Matrix-Induced Autologous Chondrocyte Implantation (MACI) Using a Cell-Seeded Collagen Membrane Improves Cartilage Healing in the Equine Model

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Background: Autologous chondrocyte implantation (ACI) using a collagen scaffold (matrix-induced ACI; MACI) is a next-generation approach to traditional ACI that provides the benefit of autologous cells and guided tissue regeneration using a biocompatible collagen scaffold. The MACI implant also has inherent advantages including surgical implantation via arthroscopy or miniarthrotomy, the elimination of periosteal harvest, and the use of tissue adhesive in lieu of sutures. This study evaluated the efficacy of the MACI implant in an equine full-thickness cartilage defect model at 1 year.

Methods: Autologous chondrocytes were seeded onto a collagen type-I/III membrane and implanted into one of two 15-mm defects in the femoral trochlear ridge of 24 horses. Control defects either were implanted with cell-free collagen type-I/III membrane (12 horses) or were left ungrafted as empty defects (12 horses). An additional 3 horses had both 15-mm defects remain empty as nonimplanted joints. The repair was scored by second-look arthroscopy (12 weeks), and necropsy examination (53 weeks). Healing was assessed by arthroscopic scoring, gross assessment, histology and immunohistology, cartilage matrix component assay, and gene expression determination. Toxicity was examined by prostaglandin E_2 formation in joint fluid, and lymph node morphology combined with histologic screening of organs.

Results: MACI-implanted defects had improved gross healing and composite histologic scores, as well as increases in chondrocyte predominance, toluidine blue-stained matrix, and collagen type-II content compared with scaffold-only implanted or empty defects. There was minimal evidence of reaction to the implant in the synovial membrane (minor perivascular cuffing), subchondral bone, or cartilage. There were no adverse clinical effects, signs of organ toxicity, or evidence of chondrocytes or collagen type-I/III membrane in draining lymph nodes.

Conclusions: The MACI implant appeared to improve cartilage healing in a critical-sized defect in the equine model compared with collagen matrix alone.

Clinical Relevance: These results indicate that the MACI implant is quick to insert, provides chondrocyte security in the defect, and improves cartilage healing compared with ACI.

Autologous chondrocyte implantation (ACI) has been used to treat focal traumatic cartilage defects for nearly 30 years[1-3]. Indications have generally been extensive cartilage defects and as definitive treatment when other surgical options have failed[4,5]. Despite generally sustained functional improvement, adverse events have been recorded, with as many as 44% of joints treated with ACI needing revision surgery[6-8]. Complications include delamination and dislodgment of the retaining periosteal flap, hypertrophy of the periosteum, and degeneration or failure of the cartilage repair in long-term

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TABLE I Synovial Fluid Analysis at 0, 12, and 53 Weeks Following Surgery*

Group	Volume *(mL)*	Total Protein *(g/dL)*	WBC *(×10³/μL)*	RBC *(×10³/μL)*
Preop.				
Normal joints	0.80 ± 0.67[a]	2.15 ± 0.38[a]	0.78 ± 0.33[a]	7.58 ± 11.71[a]
12 weeks				
MACI + Maix	0.90 ± 0.56[a]	2.53 ± 0.58[a]	0.80 ± 0.28[a]	14.64 ± 27.10[a]
MACI + empty	1.0 ± 0.61[a]	2.56 ± 0.66[a]	0.92 ± 0.36[a]	9.06 ± 12.54[a]
Paired empty	1.00 ± 0.6 [a]	2.00 ± 0[a]	0.92 ± 0.36[a]	0.30 ± 0.14[b]
53 weeks				
MACI + Maix	0.90 ± 0.48[a]	2.29 ± 1.20[a]	1.02 ± 0.88[a]	0.37 ± 0.34[a]
MACI + empty	0.70 ± 0.34[a]	2.20 ± 0.28[a]	0.81 ± 0.56[a]	3.33 ± 8.31[a]
Paired empty	1.00 ± 0.50[a]	2.33 ± 1.16[a]	0.87 ± 0.60[a]	0.97 ± 0.90[a]
Reference range	1-2	<2.5	0-0.9	<1.00

*The values are given as the mean and the standard deviation. Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them. WBC = white blood-cell count, and RBC = red blood-cell count.

studies[7-10]. Additionally, the procedure requires placement of fine sutures in the periosteal flap and cartilage around the defect periphery, which can be complex and time-consuming.

