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Osteochondral Graft Size Is Significantly Associated With Increased Force and Decreased Chondrocyte Viability

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Abstract

Background: Insertion force has been shown to significantly reduce chondrocyte viability during osteochondral allograft transplantation. How graft size influences the required insertion force and chondrocyte viability has yet to be determined.

Hypothesis/Purpose: The purpose was to characterize how graft size influences insertion force requirements and chondrocyte viability during osteochondral transplantation. The hypothesis was that larger grafts would require greater force and reduce chondrocyte viability.

Study Design: Controlled laboratory study.

Methods: Four graft sizes—15 × 5 mm, 15 × 10 mm, 25 × 5 mm, and 25 × 10 mm (diameter × depth)—were harvested from 13 thawed fresh-frozen human cadaveric distal femurs. Average, maximum, and cumulative force and number of impacts were recorded for 44 grafts by a surgical mallet embedded with a calibrated force sensor. In a separate experiment, fresh osteochondral tissues were subjected to mechanical loading. To capture a range of clinically important forces, categories were selected to correspond to impaction force data. Chondrocyte viability was assessed with confocal laser microscopy and live/dead staining.

Results: Total force for all grafts averaged 4576 N. Median number of impacts for all grafts was 20 (range, 7–116). The mean number of impacts for 5-mm-deep grafts was 14.2 (95% CI, 10.8–18.6), as compared with 26.3 (95% CI, 19.9–34.4) for 10-mm-deep grafts ($P < .001$). The mean cumulative force for 5-mm-deep grafts was 2128 N (95% CI, 1467–3087), as opposed to 4689 N (95% CI, 3232–6803) for 10-mm-deep grafts ($P = .001$). For every 1 mm in graft depth, an average of 13.1% (95% CI, 6.2%–20.3%) more impacts are required when controlling for diameter and density ($P < .001$). For every 1 mm in graft depth, the force required increases on average by 17.1% (95% CI, 7.7%–27.4%) when controlling for diameter and density ($P = .001$). There was a significant reduction in chondrocyte viability for the forces required for graft

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thickness values >10 mm. Only forces associated with graft thickness <10 mm had chondrocyte viabilities consistently >70%.

Conclusion: Insertion force increases significantly with increasing graft depth. Controlling for diameter and bone density, a 1-mm increase in graft depth is associated with 13.1% more impacts and 17.1% more force. Chondrocyte viability was significantly reduced to <70% at average forces associated with grafts thicker than 10 mm.

Keywords

osteocondral; allograft; cartilage; viability

Osteochondral allograft transplantation (OCA) is a single-stage surgical procedure used to treat large (> 2 cm²) chondral defects of the knee.[§] Advantages include the ability to restore bone stock and transplant viable chondrocytes within mature hyaline cartilage.

^{15,16,18,25,26,29} Because chondrocyte viability directly correlates with clinical success, ^{8–11,17,19,24–29} graft incorporation while preserving chondrocyte viability is important. ^{2–5,7,13,20,21,32} Gross et al¹⁵ examined the histologic features of 35 fresh OCA plugs retrieved at the time of subsequent graft revision, osteotomy, or total knee arthroplasty. The authors found that features of early graft failure included lack of chondrocyte viability and loss of matrix cationic staining. Conversely, factors associated with long-term allograft survival included viable chondrocytes, functional preservation of matrix, and complete replacement of the graft bone with the host bone.¹⁵

Borazjani et al¹ compared chondrocyte viability in cadaveric distal femurs with 15-mm-diameter osteochondral grafts. The authors found that insertion of the grafts averaged 10 impacts, generating 2.4 kN load. The impacted grafts had significantly increased cell death on the superficial 500 mm by 21% at 1 hour and 47% at 48 hours and at deeper layers within the cartilage at 48 hours. The data suggested that insertion impact of osteochondral grafts generates damaging loads that cause chondrocyte death. However, how impaction force varies with OCA plug size is not currently known.

