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Abstract

Objective: Osteochondral graft transplantation has garnered significant attention because of its ability to replace the lesion with true hyaline cartilage. However, surgical impaction of the graft to anchor it into the defect site can be traumatic and lead to cell death and cartilage degeneration. This study aimed to test the hypothesis that increasing impulse magnitude during impaction of osteochondral plugs has a direct effect on loss of cell viability. **Design:** In this controlled laboratory study, the impaction force was kept constant while the impulse was varied. Ninety-six osteochondral plugs were extracted from the trochlea of bovine stifle joints and were randomly assigned into 3 experimental and 1 (nonimpacted) control group. The transferred impulse of the experimental groups reflected the median and the lower and upper quartiles of preceding clinical measurements. Data were obtained at day 0, day 4, and day 8; at each point, cell viability was assessed using the Live/Dead staining kit and histological assessments were performed to visualize matrix structural changes. **Results:** After impaction, cartilage samples stayed intact and did not show any histological signs of matrix disruption. As expected, higher impulse magnitudes introduced more cell death; however, this relationship was lost at day 8 after impaction. **Conclusion:** Impulse magnitude has a direct effect on cell viability of the graft. Because impulse magnitude is mostly governed by the press-fit characteristics of the recipient site, this study aids in the definition of optimal insertion conditions for osteochondral grafts.

Keywords

osteochondral graft, impaction, impulse, viability

Introduction

Hyaline cartilage forms the articulating surfaces of diarthrodial joints, providing lubrication and both static and dynamic mechanical load absorption. When this articular cartilage is damaged, its mechanical properties are severely compromised. Unlike many tissues in the body, cartilage is physiologically limited in its ability to regenerate and heal itself after being structurally compromised. Therefore, small defects in cartilage tend to worsen over time and can lead to larger defects.¹

A common technique used to repair cartilage damage is osteochondral autografting. In this procedure, small osteochondral cores are harvested from sites such as the proximal anterolateral femoral trochlea to be used as donor osteochondral grafts. The chondral lesion is excised with the harvesting tube, and the osteochondral graft is inserted. Simultaneously, the donor graft is prepped before being press-fit into the lesion. The donor graft is created with a larger diameter than the recipient site to create a snug fit, eliminating the use of fixatives; however, this process requires the graft to be

forcefully inserted, typically by impacting the grafts multiple times until they are fully seated.^{2,3} This forceful insertion can lead to decreased cell viability and may affect survivorship and durability of the osteochondral graft⁴⁻⁷ and is responsible for the depth-dependent chondrocyte death shown by histological studies.⁸⁻¹¹

Borazjani et al.¹² characterized the biomechanics of the graft insertion process by seating 10 human donor plugs into condylar recipient holes at the knee and found the applied loads and impaction taps quite variable. To mechanically describe the impact intensity of the insertion process, the variable impulse (i.e., the integral of applied load over time) was used. Although this study provided valuable clues about impact insertion and the spread of cell death

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within cartilage, a relationship of impact intensity and graft viability was not reported.

Our laboratory recently evaluated osteochondral graft cell viability that underwent a constant impulse while varying load.¹³ During characterization of surgical impaction, our lab determined the median value of impulse to be 7.0 Ns. In agreement with Borazjani et al.,¹² the study concluded that impaction does decrease graft cell viability when compared with nonimpacted grafts. Although larger loads decreased cell survival more significantly in a shorter time period (0 and 4 days), there was no difference between cell survival among varying impacted groups at 8 days.¹³ This study evaluates varying impulses while maintaining a constant impaction load during insertion of the osteochondral graft in a controlled laboratory setting. This information will be helpful to determine optimal press-fit conditions of osteochondral plugs because a tighter press-fit will require a higher impulse for plug seating and vice versa. We hypothesized that a decreased impulse during impaction will lead to increased cell viability of osteochondral tissue during transplantation.

Materials and Methods

Specimen Harvest

Osteochondral plugs were obtained from the condylar trochlea of bovine stifle joints of 6-month-old calves. The joints were obtained from a local abattoir within 1 hour of sacrifice. The articular surfaces of the joints were protected from desiccation by leaving the soft tissue and synovium surrounding the joint intact prior to harvesting. Osteochondral plugs were harvested from 6 pairs (right and left joints) of trochleas using Arthrex OATS system (Arthrex, Naples, FL) with sterile technique within 2 hours of obtaining the joints. Each harvested plug had a diameter of 8 mm and depth of 10 mm in order to reach the subchondral bone. The average cartilage thickness was 2.57 mm (\pm SD, 0.59 mm). Sixteen osteochondral plugs were removed from each pair of trochleas to give a total of 96 osteochondral plugs. The 16 plugs from each pair of trochleas were randomized and distributed among subsets based on impulse and time points (Table 1). This allowed for a sample size of 8 osteochondral plugs per condition.

