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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).

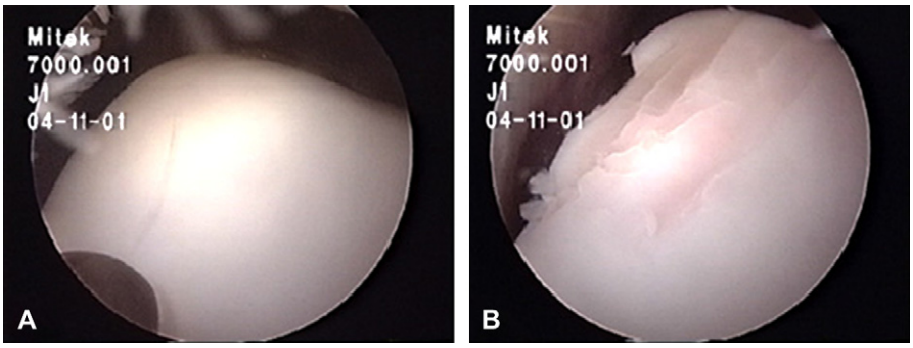

Fig. 1. Arthroscopic images (from an earlier pilot study) of pre (A) and immediately post (B) tissue harvest from lateral trochlear ridge of the distal femur.

non-oriented isotropic properties for a loaded environment like the knee. The authors' believed that the sum of these properties (natural collagen, minimal processing to preserve inherent biological properties, mechanical strength, and isotropic) made SIS a good candidate as a membrane to enable autologous cell-seeded cartilage repair. The cell-seeded SIS collagen membrane is manufactured in a continuous process by cell culture from enzymatically digested articular cartilage tissue donated by the patient. Cells were cultured in DMEM/F12 (1:1 ratio) containing 20% fetal calf serum, 70 μM gentamicin sulfate, 2.2 μM amphotericin B, and 300 μM L-ascorbic acid (Invitrogen Carlsbad, CA, USA). After 7 days cells were cultured without antibiotics. When an adequate number of cells (20×10^6) are available the cells are seeded onto the SIS collagen membrane and incubated for approximately 2–4 days to permit cell adhesion to the membranes substrate [Fig. 2(A) and (B&C) also illustrates the constructs at 3 weeks]. Approximately 4 weeks post-cartilage biopsy the newly created ACT, as well as samples of the SIS CMA without cells was shipped overnight from Verigen back to CSU for immediate implantation. The average seeding density was 1 million cells/cm^2 and these were seeded into a 20 cm^2 SIS collagen membrane. Based on the studies performed by the second author some years previously, approximately 50% (0.5 million cells/cm^2) were still viable after the 72–96 h required for international shipment in refrigerated conditions which mean that at the time of implantation approximately 10×10^6 cells were implanted. The study time line is depicted in Table II.

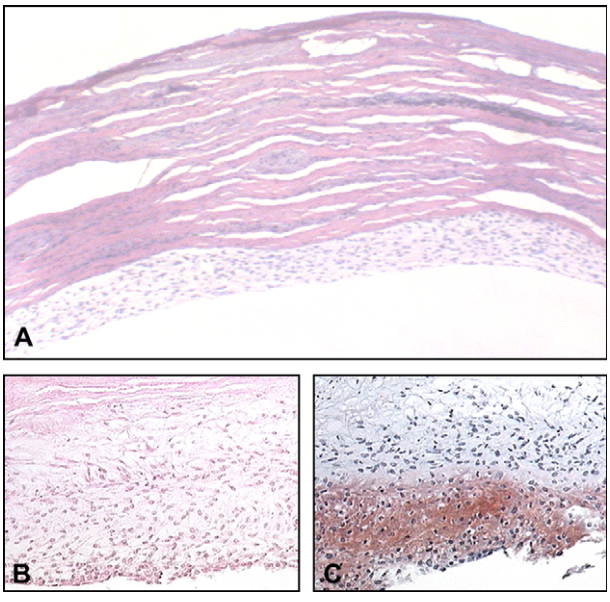

Fig. 2. Horse chondrocytes were seeded (5×10^6 cells/cm^2) onto SIS scaffolds, cultured *in vitro* for up to 3 weeks in chondrocyte growth media containing 10% fetal bovine serum (FBS), and stained with H&E and safranin-O (SO). Histological analysis confirmed that chondrocytes within 3 days attached to the surface of the SIS scaffold (Fig. A, H&E), and after 3 weeks had proliferated (Fig. B, H&E) and maintained a chondrocytic phenotype (Fig. C, SO). Note the multi-layer laminate construction of the SIS scaffold (Fig. A). Magnification 100×.

IMPLANTATION OF CARTILAGE CONSTRUCTS

Approximately 4 weeks post-cartilage biopsy *via* arthroscopy, the horses were re-anesthetized and a cranial arthrotomy approach (over the medial trochlear ridge of the femur) made to the femoropatellar joint. The arthrotomy was performed in the joint contra-lateral to the joint from which articular cartilage was harvested. Using custom 15 mm biopsy punches, an impression in the articular cartilage corresponding to the defect site was made in the medial trochlear ridge (two defects per joint and one joint per horse), with a minimum of 7 mm separating the two defects (Fig. 3). A manual curette was used to remove all cartilage within the two outlying defects, care being taken to leave the calcified cartilage intact. The site was flushed with a sterile epinephrine and normal saline solution if needed to control bleeding (at a concentration of 1/1000).

Both the ACI and CMA were sized using similar 15 mm biopsy punches and then affixed into the defects using three absorbable PDS/PGA staples (Fig. 4). The use of these staples was compared against suture fixation in an earlier pilot study. The use of staples was quicker and technically easier than suturing (approximately 5 vs 20 min) and there was no difference with construct security at follow-up examinations. Three pairs of holes (six holes per defect) were created to receive the PDS/PGA staples using a custom designed slap hammer device. The staples were then delivered using a second custom designed slap hammer device. The defects that were designated as empty chondral defects did not have any holes created. The arthrotomy incision was closed with a layer of 2-0 absorbable suture in the joint capsule, separate layers in the deep and superficial fascia were closed with 0 absorbable sutures, and the skin was closed using simple interrupted sutures of 2-0 nylon and a stent bandage sutured over the incision. No braces, slings or external fixation devices were used at any point post-operatively during the study. The overall project scheme is illustrated in Fig. 5.

3, 6, 12 AND 18-MONTH POST-CONSTRUCT IMPLANTATION ARTHROSCOPIES

At approximately 3, 6, and 12 months after implantation, diagnostic arthroscopy was performed on both femoropatellar joints to examine the defects, as well as the donor defect site (Tables II and III). During the 12-month procedure, nine horses were euthanized and the joints preserved for gross and histological analysis; the remaining six horses were survived for an additional 6 months (i.e., 18 months post-implantation) and had diagnostic arthroscopy and euthanized at this time. Horses were euthanized according to a pre-determined schedule to ensure equal treatment group size at both the 12- ($n = 6$ for each treatment) and 18-month ($n = 4$ for each treatment) time points.

