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Evaluation of autologous chondrocyte transplantation *via* a collagen membrane in equine articular defects – results at 12 and 18 months[1]

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Summary

Objective: To evaluate a technique of autologous chondrocyte implantation (ACI) similar to the other techniques using cell-seeded resorbable collagen membranes in large articular defects.

Methods: Autologous cartilage was harvested arthroscopically from the lateral trochlear ridge of the femur in fifteen 3-year-old horses. After culture and expansion of chondrocytes the newly created ACI construct (autologous chondrocytes cultured expanded, seeded on a collagen membrane, porcine small intestine submucosa) was implanted into 15 mm defects on the medial trochlear ridge of the femur in the opposite femoropatellar joint. Using two defects in each horse, the ACI technique was compared to collagen membrane alone (CMA) and empty cartilage defects (ECDs).

Results: Arthroscopic evaluations at 4, 8, 12 and 18 months demonstrated that CMA was significantly worse compared to ACI or ECD treatments, with ACI having the best overall subjective grade. Overall raw histological scores demonstrated a significant improvement with ACI compared to either CMA or ECD treated defects and ACI defects had significantly more immunohistochemical staining for aggrecan than CMA or ECD treated defects (with significantly more type II collagen in ACI and ECD compared to CMA defects) at 12 and 18 months.

Conclusions: Histologic and immunohistochemistry results from this long-term randomized study are particularly encouraging and demonstrate superiority with the ACI technique. Although there is no comparable study published with the traditional ACI technique in the horse (or with such a large defect size in another animal model), the use of a solid autologous cell-seeded-constructed implant would appear to offer considerable clinical advantages.

Key words: Equine, Autologous chondrocyte implantation, Articular defect, Collagen membrane, Cartilage resurfacing, Articular cartilage injury.

Introduction

Articular cartilage injuries have limited potential to heal and if defects are left untreated, may progress to osteoarthritis (OA)[1,2]. There are a number of different surgical procedures that have been used to treat cartilage injuries, with the ultimate aim of restoration of normal joint function, providing repair tissue in the defect that resembles hyaline cartilage, and integration of the repair tissue with surrounding cartilage and underlying bone. These surgical techniques can be divided into two categories as follows: (1) manipulation of endogenous healing, which usually involves marrow-stimulating procedures such as subchondral bone drilling, abrasion arthroplasty and microfracture and; (2) resurfacing defects with heterologous or autologous cartilage, osteochondral plug autografts and allografts or autologous chondrocyte implantation (ACI)[3,4].

ACI was first described in 1994[4] and the technique has been widely used in the United States and Europe[5–20]. ACI is the only cell-based therapy for cartilage repair that has been approved by the US Food and Drug Administration (FDA) for clinical use in the United States (Carticel®, Genzyme, USA) and involves harvesting and expanding autologous articular chondrocytes from a minor load-bearing area, with re-implantation under a periosteal flap at the defect site[4]. The technique, as it has been popularized in Europe, is the same involving culturing of articular chondrocytes and re-implantation in liquid form under a periosteal flap; the only difference being that cells are cultured by laboratories other than Genzyme.

In an Swedish study using the procedure on femoral condyles, good to excellent long-term results were considered to occur in 89% of the patients, and eight of 12 biopsy specimens showed findings consistent with hyaline tissue[15]. Other authors, however, have been skeptical with regard to autologous cartilage implantation being better than other methods[2,8,17]. In a study with 2 years follow-up, the improvement provided by ACI was considered to be inferior to that provided by osteochondral autografting[17]. On the other

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hand, a more recent randomized study concluded that clinical results were excellent or good in 88% vs 69% and arthroscopic examination demonstrated excellent or good repairs in 82% in ACI compared to 34% in mosaicplasty patients, respectively[5]. A recent paper in 80 patients with a single symptomatic cartilage defect compared the results of ACI and microfracture. At 2 years both groups had significant clinical improvement. According to the short form health survey (SF-36) physical component score at 2 years post-operatively, the improvement in the microfracture group was significantly better than in the ACI group ($P = 0.004$). Biopsy specimens were obtained from 84% of the patients and histological evaluation of repair tissue showed no significant differences between the two groups[3]. In a second recent study it was pointed out that the patients in the previous study with microfractures had lesions smaller than 4 cm^2 and the authors of the latter study reported (with 5 years follow-up) on a series of patients with average defect size of 4.9 cm^2. At 5 years, 62 patients improved, six reported no change, and 19 worsened[19]. Reasons for failure with the ACI technique include: separation of the periosteal flap from the surrounding cartilage and hypertrophy in the periosteal flap that required subsequent shaving.

Verigen® in Germany (recently acquired by Genzyme) has developed a technique of ACI that neither requires harvesting or suturing of an autologous periosteal flap, nor are the cells delivered in a liquid suspension. The technique uses a resorbable porcine collagen type I/III membrane and autologous chondrocytes are harvested and culture expanded for a period of 3–4 weeks prior to seeding on the collagen membrane. The collagen membrane is then attached into the defect with the cells toward the inside using fibrin adhesive and gentle pressure. The technique has been registered as matrix-induced ACI (MACI®). A second "solid" form of ACI has been developed by serum free cultivation of cells combined with the use of another collagen type I/III membrane called Chondro-Gide® and a third is an autologous bioengineered graft based on hyaluronan and called Hyalograft C®[10,21–24]. A special instrument has been developed to allow implantation of the graft arthroscopically.