Because OCA grafts are matched per the patient's specific clinical requirements, there is a critical need to determine how graft size influences impaction force requirements and subsequent chondrocyte viability. The purpose of this study was to characterize the amount of applied force required to transplant different-size OCA grafts into osteochondral defects of the knee and determine the relationship with chondrocyte viability. Our hypothesis was that larger-size grafts would require greater force, with a corresponding reduction in chondrocyte viability.

METHODS

Impaction Force

Sixteen fresh-frozen human cadaveric femurs were donated from the Musculoskeletal Transplant Foundation. Tissue was processed per the biosafety protocol of the Wisconsin

[§]References 1, 12, 14, 15, 18, 23, 25–29, 31.

Institute for Medical Research at the University of Wisconsin–Madison. Forty-four OCA grafts (11 per group) were collected from 13 femurs (3 femurs were excluded because of small size).

To determine bone density, each femur was subjected to a computed tomography scan before testing (GE HD 750: helical high-resolution mode at 0.5-second rotation; 40-mm detector coverage with 0.516 pitch, 20.62 speed, 1.25 thickness, 0.625 interval kV, 120 mA, 400 HD; bone kernel: 2000/350 width/level). Density of each femur was recorded as Hounsfield units. An average of 3 measurements represented the mean density of each distal femur. The images were standardized on the axial cuts by identifying the level of the distal femur where the popliteal fossa was deepest, corresponding to the approximate level of subchondral bone involved in chondral defects. The 3 measurements included the trochlea, centered above the notch at the midline of the femur, and the medial and lateral condyles centered at the level of the epicondylar axis (Figure 1).

Osteochondral grafts were harvested and inserted with the ACT system (Allograft Cartilage Transplant; Musculoskeletal Transplant Foundation Sports Medicine). To control for density, the same femur was used as both donor and recipient, with the lateral femoral condyle as donor and the medial femoral condyle as recipient (Figure 2).

The procedure followed the ACT technique guide (ConMed ACT Allograft Cartilage Transplant Instruments). The appropriate lesion gauge was used to ensure adequate room for transplant on the receipt site. A guide pin was then placed through the lesion gauge, and the cartilage was marked at 12, 4, and 8 o'clock. The appropriate lesion reamer was advanced over the guide pin to the desired depth during irrigation. The depth gauge was placed into the reamed hole to ensure appropriate depths at the 12-, 4-, and 8-o'clock positions. The donor was placed in the GraftStation with the appropriate bushing. The lesion gauge was used to optimize the fit, at which point the clamping mechanism was locked. The 12-o'clock position was marked. A power reamer advanced the appropriate Graft-Maker instrument to the desired depth during irrigation. A sagittal saw was used laterally below the maximum desired depth. The allograft donor was removed from the GraftStation and released. The donor allograft was placed into the GraftForceps and cut to the desired depth with a microsagittal saw. The graft bone was chamfered with the chamfer tool. The recipient site was dilated with the appropriate dilator. The graft was then placed into the reamed hole matching the 12-o'clock position and fit in with finger pressure. The appropriate tamp was then placed flush with the allograft plug, and the mallet was used to seat the graft.

Force data were captured with an instrumented piezoelectric mallet (model 086D05, modally tuned impulse hammer: 0–5000 lbf, 1 mV/lbf; PCB Piezotronics) connected to a signal conditioning unit (model 482C series; PCB Piezotronics) and a data acquisition unit (model cDAQ-9171; USB). Output was recorded with a custom LabVIEW graphical user interface (2015 Standard Service Program; National Instruments). A custom LabVIEW peak-picking algorithm was created to capture the peak force and raw voltage data. Data from the mallet were exported into Microsoft Excel for postprocessing.

Four different-size OCA grafts—diameters of 15 mm and 25 mm and depths of 5 mm and 10 mm—were tested (Appendix Figures A1, A2; available in the online version of this article). The total number of impacts and the average, maximum, and total cumulative force were recorded for each OCA graft and reported in newtons.