RADIOGRAPHS

Radiographic examinations were conducted at seven time periods in the study as follows: (1) at initial check-in; (2) approximately 1 week post-tissue harvest arthroscopy; (3) approximately 1 week post-construct implant arthrotomy; (4) approximately 1 week before the 3-month post-construct implant arthroscopy; (5) approximately 1 week before the 6-month post-construct implant arthroscopy; (6) approximately 1 week before the 12-month post-construct implant terminal arthroscopy ($n = 9$ horses); and (7) approximately 1 week before the 18-month post-construct implant terminal arthroscopy ($n = 6$ horses). In addition, high-detail radiographs of the repair area (Faxitron

Table II
Exercise protocols for 18 months

Study week	Duration	Activity
Week (−3)	7 days	Stall rest
Week (−2)	7 days	Stall rest
Week (−1)	7 days	Stall rest
Week 0		Unilateral biopsy surgery/stall rest
Week 1	7 days	Stall rest
Week 2	5 days	Hand walk at 5 min/day
Week 3	5 days	Hand walk at 10 min/day
Week 4−8		Unilateral arthrotomy defect creation/stall rest
Week 9−16		Hand walk progressing each week for 5, 10, 15 and 20 min through week 12. Then treadmill trot for 2 min during week 13 and 5 min for weeks 14−16. All at 5 days per week
Week 17−19		Stall rest
Week 20−21	10 days	Hand walk 10 min week 20, and 15 min week 21 at 5 days per week
Week 22−29		Treadmill trot 2 min week 22, 5 min weeks 23−25, 8 min weeks 26−27 and 10 min weeks 28−29. All at 5 days per week
Week 30−34		6-month re-look bilateral arthroscopy/stall rest weeks 31—34
Week 35−38	20 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 25−33 mph)
Week 39−46	40 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 35−38 mph)
Week 47−50	28 days	Stall rest
Week 51−55	25 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 35−38 mph)
Week 56−60		12-month re-look bilateral arthroscopy (5/27), stall rest weeks 57−60
Week 61−64	20 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 35−38 mph)
Week 65−72	40 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 35−38 mph)
Week 73−76	28 days	Stall rest
Week 77−83	35 days	Treadmill 6 min trot−gallop−trot (2 min each) at 5 days per week (trot = 8−12 mph, gallop = 35−38 mph)
Week 84		18-month re-look bilateral arthroscopy and euthanasia

images) were made post-tissue collection, but prior to sectioning bones for histological processing and examined.

12- AND 18-MONTH POST-IMPLANTATION PROTOCOL AND OTHER OUTCOME PARAMETERS

Daily observations

Throughout the study, animal care personnel assessed the horses for daily comfort, movement, and respiratory character. An SOP was established for animal care personnel to determine if an animal needed further evaluation and treatment order by a study veterinarian.

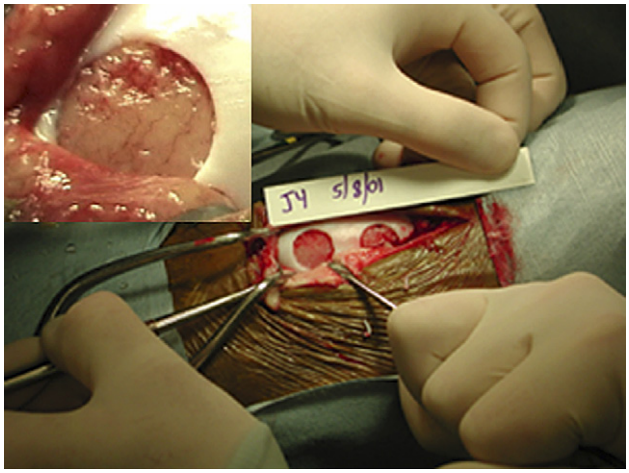

Fig. 3. Typical view of two 15 mm defects on medial trochlear ridge, demonstrating spatial relationship. The insert also shows intact blood vessels coursing below the calcified cartilage in the lower half of the defect while disruption of the vessels in the upper half of the defect is occurring secondary to partial penetration of the calcified cartilage layer. This defect is not in one of the study animals but is used to demonstrate ease of identifying penetration of the calcified cartilage.

Lameness evaluations

Lameness evaluations were performed before the tissue biopsy and implantation, and approximately every 4 weeks thereafter until the 3-month arthroscopy (excluding one lameness examination 2 weeks post-implantation). After the 3-month arthroscopy, the lameness examinations were performed approximately every 2 months for the duration of the project. These examinations included baseline movement (lameness graded 0−5, where 0 is normal), the presence of synovial effusion in the joints (grade 0−4), range of motion (0−4) and flexion tests (0−4) of the hind limb.

Exercise

Horses were exercised approximately 5 days a week according to the schedule (out to 18 months) depicted in Table II. This is a standard rehabilitation protocol for our research program and involves progression from walking to galloping.

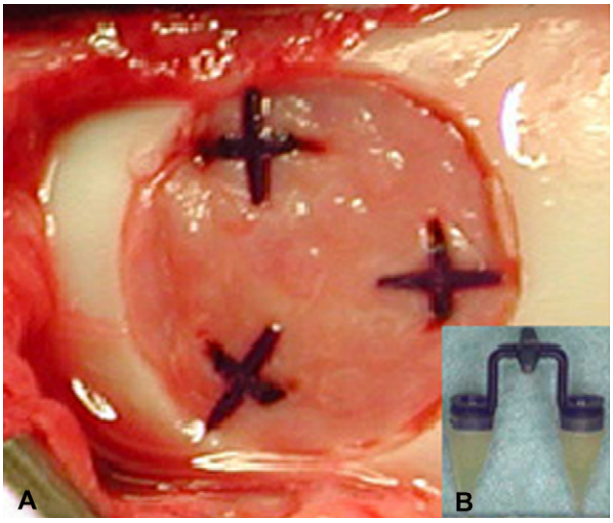

Fig. 4. Example of stapled (A) constructs from an earlier Pilot study and insert figure of absorbable PDS/PGA staple (~4 mm tip-to-tip) (B).

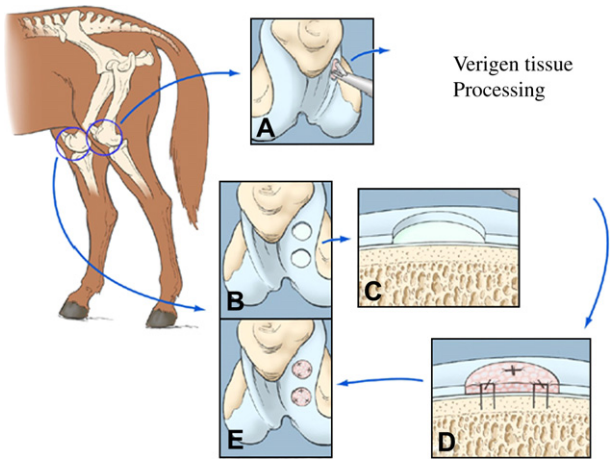

Fig. 5. Diagram of tissue harvest site (A) which was followed by processing and construct creation at Verigen. Four weeks later the opposite joint had two 15 mm defects created (B) down to the level of the calcified cartilage (C). The construct was stapled in place using three absorbable PDS/PGA staples (D and E).

Post-mortem tissue examination

After euthanasia, a necropsy was performed followed by a detailed examination of the stifle. Gross/macroscopic observations of the defect and joint surface were made of both femoropatellar articulations, including presence of osteophytes and secondary osteoarthritic change, articular surface integrity contour and congruity, any injury to opposing articular surface, level of repair fill in defect site based on surrounding "normal" cartilage surface (0–4 for even with, proud, below, dislodged, or absent [bone exposed], respectively), any irregularity of repair tissue surface, firmness of the repair tissue when palpated with a probe and compared to surrounding normal cartilage (0–4 for none, slight, mild, moderate, and marked firmness, respectively), attachment of cartilage – this category describes the degree of repair tissue attachment to the surrounding normal articular cartilage (0–4), attachment of bone – describing the degree of repair tissue attachment to the underlying bone (0–4), the area of repair tissue filling the defect expressed as a percentage, and volume of repair tissue filling the defect expressed as a percentage and examination of synovial membrane (0–4 for normal, slight, mild, moderate, and severe inflammatory change, respectively).