Complications after ACI have been reduced by second or third-generation techniques, in which collagen or synthetic membranes are substituted for periosteum[7,8]. These ACI techniques use other membranes to retain autologous chondrocytes in the cartilage defect (second generation), and cell-loaded membranes applied arthroscopically (third generation)[11-13]. The matrix-induced ACI (MACI) implant (Vericel) utilizes a porcine collagen type-I/III membrane (ACI-Maix; Vericel), seeded with autologous chondrocytes. The MACI technology implants the cell-laden membrane, using fibrin adhesive, to provide an ACI therapy, while minimizing procedure time and complexity. The MACI implant has been used with clinical success in >8,000 patients, and has been tested in several animal research trials, in which it enhanced full-thickness cartilage repair[14,15]. A positive outcome after clinical application of the MACI implant in the human knee has been described[16-27]. The MACI implant eliminates the need to suture the graft into the cartilage defect[28,29], can be applied by arthroscopic technique, and decreases the stimulus for vasculogenic hypertrophy[11,13]. Implantation using a minimally invasive technique is facilitated by securing the graft with fibrin adhesive, which has also been shown to facilitate chondrocyte migration from the membrane base to the healing tissue[17,30,31].

The literature contains little information about the survival and efficacy of the MACI implant for cartilage repair[14,15,32] Preclinical data characterizing cartilage healing are limited to a short-term study in rabbits, another in sheep, and a third in our pilot study in 6 horses[14,15,32]. The current study expanded on previous preliminary work to include 27 horses, which were used to evaluate the impact of the cell-free membrane alone and to study cartilage healing and persistence over a longer term after MACI.

Materials and Methods

Experimental Design

Autologous chondrocytes were arthroscopically harvested from 1 femoropatellar joint of 24 horses 4 weeks prior to the implantation surgery. Detailed harvest procedures and membrane seeding are described in a previous pilot study[32] and are briefly described in the Appendix. Paired full-thickness cartilage defects were formed on the lateral trochlear ridge of the femur in the contralateral limb in 27 horses. In 24 horses, an autologous chondrocyte-seeded MACI implant was secured in 1 defect, while the second defect was either repaired with a cell-free collagen type-I/III membrane (12 horses) or remained empty to heal spontaneously (12 horses). In an additional 3 horses, both defects were left empty to heal spontaneously. Detailed surgical methods, postoperative exercise, and assessment of chondrocyte viability at implantation were described in a previous study[32].

Second-Look Arthroscopy

The repaired cartilage defects in the implanted femoropatellar joints were assessed arthroscopically at 12 weeks and scored by 2 blinded observers for defect fill, repair tissue color and smoothness, pannus formation, perimeter integration, and subchondral bone attachment, as described in previous studies[32,33]. This provided interim healing data, since magnetic resonance imaging (MRI) was not possible.

Tissue Harvest and Analysis

A complete necropsy examination was performed at euthanasia 53 weeks after the index surgery. Joint tissues including cartilage and synovium, and draining lymph nodes, viscera, adrenals, kidney, liver, heart, lung, thymus, and brain were examined. Synovial fluid was sampled from both joints, the implanted femoropatellar joint was exposed, and the repair

TABLE I (*continued*)

Neutrophil *(%)*	Large Mononuclear Cells/ Macrophages *(%)*	Small Mononuclear Cells/ Lymphocytes *(%)*	Eosinophils *(%)*	Basophils *(%)*
4.48 ± 4.46[a]	78.70 ± 10.59[a]	16.57 ± 9.04[a]	0.09 ± 0.42[a]	0[a]
3.70 ± 3.95[a]	69.20 ± 13.22[a]	30.40 ± 19.59[a]	0.20 ± 0.32[a]	0[a]
4.18 ± 3.31[a]	64.91 ± 18.84[a]	30.45 ± 18.28[a]	0.09 ± 0.30[a]	0.36 ± 0.60[a]
2.00 ± 0[a]	89.50 ± 0.04[a]	8.50 ± 0.04[a]	0[a]	0[a]
0.67 ± 0.78[a]	62.33 ± 16.35[a]	37.00 ± 17.00[a]	0[a]	0[a]
1.33 ± 2.10[a]	75.33 ± 15.10[ab]	21.00 ± 10.62[ab]	0[a]	0[a]
0.33 ± 0.58[a]	93.33 ± 2.52[b]	6.33 ± 2.52[b]	0[a]	0[a]
<10	>60	<30	0	0

