In Vitro Analysis of Micronized Cartilage Stability in the Knee: Effect of Fibrin Level, Defect Size, and Defect Location

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Purpose: The purpose of the study is to assess the stability of a dehydrated cartilage allograft combined with platelet-rich plasma sealed with fibrin glue within trochlear and medial femoral condyle (MFC) chondral defects in a cadaver knee model. **Methods:** Defects were made in the trochlea (20, 25, and 30 mm) and MFC (15, 20, and 25 mm) of 6 cadaver specimens. Allograft was applied utilizing 2 different techniques: (1) proud in which the fibrin level extends beyond surrounding cartilage and (2) recessed in which the fibrin level is even with or below the surrounding cartilage. The knees were cycled by using a continuous passive motion machine through a range of motion. Defects were assessed for superficial delamination and displacement of the allograft. This was quantified as the percentage of surface delamination and/or exposed bone. Comparisons were made with regard to defect size, location, and fill. **Results:** In both the MFC and trochlea, proud application resulted in an increased rate of fibrin delamination. In the trochlea, an average of 38% delamination was detected in the recessed 20-mm defect compared with 70% in the proud 30-mm defect ($P < .05$). This effect was increased with increasing defect size. In the MFC, mean delamination of 43% and 28% exposed bone was noticed in the proud 15-mm defect compared with 95% delamination and 71% exposed bone at 25 mm. In 82% of specimens, displacement and/or delamination occurred within the first 15 minutes of testing. **Conclusions:** Increased defect size in both the trochlea and femoral condyle, as well as a proud construct application, were associated with significant delamination and displacement of the allograft/fibrin construct. **Clinical Relevance:** Proud application of allograft increases the likelihood of fibrin delamination and graft displacement in both trochlear and MFC defects. This effect is increased with increasing defect size. These data may support limiting range of motion immediately after an allograft procedure.

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It is well known that articular cartilage defects do not possess the innate ability to restore hyaline cartilage. Microfracture is a well-described and extensively studied technique in which penetration of subchondral bone and subsequent release of the underlying marrow elements lead to the formation of reparative cartilage.[1,2] Despite the technical ease and relative low cost of microfracture, the primary shortcoming of this technique is the creation of biomechanically inferior fibrocartilage, which has decreased long-term durability.[3,4] Therefore, microfracture augmentation techniques have begun to emerge as a possible method to augment the existing technique while minimizing cost.[5]

Micronized dehydrated allogenic cartilage, when combined with platelet-rich plasma (PRP) and sealed with fibrin glue, has been shown to augment the microfracture technique by promoting chondrogenesis and hyaline-like tissue formation.[6-8] This paste-like allograft compound is applied to the base of the defect after microfracture and then sealed with fibrin glue. Although some research has been done on the retention of cartilage scaffolds with fibrin sealant, not much is

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known about technical factors that could improve fixation such as the level of fibrin fill.[9,10] Also, none of these studies have evaluated a micronized application as evaluated in this setting. Similarly, it is unclear what contribution other factors, such as defect size and location, may have. Factors such as these may affect the stability of the allograft construct with early postoperative range of motion (ROM). The stability of the allograft construct is important because disruption and/or displacement of the compound may significantly affect the ability of the allograft to promote cartilage regrowth in the area of the chondral defect. The purpose of the study is to assess the stability of a dehydrated cartilage allograft combined with PRP sealed with fibrin glue within trochlear and medial femoral condyle (MFC) chondral defects in a cadaver knee model. We hypothesize that proud application of allograft, increasing defect size, and the location of the defect (MFC vs trochlea) will have an impact on the stability of the allograft construct.