Synovial membrane was collected to be submitted for hematoxylin and eosin (H&E) examination. Faxitron images were graded by an individual unaware of the treatment groups for, sclerosis (presence or absence of subchondral bone sclerosis in the area of the chondral defect as compared to adjacent radiographic bone densities on a 0–4 scale, absent to severe, respectively), lysis (presence or absence of bone lysis in the perceived area of the chondral defect as compared to adjacent radiographic bone densities using a similar 0–4 scale), subchondral bone irregularities (presence or absence of subchondral bone irregularities in the perceived are of the chondral defect on a similar 0–4 scale). A total Faxitron score was also calculated by summing each of the categories allowing a maximum score of 12.

Histology and immunohistochemistry

After necropsy procedures were completed, the tissues were trimmed using a standard band saw and Exakt bone saw with a diamond chip blade (Exakt Technologies, Oklahoma City, OK, USA). The defect was trimmed to fit in histological cassette and the specimens fixed in 10% neutral buffered formalin for a minimum of 2 days. Bone samples were then decalcified and processed with a custom procedure for large, thick bone samples. Both cartilage and bone samples were then embedded in paraffin and sectioned at 5 μm. The bone and cartilage samples were sectioned at two levels within the defect area as follows: the first level of histological samples was taken from an area approximately 3 mm and the second level 8 mm from the proximal edge of the defect. Both samples had ample surrounding tissue to allow evaluation of repair tissue integration. Equine trachea was used as a positive control for safranin-O fast green staining (SOFG).

The cartilage and bone samples were stained with H&E, toluidine blue, and SOFG. Fixation of the implants, the host response to the implants, and the quality of the repair tissue were determined microscopically by a histopathologist unaware of treatment group assignments. A modified histological grading system[26,27] was used to generate scoring for non-parametric analysis of the three treatment groups (Table IV). Quantitative histomorphometry was also performed using a commercial software program [Image-Pro Plus (version 4.5.1.29) Media Cybernetics Inc., Silver Spring, MD 20910, USA].

The synovial tissues were processed using standard programs for soft tissues, embedded in paraffin, and sectioned at 5 μm. These sections were stained using H&E. The synovial membrane sections were scored for cellular inflammation, intimal hyperplasia, subintimal edema, subintimal fibrous and increase vascularity using a 0–4 scale (0 representing normal and 4 severe change) by an observer unaware of treatment assignments.

Immunohistochemical analysis was performed on fresh frozen sections harvested from two locations adjacent to histologic harvest sites. For immunohistochemical examination, antibodies against type I and II collagens, aggrecan, and the chondrocyte specific protein S-100 were used. The methods for staining and preparation of negative controls have been previously described by the authors[28]. The immunohistochemistry sections were graded by a histologist unaware of the treatment assignments using a 0–3 scale: a grade 0 indicated that immunoreactivity was not present, a grade 1 represented at least some immunoreactivity was present, a grade 2 indicated that immunoreactivity extended focally either from top to bottom or from side to side in the graft, and a grade 3 indicated that immunoreactivity involved the entire graft (top to bottom and side to side).

Data analysis

Data were analyzed using one or a combination of descriptive statistics, non-parametric frequency tables, and mixed model analysis of variance (AOV). When main or interaction effects had *P*-values that were considered significant (*P*-value < 0.05) or a trend (*P*-value <0.10 and >0.05) then individual comparisons were made using the least square means procedure. The horse was considered a random effect in the AOV models. A commercially available software package was used for all analysis, SAS version 8E (Cary, NC, USA). Graphs were represented by their means ± 1 standard error of the mean (S.E.M.) with statistical significance noted by letter connecting the statistical comparison.

Results

CARTILAGE HARVEST

No complications were encountered from the harvest of autologous articular cartilage samples and approximately 300 mg of tissue was easily obtained from the lateral trochlear ridge of the femur. Follow-up arthroscopy at 12 or 18 months study period demonstrated a nominal amount of tissue formed in the harvest sites. The clinical examination showed that for all time periods each of the clinical parameters in the biopsy limb was on average, below grade 1 indicating less than a slight change in any of the parameters.

IMPLANTATION OF CARTILAGE CONSTRUCTS

No significant technical problems were encountered at the time of construct implantation. Punctuate bleeding was noted in one of the 30 defects during creation: the area was confined to $<10\%$ of the total defect area and was stopped using local epinephrine and based on gross photographs of repair tissue, did not impact later repair tissue formation. Slight lifting from the base of the defect and retraction of the implanted construct from the edge of the defect margin (<1 mm retraction from the defect margin for under 40% of the defect circumference) occurred in one location (Fig. 6). The retraction appeared to be secondary to staple placement, causing increased tension in the area of construct retraction, but based on arthroscopic follow-up and gross observations, when retraction was noted, no long-term differences were observed (Fig. 6).

3-, 6-, AND 12-MONTH POST-CONSTRUCT IMPLANTATION

No complications or significant abnormalities were encountered during the arthroscopic evaluations of either the biopsy or implantation joints of any of the horses. Overall

Table III
Grading criteria for arthroscopic evaluation

Cartilage attachment – This category describes on average the defect repair tissue attachment with the surrounding normal cartilage. Possible responses were:
0 = Normal attachment
1 = Moderate attachment
2 = Mild attachment
3 = Slight attachment
4 = No attachment

Bone attachment – This category describes the firmness of the repair tissue attachment to the bone at the base of the defect. Possible responses were:
0 = Normal attachment
1 = Moderate attachment
2 = Mild attachment
3 = Slight attachment
4 = No attachment

Firmness – This category describes on average the firmness of the repair tissue to normal surrounding articular cartilage based on palpation with a microscopic probe. Possible responses were:
0 = Similar to surrounding normal articular cartilage
1 = Slightly soft compared to normal surrounding articular cartilage
2 = Mildly soft compared to normal surrounding articular cartilage
3 = Moderately soft compared to normal surrounding articular cartilage
4 = Marked softening compared to normal surrounding articular cartilage

Blood – This category describes the presence or absence of hemorrhage associated with the defect area or its periphery. Possible responses were:
1 = Fresh blood: active hemorrhage at time of surgery
2 = Old blood: no active hemorrhage at the time of surgery
3 = No blood visualized at the time of surgery

Shape – This category describes the margin of the defect as it relates to the original geometry at time 0 (creation). Possible responses were:
0 = No apparent increase in damage tissue beyond original defect margins
1 = Degeneration of tissue beyond original defect margins

Grade – This category describes the overall subjective evaluation of the repair tissue by the evaluator. Criteria used to determine the grade were:
(1) Attachment of repair tissue to the surrounding normal articular cartilage
(2) Level (height) and undulation of the repair tissue surface as compared to the surrounding normal articular cartilage
(3) Color of the repair tissue, where white homogenous tissue without a fibrous like appearance is used as the ''gold standard''.
Possible responses were:
0 = Tissue not present to grade
1 = Poor
2 = Fair
3 = Good
4 = Excellent

Level – This category describes the level of repair tissue filling in association with the surrounding normal articular cartilage. Possible responses in relation to the surrounding normal articular cartilage were:
1 = Mildly recessed
2 = Slightly recessed
3 = Leveled
4 = Slightly elevated
5 = Mildly elevated
6 = Moderately elevated

Color – This category describes the color of the repair tissue. When repair tissue is characterized by two colors, the predominate color is indicated first. Possible responses were:
1 = Red
2 = White/red
3 = Yellow
4 = Yellow/white
5 = White/yellow
6 = White

Surface – This category describes the relative undulation of the repair tissue surface. Possible responses were:
1 = Non-undulating
2 = Slightly undulating
3 = Mildly undulating
4 = Moderately undulating

Staple – This category describes if a PDS/PGA staple was visible to the observer. Possible responses were:
0 = Absent
1 = Present
''.'' = No staple was used, so data were not included in statistical calculation

Better – This category compares which of a given defect in a joint has subjectively more desirable repair tissue tilling. Possible responses were:
1 = Better
2 = Worse

Table IV
Modified subjective histopathologic grading system for evaluation of repair