Controlled Mechanical Impaction

Impaction was delivered to each osteochondral plug using a SmartImpactor™ (Rush University, Chicago, IL),¹⁴ a pneumatically driven device. The impaction device delivered flush, consistent loads and loading rates to the articular surface of osteochondral plugs that were held in quasi-unconfined compression by a custom-made plug

Table 1. Block Randomization Matrix to Plan the Removal of 16 Osteochondral Plugs from Each Animal (2 Knees)^a

Impulse (Ns)	Day 0	Day 4	Day 8
Control	1	2	3
3.1	4	5	6
7.0	7	8	9
9.5	10	11	12

^aThe randomization matrix is based on impulse and day categories.

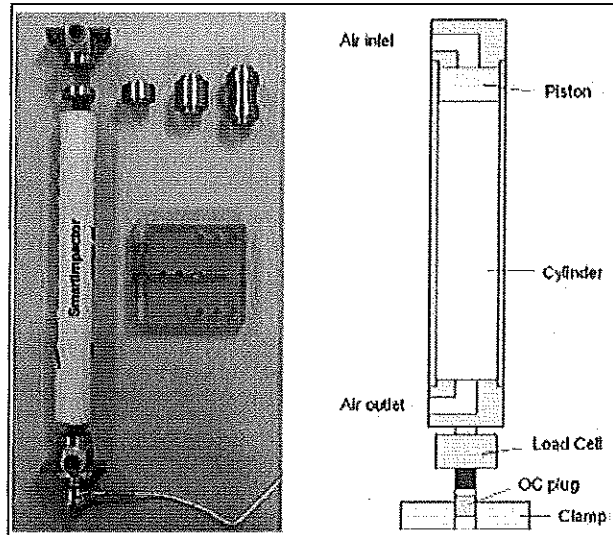


Figure 1. Exploded view (left) and working principle (right) of the pneumatic impaction device used to impact osteochondral plugs with consistent loads. By applying alternating air pressure to the chambers of the cylinder, a steel piston inside the tube is actuated. Its speed is controlled by adjusting the time profile of the compressing and sucking pressure. Each time the piston hits the end cap with defined speed, a mechanical impulse is generated and transfers through the impactor tip onto the specimen. The impulse can be varied by adjusting the piston's weight and air pressure.

clamp (Fig. 1). The impaction device was fitted with a load cell and an end piece of a plastic surgical tamp to mimic the impaction interface in the clinical setting. Based on a previous study,¹³ in which the surgical implantation of osteochondral plugs with the OATS system had been characterized, a load of 50 N was chosen. This level of load reflected the lower end of applied forces during surgical implantation and produced the most favorable results in the subsequent laboratory test, where loads were varied keeping a constant impulse. Osteochondral plugs received varying levels of impulse (control, 3.1 Ns, 7.0 Ns, and 9.5 Ns) by varying the number of impaction cycles (11, 24, and 32). Also, the impulse values were determined from the previous study and reflect 1st-quartile, median, and 3rd-quartile

levels of impulse placed on cartilage samples during impaction in a clinical setting.¹³ Each impaction cycle lasted approximately 3 ms, and loading rate was typically 40 kN/s, similar to previous findings.^{11,13} Nonimpacted plugs served as control (Table 1).

Articular Cartilage Culture

Following impaction, the articular cartilage was separated from the subchondral bone using a scalpel blade and was prepared for culture. The remaining cartilage disc was cultured in 3 mL of minimal essential media with 10% fetal bovine serum, glutamate, nonessential amino acids, antifungal agents, and antimicrobial agents at 37 °C for 4 or 8 days. Medium was replaced every 3 days.

Endpoints

The cartilage discs were assayed for cell viability at days 0, 4, and 8. Cell viability assessments at day 0 were conducted within 3 hours of impaction. In addition, plugs were stained using Safranin-O for histological examination. These examinations were performed to rule out the possibility of any matrix damage, which should not be expected with the amount of load applied.¹³ Any matrix damage would point to a technical error during load application (e.g., load applied off-centric, nonflush with tapping device, etc.).