Methods

Institutional review board exempt status was obtained for this cadaver study. Fresh frozen adult cadaver knees were obtained from Biologic Resource Center of Illinois. The knees were assessed by the senior author (A.B.Y.) who was an orthopaedic sports medicine fellow at the time of the study. Knees with evidence of ligamentous or chondral injury were excluded from analysis. Six unmatched knees without evidence of ligamentous and/or chondral injury were selected and thawed over 24 hours. A medial arthrotomy was performed; and focal, circular, full-thickness chondral defects were created by using the Osteochondral Allograft Transfer System (Arthrex, Naples, FL) scoring reamer and a curette. Defect preparation included removal of the calcified chondral layer and creation of vertical walls with a curette. A 0.0625″ Kirschner wire (1.6 mm) was used to create evenly spaced microfracture holes (3 to 4 mm apart) at the base, avoiding coalescence (Fig 1). Defects were centered over the weight-bearing portion of the MFC and the central trochlea. For each specimen, 3 sequentially larger defects were created and then tested individually at each location; defects included MFC defects of 15-mm, 20-mm, and 25-mm in diameter and trochlear defects of 20-mm, 25-mm, and 30-mm in diameter. Within those defects, the stability of proud versus recessed application of the allogenic/cartilage construct was assessed. A total of 12 individual tests were performed on each specimen. Each given defect location, size, and application method (proud vs recessed) was tested a total of 6 times, 1 time for each of the 6 cadaver knees.

The BioCartilage (Arthrex, Inc, Naples, FL) was prepared according to the manufacturer's guidelines. Equal parts of micronized allogenic cartilage were combined with equine PRP, ensuring that the consistency was paste-like. Barium (0.5 g) was added to allow for monitoring of displacement by means of fluoroscopy. Care was taken to ensure that the barium did not alter the overall consistency of the construct. The amount of barium selected was found to be the minimum amount necessary to adequately visualize the allograft construct with fluoroscopy. If necessary, additional PRP was added until the final consistency was similar to a paste that would be used clinically. Previously tested allograft was not reused after being tested, and a new batch was created by means of the previously described technique for each defect tested. The allograft was applied to the defect using 1 of 2 techniques, proud or recessed. The proud technique involved application of the allograft to the level of the surrounding cartilage, resulting in the fibrin (Evicel fibrin sealant, Ethicon, Somerville, NJ) being relatively proud (Fig 2A). With the recessed technique, the allograft was added such that it fully covered the exposed bone but remained below the level of the surrounding cartilage. Fibrin was then added to maintain a flush level of fill (Fig 2B). The fibrin was

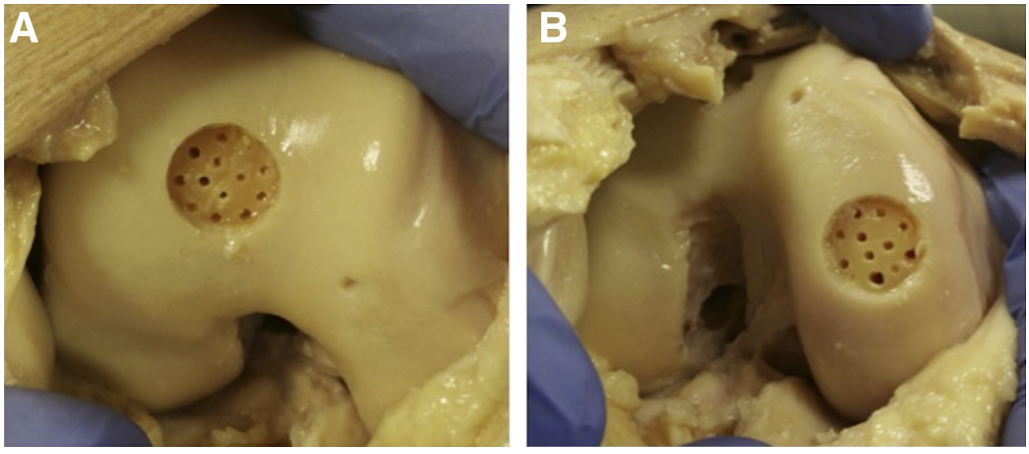

Fig 1. Right knee specimen demonstrating a trochlear (A) and medial femoral condyle defect (B) created with the Osteochondral Allograft Transfer System harvester and subsequent microfracture with a 0.0625″ Kirschner wire.