Category	Score
Nature of the repair tissue (include average thickness of reparative cartilage)	
Hyaline cartilage	4
Hyaline-like cartilage	3
Mostly fibrocartilage	2
Mostly non-cartilage	1
Non-cartilage only	0
Matrix staining	
Normal or nearly normal staining	3
Moderate (or increased) staining	2
Slight staining	1
None	0
Structural integrity (morphologic zone reconstitution)	
Normal structure	2
Slight disruption	1
Severe disruption	0
Surface regularity	
Smooth and intact	3
Superficial, horizontal lamination	2
Slight disruption, fissures	1
Severe disruption, fibrillation	0
Filling of the defect	
100%	3
75–100% or >100%	2
50–75%	1
<50%	0
Bonding to host tissue: integration with adjacent cartilage	
Bonded	2
Partially bonded	1
Not bonded	0
Bonding to host tissue: integration with underlying subchondral bone	
Bonded	2
Partially bonded	1
Not bonded	0
Degenerative changes of the repair tissue	
Severe degeneration	3
Normal cellularity and cell morphology	2
Mild hypocellularity and cell cluster	1
Moderate hypocellularity and cell degeneration	0
Degenerative changes in an adjacent cartilage	
Normal cellularity	3
Slight hypocellularity and cell morphology	2
Moderate hypocellularity and cell degeneration	1
Severe degeneration	0
Maximum score	25

the repair tissue attachment to surrounding normal cartilage and bone (independent of defect treatment) was good but slightly less than the normal (grade 1 of 4 where 4 is completely detached). Significant difference was seen over time related to cartilage and bone attachment of the repair tissue characterized by progressively better attachment up to 12 months followed by a decrease in attachment in the CMA treated defects. The overall arthroscopic grade (fair to good, with the ACI treatment group having the best average score) also demonstrated that CMA was significantly worse compared to ACI or ECD treatments on average, independent of time or defect location (Fig. 7). On average, independent of the defect treatment, the firmness of the repair tissue was found to be mildly moderately softer on average compared to the surrounding articular cartilage. No

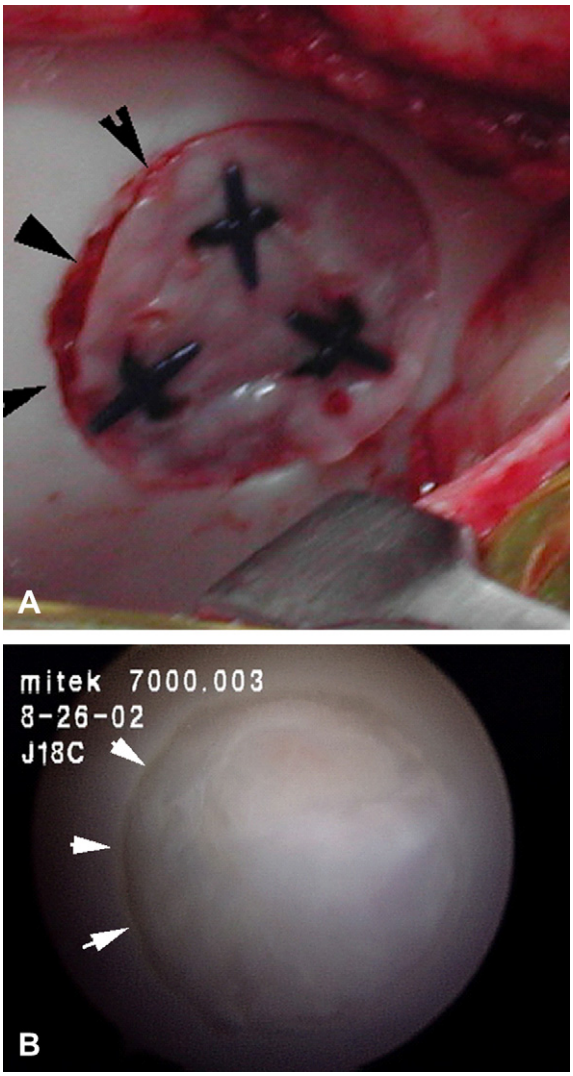

Fig. 6. Implantation picture of defect with construct edge retraction at implantation (A), but without long-term arthroscopic ramifications at 12 months (B).

significant differences were noted for firmness, presence of blood, shape, level or color in association with the location of the defect (proximal or distal) treatment (ECD, CMA, and ACI) or study period. The significant differences held throughout to the 18-month period.

RADIOGRAPHIC EXAMINATIONS

All radiographic examinations of the stifles were normal at the beginning of the study. Subjective radiographic changes consisting of areas of radiolucency associated with the subchondral plate and staple placement, as well as areas of sclerosis were observed. These abnormalities were noted throughout the study in areas where defects were created and were subjectively graded and analyzed based on high-detail radiographs (Faxitron images) at the end of the study. There were significant changes based on treatment group for lysis (Fig. 8) and sclerosis with CMA treated defects at 12 months having the most pathologic scores.

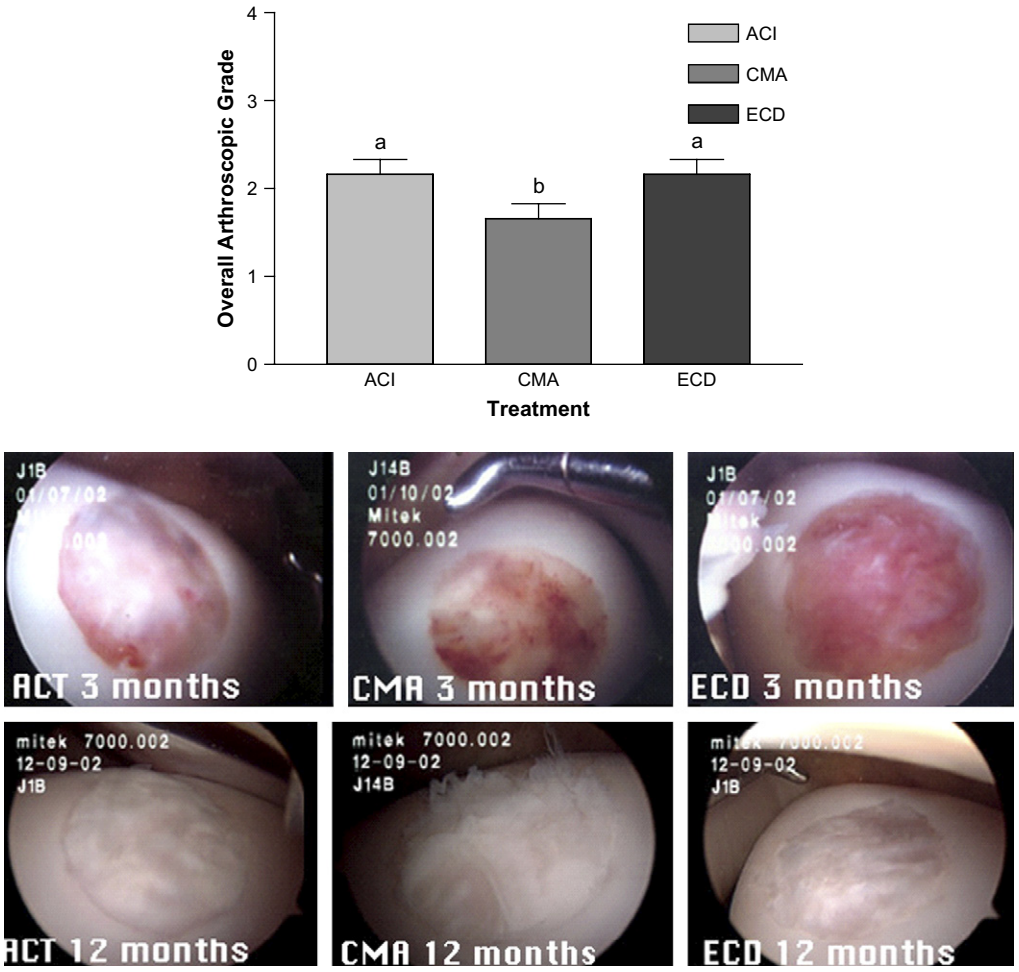

Fig. 7. Plot of overall arthroscopic grade and treatment group. Different letters indicate a significant difference between groups averaged over time. Lower panel shows arthroscopic view of representative repair tissue from each treatment group of study horses at 3- and 12- month time points. The letters on the photos could not be changed due to GLP requirement, ACT = ACI.