The cartilage discs were axially bisected, and one of the halves was stained for cell viability (and the other used for histology). The cut edge, located in the center of the original plug, was examined. The sections were incubated in 4 μ M calcein-AM, 8 μ M ethidium homodimer-1 (Molecular Probes Inc., Eugene, OR), and 1 mL phosphate-buffered saline at 37 °C for 30 minutes. The cartilage discs were inspected using a confocal laser-scanning microscope (MRC-1000; BioRad, Hemel Hempstead/Cambridge, England), and digital images were captured using Metamorph software (version 6.1; Molecular Devices Corporation, Sunnyvale, CA). The calcein-AM (green fluorescence) and the ethidium homodimer-1 (red fluorescence) labeled the live and dead cells, respectively. Using Metamorph software, the cell viability ratio was calculated from the full-thickness section located at the center of the plug. The cell viability ratio was defined as the number of live cells divided by the total number of cells, both live and dead cells.

Data Analyses

Statistical analyses were performed using nonparametric analyses. Kruskal-Wallis tests were conducted followed by Mann-Whitney pairwise comparisons. Level of significance was set at $P \leq 0.05$. Analyses were conducted with

SPSS version 11.5 (SPSS, Inc., Chicago, IL). Grubbs' test was used to identify outliers using a 95% confidence interval, of which there were none.

Results

Histological analysis of all samples demonstrated intact cartilage without matrix disruption. However, the loaded plugs showed lower counts of live cells and higher dead cells when compared with control. Example images from day 4 are shown in Figure 2. At day 0, there was a significant difference between all the varying conditions: between control and 3.1 Ns ($P = 0.008$), control and 7.0 Ns ($P < 0.001$), control and 9.5 Ns ($P < 0.001$), between 3.1 Ns and 7.0 Ns ($P = 0.004$), between 3.1 Ns and 9.5 Ns ($P < 0.001$), and between 7.0 Ns and 9.5 Ns ($P < 0.001$). Similarly at day 4, there were significant differences among cell viability between the control and 7.0 Ns ($P = 0.004$), control and 9.5 Ns ($P < 0.001$), and 3.1 Ns and 9.5 Ns ($P = 0.026$). There were no significant differences between varying impulses at day 8. Longitudinal analyses of the results also show that among each level of varying impulse (control, 3.1 Ns, 7.0 Ns, and 9.5 Ns), the cell viability between day 0 and day 8 was always significantly different ($P \leq 0.018$). There was also a significant difference between day 0 and day 4 for control ($P < 0.001$), 3.1 Ns ($P = 0.006$), and 7.0 Ns ($P < 0.001$) and between day 4 and day 8 for control ($P = 0.001$) and 3.1 Ns ($P = 0.031$). Figures 3 and 4 summarize the cell viability results among days and impulse levels, respectively.

Discussion

This study examined the biologic effects of applying a controlled mechanical load at varying impulses to osteochondral plugs from bovine samples, with a constant load and impulse range consistent with that of clinical values determined from a previous study by our laboratory. It was found that a decreased impulse during impaction will lead to increased cell viability of osteochondral tissue during transplantation. This is an important result, as increased cellular viability of osteochondral grafts is considered to lead to its increased survivorship and durability.⁷ However, a certain minimum impulse will be necessary to guarantee initial stability of the graft to provide acceptable anchorage. These optimal insertion conditions have yet to be defined.

The investigation required the use of a pneumatically controlled impaction device (SmartImpactor™, Rush University Chicago, IL) to deliver a consistent maximal load and impulse.¹³ To improve the efficiency of the device, new operational and monitoring software has been developed and was used in this study. Previous investigators in our lab have developed this device for a study with a