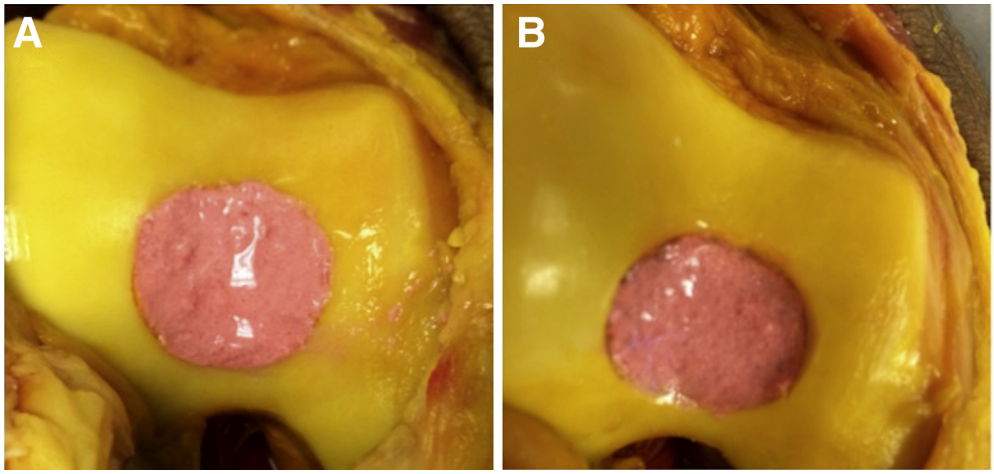

Fig 2. Right knee specimen demonstrating a 25-mm trochlear defect with proud (A) application of BioCartilage and fibrin above the level of the surrounding cartilage and recessed (B) application of BioCartilage and fibrin below the level of the surrounding cartilage.

allowed to dry for a minimum of 5 minutes before any additional manipulation of the knee.

For testing, each knee was positioned and secured on a continuous passive motion machine. The arthrotomy was sutured closed, and 5 cc of normal saline solution was injected into the joint to simulate synovial fluid. The quadriceps tendon was tensioned with a 10-lb weight to simulate an active extensor mechanism (Fig 3). The continuous passive motion machine was used to cycle the knees through a specified ROM: 0° to 120° for MFC defects and 0° to 40° for trochlear defects. After closure of the arthrotomy and tensioning of the quadriceps, we did not note any abnormal patellar tracking in our specimens. Knees were assessed with fluoroscopy after 15, 30, and 60 minutes of cycling. If displacement was noted with fluoroscopy, the arthrotomy was opened, and the status of the construct was documented. Both the percentage of fibrin delamination and the percentage of allograft displacement resulting in exposed bone were assessed by means of visual inspection. Photographs of each defect were taken after testing and then visually inspected to estimate the degree of fibrin delamination and allograft displacement. The arthrotomy was then closed and cycling continued. With the exception of defects with 100% early displacement, all knees underwent 60 total minutes of cycling. At the end of cycling, the arthrotomy was opened, and the final degree of fibrin delamination and/or exposed bone was recorded.

Statistical Analysis

Statistical analysis was performed in Microsoft Excel (Microsoft, Redmond, WA) by using XLSTAT (Addinsoft, New York, NY). Descriptive statistics included average and standard deviations. Comparative statistics were performed utilizing nonparametric testing (Mann-Whitney U test) and significance set at $P < .05$.

Results

Proud Versus Recessed Application

Proud application generally led to an increase in fibrin delamination and exposed bone. In the MFC, there was

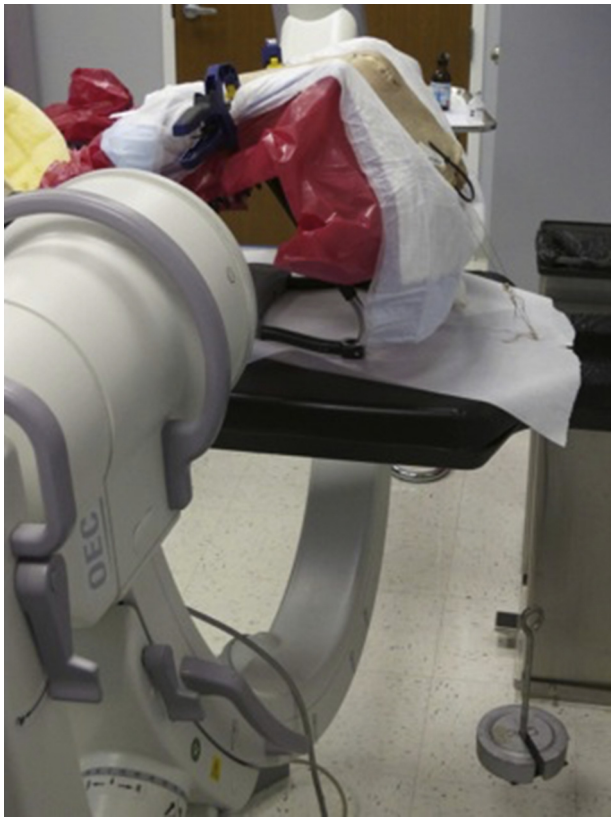

Fig 3. The knee was positioned in the continuous passive motion machine. Ten pounds of traction was added to the quadriceps tendon to stimulate an active extensor mechanism. Trochlear defects were cycled from a range of 0° to 40° of flexion, and medial femoral condyle defects were cycled from 0° to 120° of flexion.