Priori Power Analysis

From Borazjani et al,¹ the insertion of a graft sized 15×10 mm took a mean (SD) 10 (4) impacts with a force of 2.4 (0.9) kN. Therefore, the total force was approximately 24 (9) kN. If the assumption is that an average total force for 15×5 mm will be one-third less (16 [6]) than the total expected force for 15×10 mm, that 25×5 mm will be the same (24 [9]), and that 25×10 mm will be one-third more (32 [12]), a total of 40 grafts (10 per group) would be needed to achieve 88% power with an analysis of variance (ANOVA) test having a 5% significance level.

Analysis Methods

Differences among the 4 plug conditions were assessed with repeated measures ANOVA accounting for multiple measures within each femur while controlling for density of the femur. *P* values were adjusted with the Tukey test for a family of 4 means. Linear regression modeled total force and number of impacts while controlling for clinically important covariates. Linear regression assumptions were assessed. A *P* value $< .05$ was considered statistically significant. Statistical analysis was performed with RStudio (v 1.0.143; RStudio, Inc).

Chondrocyte Viability.—To determine the relationship of graft size and chondrocyte viability, fresh osteochondral tissue was used. Fresh tissue was obtained as surgical waste from patients undergoing total knee replacement. This model has been validated in previous studies.²² Fresh osteochondral tissue was subjected to 2250 N, 4450 N, 6670 N, and 8900 N. Three biological replicates were used for each category. Force was standardized with a custom weight drop system with polyvinyl chloride piping and tungsten weights. With a tungsten weight of known mass, the height was calculated from which it would need to be dropped to apply the desired force. This calculation was then verified with a force simulator platform.

This platform was constructed with a flat strike surface and an attached cylindrical piston, with conformal polyethylene bushing to guide it. The force sensor and polyethylene bushing were mounted to a metal baseplate attached to a ball-and-socket mount, which allowed for positional adjustment in 3 planes. The same piezoelectric force sensor and data acquisition methods discussed previously were used.

Chondrocyte viability was determined with confocal laser microscopy (Nikon A1RS Confocal Microscope; Optical Imaging Core, University of Wisconsin-Madison) and live/dead staining assay (catalog No. L3224, LIVE/DEAD Viability/Cytotoxicity Kit for Mammalian Cells: calcein AM and ethidium homodimer-1 [EthD-1]; ThermoFisher Scientific). The live/dead reagent stock solution was warmed to room temperature. A 4mM EthD-1 solution was made by adding 20 μ L of 2mM EthD-1 stock solution (component B)

to 10 mL of sterile tissue culture–grade phosphate-buffered saline. This was vortexed to ensure thorough mixing. The working solution was then made by adding 5 μ L of the supplied 4mM calcein AM stock solution (component A) to the 10 mL of EthD-1 solution.

Osteochondral tissue samples were cut to a width of 1.0 mm with a low-speed saw (Buehler; Isomet 2000) and incubated in the working solution for 15 minutes. Tissue was then placed on a coverslip and evaluated by confocal laser microscopy. Images captured included fluorescein, tetramethylrhodamine, and red-green-blue channels. Cell counts were determined with Fiji (<https://figi.sc>).³⁰ Images were first captured for each channel and then counted with the automated counting of single color images and exported into Microsoft Excel. The live cell counts (fluorescein) were divided by the dead cell counts (tetramethylrhodamine) to calculate the percentage of chondrocyte viability.

Before mechanical impaction, the chondrocyte viability was determined to ensure live cells, and this also served as control. The fresh osteochondral tissue was then subjected to mechanical force with the force simulator platform as described. Because fresh osteochondral tissue was limited, the most parsimonious groupings were determined to accurately capture clinically important measures as determined by the impaction analysis.

