Introduction: Intrinsic repair of articular cartilage is unsatisfactory, and untreated focal defects (without extensive damage to the subchondral bone) impair quality of life to the same extent as do more widespread osteoarthritic (OA) changes to the joint (1). As such, there is substantial interest in treating localized defects in certain patient populations (2, 3). Pre-clinical large animal models, such as the goat, sheep, and pig, have provided a wealth of information on the efficacy of potential treatments (4-8). Some studies have noted a marked remodeling of the subchondral bone following the creation of a purely chondral defect; however, the mechanisms underlying this response have not been well characterized (9). One hypothesis is that the surgical procedure creates microscopic damage to the underlying bone, instigating a remodeling response. Alternatively, the lack of mechanical function in the repair tissue or implant material could lead to subchondral remodeling due to decreased load transfer to the bone. To gain further insight into this issue, the objective of this study was to compare the healing response and subchondral remodeling in models of cartilage injury that do and do not create microdamage to the bone, both in the context of naturally forming repair tissue and with treatment using a cartilage autograft that provides functional load transfer to the subchondral bone.

Methods: In seven Yucatan minipigs, chondral defects (4 mm diameter) were created bilaterally in the trochlear groove of the stifle joint. Five experimental groups were compared: 1) an untreated full thickness defect (untreated FTD, n=14), 2) a full thickness defect treated with microfracture (FTD-MF) (n=6), 3) a full thickness defect treated with transfer of autologous cartilage (FTD-ACT) (n=7), and 4) an untreated partial thickness defect (untreated-PTD, n=3). Normal cartilage served as a positive control (n=14). Other groups not reported here were also performed, giving rise to the unequal sample sizes. At 6 weeks, animals were euthanized. Bone morphometry under the defect site was determined using microcomputed tomography (μCT). Bone volume per total volume (BV/TV) was calculated for the first 2 mm and for a region 3-5 mm beneath the original defect. Histological evaluation included cell morphology (hematoxylin & eosin) and matrix staining (proteoglycan and collagen via Safranin O/fast green). Samples were scored using a modified ICRS-II system (7). BV/TV and histological scores between groups was compared via ANOVA with Games-Howell post-hoc tests to account for the unequal variances between groups (p<0.05).

Results: At the time of surgery, a small amount of bleeding from the subchondral bone was noted following the creation of all full thickness defects, while no bleeding was observed when creating the partial thickness defects. Six weeks after surgery, bone morphology of the groups involving a full thickness cartilage defect showed evidence of bone remodeling and resorption beneath the defects, with regional differences (Fig. 1A). Quantitatively, within 2mm of the cartilage/bone interface the BV/TV for these groups were 55-61% lower than normal (p<0.05) and 56-62% (p0.05). In terms of histologic appearance (Fig. 2A), the untreated FTD group filled incompletely with a mostly fibrous tissue. MF treatment led to a similar appearance, with some samples showing more robust staining for proteoglycans. ACT treatment resulted in fill of the vast majority of the defect space with tissue that stained well for proteoglycans; however, these constructs were quite variable in their ability to integrate with the surrounding tissue. From ICRS-II scoring (Fig. 3), the mean overall values for the FTD groups were 12-57% lower than normal (p<0.05). Additionally, the untreated FTD group was 48% and 51% lower than the untreated PTD and FTD-ACT groups, respectively (p<0.05). In terms of matrix staining, the untreated FTD and FTD-MF groups were 57% and 43% lower than normal, respectively (p<0.05). Additionally, the untreated group was 55% lower than the FTD-ACT group (p<0.05). Finally, in terms of cellular morphology, the full thickness defect groups were 12-65% lower than normal, and the untreated group was 60% lower than the FTD-ACT group (p<0.05). Additionally, no differences were found between the untreated PTD and any of the full thickness defect groups for matrix staining and cellular morphology (p>0.05).

Discussion: In this study, we quantitatively assessed the role of the severity of a focal cartilage injury as well as potential treatments on the healing response of cartilage as well as the remodeling of the subchondral bone in a porcine model. Interestingly, substantial bone remodeling occurred when a full thickness defect was created. This effect was independent of treatment group, as similar levels of bone remodeling occurred if the defect was left untreated, was treated with microfracture, or was treated with an autologous cartilage plug. On the other hand, creation of a partial thickness chondral defect had no impact on the underlying bone. Together, these results suggest that the bony remodeling observed is a result of the injury to the subchondral bone surface and not treatment (or capacity for load transmission). Indeed, even filling the defect with an autologous cartilage plug, which should allow transfer of mechanical loads to the bone (10), could not prevent remodeling, while
the partial chondral injury (which did not allow for mechanical load transmission) showed little bony remodeling. One limitation of this study is the use of an adolescent porcine model, which lacks a layer of calcified cartilage in the trochlear groove. Thus, creation of a full thickness defect resulted in unavoidable microscopic damage to the subchondral bone and bleeding within the defect (5). Other animal models with a layer of calcified cartilage may allow the creation of full thickness defects without bony remodeling, although some studies in the skeletally mature goat model suggest otherwise (9). Despite the remodeling, transfer of autologous cartilage was able to restore the histological appearance of the native cartilage, with histological scores substantially higher than the untreated or MF groups, which filled with a fibrocartilaginous tissue. These data indicate that the type of cartilage injury should be carefully controlled in future studies to evaluate tissue engineering or regenerative medicine approaches. Longer-term studies are also warranted to determine whether such subchondral abnormalities resolve towards the reestablishment of a patent subchondral architecture if provided a longer time course for healing and remodeling.

**Significance:** The severity of a focal chondral defect dictates the amount of bony remodeling in the porcine model. These data will guide future work in the evaluation of tissue engineering and regenerative medicine strategies for cartilage repair using this animal model.

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Figure 1. Typical 3-D μCT reconstructions of bone (A) (centered under defect, scale bar = 2 mm). Bone Volume/Total Volume in regions adjacent to (0-2 mm) and further removed (3-5 mm) from the cartilage interface (B) (*p<0.05 vs. normal, bars-p<0.05 between groups). (FTD=full thickness defect; PTD=partial thickness defect; MF= microfracture; ACT=autologous cartilage transfer)
Figure 2. (A) Histological staining for proteoglycans (red) and collagen (green) following 6 weeks in-vivo (scale=2mm, overall score shown). (B) Histological scoring: Overall assessment (A), matrix staining (B), and cellular morphology (C) (*p<0.05 vs. normal, bars-p<0.05 between groups).

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