

# Age Dependent Cartilage Repair and Subchondral Bone Remodeling in a Minipig Defect Model

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## Introduction

Given the prevalence of focal cartilage injuries, and the propensity for such defects to instigate the early onset of osteoarthritis, there exist a number of surgical treatments and options to enhance repair. While such treatments can positively impact regeneration of cartilage, they may have unwanted effects on adjacent structures. For example, after treatment by microfracture or autologous chondrocyte implantation (ACI), subchondral bony remodeling has been reported, ranging in severity from upward bony migration<sup>1,2,3,4</sup> to the formation of intralesional osteophytes or cysts.<sup>5,6,7</sup> Similar to the human condition, large animal models also show changes in subchondral bone during repair of chondral defects.<sup>8,9,10,11</sup> In studies involving microfracture, upward subchondral bone plate migration is commonly reported.<sup>12,13</sup> Here, unloading of the underlying bone (due to the lack of load transmission through the cartilage in the defect) may contribute to this atypical remodeling.<sup>10</sup> Given these findings, and because such remodeling could compromise outcomes of tissue engineered cartilages in repair approaches, the objective of this study was to evaluate subchondral bone remodeling as a function injury type and repair scenario in a Yucatan minipig. Further, since skeletal maturity likely impacts both bone remodeling (due to changes in subchondral bone activity) and inherent cartilage repair capacity (likely due to changes in endogenous stem cell populations and reduced cell number), both skeletally mature and immature groups were evaluated.

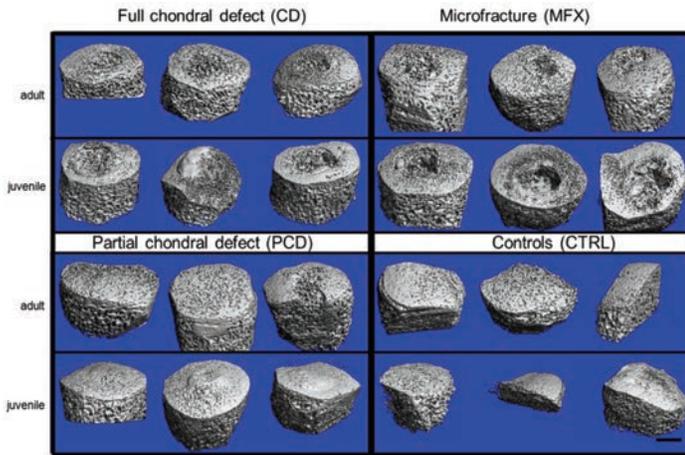
## Methods

To carry out this study, three juvenile (6 months, male, 32.9-37.7kg) and three skeletally mature (18 months of age, male, 62.0-64.0kg) Yucatan minipigs were used with IACUC approval from the Philadelphia VA Medical Center and the University of Pennsylvania. In each animal, 4mm diameter defects were created bilaterally in the trochlear groove [10]. Treatment conditions included an untreated full thickness chondral defect (CD, n = 3adult/3juvenile), a partial thickness (~50%) chondral defect (PCD,

n = 3/3), and a full thickness chondral defect treated with microfracture (MFX, n = 3/3). After 6 weeks post-operatively, animals were euthanized and joints harvested. Following imaging the gross appearance of each lesion, osteochondral samples containing the lesion site were removed from the trochlear groove and imaged by micro-CT (55kVp and 145μA). Bone volume per total volume (BV/TV), trabecular thickness, trabecular number and trabecular spacing were quantified as a function of distance from the cartilage interface (Fig. 2a). Samples then were scanned a second time after incubation with Lugol's solution for 48 hours in order to visualize defect fill. Samples were next decalcified, cut to 6μm thin sections, stained with Safranin O/Fast-Green or H&E and scored by 6 blinded reviewers using the ICRS II scoring system.<sup>13</sup> To analyze the quality of the formed tissue, immunohistochemical staining for type II collagen was also performed. One adjacent osteochondral plug per joint served as control. Statistical analysis was carried out using two-way ANOVA with individually performed posthoc tests to maintain overall alpha level at  $p < 0.05$ .