Analysis Methods.—Methods for the analysis of the viability data included conducting repeated measures ANOVA with viability as the outcome; with force (low, medium, high, or maximum), condition (control or experimental), and their interaction as fixed effects; and with bone as a random effect. *P* values were adjusted with the Tukey test for 4 comparisons. A *P* value <.05 was considered statistically significant. Statistical analysis was performed with RStudio (v 1.0.143; RStudio, Inc).

RESULTS

Impaction Force Analysis

Table 1 presents a summary of the descriptive data.

The small-diameter (15 mm), large-depth (10 mm) graft was associated with the largest total force (mean $\pm$ SE: 6953 $\pm$ 6797 N). There were no differences in the maximum force or average force among any of the grafts, indicating uniform impacts within and among graft sizes (Tables 2 and 3).

Cumulative insertion force (total force) varied among the 4 groups. There was a clear trend toward increased force required with deeper grafts, with a near-significant difference between 25 $\times$ 10-mm grafts (4459 N; 95% CI, 2716–7321 N) and 15 $\times$ 5-mm grafts (1991 N; 95% CI, 1213–3268 N) (*P* = .051) and with significantly more cumulative force in the 15 $\times$ 10-mm grafts (4931 N; 95% CI, 3004–8095 N) than in the 15 $\times$ 5-mm grafts (*P* = .022) (Figure 3).

The number of impacts demonstrated a clear trend toward increasing number as graft depth increased. There was a significantly greater number of impacts between 25-mm diameter $\times$ 10-mm deep (26.2; 95% CI, 18.2–37.8) and 15-mm diameter $\times$ 5-mm deep (13.1; 95% CI,

9.1–18.9) ($P = .018$), as well as 15-mm diameter $\times$ 10-mm deep (26.1; 95% CI, 18.1–37.6) and 15-mm diameter $\times$ 5-mm deep ($P = .018$) (Figure 4).

Both the number of impacts and the cumulative force were significantly greater for plugs 10 mm deep than 5 mm deep, when controlling for diameter. The geometric mean cumulative force for 5-mm-deep grafts was 2128 N (95% CI, 1467–3087 N), as opposed to 4689 N (95% CI, 3232–6803 N) for 10-mm-deep grafts ($P = .001$) (Figure 5).

Similarly, the geometric mean number of impacts for 5-mm-deep grafts was 14.2 (95% CI, 10.8–18.6), as opposed to 26.2 (95% CI, 19.9–34.4) for 10-mm deep grafts ($P < .001$) (Figure 6).

Given the significance of depth on both total force and number of impacts, a mixed effect linear regression model was used to predict the relative change of total force and number of impacts with increasing graft depth. When controlling for density and diameter, total force increased an average of 17.1% for every 1-mm increase in graft depth (Table 4).

Similarly, the number of impacts increased an average of 13.1% for every 1-mm increase in graft depth when controlling for density and diameter (Table 5).

Mixed effect linear regression predicted total force and number of impacts for sizes between 5 mm and 10 mm deep by 1-mm increments (Table 6).

Cartilage Viability Analysis

All fresh grafts had chondrocyte viabilities $>93\%$ (93%–99%). To cover the clinically important range of impaction force, the control conditions were compared with the experimental conditions under low total force (2225 ± 57 N), medium total force (4450 ± 82 N), high total force (6670 ± 144 N), and max total force (8900 ± 125 N). The total force was achieved by repeatedly loading the graft at the mean force of 165 N. Qualitative data from confocal laser microscopy are shown in Figure 7.