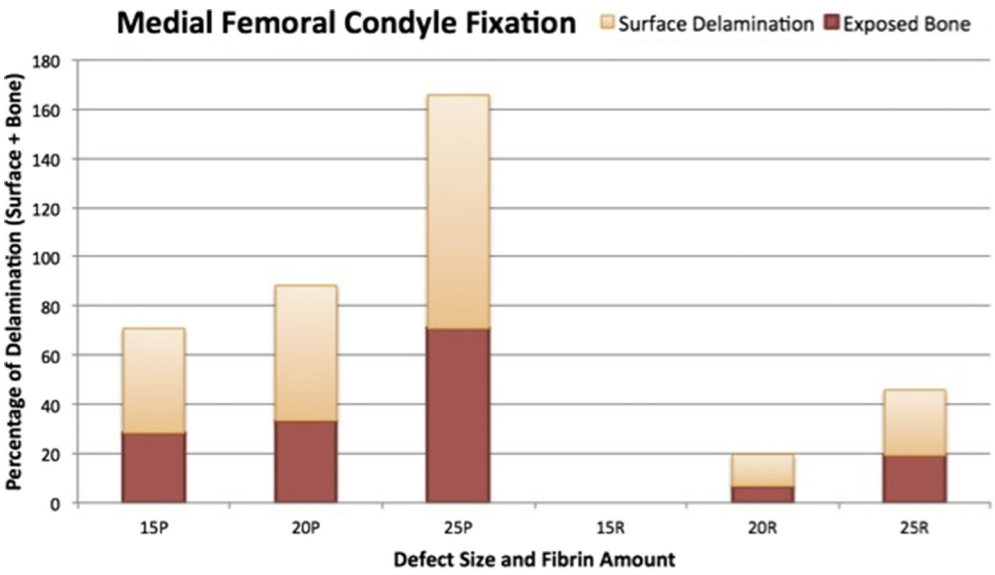

Fig 4. Percentage of fibrin delamination and exposed bone seen with increasing defect size for both proud and recessed application of the BioCartilage fibrin construct in the medial femoral condyle.

a trend toward increased surface delamination in both the 15-mm (proud: 43% delamination vs recessed: 0% delamination, $P = .06$) and 20-mm (20-mm proud: 55% delamination vs 20-mm recessed: 13% delamination, $P = .055$) defects. The impact of proud versus recessed application was more pronounced at the largest defect size (25 mm) where there was a significant increase in both surface delamination (proud: 95% delamination vs recessed: 27% delamination, $P = .03$) and bone exposure (proud: 70% bone exposure vs recessed: 19% bone exposure, $P = .045$) (Fig 4) (Table 1).

In the trochlea, proud application of allograft generally increased the amount of fibrin delamination. Proud application increased surface delamination in the 20-mm (proud: 38% vs recessed: 7%, $P = .03$) defect (Fig 5). However, proud application of allograft in the trochlea had no significant impact on the amount of exposed bone (Fig 6) (Table 2).

Impact of Defect Size

Increasing defect size resulted in increased delamination and bone exposure in both the proud and recessed defects and in both the MFC and the trochlea. In the recessed MFC defects, there was an increase in surface delamination in the 20-mm defect compared with the 15-mm defect (13% delamination vs 0% delamination, $P = .02$). There was no significant difference in exposed bone among the recessed MFC defects. A similar trend was seen in the trochlea where increased size of recessed defects led to an increase in surface delamination but no significant increase in allograft displacement with exposed bone (20 mm: 7% vs 25 mm: 38%, $P = .06$) (20 mm: 7% vs 30 mm: 38%, $P = .045$).

