein et al<sup>6</sup> reporting their use of fresh allografts in the treatment of large, traumatic defects of the femoral condyle and tibial plateau. Despite the high interest in the behavior of cartilage preserved for cartilage transplantation, little has been done to examine the effects of the surgical pro-

cedure itself on the transplanted tissue. Repair of an osteochondral defect with an osteochondral plug involves significant physical manipulation of the tissues where the cartilage is impact loaded by the devices during retrieval, manipulation, and placement of the plug. Numerous studies have addressed the effects on cell viability of articular cartilage harvested for osteochondral plugs; however, less is known about the effects of implantation of these grafts.<sup>1-4,7,17,20</sup>

During implantation the donated osteochondral allograft material potentially undergoes acute trauma as the surgeon taps on the articular surface with a surgical mallet delivering an impact force to the articular surface. The force that each surgeon uses during this process varies and has yet to be studied or quantified. More specifically, how this impact affects the cell viability of the implanted donor articular cartilage has not been investigated. It has been demonstrated that cartilage homeostasis is disrupted

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**Figure 1.** Osteochondral plug 8-mm in diameter. The plug was trimmed to 10 mm in length.



**Figure 2.** Modified tamp device holding a light weight 1 kN load cell (Entran).



**Figure 3.** The plugs were inserted into previously prepared recipient holes using the modified tamp device.

with mechanical trauma,<sup>9,10,16</sup> which can lead eventually to degeneration. The present study describes the mechanical conditions of osteochondral plugs that undergo loading by the instruments used in preparation of the cartilage for transplantation and focuses on cell viability in cartilage after impaction.

## MATERIALS AND METHODS

### *Articular Cartilage Sampling and Culture*

Articular cartilage samples were taken from adult bovine tibial plateaus obtained immediately after sacrifice (within 3 hours of death) to preserve cell viability. Samples were removed using sterile technique, minimizing contamination for future culture steps. Each sample consisted of an 8-mm diameter osteochondral plug obtained by using the Arthrex OATS system (Naples, Fla) (Figure 1). Eighteen samples were harvested from the bovine tibial plateaus. Once these plugs were obtained, the bone was cut so