Chondrocyte viability was reduced from a mean (SD) 95% (4.6%) to 84% (9.2%) under low total force (2225 N), 93% (3.9%) to 66% (7.4%) under medium total force (4450 N), 99% (8.7%) to 55% (13.9%) under high total force (6670 N), and 94% (6.8%) to 56% (13.5%) under maximum total force (8900 N). The mean differences between the control and experimental conditions were as follows: for low total force, -0.10 (95% CI, -0.27 to 0.06 ; $P = .176$); for medium total force, -0.27 (95% CI, -0.43 to -0.11 ; $P = .005$); for high total force, -0.44 (95% CI, -0.61 to -0.28 ; $P < .001$); and for maximum total force, -0.38 (95% CI, -0.54 to -0.22 ; $P = .001$). To ensure that grafts maintained chondrocyte viability $>70\%$, total force <4450 N was required (Figure 8).

DISCUSSION

These data support our hypothesis that larger OCA graft size, specifically depth (thickness), is associated with greater force during insertion. There was a significant reduction in chondrocyte viability when the total force exceeded 4450 N.

This total force was associated with average force requirements for grafts 10 mm thick. Moreover, only tissue subjected to total forces <4450 N consistently maintained chondrocyte viability >70%.

The average force per impact in the current study ranged from 148 to 189 N and is lower than that reported by Borazjani et al¹ but is similar to that reported by Kang et al.¹⁸ Similarly, average cumulative force in this study ranged from 1993 to 4929 N, providing initial data regarding applied force during an osteochondral transplant surgical procedure. Based on these data, it is predicted that force requirements will increase linearly between clinically important graft depths (thicknesses). Controlling for important covariates, including density and graft size, the average total force of a 5-mm graft is expected to be approximately 2128 N, increasing 17.1% for every 1-mm increase in graft depth, to 4686 N for a 10-mm graft. Interestingly, this range of force was associated with chondrocyte viability between 84% and 66%, respectively. Given the data of Cook et al,⁶ which suggest that >70% chondrocyte viability at the time of implantation is necessary for a successful outcome, preventing excessive force is likely to improve graft viability. Because total forces >4450 N were associated with a significant reduction in chondrocyte viability <70%, ways to limit traumatic forces across fresh OCA grafts are needed. Because increased depth requires a linear increase in force, chondrocyte viability is expected to decrease accordingly, potentially compromising graft viability. Although the ideal graft size has yet to be determined, graft depths <10 mm thick are suggested to maintain chondrocyte viability >70%. Furthermore, grafts that are 10 mm deep (thick) can be difficult to orient, owing to their long cylindrical shape. This is especially true of small-diameter grafts of large depths. These data suggest that although mean values are less than those associated with viabilities of >70%, larger total forces can be needed when graft orientation is difficult.

Although there are benefits to using deeper grafts, such as a larger bone surface area for incorporation, depth is associated with greater force requirements, which may contribute to a corresponding reduction in chondrocyte viability. Of course, the clinical concern of a graft that is too shallow is the lack of bone incorporation or instability. This is especially true when sclerotic bone is encountered during OCA surgery. Although further research is needed to determine how to reduce these requirements and optimize graft viability, one consideration is to trephine the recipient bone with a small drill to gain a bleeding surface, allowing for a thinner OCA graft while attempting to preserve graft incorporation.

The primary strength of this study was the simulation of surgical technique and chondrocyte viability data. In contrast to previous laboratory studies that used different impaction devices and xenografts, our experiment utilized a surgical mallet and equipment used during OCA surgery. Moreover, 2 experienced sports fellowship-trained surgeons familiar with this procedure performed the transplantation in the same manner.

Despite the effort to simulate surgery in a controlled laboratory environment, this study was not without limitations. Primarily, fresh-frozen allografts were used, which might have different biomechanical properties as compared with fresh grafts. Furthermore, a single insertion handle was used during this experiment, and the requirements for OCA graft insertion may indeed vary with different interfaces. Moreover, variation in OCA systems and

surgical technique may affect the force requirements—for example, undersizing the donor graft, thereby permitting greater finger pressure insertion before impaction and reducing cumulative force. Another limitation was that the viability experiments were not performed on the specimens used to gather the force data. Although ideal, this was not feasible owing to the availability of fresh grafts.