## Results

Micro-CT analysis showed marked differences between adult and juvenile minipigs in terms of BV/TV in the subchondral regions of cartilage lesions. CD and MFX groups showed increased bone loss in juveniles compared to adults, while the PCD group showed a slight increase in BV/TV in juveniles (Figure 1 and Figure 2c). Results reached significance ( $p < 0.006$ ) between defect groups in range 1 (see Figure 2c). Defect fill (Figure 2b) assessed from post-Lugols micro-CT was not significantly different between animals or groups, but tended to be higher in juveniles compared to adults. Histology showed qualitatively better fill in juveniles, with some evidence of Safranin O positive staining. Quantification of this histology using the ICRS II scoring system showed equal overall assessment for the CD groups, better overall assessment for the juvenile MFX groups compared to adult MFX, and values close to the control samples for the PCD groups (Figure 3b). Furthermore, for the CD



**Figure 1.** Micro-CT imaging of adult and juvenile subchondral bone after cartilage injury and repair. Juvenile minipigs showed significant bone loss 6 weeks postoperatively in both the full chondral defects (CD) and microfracture (MFX) groups, while osseous overgrowth was visible in partial chondral defects (PCD). Adults showed little change in subchondral bone in any group aside from MFX.

group, there was less alteration in the subchondral bone and a slightly better basal integration noted in adults compared to juveniles. Likewise, the MFX group showed decreased basal integration in juveniles ( $p < 0.01$ ) compared to adults (Figure 3b). Staining for collagen II showed more intense signal in juvenile CD and MFX groups compared to the same repair groups in adults (data not shown).

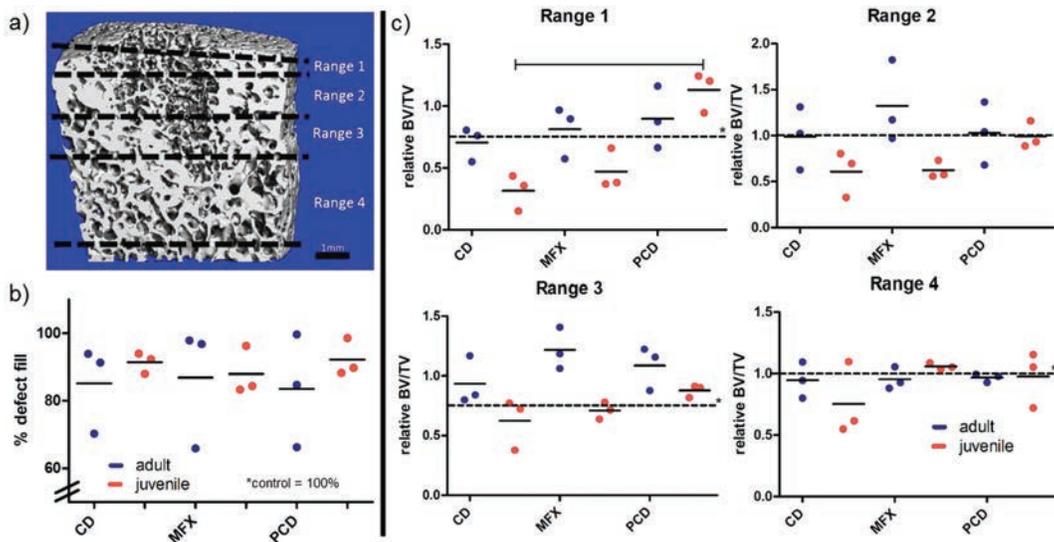
## Discussion

This study showed more intense subchondral bone remodeling in juvenile minipigs compared to adults, even when the cartilage injuries did not physically perturb the subchondral plate. Indeed, while full chondral and MFX groups showed a substantial loss in bone beneath the defect,