LAMENESS EVALUATIONS

Limbs from which biopsies were taken from the femoropatellar joint only showed a slight (<grade 1) degree of lameness beginning 2 months following biopsy. Limbs containing defects/implanted joints demonstrated a significant increase in lameness, response to flexion and synovial effusion scores at the first time point post-defect creation/implantation and significantly improved throughout the remainder of the study. Because two different treatment groups were in each joint/limb, differentiating which treatment group may have contributed to a lameness is not possible and no patterns appeared to exist throughout the study.

POST-MORTEM TISSUE EXAMINATIONS

There was no infection in any joints and no significant difference in the amount of secondary osteoarthritic change in the joints (OA change was only noted in 2/15 biopsy and 1/15 treated joints). Likewise, there was no significant difference noted between the groups with regard to articular damage to the opposing surface. Only 3/30 joints had some damage noted and it was considered to be not clinically relevant. No significant differences were noted in the degree of synovial inflammation, with the highest average score being

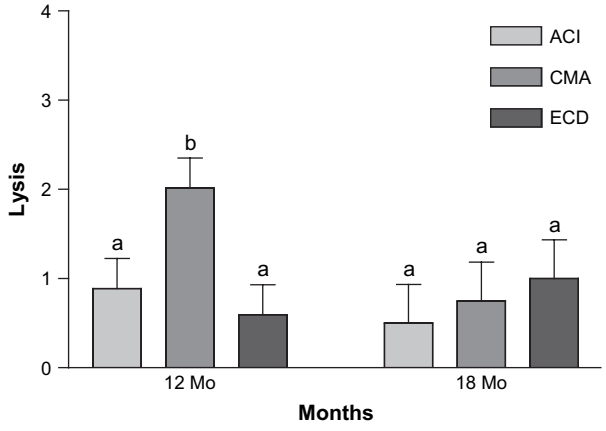

Fig. 8. Plot of Faxitron lysis score by study period and treatment group. Different letters indicated significant differences averaged over time.

0.7 ± 0.2. One horse had a loose body in a biopsy/donor joint and this was considered to be likely from a harvested cartilage fragment that was not retrieved at the time of surgery.

Specific examination of the repair tissue in the defect showed no significant difference in repair fill compared to the surrounding normal cartilage based on treatment group, although on average the proximal defects had inferior cartilage attachment (slight compared to mildly less than what was expected for normal tissues) and defect filling compared to the distal location. The earlier weight-bearing, and therefore harsher environment of the proximal compared to the distal location, most likely explains this finding. No significant difference was noted in repair tissue firmness, repair tissue surface irregularities or attachment to bone in any analysis. The firmness of the tissue on average was graded to be mildly less than normal tissue for ACI and ECD treated defects, but moderately less than normal tissue for CMA, with mild surface irregularities in all treatment groups.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Synovial membrane histology

Cellular infiltration scores (perivascular mononuclear cells) were as follows: ACI + CMA = 2.7 ± 0.41, ECD and CMA = 1.54 ± 0.37, and ECD + ACI = 0.86 ± 0.37. It is note worthy that two treatment groups would have been contributed to this score although even with this limitation it was possible to conclude that the cellular infiltration scores were highest when CMA was present in the defects and lowest when ACI was present.

Repair tissue histology and immunohistochemistry

Analysis of the overall raw histological scores demonstrated a significant improvement with ACI compared to either CMA or ECD treated defects independent of location (Figs. 9 and 10). The nature of the repair tissue was graded as being more hyaline-like in the ACI treated defects (and the overall histology score was significantly improved) compared to the CMA and ECD treated defects. Likewise the surface regularity was significantly worse in the CMA compared to either the ACI or ECT treated defects (Fig. 10). Better bonding of the repair tissue to the surrounding articular cartilage was noted in the ACI and CMA treatment groups at 18 months compared to the ECD treated defects (there was deterioration of bonding in ECD at 18 months compared to 12 months). The bonding was also noted to deteriorate in the ECD treated defects when the 12- vs the 18-month tissues were compared, while the other treatment groups improved (ACI) or did not significantly change (CMA) (Fig. 9). It is of interest that during the course of 18 months the calcified cartilage was reabsorbed and replaced by bone. In most defects the subchondral bone plate was still present. In all defects a subchondral bone plate of some form is present but it is typically not thick when it is created in normal joints. There was no significant difference noted in SOFG staining although the matrix staining with toluidine blue decreased significantly for all treatment groups between 12 and 18 months. The level of defect fill was not significantly different between the treatment groups, although ACT treated defects had the highest numeric score. Bonding of the repair tissue to the bone did not show differences, but similar trends were seen as compared to the repair tissue bonding to the articular cartilage. There was no significant difference in cellular morphology noted for any statistical comparisons in either the repair or surrounding host tissues. Significant differences were not seen in the quantitative histomorphometric analysis (Table V).

Results of immunohistochemical analysis indicated that ACI and ECD had significantly more type II collagen when compared to the CMA treated defects and significantly more aggrecan was noted in the ACI compared to either the CMA or ECD treated defects (Figs. 11 and 12). No other significant differences were noted.

Discussion

The therapeutic strategy of ACI seems to have been improved recently by the use of biocompatible scaffolds which allow better fixation of the cells inside the defect while retaining the original cellular phenotype[2,29,30].

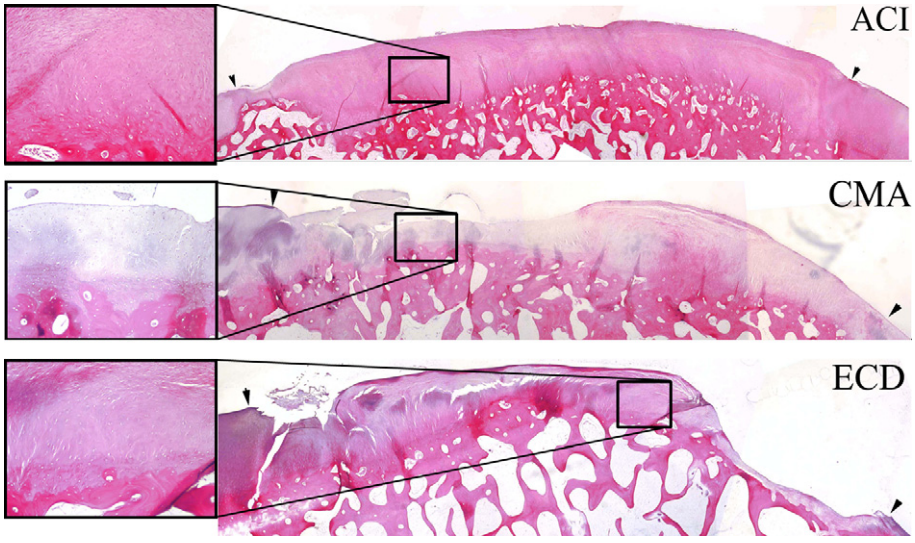

Fig. 9. Histologic photomicrographs of repair tissue at 18 months for ACI, CMA and ECD. Arrows indicate defect margins on the 2× magnification and each insert shows a 10× magnification.