Further study is needed to fully characterize the association of important variables, including defect location, interface used for insertion, and adjacent graft placement. However, it is important for surgeons to be aware of the larger applied insertion force required for OCA grafts of greater depth, irrespective of diameter. Further data are needed with this model to improve the long-term viability of this procedure. It is likely important to determine how the number of subthreshold impacts and maximum applied force over a single impact affect graft viability within the range that occurs during surgery.

In summary, the current cumulative applied force data suggest that graft depth significantly influences force of impaction, with greater force associated with significant reduction in chondrocyte viability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Borazjani BH, Chen AC, Won CB, et al. Effect of impaction on chondrocyte viability during insertion of human osteochondral grafts. *J Bone Joint Surg Am*. 2006;88(9):1934–1943. [PubMed: 16951108]
2. Borrelli J Jr, Tinsley K, Ricci WM, Burns M, Karl IE, Hotchkiss R. Induction of chondrocyte apoptosis following impact load. *J Orthop Trauma*. 2003;17:635–641. [PubMed: 14574191]
3. Borrelli J Jr, Torzilli PA, Grigien R, Helfet DL. Effect of impact load on articular cartilage: development of an intra-articular fracture model. *J Orthop Trauma*. 1997;11:319–326. [PubMed: 9294794]
4. 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. [PubMed: 15639638]
5. 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;19:703–711. [PubMed: 11518282]
6. Cook JL, Stannard JP, Stoker AM, et al. Importance of donor chondrocyte viability for osteochondral allografts. *Am J Sports Med*. 2016;44(5):1260–1268. [PubMed: 26920431]

7. Costouros JG, Dang AC, Kim HT. Inhibition of chondrocyte apoptosis in vivo following acute osteochondral injury. *Osteoarthritis Cartilage*. 2003;11:756–759. [PubMed: 13129695]
8. 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:S90–S99.
9. 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):22–24. [PubMed: 11685839]
10. 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;393:302–309.
11. Eckstein F, Faber S, Muhlbauer R, et al. Functional adaptation of human joints to mechanical stimuli. *Osteoarthritis Cartilage*. 2002; 10:44–50. [PubMed: 11795982]
12. Flanigan DC, Harris JD, Trinh TQ, et al. Prevalence of chondral defects in athletes' knees: a systematic review. *Med Sci Sports Exerc*. 2010;42(10):1795–1801. [PubMed: 20216470]
13. 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;9:582–592. [PubMed: 11520173]
14. Ghazavi MR, Pritzker KP, Davis AM, et al. Fresh osteochondral allografts for post-traumatic osteochondral defects of the knee. *J Bone Joint Surg Br*. 1997;79:1008–1013. [PubMed: 9393922]
15. Gross AE, Kim W, Las Heras F, et al. Fresh osteochondral allografts for posttraumatic knee defects: long-term followup. *Clin Orthop Relat Res*. 2008;466(8):1863–1870. [PubMed: 18465182]
16. 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.
17. Haut RC. Contact pressures in the patellofemoral joint during impact loading on the human flexed knee. *J Orthop Res*. 1989;7:272–280. [PubMed: 2918426]
18. Kang RW, Friel NA, Williams JM, Cole BJ, Wimmer MA. Effect of impaction sequence on osteochondral graft damage: the role of repeated and varying loads. *Am J Sports Med*. 2010;38(1):105–113. [PubMed: 19915099]
19. 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;251:580–585. [PubMed: 9792816]
20. 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. [PubMed: 12919877]
21. Loening A, Levenston M, James I, et al. Injurious compression of bovine articular cartilage, induces apoptosis before detectable mechanical damage. *Trans Orthop Res Soc*. 1999;24:42.
22. Lu Y, Edwards RB, Kalscheur VL, et al. Effect of bipolar radiofrequency energy on human articular cartilage: comparison of confocal laser microscopy and light microscopy. *Arthroscopy*. 2001;17(2): 117–123. [PubMed: 11172239]
23. 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.
24. 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. [PubMed: 15652547]
25. Pallante AL, Chen AC, Ball ST, et al. The in vivo performance of osteochondral allografts in the goat is diminished with extended storage and decreased cartilage cellularity. *Am J Sports Med*. 2012; 40(8):1814–1823. [PubMed: 22707746]
26. Pallante AL, Gortz S, Chen AC, et al. Treatment of articular cartilage defects in the goat with frozen versus fresh osteochondral allografts: effects on cartilage stiffness, zonal composition, and structure at six months. *J Bone Joint Surg Am*. 2012;94(21):1984–1995. [PubMed: 23138239]
27. Patil S, Butcher W, D'Lima DD, Steklov N, Bugbee WD, Hoenecke HR. Effect of osteochondral graft insertion forces on chondrocyte viability. *Am J Sports Med*. 2008;36(9):1726–1732. [PubMed: 18490471]
28. Patwari P, Fay J, Cook MN, et al. In vitro models for investigation of the effects of acute mechanical injury on cartilage. *Clin Orthop Relat Res*. 2001;291:S61–S71.