was scored (defect fill, integration with surrounding cartilage, integration to subchondral bone, repair tissue color, surface smoothness, and pannus). The implanted defect was divided into 3 sagittal segments, with the central 3-mm osteochondral block fixed for histologic analysis, the cartilage removed from an adjacent third for biochemical and gene expression assays, and a lateral osteochondral block frozen for biomechanical testing[34].

Histologic Analysis

Detailed histologic and immunohistologic methods and scoring for hematoxylin and eosin, toluidine blue, and collagen type-II immunohistochemistry have been published previously[32,33] (see Appendix). Slides were prepared and scored in a blinded manner. Data for histologic scoring were analyzed to compare MACI-implanted defects and control defects, in which a cell-free collagen membrane had been implanted or that were left empty. The level of significance was set at $p < 0.05$.

Proteoglycan and DNA Analysis

Frozen cartilage was milled and lyophilized. Repair tissue glycosaminoglycan (GAG) content in MACI-implanted and control cartilage defects was assayed after papain digest using

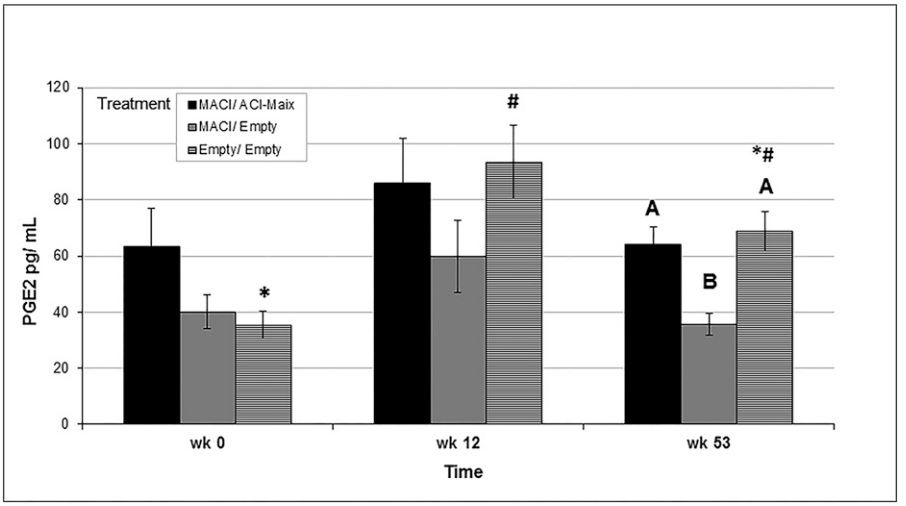

Fig. 1

PGE2 data from synovial fluid of implanted joints obtained at the time of implantation (wk 0), at the 12-week second look (wk 12), and at termination (wk 53). Significant differences (where $p < 0.05$ on pairwise analysis) within each time point are denoted by different letters (A or B). Within each treatment type, significant differences ($p < 0.05$ on post hoc pairwise analysis after analysis of variance) over time are denoted by different symbols (*, #). The graph is arranged by time and shows no difference in PGE2 levels among joints receiving MACI and cell-free ACI-Maix, MACI and empty defects, or paired empty defects, at week 0 or 12 weeks. At 53 weeks, PGE2 levels in joints implanted with MACI and empty defects were less than those in joints implanted with MACI and cell-free ACI-Maix or paired empty joints.