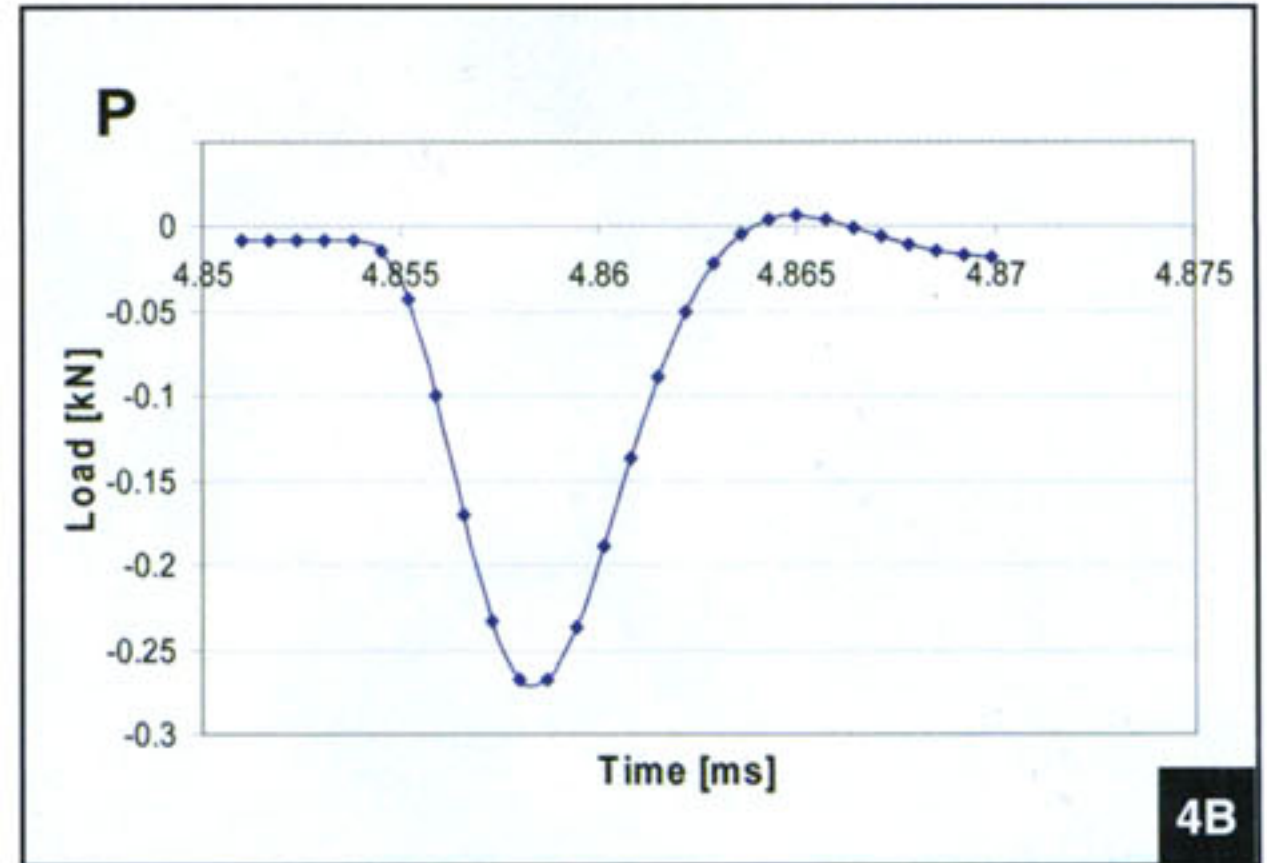
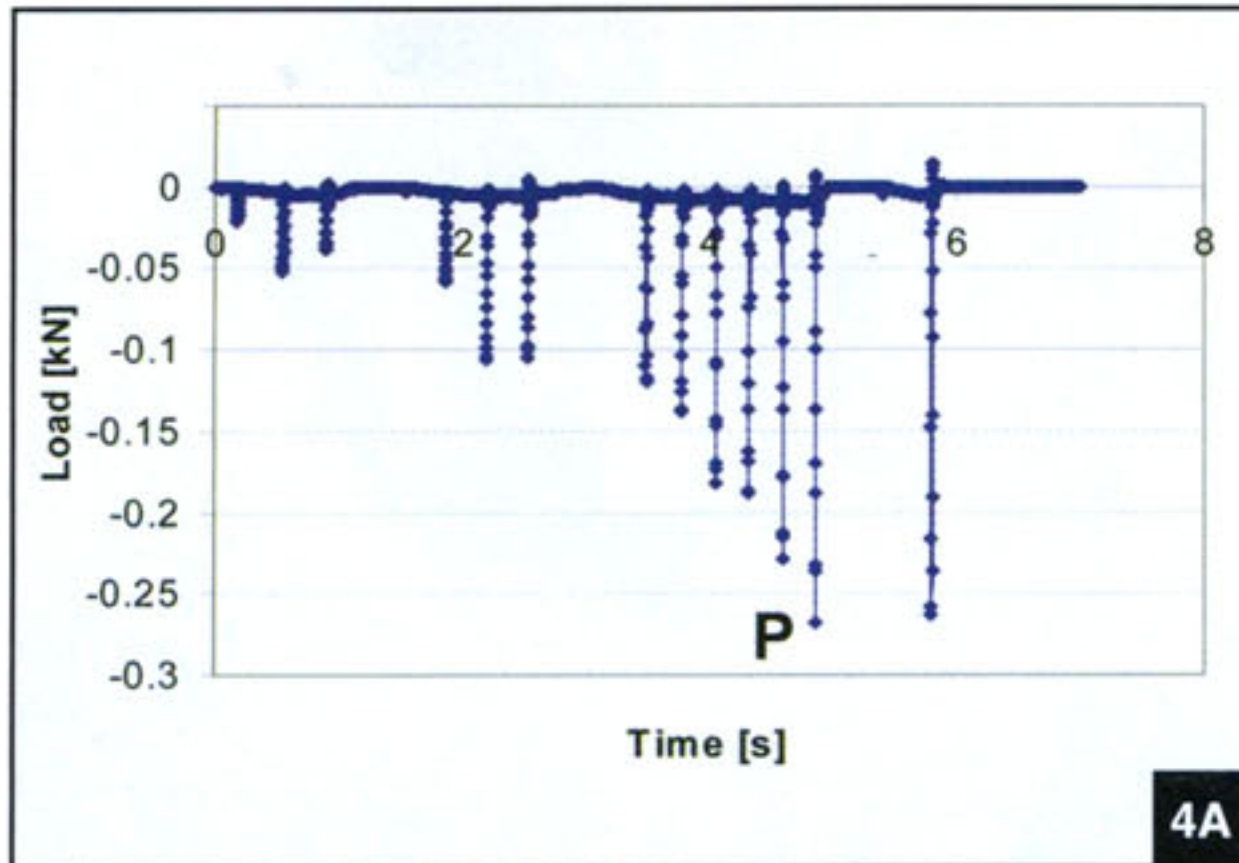
that the entire depth of the plug was 10 mm. The recipient holes were made using the recipient harvester, with a total depth of >10 mm. The plugs from making these recipient holes were saved and used as controls ("removal only" control plugs). Tissue culture involved placing the samples in 3 mL of minimal essential media with 10% fetal bovine serum, glutamate, nonessential amino acids, anti-fungal agents, and antimicrobial agents at 37°C for 48 hours or 7 days. Media was changed every 3 days.