29. Repo RU, Finlay JB. Survival of articular cartilage after controlled impact. *J Bone Joint Surg Am.* 1977;59:1068–1076. [PubMed: 591538]
30. Schindelin J, Arganda-Carreras O, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012;9(7):676–682. [PubMed: 22743772]
31. Sherman SL, Garrity J, Bauer K, Cook J, Stannard J, Bugbee W. Fresh osteochondral allograft transplantation of the knee: current concepts. *J Am Acad Orthop Surg.* 2014;22:121–133. [PubMed: 24486758]
32. 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;87:1285–1291. [PubMed: 16129760]

Clinical Relevance:

Based on the current data, graft depth is an important consideration for surgeons when sizing osteochondral allograft transplant for chondral lesions of the knee.

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Figure 1.
Computed tomography scan of distal femur. Circles denote where density measurements were taken (in Hounsfield units).

Figure 2.
The lateral condyle (left) was used as donor, and the medial condyle (right) was prepared as recipient.

Figure 3.
Log-transformed total force (N) among groups. Values are presented as mean (95% CI).

Figure 4.

Log-transformed number of impacts among groups. Values are presented as mean (95% CI).

Figure 5. Log-transformed total force comparing 5- and 10- mm-deep graphs. Values are presented as mean (95% CI).

Figure 6. Log-transformed number of impacts between 5- and 10-mm-deep grafts. Values are presented as mean (95% CI).

Figure 7.

Confocal microscopy with live/dead stain: (A) control group (4 $\times$) and (B) experimental group (4 $\times$). FITC, Fluorescein isothiocyanate; TRITC, Tetramethylrhodamine.

Figure 8.
Cell viability before and after loading. Values are presented as mean (95% CI).

TABLE 1

Descriptive Data^a

	All	Diameter:Depth, mm (n = 11 per Group)			
		25:10	15:10	25:5	15:5
Density, HU					
Mean ± SE	1258 ± 401	1305 ± 422	1219 ± 398	1289 ± 435	1219 ± 398
Median (IQR)	1173 (1025–1472)	1198 (1062–1629)	1148 (1025–1357)	1198 (1025–1629)	1148 (1025–1357)
Maximum force, N					
Mean ± SE	399 ± 265	369 ± 83	530 ± 437	356 ± 184	341 ± 213
Median (IQR)	344 (244–473)	353 (313–419)	282 (366–619)	338 (190–508)	291 (230–361)
Minimum force, N					
Mean ± SE	57 ± 13	56 ± 10	50 ± 4	65 ± 20	58 ± 10
Median (IQR)	53 (49–61)	56 (50–60)	50 (47–51)	60 (51–70)	56 (49–66)
Total force, N					
Mean ± SE	4576 ± 4471	5218 ± 2940	6953 ± 6797	3198 ± 2705	293 ± 3479
Median (IQR)	3187 (1700–5777)	4524 (3674–6027)	3754 (2816–8078)	2033 (1210–4150)	1935 (1139–3020)
No. of impacts					
Mean ± SE	23.9 ± 19.0	29.8 ± 14.9	28.1 ± 11.5	23.5 ± 31.5	14.4 ± 7.4
Median (IQR)	20.0 (10.0–30.5)	30.0 (19.5–36.5)	26.0 (23.0–32.5)	10.0 (9.5–20.5)	13.0 (9.5–15.0)
Average force, N					
Mean ± SE	183 ± 111	174 ± 23	225 ± 183	168 ± 93	167 ± 87
Median (IQR)	165 (120–196)	167 (161–185)	174 (128–238)	132 (106–210)	149 (113–187)