TABLE II Arthroscopic Second-Look Repair Defect Cartilage Scores at 12 Weeks*

Defect Treatment	Smooth White Repair Tissue *(0-4 points)*	Percent Filling *(0-4 points)*	Repair Tissue Integration *(0-3 points)*	Tissue Color *(0-3 points)*	Pannus *(0-3 points)*	Total *(0-17 points)*
MACI (n = 24)	2.63 ± 0.13^{ab}	0.92 ± 0.19^{a}	0.63 ± 0.16^{a}	3.08 ± 0.21^{ab}	0	7.25 ± 0.44^{a}
Cell-free ACI-Maix (n = 12)	2.25 ± 0.25^{a}	0.83 ± 0.35^{a}	1.17 ± 0.44^{a}	2.67 ± 0.38^{a}	0	6.92 ± 0.93^{a}
Ungrafted empty (n = 12)	2.67 ± 0.19^{ab}	1.42 ± 0.42^{ab}	1.25 ± 0.28^{a}	2.58 ± 0.40^{a}	0	7.92 ± 1.00^{a}
Paired empty (n = 6)	3.00 ± 0^{b}	2.50 ± 0.43^{b}	1.33 ± 0.21^{a}	3.83 ± 0.17^{b}	0	10.67 ± 0.72^{b}

*The values are given as the mean and the standard deviation. The total score ranged from 0 (indicating normal) to 17 (poorest healing). Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them.

the dimethylmethylene blue (DMMB) microplate technique[35]. An aliquot of papain digest was used for DNA quantification using fluorometric bisbenzimide assay[36].

Synovial Fluid Analysis

Serial synovial fluid samples were analyzed using a Coulter counter and Giemsa-stained cytology to determine total and differential cell counts, and protein content was determined by refractometer. Prostaglandin E_2 (PGE2) content was determined by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Assay Designs).

Gene Expression

Gene expression of aggrecan and collagen type II in cartilage repair tissue and surrounding cartilage was assessed by quantitative polymerase chain reaction (see Appendix). Total copy number of mRNA was obtained for each gene of interest from a concurrently performed standard curve, and these values were normalized to 18S RNA expression.

Statistical Analysis

Details on statistical analysis are provided in the Appendix.

Results

Chondrocyte Viability

Implanted MACI membranes were assessed for in situ chondrocyte viability after shipping and implantation. The implanted MACI membranes had a chondrocyte viability mean of 82%. No viable chondrocytes were identified

TABLE III Gross Healing Scores at 53-Week Termination*

Treatment Group	Smooth White Repair Tissue *(0-4 points)*	Percent Filling *(0-4 points)*	Repair Tissue Integration *(0-3 points)*	Tissue Color *(0-3 points)*	Pannus *(0-3 points)*	Total *(0-17 points)*
MACI group 1 (n = 12)	0.92 ± 0.19^{a}	0.50 ± 0.20^{a}	0.42 ± 0.19^{ac}	0.92 ± 0.19^{a}	0	2.75 ± 0.55^{a}
ACI-Maix group 1 (n = 12)	2.08 ± 0.26^{b}	1.50 ± 0.23^{b}	1.25 ± 0.33^{b}	2.42 ± 0.38^{b}	0	7.25 ± 0.84^{b}
MACI group 2 (n = 12)	1.42 ± 0.22^{ac}	0.75 ± 0.22^{a}	0.17 ± 0.11^{c}	1.42 ± 0.26^{ac}	0	3.75 ± 0.63^{ac}
Empty group 2 (n = 12)	1.83 ± 0.24^{bc}	1.00 ± 0.21^{ab}	1.08 ± 0.29^{ab}	1.92 ± 0.34^{bc}	0	5.83 ± 0.74^{b}
Paired empty (n = 6)	1.83 ± 0.31^{bc}	1.17 ± 0.17^{ab}	1.00 ± 0.26^{abc}	1.67 ± 0.33^{abc}	0	5.67 ± 0.67^{bc}

*The values are given as the mean score and the standard deviation. The total score ranged from 0 (indicating normal) to 17 (poorest healing). Group-1 defects were implanted with MACI and cell-free ACI-Maix membrane. Group-2 defects were implanted with MACI and the other defect remained empty. Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them.

in cell-free collagen type-I/III membranes or snap-frozen MACI implants.

Clinical Findings

Lameness associated with the surgical implantation declined to zero within 21 days after surgery. The miniarthrotomy incisions healed without dehiscence. Semiquantitative assessment of lameness at 12 and 53 weeks after implantation revealed no differences in lameness between the limbs with the MACI implant, cell-free collagen membrane, or empty defects at 12 weeks. None of the horses showed lameness 53 weeks after implantation.

