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

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 bone remodeling has been reported, ranging in severity from upward boney migration 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, 32.9-37.7 kg) and three skeletally mature (18 months of age, male, 62.0-64.0 kg) Yucatan minipigs were used with IACUC approval from the Philadelphia VA Medical Center and the University of Pennsylvania. In each animal, 4-mm 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)

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-Lugol's 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...
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.\(^9\)

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 bone 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 subchondral plate may likewise increase the risk of treatment failure for cell based cartilage repair\(^3\) 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 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.
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References