### *Implantation and Measurement of Impact Force*

Using a plastic tamp device, modified to include a 1 kN load cell (Entran, Measurement Specialists Inc, Hampton, Va; error <0.41%), the donor plugs were placed into the recipient holes (Figures 2 and 3). The plugs were then tapped into the holes using this tamp and a surgical hammer, with the load cell measuring the force during implantation at a sampling frequency of 1.5 kHz. Once these plugs were inserted, they were carefully retrieved immediately without interference with the cartilage layer and placed in tissue culture as described above for 48 hours or 7 days. Those 18 plugs became the experimental group, which was compared to the 18 plugs from the previously described control group. In both groups, 6 plugs each were randomly assigned to be investigated at 3 different time points (0 hours, 48 hours, and 7 days).

### *Cell Viability Measurement*

Cell viability was measured using confocal microscopy<sup>12</sup> at three time points: 0 hours, 48 hours, and 7 days. At the end of each time point, the respective osteochondral plugs were removed, the subchondral bone was cut away, and a 2×2-mm portion in the center of the plug was stained to assess cell viability. The 2×2-mm slices were placed in 4 μM calcein-AM and 8 μM ethidium homodimer (Molecular Probes Inc, Portland, Ore) at room temperature for 30-60 minutes. Samples were then ex-



**Figure 4.** Example of a force-time plot showing the load profile during insertion consisting of 13 taps (A). Typically, the operating surgeon started carefully with the first hit (21 N). Then the applied load at each individual hit increased to a maximum (268 N) before it decreased again towards the end. Blow-up of the peak load (P) in a millisecond time scale (B). Note the steep increase of the loading rate and its short duration.

amined on a confocal laser-scanning microscope (MRC-1000; BioRad, Hemel Hempstead/Cambridge, England) equipped with an argon laser and necessary filters (fluorescein and rhodamine). Digital images were captured and stored on a PC. This technique is used to identify living cells labeled with calcein-AM (green fluorescence) and dead cells labeled with ethidium homodimer (red fluorescence). Estimates of living and dead cells were obtained for the upper one-third, middle one-third, and deep one-third of the uncalcified layer of cartilage and related to the total cell count of the specimen. All cells, living and dead, from all layers added up to 100%.

Statistical analysis was performed using SPSS for Windows (version 10.0; SPSS Inc, Chicago, Ill). Linear regression analyses were performed to identify relationships between loading parameters. Non-parametric tests were conducted to determine whether differences occurred between testing groups with respect to cell viability. The level of statistical significance was set to a type I error of 5%.