Synovial Fluid Analysis

Synovial fluid white blood-cell and red blood-cell counts, protein, and viscosity were determined on samples taken before implantation, at 12 weeks, and at termination 53 weeks after implantation. Protein content and cell counts were normal before surgery and were normal or slightly increased at 12 and 53 weeks (Table I). There were no significant differences in cell count or protein levels in joints implanted with MACI and cell-free membrane, MACI and empty, or paired empty defects. Synovial fluid PGE2 levels prior to implantation, at 12 weeks, and at 53 weeks are presented in Figure 1 and in the Appendix. PGE2 levels in synovial fluid were not significantly elevated in MACI or cell-free collagen type-I/III membrane-implanted groups at 12 or 53 weeks after surgery.

Arthroscopic Second Look at 12 Weeks

Cartilage repair tissue scores for MACI-implanted defects, collagen type-I/III implanted defects, and empty defects showed few differences in the overall defect repair (Table II). The observers were not blinded to treatment group. There were no significant differences for any individual parameter or for total scores between the 3 treatment groups. Total and individual scores in treated joints (proportion hyaline-like tissue, percent fill, and tissue color)

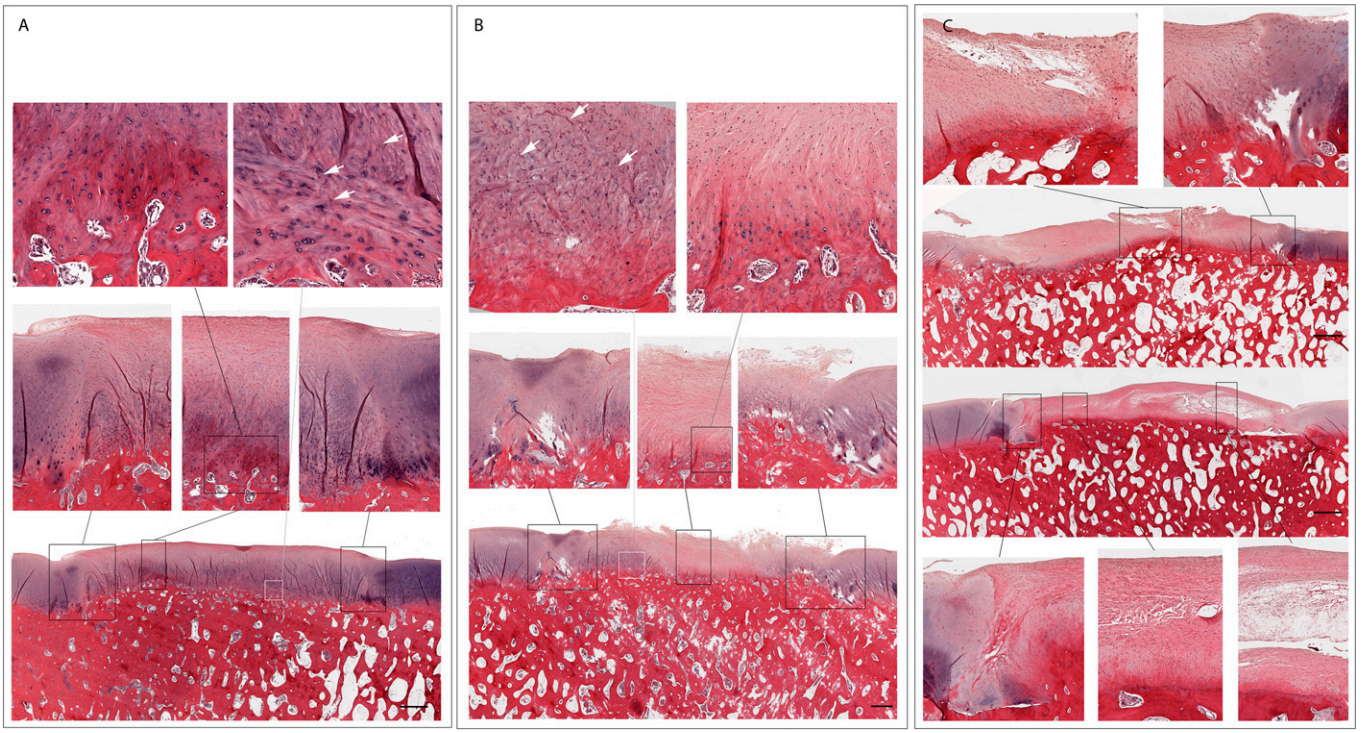

Fig. 2

Figs. 2-A, 2-B, and 2-C Photomicrographs of hematoxylin and eosin-stained sections from healing defects at 53 weeks. **Fig. 2-A** MACI-implanted defect 53 weeks after repair. Insets (boxed) show attachment to cartilage perimeter (left), chondrocyte-rich region (center), and attachment to perimeter cartilage (right). Top panels are higher magnification (×20) showing chondrocyte predominance (left), and minor residual collagen membrane (arrows; right). (Link to the digital whole-slide image: Section of MACI-Implanted Defect at 53 Weeks.) **Fig. 2-B** Cell-free collagen type-I/III membrane-implanted defect 53 weeks after repair. Inset (boxed) shows attachment to cartilage perimeter (left) and chondrocyte-poor region (center). Top panels are higher magnification (×20) showing minor residual collagen type-I/III membrane in the base of the defect (arrows; left), and minor chondrocyte populations in the center of the healing defect (right). (Link to the