

IN VIVO EVALUATION OF A ONE STEP AUTOLOGOUS CARTILAGE RESURFACING TECHNIQUE IN A LONG TERM EQUINE MODEL

+*Frisbie, DD; **Lu, Y; *Colhoun, HA; * Kawcak, CE; **Binette F; *McIlwraith, CW
 *Orthopaedic Research Laboratory, Colorado State University, Fort Collins, CO
 ** DePuy Biologics, DePuy Spine, a Johnson & Johnson Company, Raynham, MA
 dfrisbie@colostate.edu

INTRODUCTION

Damaged articular cartilage has a poor capacity for spontaneous healing and lesions left untreated often leads to osteoarthritis. Currently autologous chondrocyte implantation (ACI) is the preferred cell based cartilage resurfacing technique [1]. Current ACI techniques require two surgical procedures, the 1st for harvest of autologous chondrocytes, followed by in vitro cell expansion and a second surgical procedure for re-implantation of cells. Some of the ACI procedures also rely on harvest of autologous periosteum, which is associated with some morbidity. The current study evaluates a one step surgical procedure that does not require the use of autologous periosteum. It utilizes a similar amount of harvested autologous cartilage compared to current ACI techniques but minces the cartilage into small fragments for immediate implantation after the fragments are glued to a reabsorbable synthetic scaffold. This study is designed to compare long term result of this novel one step intra-operative technique to both a previously described ACI technique [2] and empty or untreated cartilage defects.

METHODS

Sixteen young skeletally mature horses free of musculoskeletal lesions were entered into an Animal Care & Use Committee approved study. Two clinically relevant defects (15mm diameter) were created in one limb. Cartilage was debrided to the level of the calcified cartilage (Outerbridge grade III) on the medial trochlea of the distal femur. Four different scaffold materials were tested with or without the presence of minced cartilage and compared to empty defects or those treated with a modified ACI procedure. For the purpose of this study the ACI control group consisted of the arthroscopic harvest of 300mg of articular cartilage one month prior to implantation. The chondrocytes were expanded and seeded onto a Porcine Small Intestinal Submucosa (SIS) scaffold and were allowed to attach for 5 days prior to implantation (2). All other construct preparation was performed at the time of defect creation. A small fraction of the cartilage recovered during defect creation was minced into fragments and attached to either scaffold made of PGA/PCL foam reinforced with PDS mesh (fragments on PDS reinforced foam [FP]), Porcine derived scaffold-SIS (fragments on SIS [FS]) or Panacryl non-woven (fragments on Panacryl [FY]) using fibrin glue (Tisseal) as the cohesive support. Defects were left empty untreated (Empty [E]) or filled with PDS reinforced foam (PDS [P]) or Panacryl non-woven (Panacryl [Y]) alone as control treatments. When constructs were used in a defect, they were affixed using three PDS/PGA staples per defect; no staples were placed in empty defects. Defect healing was assessed arthroscopically at 4, 8 and 12 months, and at 4 months a 4mm osteochondral biopsy of defect and adjacent normal tissue was harvested for histologic examination. All horses were humanely sacrificed following the 12 month arthroscopic evaluation. Magnetic resonance imaging (MRI), high detail radiographs, gross, histologic (morphometric and histochemical) and immunohistochemical evaluation of the defect and surrounding tissues were performed at 12 months. Clinical evaluation of pain was also performed at 2 month intervals throughout the study.

Data were analyzed using one or a combination non-parametric frequency tables and Mixed model analysis of variance. When individual comparisons were made a Least Square Means procedure was used, a p-value < 0.05 was considered significant. Data is presented by the means +/- standard error.

RESULTS

No significant abnormalities were encountered with any of the surgical procedures. Harvesting 300mg of articular cartilage did not appear to be associated with any significant long-term morbidity as assessed by the outcome parameters of this study. Defect creation and implantation of constructs were also completed without significant problems. Arthroscopic evaluation of repair tissue demonstration continued maturation of repair tissue throughout the study period. Furthermore, the overall repair tissue score was better for Fragments on PDS reinforced foam and worse for Panacryl alone when compared to Empty

defects at all evaluation periods. Defects within a particular joint were also compared to each other and a defects receiving Fragments on PDS reinforced foam were judged as being "better" 80% of the time, this proportion was significantly greater than any other treatment group. Clinical examinations of pain, which included visual gait abnormalities, response to limb/joint flexion and joint effusion, did not demonstrate significant differences between treatment groups with the exception of joints containing Panacryl only defects which were worse compared to all other treatment combinations. Neither high detail radiographs nor MRI were sensitive enough to detect a significant treatment effect but did support the arthroscopic findings. Necropsy and histologic examinations had similar statistical findings as the arthroscopic examinations (Figure 1). The overall histologic grade (modified O'Driscoll) also demonstrated that defects treated with cell based therapies as a whole performed better compared to non cell based treatments with scaffolds. Furthermore, Panacryl alone was significantly more hypocellular, associated with more adjacent tissue degeneration and subchondral bone inflammation as well as worse proteoglycan staining compared to empty and most cell based treatments.

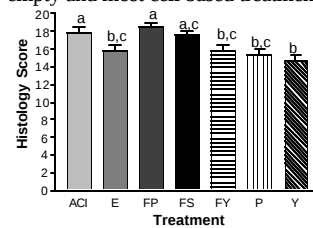


Figure 1 – Plot of total histologic score by treatment group. Different letter indicate statistical differences.

CONCLUSION

This study demonstrated the feasibility and ease of using a one step surgical procedure for cartilage resurfacing. No significant side effects were noted and all treatments were safe. Maturation of repair tissue was observed arthroscopically and histologically throughout the study. Panacryl alone performed worse than the other treatment groups using multiple different outcome parameters. Conversely, Fragments on PDS reinforced foam performed best compared to the other treatment groups and was significantly better than empty defects and similar to ACI treatment. Histologically, areas of hyaline-like tissue were present in some of the cell based treatment conditions, especially for Fragments on PDS reinforced foam condition at 12 months post implantation. In conclusion this novel treatment provides a similar repair tissue to accepted ACI techniques in a one step surgical procedure and warrants further clinical evaluations.

REFERENCES

1. Bentley, G., et al., *A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee*. J Bone Joint Surg Br, 2003. **85**(2): p. 223-30.
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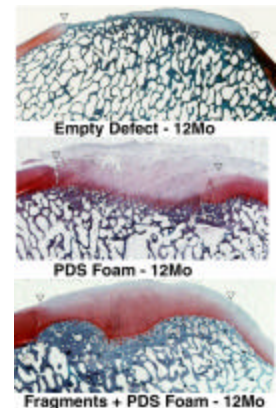


Figure 2 – SOFG stained sections of repair tissue at 12 months post-implantation, arrows demarcate defect margin.