## RESULTS

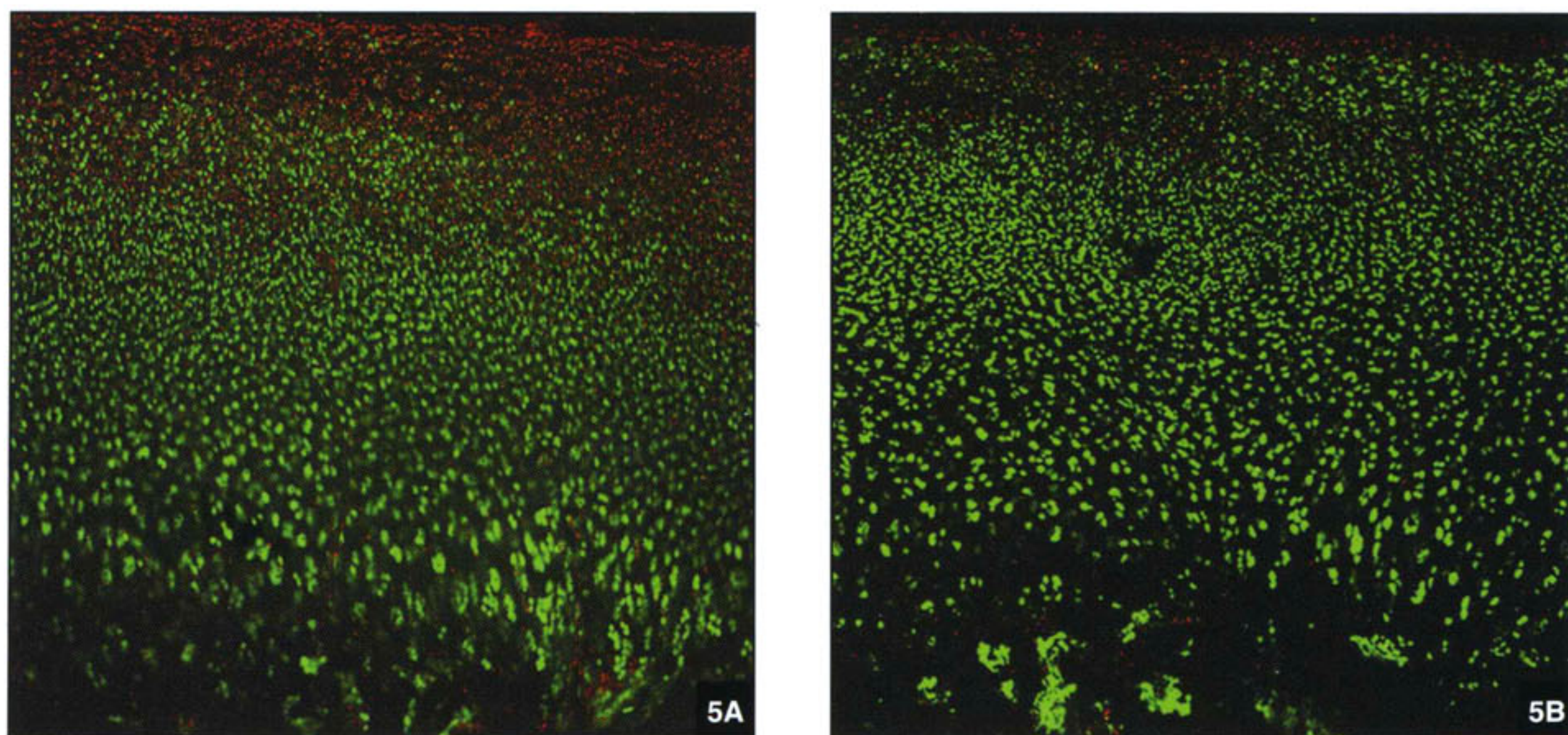
Each plug was reimplanted with 18 taps on average in  $5.8 \pm 0.8$  seconds. During insertion, the force was low at the beginning ( $25 \pm 6$  N) and increased with time to a peak of  $307 \pm 84$  N (range: 256-495 N) before it decreased again towards the end of the surface matching process with the host tissue (Figure 4). The applied impulse (ie, the integral of force over time during insertion) ranged from 5.7-17.8 N between individual plugs. Peak force and applied impulse were highly correlated ( $R^2=0.76$ ,  $P<.001$ ). Typically, a loading cycle during tapping lasted  $<10$  milliseconds with peak loading rates up to  $133 \pm 25$  kN/s for each individual plug. The loading rate was dependent on the peak force, ie, the higher the applied load, the higher the rate.