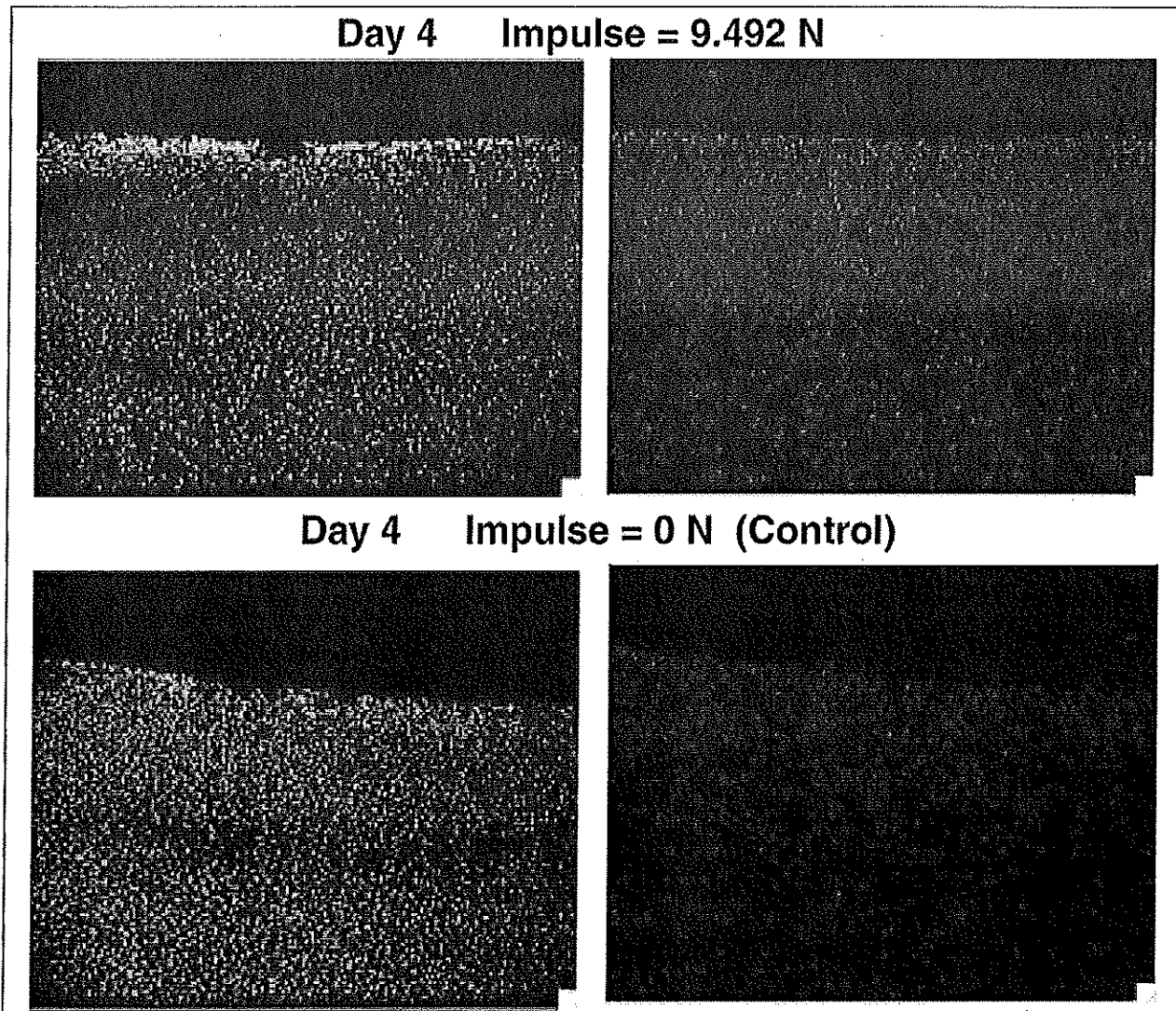


Figure 2. Cell viability example images taken at day 4 for an osteochondral plug impacted at 50 N with an impulse of 9.5 Ns shown against a control plug (D). Note the increase of cell viability (and increase of cell death) in the superficial zone compared with control. Live cells are stained green (left image), and dead cells are stained red (dead image). Cells on both images were counted, and a cell viability ratio of number of live cells to number of total cells was computed.

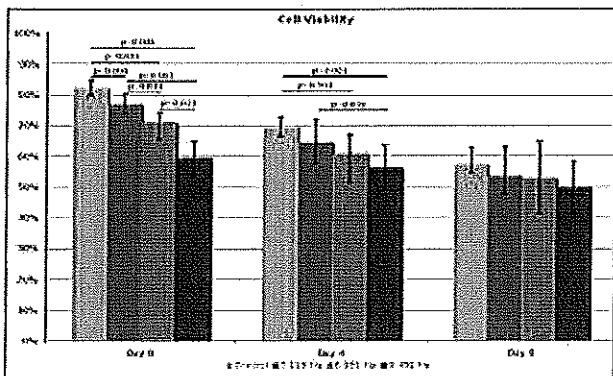


Figure 3. Summary of cell viability results (mean \pm SD) grouped by day. Significant differences of cell viability between impulses are highlighted.