This current paper examines a technique similar to other techniques using cell-seeded resorbable collagen membranes where autologous chondrocytes were culture expanded, seeded on a collagen membrane [small intestine submucosa (SIS)] and then re-implanted into large cartilage defects in the horse.

Method

The Animal and Care Use Committee at Colorado State University (CSU) approved that all aspects of this study were carried out following good laboratory practice (GLP) guidelines. Fifteen horses were purchased from a commercial vendor. Horses were 2–3 years of age (skeletally mature) and were free of musculoskeletal disease (based on clinical examination and radiographs). Horses were housed in 15 m^2 stalls located at the Orthopaedic Research Center at CSU and remained in these stalls except when undergoing surgery, treadmilling, weighing, or at the time of sacrifice.

EXPERIMENTAL DESIGN

The horses were randomized for treatment by assignment of horse numbers as indicated in Table I. Each 15 mm diameter defect ($n = 2$) in each horse randomly received one of three treatments: Group I = ECD (empty cartilage defect), Group II = ACI (autologous chondrocyte implantation attached with three absorbable polydioxanone (PDS)/polyglycolic acid (PGA) staples) and Group III = collagen membrane alone [CMA (no cells) attached with three absorbable PDS/PGA staples]. No horse received the same treatment in both defects. The study design enabled 10 data points for each of the three treatment options (it is to be noted that if each horse had an empty defect for one of its two defects, there would be fewer opportunities to evaluate the ACI and CMA – not more than 15 data points total).

CARTILAGE HARVEST

On day 0 of the studies horses had pre-operative medications of 4.5 mg/kg of phenylbutazone (this was continued once a day for 5 days), as well as antibiotics (ceftiofur, 2.2 mg/kg IM twice daily for 3 days). A catheter was placed, the horses pre-medicated with xylazine (0.5–1.1 mg/kg IV) or detomidine (5–10 mcg/kg IV) ± butorphanol (0.02–0.05 mg/kg IV) and then general anesthesia was induced with ketamine (2.2 mg/kg IV), and valium (0.1 mg/kg IV). Anesthesia was maintained with halothane in 100% oxygen through a semi-closed breathing system. After surgical preparation, and draping, arthroscopic surgery was done on one randomly selected femoropatellar joint using a previously described technique[25]. To obtain cartilage for ACI, approximately 300 mg of articular cartilage was harvested from the proximal aspect of the lateral trochlear ridge of the femur using Ferris-Smith intervertebral disc rongeurs (Fig. 1). During the same surgical procedure, approximately 100 ml of blood was collected from the horse and the harvested tissue and blood packaged in a specifically designed insulated transport kit to be transported overnight from the surgery site to Verigen for processing. The harvested cartilage was transported in 50 ml of Dulbecco's Modified Eagle Medium (DMEM)/F12 transport media.

The ACI construct is a combination biological and devised product comprised of cultured autologous chondrocytes seeded onto a porcine SIS collagen membrane. The SIS collagen membrane is currently used in a product marketed by DePuy Inc. a Johnson and Johnson Company under the name Restore Orthobiologics Soft Tissue Implant (however, there is a difference in size between the SIS membrane used in this study and the Restore product). SIS is a naturally – derived terminally – sterilized avascular collagen material (primarily type I) that is minimally processed during the creation of the 4 × 5 cm membrane. Its laminated construction (10 layers of individual SIS membranes) provides sufficient mechanical strength and

Table I
Randomized assignment of horses into treatment groups

Study groups			Horse #	Left stifle		Right stifle	
I	II	III	—	Proximal	Distal	Proximal	Distal
ECD	ACI	—	J16C	Biopsy		ECD	ACI
ECD	—	CMA	J17C	ECD	CMA	Biopsy	
—	ACI	CMA	J18C	Biopsy		ACI	CMA
ECD	ACI	—	J19C	ACI	ECD	Biopsy	
ECD	—	CMA	J33C*	Biopsy		CMA	ECD
—	ACI	CMA	J21C	CMA	ACI	Biopsy	
—	ACI	CMA	J22C	Biopsy		CMA	ACI
ECD	ACI	—	J23C	ECD	ACI	Biopsy	
ECD	—	CMA	J24C	Biopsy		ECD	CMA
—	ACI	CMA	J25C	ACI	CMA	Biopsy	
ECD	ACI	—	J26C	Biopsy		ACI	ECD
ECD	—	CMA	J35C	CMA	ECD	Biopsy	
ECD	ACI	—	J36C	Biopsy		ECD	ACI
ECD	—	CMA	J39C	Biopsy		CMA	ECD
—	ACI	CMA	J42C	ACI	CMA	Biopsy	

I: ECD (empty); II: ACI (cells + staple + membrane). III: CMA (staple + membrane).