Thirty plugs were available for detailed microscopic analysis. There were at least five samples per investigated group per given time point except for the experimental group at day 7. In this subgroup, only three plugs were available for analysis. With  $>60\%$ , cell death was highest in the upper zone for all groups at all time points (Figure 5). Cell death was considerably lower for the middle and deep zone where it approximated 20%. In general, cell death slightly increased with culturing time. In particular, the upper and deep zone appeared to be affected. The above observations were true for the experimental and control groups (Figure 6).

Although cell death appeared to be higher in all zones of the experimental group, this effect vanished with time and could not be manifested by statistical means (Figure 6). Investigating the whole plug—neglecting zonal divisions—at time 0 hours, cell viability was significantly lower for the impacted plugs compared to control plugs (dead:  $P<.02$ , living:  $P=.05$ ). After 48 hours, as well as after 7 days, mean cell viability remained better for the nonimpacted grafts; however, this difference was no longer significant.

## DISCUSSION

The data in the present study indicated a significantly higher death of chondrocytes at time 0 hours in osteochondral plugs manipulated with the retrieval and placement instruments. Although the trends continued with 48 hours and 7 days in culture, these differences were no longer statistically significant (which might be related to a decreased sample size at day 7). These results are similar to those obtained by Nabavi-Tabrizi et al<sup>14</sup> who demonstrated significant death levels of chondrocytes 24 hours after impaction. Impact loading can be deleterious



**Figure 5.** Overlays of images from the confocal microscope taken with fluorescein and rhodamine filters. Living cells appear green and dead cells red. Specimen of the time point zero experimental group (A). Specimen of the time point zero control group (B). Note the presence of cell death in the upper layer of cartilage in A compared to B.

to articular cartilage cells, resulting ultimately in cartilage degeneration. Nabavi-Tabrizi et al<sup>14</sup> studied metal versus plastic surfaces in the impaction devices and found no differences but did not monitor the applied load on the cartilage. The present study measured the applied load. Further testing of the load ranges and variations is being performed to determine the parameters of loading during manipulation of the osteochondral plug. Such data would be useful in the refinement of instruments used in the manipulation of cartilage tissue for implantation.