In the proud MFC defects, increasing defect size led to a significant increase in both surface delamination and exposed bone. A significant increase in exposed bone was noted at each increase in defect size (15 mm: 28% vs 20 mm: 33%, $P = .04$) (20 mm: 33% vs 25 mm: 70%, $P = .049$). In the trochlea, increasing defect size did not affect the degree of surface delamination with proud allograft application. However, there was a significant increase in bone exposure seen in the proud 30-mm trochlear defect compared with the smaller defects (30 mm: 32% vs 25 mm: 12%, $P = .03$).

Table 1. Percentage of Fibrin Delamination and Exposed Bone Seen With Increasing Defect Size for Both proud and recessed Application of the BioCartilage Fibrin Construct in the Medial Femoral Condyle

	Medial Femoral Condyle Fixation		
	Defect Size (mm)	Surface Delamination (%)	Exposed Bone (%)
recessed	15	0 ± 0	0 ± 0
	20	13 ± 11	7 ± 9
	25	27 ± 35	19 ± 32
proud	15	42.5 ± 44	28 ± 43
	20	55 ± 40	33 ± 44
	25	95 ± 15	70 ± 34

Timing of Displacement

In the majority of specimens (82%), the fibrin delamination and/or allograft displacement occurred within the first 15 minutes of testing. Additional delamination and/or displacement was noted in the remaining 54% of these specimens after the full 60 minutes of testing. In 18% of specimens, displacement and/or delamination did not occur until after 60 minutes of testing.

Discussion

Fibrin level and defect size had a significant impact on the stability of the allogenic cartilage/fibrin construct in

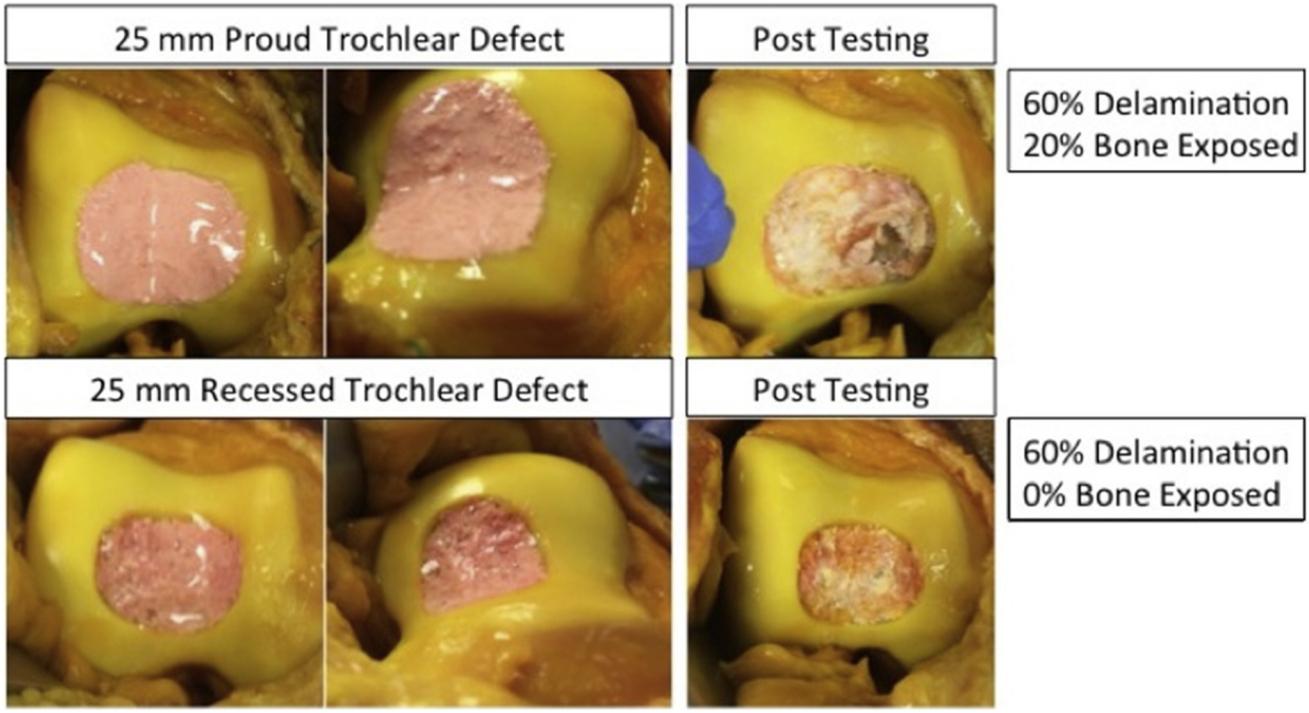

Fig 5. Comparison of fibrin delamination and bone exposure in a right knee 25-mm trochlear defect treated with proud versus recessed application of the BioCartilage fibrin construct.