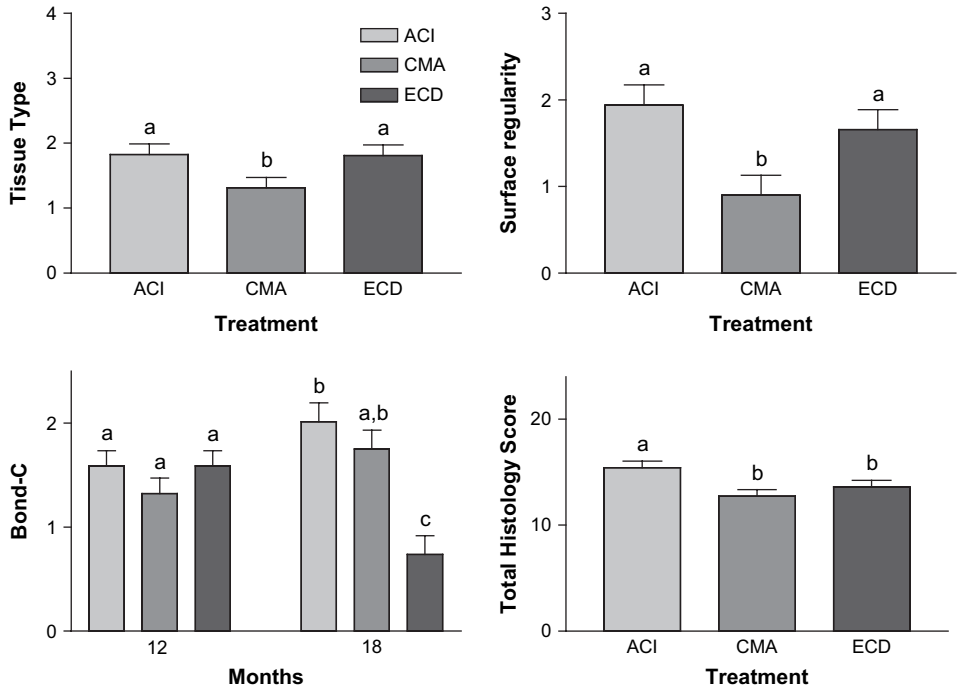

Fig. 10. Plots of histologic grading for tissue type, surface regularity, repair tissue bonding to surrounding cartilage (bond-C), and total histology score (sum of all response categories) by treatment group and by time post-implantation. There was no difference between 12 and 18 months, so these figures represent all defects.

Although the most common adverse event reported with ACI is intra-articular adhesions (2%),[31] the next most common adverse events include detachments/delamination and hypertrophy of the periosteum. The cartilage registry report, an international multi-center observation assessment of patients treated with ACI has revealed by patient assessment that 78% of all defects treated with ACI had improvement, and 81% of isolated femoral condyle defects had improved[32].

The increased cellular infiltration scores in the synovial membrane from joints in which CMA was present imply that there is more reaction to the porcine collagen membrane without the cellular cover. This conclusion is based on joints where there was an empty defect and an ACI defect having low scores compared to the other two groups of joints that contained a CMA as one of the treatments. Previous work[33] has demonstrated that porcine SIS is not an acellular collagenous membrane and contains porcine DNA and the potential for such reaction in humans would tend to argue for choosing another membrane.

The technique used in this equine study resembles the MACI® approach, the principal difference being that the MACI chondrocytes are seeded on a porcine collagen type I/III membrane, whereas the construct we used is

Table V

Histomorphologic and subjective histologic analysis of repair tissue at both 12 and 18 months reported by treatment group. Mean ± S.E.M. Different letters indicate a statistical difference between groups within a row. No significant effect of time was seen between 12 and 18 months in the histology outcome parameters with the exception of bonding to surrounding cartilage (see Fig. 10)

Histomorphologic analysis of repair tissue	Empty chondral defect	Collagen membrane	Autologous chondrocyte transplant
% Repair filling tissue defect	60.6^{a}	62.2^{a}	64.8^{a}
% Fibrous tissue in total repair tissue	59.3^{a}	53.6^{a}	51.3^{a}
% Fibrocartilage in total repair tissue	35.6^{a}	38.8^{a}	41.9^{a}
% Hyaline cartilage in total repair tissue	5.0^{a}	4.9^{a}	6.4^{a}
% Calcified cartilage in total repair tissue	0.9^{a}	0^{a}	0^{a}
Subjective histology scores			
Nature of the repair tissue	$1.8^{a} \pm 0.2$	$1.3^{b} \pm 0.2$	$1.8^{a} \pm 0.2$
Matrix staining	$1.2^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$1.2^{a} \pm 0.1$
Structural integrity	$0.8^{a} \pm 0.1$	$0.6^{a} \pm 0.1$	$0.8^{a} \pm 0.1$
Surface regularity	$1.7^{a} \pm 0.2$	$0.9^{b} \pm 0.2$	$1.9^{a} \pm 0.2$
Filling of the defect	$1.6^{a} \pm 0.2$	$1.6^{a} \pm 0.2$	$2.0^{a} \pm 0.2$
Bonding to host tissue: integration with adjacent cartilage	$1.2^{a} \pm 0.1$	$1.5^{b} \pm 0.1$	$1.8^{b} \pm 0.1$
Bonding to host tissue: integration with underlying subchondral bone	$1.6^{a} \pm 0.1$	$1.6^{a} \pm 0.1$	$1.9^{a} \pm 0.1$
Degenerative changes of the repair tissue	$2.0^{a} \pm 0.0$	$2.0^{a} \pm 0.0$	$2.0^{a} \pm 0.0$
Degenerative changes of adjacent cartilage	$2.0^{a} \pm 0.0$	$2.0^{a} \pm 0.0$	$2.0^{a} \pm 0.0$
Total	$13.6^{a} \pm 0.6$	$12.8^{a} \pm 0.6$	$15.4^{b} \pm 0.6$

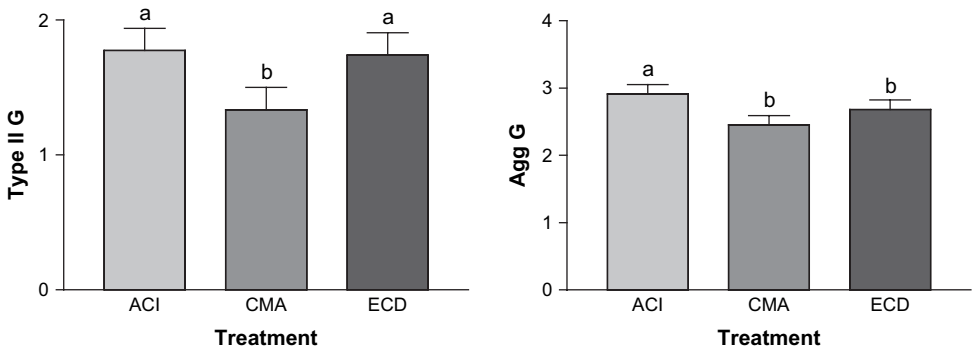

Fig. 11. Plot of immunohistochemistry results for both type II collagen and aggrecan for the repair tissue or "graft tissue (G)" by treatment groups. Different letters indicate a statistical difference between groups averaged over time.

seeded on a collagen membrane derived from 10 layers of porcine SIS. In previous work of the authors suturing of the collagen membrane was compared to the use of absorbable PDS/PGA staples. Both methods provided good fixation, but suturing is much more tedious and time consuming and is more difficult to adapt to arthroscopic technique in human clinical patients[34].