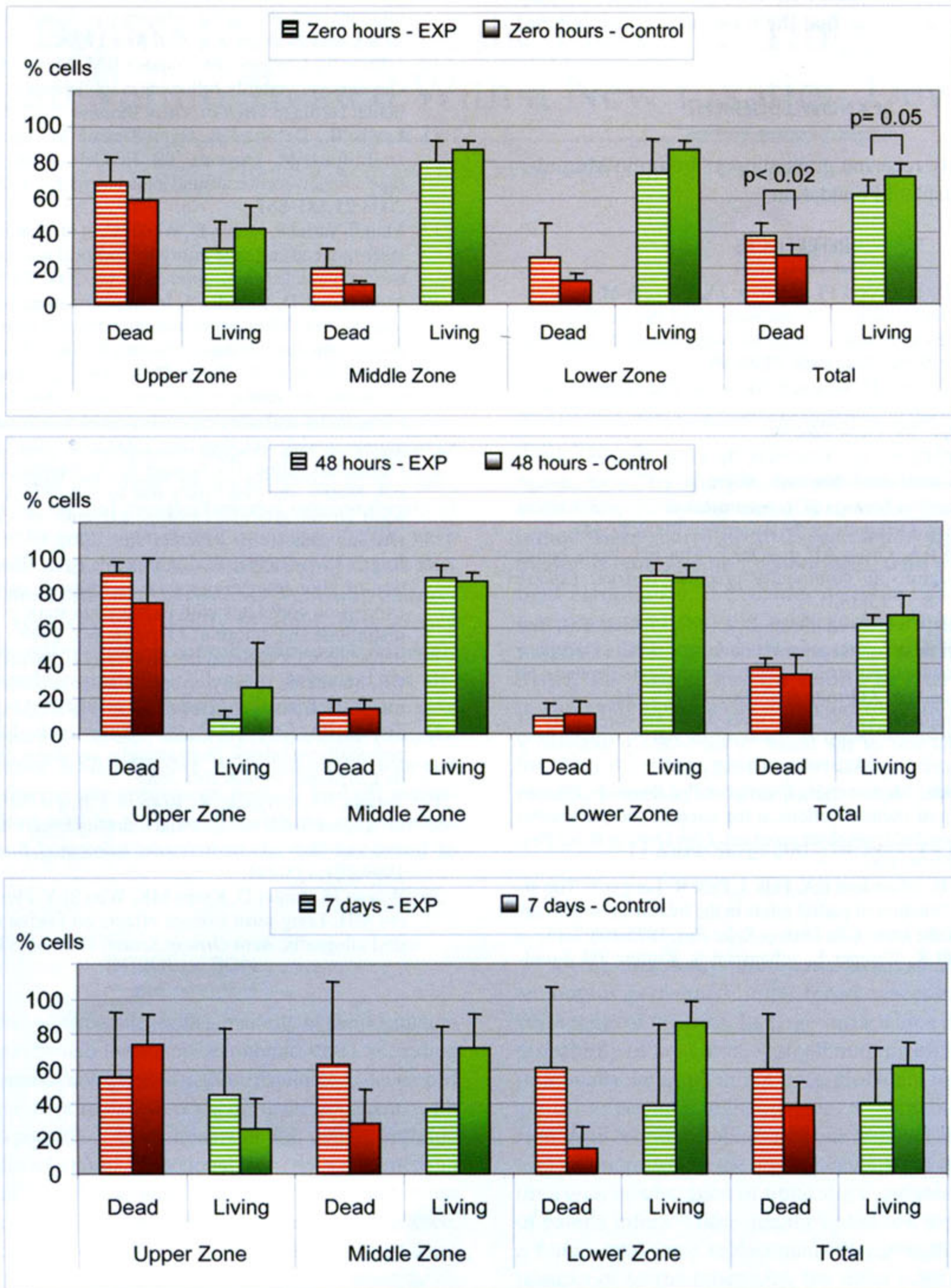
The results of the present study add to the large body of literature indicating that there is a limit to which articular cartilage can be mechanically loaded beyond which results in cell death and failure of the cartilage. Matrix changes and death have been examined in a similar fashion to the present study.<sup>18,19</sup> Within 24 hours following impaction of the cartilage, 18% of the chondrocytes had died. Quinn et al<sup>15</sup> applied strain rates from  $3 \times 10^{-5}$  to  $0.5 \text{ s}^{-1}$ . They found that at the slowest rates there was excessive cell death throughout the whole tissue without visible matrix damage, whereas at faster rates cell death was confined to the superficial layer and occurred adjacent to fissures.

Few studies investigated loading rates (rather than strain rates). Those investigations allow a better comparison with the data of this study as only forces were measured during tapping. Lewis et al<sup>11</sup> generated blunt trauma with a single impact of 53 MPa at 212 MPa/s. They found that cell death correlated with the degree of mechanical disruption of the matrix. Ewers et al<sup>5</sup> loaded cartilage explants in unconfined compression with 40 MPa using two

different loading rates (40 and 900 MPa/s). Greater matrix damage was found with the high loading rate but less cell death was generated when compared to the low loading rate. In a study by Milentijevic and Torzilli,<sup>13</sup> the effect of contact stress was systematically investigated using bovine cartilage in confined compression. Applying peak stresses from 10-60 MPa at a loading rate of 350 MPa/s, they observed that cell death increased in depth with increasing peak stress.

In this study, the (nominal) peak stresses (ie, measured peak forces per cross-sectional plug area) scattered between 5 and 10 MPa and thus are considerably lower than what has been studied previously. Yet, the loading rates, which ranged from 2000-3600 MPa/s, are drastically larger. Although cell death due to impact occurred in all tissue layers, it was highest in the superficial zone. These results can be compared to the most recent study of Milentijevic and Torzilli<sup>13</sup> who investigated the mechano-biological response of articular cartilage to varying loading rates (25-1000 MPa/s) and load magnitudes (10-40 MPa). In their study, both variables affected the results in that the depth of cell death from the articular surface increased with peak stress and decreased with increasing stress rate.