our cadaver study of microfracture augmentation. Proud application of fibrin above the level of the surrounding cartilage resulted in an increased rate of fibrin delamination and displacement of the allogenic cartilage/fibrin construct resulting in exposed bone. This effect was seen in both the trochlea and the MFC and was more pronounced with increasing defect size. Even with recessed application of the allogenic cartilage/fibrin construct, there was evidence of fibrin delamination at larger defect sizes. These data may support limiting ROM immediately after the allograft procedure.

Microfracture is a commonly performed procedure for defects <2 cm^2; however, results are limited because the resulting fibrocartilage is less resistant to shear force and thus less durable compared with native hyaline cartilage. Augmentation of microfracture has been explored with promising results. Improved clinical

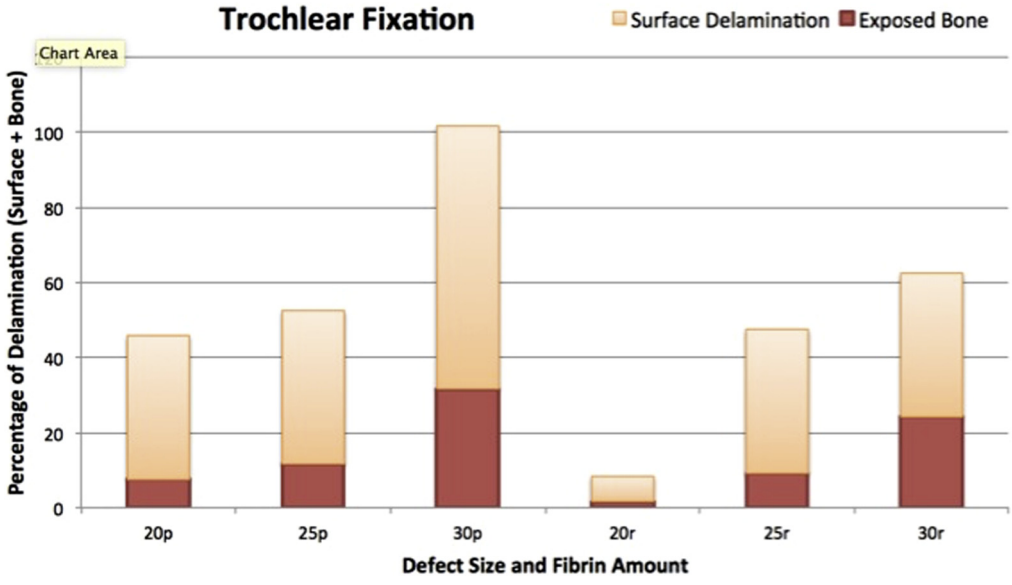

Fig 6. Percentage of fibrin delamination and exposed bone seen with increasing defect size for both proud and recessed application of the BioCartilage fibrin construct in the trochlea.

Table 2. Percentage of Fibrin Delamination and Exposed Bone Seen With Increasing Defect Size for Both proud and recessed Application of the BioCartilage Fibrin Construct in the Trochlea

	Trochlea Fixation		
	Defect Size (mm)	Surface Delamination (%)	Exposed Bone (%)
recessed	20	7 ± 10	2 ± 4
	25	38 ± 27	9 ± 10
	30	38 ± 34	24 ± 31
proud	20	38 ± 33	8 ± 12
	25	41 ± 29	12 ± 10
	30	70 ± 28	32 ± 17

outcomes have been shown in PRP-enhanced microfracture of the talus.[11]

Several studies have included reports on the potential benefit of microfracture augmentation with porous scaffolds derived from articular cartilage. These scaffolds function to promote differentiation of stem cells toward a chondrogenic phenotype.[5,12,13] Xing et al.[5] demonstrated a higher percentage of type II collagen and improved glycosaminoglycan content in trochlear lesions when microfracture was augmented with osteochondral autograft paste harvested from the intercondylar notch.