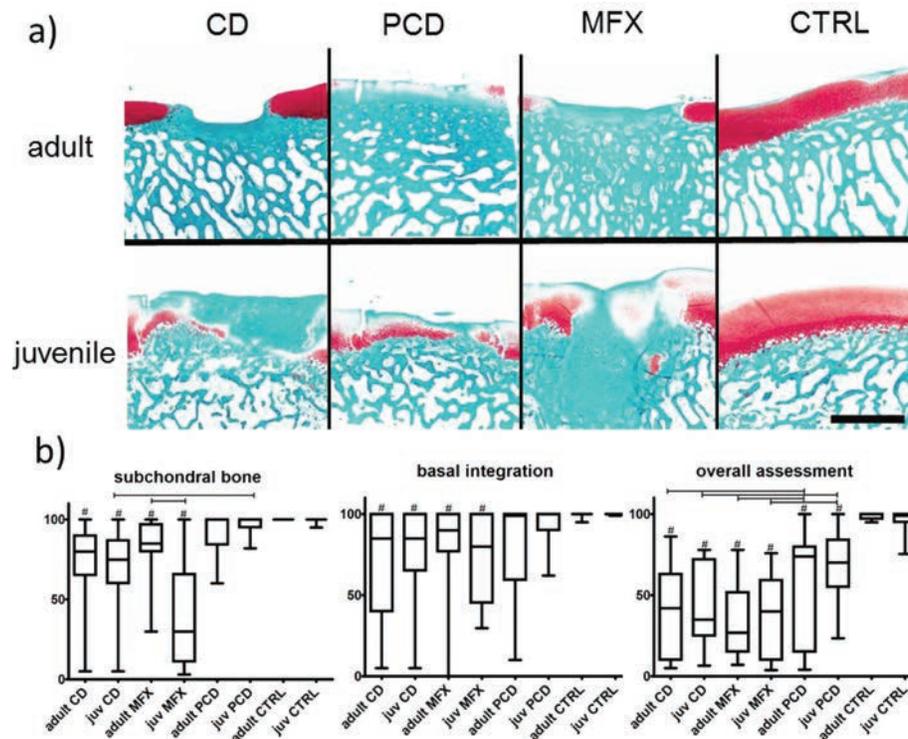
PCD groups showed some evidence of overgrowth. These findings are consistent with previous reports in the literature.<sup>9</sup> We also found that, while defects of both ages filled to some extent with fibrous tissue, defects in juvenile animals filled to greater extent and were more likely to contain PG and type II collagen, indicative of better quality repair. Additional time points are required to fully elucidate the spatiotemporal pattern of boney remodeling and defect fill, and further studies are required to understand the causative mechanism of the bone remodeling in juveniles and the apparent decrease in cartilage formation in the adults. Based on these findings, it is recommended that both pre-clinical and clinical studies of cartilage repair carefully evaluate and monitor changes in subchondral bone, for instance using novel MRI imaging methods to avoid radiation exposure in patients. Regardless of the cause, the boney remodeling needs to be addressed if the minipig is to be used for the study of cartilage repair techniques. That is, it will be difficult to interpret findings of even the best engineered cartilage in a repair site if it is placed atop a subchondral bone plate undergoing marked remodeling. A remodeling suchondral plate may likewise increase the risk of treatment failure for cell based cartilage repair<sup>3</sup> and so speed the onset of osteoarthritis as a consequence of altered biomechanical signals in the cartilaginous repair tissue.

## Significance

This large animal study of cartilage repair shows the significant impact that skeletal maturity has on the on the propensity of subchondral bone to remodel as result of chondral injury. This is important finding to consider as a selection criteria for studying cartilage repair in animal models, and could likewise direct new analyses and understanding of human patient's cartilage repair outcomes and be an important factor in the effectiveness of new therapeutic approaches.



**Figure 2.** (A) Schematic of micro-CT analysis in each range (as a function of distance from the bone-cartilage interface). (B) Defect fill of cartilage lesions showed a trend towards better fill in juvenile minipigs. The three lower values in the adult minipigs were derived from different animals. (C) Analysis of bone volume per total volume (BV/TV) as a function of distance from the cartilage-bone interface and lesion type. Cartilage defects led to marked decreases in BV/TV in range 1 in juvenile animals, while little changes occurred in the adults.



**Figure 3.** (A) Safranin O/Fast Green staining of representative samples for each group and age. (B) ICRS II score for overall assessment, integrity of the subchondral bone, and basal integration ( $p < 0.006$  compared to age related ctrl group).

## Acknowledgements

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