There is minimal follow-up published with other cell-seeded resorbable membranes, but it was the subject of a report at the fifth International Cartilage Research Society (ICRS) Symposium in 2004 where the authors described an arthroscopic technique. They presented 40 defects in 36 patients and had re-assessed the first 20 knee joints with a mean follow-up of 24 months (minimum 12 and maximum 36 months)[35]. All patients improved after surgery and at clinical evaluation, the Modified Cincinnati Knee Score improved from a baseline value of 2.9 ± 1.1 to a score of 8.1 ± 1.4, 8.7 ± 1.0, and 9.3 ± 0.8 at 12, 24, and 36 months, respectively. The authors considered that the arthroscopic technique was easier and faster and gave a quicker functional recovery, reduced hospital stay, and better compliance with the surgical and rehabilitative procedures. They also considered that the mechanical characteristics of the collagen membrane were helpful, as the scaffold is extremely resistant to tears, even after prolonged exposure to arthroscopic fluids. As mentioned previously, an arthroscopic technique has also been described for the Hyalograft C procedure, but it has been noted that there are technical limitations in treating lesions located in the patellar groove, posterior portion of the femoral condyles, or the tibial plateau[10]. In a cohort study of 67 patients, 97% of the patients improved, based on their subjective evaluation, 94% had improvement in quality of life, 87% of the patients improved in a knee functional score and arthroscopic evaluation of cartilage repair showing 96.7% biologically acceptable. They also noted that on histologic assessment of the

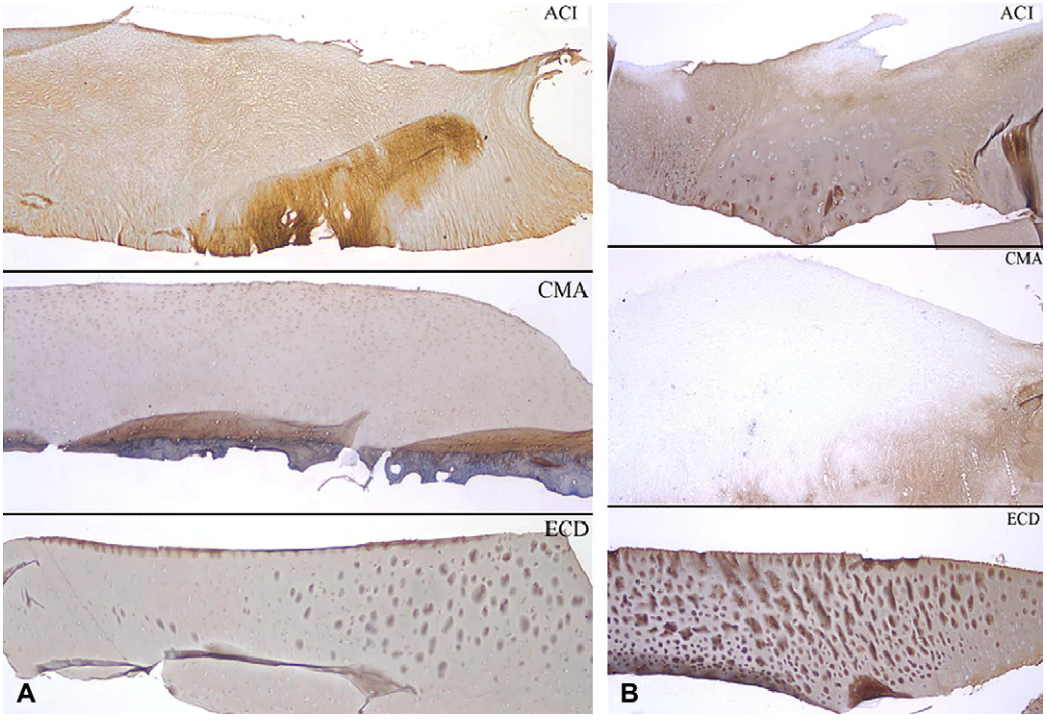

Fig. 12. Representative pictures (2×) of ACI, CMA and ECD treated defects stained for aggrecan (A) and type II collagen (B).

grafted site, the majority of the specimens were considered hyaline-like[23].

This current study allowed us to evaluate a technique closely resembling MACI® in large defects in an equine model. As has been noted before, the major indication for ACI is symptomatic, large full-thickness chondral lesions located on the femoral condyle and trochlear groove, including osteochondritis dissecans (OCD)[31]. The horse gives us the opportunity to emulate large defects such as are treated in humans with ACI. While it is recognized that most clinical human defects are in loaded areas on the medial femoral condyle, this model used the femoral trochlea in part because of the ability to create two 15 mm diameter defects in one joint and decrease the number of horses. The loading is different in the patellofemoral articulation but is still significant and the control defects are in the same location. Furthermore, loading studies conducted in the defect locations suggest that the proximal location represents an area that is consistently loaded at all levels of exercise. This is in contrast to the distal location which is loaded based on the exercise regime of the horse in particular, this area only shows contact when the joint is at or equal to 120° of flexion. The exercise regime followed in the current study was designed to provide minimal loading of the distal defect until about 4 months post-treatment based on flexion angles. The authors have also studied defects on the medial femoral condyle (the most common location treated with ACI) in the evaluation of subchondral microfracture[28,36]. The defects on the medial femoral condyle are 1 cm^2. The thickness of the cartilage in the horse is also comparable to that of humans[37].

Arthroscopic evaluation enabled us to follow both the firmness and appearance of the repair tissue, as well as its attachment. It is to be noted that our experiment was designed to eliminate any potential differences between proximal and distal locations and there was no significant difference for the arthroscopic parameters between the proximal or distal defects. It also should be noted that calcified cartilage was retained in all defects, in order to address FDA concerns that beneficial results from this technique could be attributed to the ACI rather than coming from subchondral elements. The PDS/PGA staples were only partially cannulated (to enable insertion), and therefore did not enable subchondral elements to migrate into the defect site. In other work from the authors' laboratory, it has been demonstrated that the normal healing response for full-thickness defects is considerably inferior when the calcified cartilage is retained[38]. Based on the results of that study, removal of the calcified cartilage with debridement down to the subchondral bone plate could potentially improve the quality of the repair tissue, as well as the attachment of the repair tissue. On the other hand, it is possible that retention of the calcified cartilage layer could have shielded the construct from an immune response. The overall subjective tissue grade arthroscopically was fair to good, with the ACI treatment group having the best average score.

As previously noted on histology that there was cellular infiltration of the synovial membrane in both the CMA and ACI groups, with a larger contribution coming from CMA treatment (this was attained by comparing the joints with no CMA treatment to the joints with CMA treatment). This observation could imply that there is some reaction to the porcine collagen membrane. We considered this inflammatory cell infiltration to be clinically insignificant based on what we see in equine clinical cases. Histological evaluation of the repair tissue showed a significant improvement in total histology scores with ACI compared to either CMA or ECD treated defects and the repair tissue with ACI was also graded as being more hyaline-like (all sections were graded in a blind fashion). When quantitative histomorphometry was performed, no statistical differences were observed in repair tissue character. This may seem in conflict with the subjective grading of tissue type a larger continuum was used in determining tissue character with the subjective grading of tissue type when compared to the histomorphometry. The overall total histologic score is still valid in the authors mind given only one component of this score represented tissue character. Note that when considering the differences between hyaline cartilages, only considering percent filling by fibrous tissue vs fibrous cartilage vs hyaline cartilage that the differences are insignificant both subjectively and with quantitative histomorphometry.

Immunohistochemistry revealed significantly more type II collagen in the ACI and ECD defects compared to CMA defects and there was significantly more aggrecan noted in the ACI group compared to either the CMA or ECD treated defects. These findings are encouraging in that, in all previous studies done in the equine model, the type II collagen content was relatively good for a full-thickness defect at 12 months and improved when subchondral microfracture is done as well[28,36], but the aggrecan content is usually half that of normal articular cartilage and upregulating aggrecan content is a particular challenge[28]. In a recent study looking at human cartilage biopsies 2 years after ACI, it was shown that type II collagen mRNA content as well as aggrecan mRNA expression was at lower levels in the treated samples vs controls, but this is not surprising in view of the 2-year time period and it would hardly be expected to see upregulation of mRNA. However, the immunohistochemistry results were rather confusing in that type II collagen was found in all of the samples and confined to the cells, while controls showed positive diffuse staining in the extra-cellular matrix[39].