digital whole-slide image: Section of Cell-Free Collagen Membrane Defect at 53 Weeks.) **Fig. 2-C** Two examples of paired empty defects at 53 weeks, in which both defects were allowed to heal spontaneously. Inset magnified (×4) areas (boxed) show gaps in perimeter integration, separation of the healing tissue from underlying subchondral bone, and fibrillation of the surface. In low-power specimen, bar = 1 mm. (Link to the digital whole-slide images: Sections of Paired Empty Defects at 53 Weeks – Example 1 and Example 2.)

TABLE IV Histologic, Histochemical, and Immunohistochemical Scores Listed by Parameter and Treatment Group with MACI-Implanted, Cell-Free ACI-Maix-Implanted, and Empty Defects*

Treatment Group	Defect Fill	Chondrocyte Predominance	Perilesional Chondrocyte Cloning	Subchondral Bone Attachment	Perimeter Integration
MACI (n = 24)	0.79 ± 0.23[a]	1.75 ± 0.16[a]	1.50 ± 0.14[a]	0.46 ± 0.22[a]	0.63 ± 0.12[a]
ACI-Maix (n = 12)	1.08 ± 0.42[ab]	2.92 ± 0.08[b]	2.08 ± 0.19[a]	1.75 ± 0.51[a]	1.17 ± 0.30[a]
Empty (n = 12)	1.50 ± 0.29[ab]	2.67 ± 0.14[b]	2.17 ± 0.21[a]	0.58 ± 0.26[a]	1.17 ± 0.27[a]
Paired empty (n = 6)	2.50 ± 0.22[b]	3.00 ± 0[b]	2.17 ± 0.31[a]	1.50 ± 0.67[a]	1.17 ± 0.17[a]

*The values are given as the mean score and the standard deviation. Lower scores indicate more normal findings, with a total minimum score of 0 and a maximum of 32. Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them.

were significantly better than scores for paired empty defects.

Gross Appearance

The gross appearance of the implanted defects at termination was scored by 2 blinded observers. The individual parameter scores and the total composite score are presented in Table III. MACI-implanted defects had significantly improved scores for defect fill, tissue color, and perimeter integration. The cartilage-healing total gross score for MACI showed improved healing compared with cell-free collagen membrane, single empty defect, and dual empty defects. Composite scores for both implanted defects were improved compared with empty defects. With respect to individual parameters, MACI was significantly better than cell-free collagen type-I/III membrane alone in every category. None of the implanted cell-free collagen or MACI membranes protruded from the defect or were free in the joint.