A growing body of literature is now developing, which has indicated that the range of loading used in the manipulation of cartilage tissue in transplantation must be carefully considered if cell preservation is to be maintained. Because osteochondral grafts are anchored using press-fit conditions, the optimal insertion parameters (ie, maximum load, loading rate, and overall impulse) are yet to be



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**Figure 6.** Cell viability of experimental group (hatched bars) versus control group (full bars). Living cells are held in green, dead cells appear in red. Cell counts have been normalized to the total cell number of each plug and are presented as percentages of the total number. The bar length reflects the average value of the investigated group; the error bars represent the standard deviation. Each row of data stands for a certain time point (0 hours, 48 hours, and 7 days) of the investigation. Data are broken down into cell viability measurements of the upper, middle, and deep layer of the tissue in each row. A total cell count, without subdivision into layers, is presented at the end of each row.

determined. Future studies should focus on the interplay of these parameters so that the fixation of osteochondral plugs is without trauma.

### ACKNOWLEDGEMENT

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### REFERENCES

1. Amiel D, Harwood FL, Hoover JA, Meyers M. A histological and biochemical assessment of the cartilage obtained from in vitro storage of osteochondral allografts. *Connect Tissue Res.* 1989;23:89-99.
2. Ball ST, Amiel D, Williams SK, et al. The effects of storage on fresh human osteochondral allografts. *Clin Orthop Relat Res.* 2004;418:246-252.
3. Ball ST, Chen AC, Tontz WL Jr, et al. Preservation of fresh human osteochondral allografts: effects of storage conditions on biological, biochemical and biomechanical properties. *Orthopaedic Transactions.* 2002;27:441.
4. Czitrom AA, Gross AE, Langer F, Sim FH. Bone banks and allografts in community practice. *Instruct Course Lect.* 1988;37:13-24.
5. 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;19:779-784.
6. Finkelstein JA, Gross AE, Davis A. Varus osteotomy of the distal part of the femur. A survivorship analysis. *J Bone Joint Surg Am.* 1996;78:1348-1352.
7. Gross AE, McKee NH, Pritzker KP, Langer F. Reconstruction of skeletal deficits at the knee. A comprehensive osteochondral transplant program. *Clin Orthop Relat Res.* 1983;174:96-106.
8. 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.
9. Holmvall K, Camper L, Johansson S, Kimura JH, Lundgren-Akerlund E. Chondrocyte and chondrosarcoma cell integrins with affinity for collagen type II and their response to mechanical stress. *Exp Cell Res.* 1995;221:496-503.
10. 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.
11. 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;21:881-887.
12. Mainil-Varlet P, Monin D, Weiler C, et al. Quantification of laser-induced cartilage injury by confocal microscopy in an ex vivo model. *J Bone Joint Surg Am.* 2001;83:566-571.
13. 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.
14. Nabavi-Tabrizi A, Turnbull A, Dao Q, Appleyard R. Chondrocyte damage following osteochondral grafting using metal and plastic punches: comparative study in an animal model. *J Orthop Surg.* 2002;10:170-172.
15. 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;19:242-249.
16. Ragan PM, Badger AM, Cook M, et al. Down-regulation of chondrocyte aggrecan and type-II collagen gene expression correlates with increases in static compression magnitude and duration. *J Orthop Res.* 1999;17:836-842.
17. Schachar NS, Cucheran DJ, McGann LE, Novak KA, Frank CB. Metabolic activity of bovine articular cartilage during refrigerated storage. *J Orthop Res.* 1994;12:15-20.
18. Torzilli PA, Grigiene R. Continuous cyclic load reduces proteoglycan release from articular cartilage. *Osteoarthritis Cartilage.* 1998;6:260-268.
19. 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;121:433-441.
20. Wayne JS, Amiel D, Kwan MK, Woo SLY, Fierer A, Meyers MH. Long-term storage effects on canine osteochondral allografts. *Acta Orthop Scand.* 1990;61:539-545.