AGE-RELATED DECLINE IN OSTEOCHONDRAL REPAIR IN A RAT MODEL

INTRODUCTION
 Damaged articular cartilage rarely heals or regenerates in middle-aged and elderly adults, who are at increased risk for osteoarthritis following articular trauma (1,2). Although the mechanism of this apparent aging effect is unclear, our recent work with bone marrow-derived mesenchymal stem cells (BMSCs) in an in vitro chondrogenesis model indicated that cartilage regeneration may be impaired by intrinsic age-related declines in the chondrogenic potential of BMSCs (3-4). We hypothesized that this decline would impair cartilage regeneration in older adults. To test this we developed an osteochondral (OC) defect model in the rat. The effect of aging on the ability to regenerate a hyaline cartilage-like matrix in OC defects was studied by comparing the repair responses of juvenile rats (1-month-old), young adult rats (3-month-old), and old adult rats (12-month-old). Cartilage extracellular matrix production within the defects was assessed by safranin-O histology and image analysis at 6-weeks post-injury.

METHODS
 Sprague Dawley rats of varying ages (4 per age group) were anesthetized and a knee arthrotomy was performed to expose the distal femur. A 0.75 mm drill bit was used to create an OC defect to a depth of 1.5-2 mm below the joint surface in the femoral condylar groove (Figure 1). The rats were housed for 6 weeks after surgery. All animal procedures were performed in accordance with the regulations of the University of Iowa Animal Care Committee.

Distal femurs were dissected free of extraneous tissue, fixed in formalin and prepared for paraffin histology. Sections cut through the defects were mounted on slides and stained with safranin-O/fast green. The sections were scanned at high resolution (1.1 pixels per μm) and a custom MATLAB-based (Mathworks) image analysis program was used to measure the intensity of safranin-O (Saf-O) staining within the defects. Stain intensity within defects was normalized to stain intensity in the same area on the contra lateral (non-operated) femur (Relative Intensity). One-way analysis of variance was used to test for significant differences between the age groups.

RESULTS

Figure 1. Osteochondral Defect Model (A) The surface of the distal femur was exposed and a 0.75 mm drill bit was used to create a hole in the condylar groove (indicated by the arrow) through the articular cartilage surface and subchondral bone. (B) Saf-O stained section of a rat distal femur showing a defect immediately after it was made. The original cartilage surfaces are seen above and below the defect, which was filled with a fibrin clot at this stage. Bar = 1 mm.

Figure 2. Saf-O Staining Intensity of Repair Tissue Columns and error bars indicate means and standard deviations based on at least 4 rats per age group. Staining intensity was significantly higher in 1-month-old (1-Mo) and 3-month-old (3-Mo) rats than in 12-month-old (12-Mo) rats.

DISCUSSION
 This study showed a marked age-related decline in the potential for cartilage regeneration in OC defects in rats. Defects in 1-month-old and rats were filled with a hyaline cartilage-like matrix by six weeks after injury. Defects in 3-month-old rats were also filled with hyaline cartilage but there was a trend toward lower PG density than was found in 1-month-old rats. In both cases the repair tissue was well integrated with subchondral bone and lateral cartilage surfaces. In contrast defects in 12-month-old rats contained significantly less PG than in younger rats and the repair tissue was not well integrated with surrounding tissues.

The results of this in vivo study are consistent with previous findings, which showed an age-related loss of chondrogenic activity in rat BMSCs in vitro. This suggests that the lack of healing in older rats that we observed in this study was attributable to aging changes that restrict the ability of BMSCs to differentiate appropriately for hyaline cartilage formation. Such a deficit might explain the age-related failure of spontaneous cartilage regeneration in humans following articular injury. Moreover, aging effects could hamper efforts to augment OC defect repair in older patients by delivery of autologous BMSCs to the injury site.

REFERENCES

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