The histologic and immunohistochemistry results from this long-term, randomized, histologically blinded large animal GLP study are particularly encouraging suggesting superiority with the ACI technique. There were no complications, even with 18 months follow-up and rigorous physical rehabilitation. 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 construct implanted with absorbable PDS/PGA staples would appear to offer considerable clinical advantages.

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References

1. Buckwalter JA, Mankin HJ. Articular cartilage. Part II: generation and osteoarthrosis, repair, regeneration and transplantation. J Bone Joint Surg Am 1997;79:612–32.
2. Hunziker EB. Articular cartilage repair. Basic science and clinical progress. A review of the current status and prospect. Osteoarthritis Cartilage 2002;10:432–63.

3. Knutsen G, Engebertsen L, Ludbigsen TC, Drogset JO, Grontvedt T, Solheilm E, *et al*. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg (Am) 2004;86A:455–64.
4. Brittberg M, Lindhal A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889–95.
5. Bentley B, Biant LC, Carrington RW, Akmal M, Goldberg A, Williams AM, *et al*. A prospective, randomized comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg Br 2003;85:223–30.
6. Brittberg M. Autologous chondrocyte transplantation. Clin Orthop Relat Res 1999;367(Suppl):S147–55.
7. Brittberg M, Tallhede NT, Sjogren-Jansson B, Lindahl A, Peterson L. Autologous chondrocytes used for articular cartilage repair: an update. Clin Orthop Relat Res 2001;391(Suppl):S337–48.
8. Gillogly SD, Voight M, Blackburn T. Treatment of articular cartilage defects of the knee with autologous chondrocyte implantation. J Orthop Sports Phys Ther 1998;28:241–51.
9. Knutsen G, Solheim E, Johansen O. Treatment of focal cartilage injuries in the knee. Tidsskr Nor Laegeforen 1998;18:2493–7.
10. Marcacci M, Saffagnini S, Kon E, Visani A, Iackono F, Loreti I. Arthrosopic autologous chondrocyte transplantation: technical notes. Knee Surg Sports Traumatol Arthrosc 2002;10:154–9.
11. Micheli LJ, Browne JE, Erggelt C, Fu F, Mandelbaum B, Moselely JB, *et al*. Autologous chondrocyte implantation of the knees: muticenter experience and minimum 3-year follow-up. Clin J Sport Med 2001; 11:223–8.
12. Minas T, Peterson L. Advanced techniques in autologous chondrocyte transplantation. Clin Sports Med 1999;18:13–44.
13. Muellner T, Knopp A, Ludvigsen TC, Engebretsen L. Failed autologous implantation. Complete atraumatic graft delamination after 2 years. Am J Sports Med 2001;29:516–9.
14. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. 2- to 9- year outcome after autologous chondrocyte implantation of the knee. Clin Orthop 2000;374:212–34.
15. Peterson L, Brittberg M, Kibiranta I, Akerlund EL, Lindahl A. Autologous chondrocyte transplantation. Biomechanics and long-term durability. Am J Sports Med 2002;30:2–12.
16. Richardson JB, Caterson B, Evans EA, Ashton BA, Roberts S. Repair of human articular cartilage after implantation of autologous chondrocytes. J Bone Joint Surg Br 1999;81:1064–8.
17. Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am 2003;85:185–92.
18. Messner K, Gillquist J. Cartilage repair. A critical review. Acta Orthop Scand 1996;67:523–9.
19. Browne JE, Anderson AF, Arciero R, Mandelbaum B, Mosely JB, Michele J, *et al*. Clinical outcome of autologous chondrocyte implantation at 5 years in US subjects. Clin Orthop 2005;436:237–45.
20. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at 2–10 years. J Bone Joint Surg Am 2003;85(Suppl 2):17–24.
21. Solchaga LA, Dennis JE, Goldberg VM, Caplan AI. Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage. J Orthop Res 1999;17:205–13.
22. Solchaga LA, Yoo JU, Lundberg M, Dennis JE, Huibreg TSE, Goldberg VM, *et al*. Hyaluronic acid-based polymers in the treatment of osteochondral defects. J Orthop Res 2000;18:773–80.
23. Pavesio A, Abatangelo G, Borrione A, Borccta D, Hollander AP, Kon E, *et al*. Hyaluronan-based scaffolds (Hyalograft® C) in the treatment of knee cartilage defects: preliminary clinical findings. Cartilage and Bone. Novartis Found Symp 2003;249:203–17.
24. Grigolo B, Lasignoli G, Piacentini A, Fiorini M, Gobbi P, Mazzotti G, *et al*. Evidence for re-differentiation of human chondrocytes grown on an hyaluronan-based biomaterial (Hyaff® II): molecular, immunohistochemical and ultrastructural analysis. Biomaterials 2002;23: 1187–95.
25. McIlwraith CW. Diagnostic and Surgical Arthroscopy in the Horse. 2nd edn. Philadelphia, PA: Lea & Fibiger; 1990.
26. O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg (Am) 1986;68:1017–35.
27. O'Driscoll SW, Keeley FW, Slater RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion. A follow-up report at one year. J Bone Joint Surg (Am) 1998;76:595–606.
28. Frisbie DD, Oxford JT, Southwood L, Trotter GW, Rodkey WG, Steadman JR, *et al*. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop 2003;407:215–27.
29. Wu D, Razzano P, Grande DA. Gene therapy and tissue engineering in the repair of the musculoskeletal system. J Cell Biochem 2003;88: 467–81.
30. Solchaga LA, Gau J, Dennis JE, Awadallah A, Lundberg M, Caplan AI, *et al*. Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. Tissue Eng 2002;8: 337–47.
31. Gillogoly SD. Treatment of large full-thickness chondral defects of the knee with autologous chondrocyte implantation. Arthroscopy 2003; 19:147–53.
32. Mandelbaum B, Browne J, Fu F, Micheli L, Mosely B, Erggelet C. Current concepts in articular cartilage of the knee. Am J Sports Med 1998;26:853–61.
33. Zheng MH, Chen J, Kirilak Y, Willers C, Xu J, Wood D. Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: possible implications in human implantation. J Biomed Mater Res B Appl Biomater 2005;73: 61–7.
34. Frisbie DD, Colhoun HA, Bowman S, McIlwraith CW. PDS/PGA staples compared to suture fixation of autologous chondrocyte construct. [Abstract], Orthopaedic Research Socitey, New Orleans 2003: p. 712.
35. Scorrano A, Biggi F. Arthroscopic autologous chondrocyte implantation: mid-term results. [Abstract], 5th ICRS Symposium 2004.
36. Frisbie DD, Trotter GW, Powers BE, Rodkey WG, Steadman JR, Howard RD, *et al*. Arthroscopic subchondral bone plate microfracture technique augments healing of large osteochondral defects in the radial carpal bone in medial femoral condyle of horses. Vet Surg 1999; 28:242–55.
37. Frisbie DD, Cross M, McIlwraith CW. A comparative study of articular cartilage thickness in the style of animal species used in pre-clinical studies compared to articular cartilage thickness in the human knee. Vet Comp Orthop Traumatol 2006;19:142–6.
38. Frisbie DD, Rodkey WG, Steadman JR, McIlwraith CW. Effects of calcified cartilage on healing on chondral defects treated with microfracture in horses. Am J Sports Med 2006;34(11):1824–31.
39. Grigolo V, Roseti L, de Franceschi L, Piacentini A, Cattini L, Manfredini M, *et al*. Molecular and immunohistological characterization of human cartilage 2 years following autologous cell transplantation. J Bone Joint Surg (Am) 2005;87:46–57.