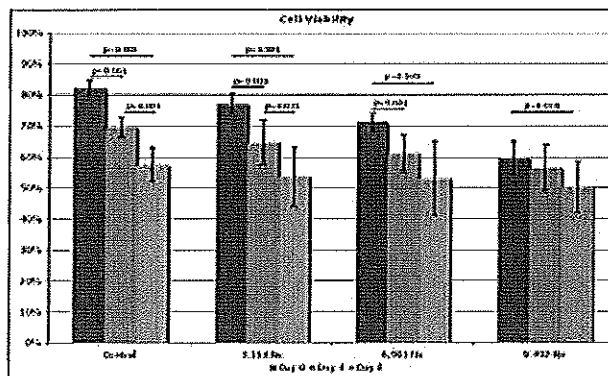


Figure 4. Summary of cell viability results (mean \pm SD) grouped by impulse level. Significant differences of cell viability between days are highlighted.

similar setup. In that study, different load levels were targeted to insert osteochondral plugs into an experimental recipient device. Ten plugs per load level, 8 mm in diameter, 10 mm long, with 2 ± 1 mm cartilage thickness were exposed to a series of 7 taps. The maximum force during each hit was measured and the variation determined. Based on these measurements, the coefficient of variation for load levels of 37 and 75 N was $<2\%$.¹⁴ Similar variation was found in this study using 50 N as target load.

Previous studies have shown that impaction of osteochondral grafts generates damaging loads that cause chondrocyte death, particularly in the superficial zone.¹² Higher load magnitudes were associated with higher cell death when the overall impaction impulse was kept constant.¹³ Hence, it was suggested that lower load magnitudes and a greater number of hits be used to ensure proper fit. In this study, however, it was shown that loads as small as 37 N (requiring 74 hits for complete insertion) can still have deleterious effects on chondrocyte viability. It is evident from the above and our study that the overall impaction impulse plays a major role. The design of our study involved multiple impaction cycles on each osteochondral plug, at a constant load and loading rate, to reach varying values of impulse. Both the applied load and impulse levels represented clinically relevant values.¹³ In a clinical setting, the necessary impulse for plug seating is dependent on recipient hole tolerances, subchondral bone quality, and bone thickness.

As expected, our study demonstrated an inverse relationship between impulse and chondrocyte viability. As the impulse submitted to the osteochondral plugs was increased, the cell viability of the chondrocytes decreased, as was expected. This relationship was seen on day 0 and to an extent on day 4, but was lost by day 8. This might be due to the culturing technique but could also be related to the plug retrieval process. It was shown earlier that blunt trauma generated with a trephine causes cell death adjacent to the lesion edge.¹⁵ The tissue reaction following trauma may have compromised the cartilage during culturing.

Our study had several additional limitations. This study focused on osteochondral plug characteristics of autografts, which have a smaller surface area (<0.5 cm²) than allografts (>2 cm²).³ The decreased diameter cannot distribute the loads to a large surface area, which may lead to increased stress magnitudes. However, the general principle of the surgical impaction effects on the biology of the osteochondral plugs is the same for both autografts and allografts. In particular, the average impulse per hit was strikingly similar in clinical insertion studies in our laboratory with autograft¹³ and others who used allograft.¹² In both cases, an average impulse of 0.6 Ns per hit was measured. We were also unable to study stress-strain relationships during the insertion process because displacements are not recorded using the pneumatic impaction device. Such a

device was used because conventional material-testing machines are incapable of producing consistent load magnitudes at high loading rates for the experimental setup of this study.¹⁶ Last but not least, the study was also conducted *in vitro*, which is a different surrounding than *in vivo*, omitting the contributing factor of other tissues in the repair process. For example, the utilized plug length of 10 mm might be too short for stable fixation *in vivo*. Also, this study did not evaluate the process of plug harvesting, which is similarly important for the viability of the graft.¹⁷

The clinical significance that can be extrapolated from this study is that a lower impulse during impaction will lead to an increased cell viability of osteochondral plugs. This increased cell viability should lead to a higher degree of biomechanical function of the transplant and facilitate improved incorporation into the host tissue. Further studies should look into defining necessary press-fit conditions to ensure safe initial anchorage of the plug while maintaining the highest cell viability possible. It is ultimately the press-fit characteristics that define the required impulse to seat the plug and require appropriate instruments, taking the biological tolerances into account.¹⁸

Declaration of Conflicting Interests

Brian J. Cole is a consultant for Arthrex, Inc., Naples, FL.

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