^aHU, Hounsfield units; IQR, interquartile range.

TABLE 2
Average Force of Impaction Based on Osteochondral Allograft Transplant Graft Size Indicating Uniform and Consistent Impaction Force

Diameter, mm	Depth, mm	Average Force, N ^a	Contrast	P Value ^b
15	5	676 (507–903)	15 × 5 to 25 × 5	.998
25	5	658 (494–881)	15 × 5 to 15 × 10	.552
15	10	841 (632–1121)	15 × 5 to 25 × 10	.913
25	10	756 (565–1005)	25 × 5 to 15 × 10	.460
			25 × 5 to 25 × 10	.842
			15 × 10 to 25 × 10	.911

^aMean (95% CI).

^bAdjusted with the Tukey test for a family of 4 means.

TABLE 3
Maximum Force Based on Osteochondral Allograft Transplant Size Indicating Relatively Uniform and Consistent Impaction

Diameter, mm	Depth, mm	Maximum Force, N ^a	Contrast	P Value ^b
15	5	1317 (956–1810)	15 × 5 to 25 × 5	.992
25	5	1379 (1005–1895)	15 × 5 to 15 × 10	.131
15	10	1908 (1388–2625)	15 × 5 to 25 × 10	.748
25	10	1557 (1130–2135)	25 × 5 to 15 × 10	.228
			25 × 5 to 25 × 10	.886
			15 × 10 to 25 × 10	.612

^aMean (95% CI).

^bAdjusted with the Tukey test for a family of 4 means.

TABLE 4

Linear Regression Model of Total Force

	Estimate^a	95% CI	P Value
Diameter per mm	0.2	(−4.0 to 4.5)	.932
Depth per mm	17.1	(7.7 to 27.4)	.001
Density per HU	0.3	(−0.0 to 0.6)	.064
Intercept	92.2	(23.9 to 355)	<.001

^aPercentage increase in geometric mean of cumulative force per 1-unit change: $\log(\text{total force}) \sim \text{diameter} + \text{depth} + \text{density}$. Bold indicates significance, $P < .05$. HU, Hounsfield units.

TABLE 5

Linear Regression Model of Number of Impacts

	Estimate ^a	95% CI	P Value
Diameter per mm	0.8	2.3 to 4.1	.587
Depth per mm	13.1	6.2 to 20.3	< .001
Density per HU	0.2	−0.0 to 0.4	.117
Intercept	3.9	1.5 to 10.6	.009

^aPercentage increase in geometric mean of cumulative force per 1-unit change: $\log(\text{impacts}) \sim \text{diameter} + \text{depth} + \text{density}$. Bold indicates significance, $P < .05$. HU, Hounsfield units.

TABLE 6

Linear Regression Model: Predicted Total Force and Number of Impacts of Grafts Between 5 and 10 mm

Depth ^a	5 mm	6 mm	7 mm	8 mm	9 mm	10 mm
Total force, N	2128	2492	2918	3417	4001	4686
Impacts	14.2	16.1	18.2	20.5	23.2	26.3

^aReported as expected geometric mean.