Another particulated juvenile cartilage technique (DeNovo NT) has shown early promising results. With DeNovo NT, fibrin glue is applied to subchondral bone at the base of the defect. The juvenile cartilage is than applied on top of the glue, and the entire construct is then covered with an additional layer of fibrin glue. In addition to improved clinical outcomes, use of this technique has been associated with varying amounts of hyaline cartilage in repaired lesions as determined by histologic analysis. During an investigation by Farr et al.,[14] 2 of 11 patients showed delamination on arthroscopic evaluation. The allograft is a dehydrated, micronized articular cartilage substrate obtained from juvenile donor cadavers. Hirahara and Mueller[8] describe micronization as a potential benefit over DeNovo NT because it increases the effective surface area of cartilage matrix, allowing for a more even distribution of hyaline cartilage regrowth.

The micronized allogenic cartilage and PRP combination product must be used in contained defects with healthy surrounding native cartilage to allow for stability of the blood clot formed with bone marrow stimulation. In our study, the stability of the allograft was affected by the amount of allograft used, the size of the defect, and the location of the defect. In general, proud application of the allograft construct above the level of the surrounding articular cartilage leads to an increased rate of fibrin delamination and displacement of the allograft with exposure of the underlying bone. This effect is more pronounced in the MFC compared with the trochlea and in larger defects compared with smaller ones.

Our current recommendations for allograft recipients during the postoperative period are supported by the findings of this study and include initiation of ROM early in the postoperative course to maximize healing potential. Evidence has shown that early ROM allows for increased synovial fluid motion and thus improved nutrition to the cartilage repair.[15] For treatment of both femoral condyle and trochlear lesions, continuous passive motion is started 48 hours after the procedure (allowing for clot stabilization) for 6 hours daily and continued for 6 weeks. We begin condyle lesions at 0° to 40° and advance 5° to 10° daily as tolerated. Trochlear lesions begin at 0° to 30°, advancing an additional 30° every 2 weeks. Passive and active assisted ROM are started after 2 weeks. Weight bearing is limited for patients with condyle lesions to decrease potential overgrowth. After 6 weeks, the patient begins graduated weight bearing over 4 weeks. Trochlear lesions are full weight bearing with brace locked in extension.

Limitations

This study has several limitations, including lack of a power analysis. Despite testing of each state (a given defect size with both proud and recessed application) a total of 6 times, it is possible that a type II error may have occurred, resulting in a nonsignificant difference between 2 given testing states when 1 did truly exist. This study is an in vitro assessment in which a cadaver model was used to replicate the stresses associated with postoperative ROM. The true effect of the micronized cartilage allograft application, defect size, and defect location on the construct stability in an in vivo model is not known. Clot formation and other in vivo factors likely change the consistency of the construct over the first several days after implantation. Application of the allogenic cartilage construct was performed in a manner intended to mimic what would be done clinically. The main distinction in application was a recessed application versus a proud application as described in the Methods section. The exact volume of the allogenic cartilage construct was not quantified objectively. It is possible that some of the "proud" constructs may have been more proud than others, and the same may be true of the "recessed" constructs. This discrepancy may have potentially affected the results of the study. Defects were placed centrally within the MFC and trochlea. Some variability in defect position may exist between specimens, which may have affected the results. In a given knee, 3 different defect sizes were tested, resulting in the arthrotomy being opened and closed for each new testing session. It is possible that repeated opening and closing of the capsule may have altered the soft tissue environment and affected the

results. The degrees of cartilage displacement and fibrin delamination were also subjective and estimated from visual inspection of the cartilage/fibrin construct during testing. This study assesses the rate of delamination and/or displacement of the allograft, but full clinical consequences are not known. There is likely a threshold percentage of disruption at which the ability of the allograft to augment chondral regeneration becomes negatively affected. In our study, post-treatment passive ROM was limited to 30° of knee flexion. This value was chosen because it reflects the postoperative parameters of the senior author for management of articular cartilage procedures involving the patellofemoral joint. The impact of passive ROM parameters outside of this range on the stability of an allograft in a trochlear defect is not known and may differ from what was observed in our study. For the purpose of monitoring displacement of the construct with intraoperative fluoroscopy, barium was added. This is not done clinically, and although we did not note a change in consistency in the final construct, it is possible that it may have been altered by the addition of the barium and may have affected our results.

Conclusions

Increased defect size in both the trochlea and femoral condyle, as well as a proud construct application, was associated with significant delamination and displacement of the allograft/fibrin construct.

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