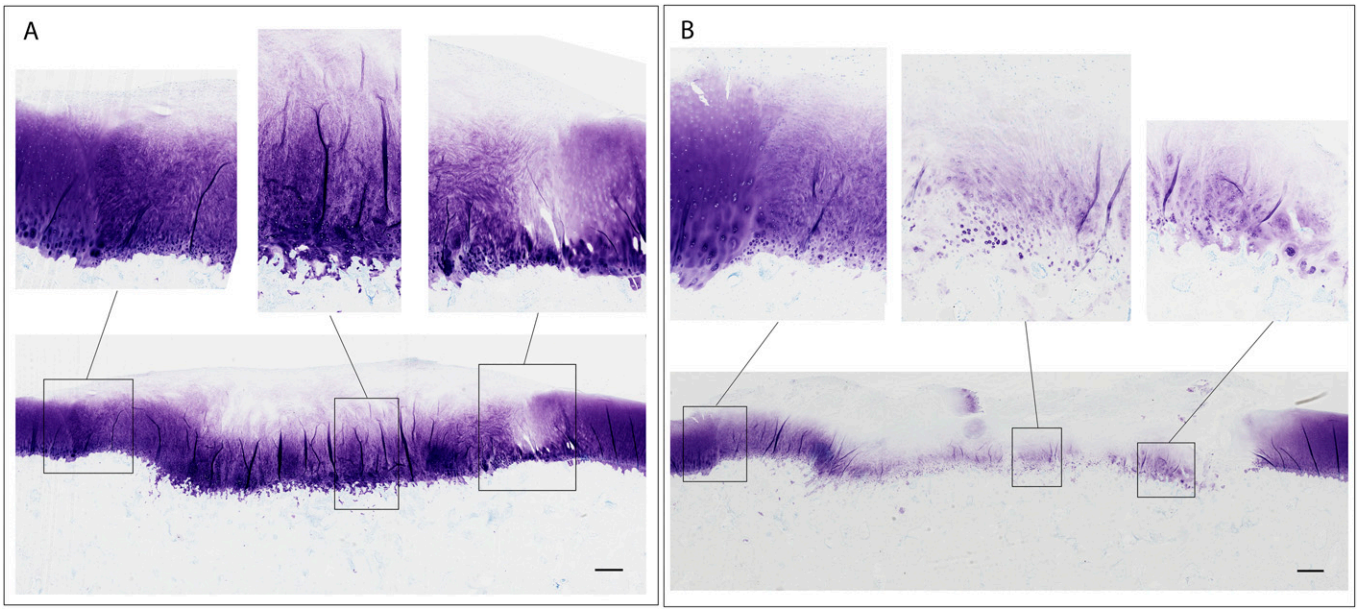

Fig. 3

Figs. 3-A and 3-B Photomicrographs of toluidine blue-stained healing defects. **Fig. 3-A** MACI-implanted defect 53 weeks after repair, showing extensive proteoglycan-rich zone in the base of the defect and well-integrated ends. Inset (×2.5 magnification) (boxed) shows attachment to cartilage perimeter (left), center inset shows toluidine blue histochemical localization of a deep layer of proteoglycan-containing matrix, and right inset shows minor perimeter detachment of the defect repair tissue. (Link to the digital whole-slide image: Toluidine Blue-Stained Healing of MACI-Implanted Defect.) **Fig. 3-B** Empty defect 53 weeks after spontaneous repair, from the same animal as in Figure 3-A, showing thin proteoglycan-containing zone in base of defect. Inset (×2.5 magnification) (boxed) shows attachment to cartilage perimeter (left), and center and right insets show toluidine blue histochemical localization of a thin layer of proteoglycan-containing matrix. In low-power specimen, bar = 1 mm. (Link to the digital whole-slide image: Toluidine Blue-Stained Healing of Empty Defect.)

TABLE IV (*continued*)

Surface Fibrillation	Tidemark	Toluidine Blue	Collagen Type II	Total
1.08 ± 0.25[a]	2.50 ± 0.12[a]	2.83 ± 0.18[a]	2.33 ± 0.22[a]	13.88 ± 0.93[a]
1.83 ± 0.35[a]	2.92 ± 0.08[a]	3.83 ± 0.11[b]	3.50 ± 0.20[b]	21.08 ± 1.36[b]
2.08 ± 0.26[a]	2.75 ± 0.13[a]	3.75 ± 0.18[b]	3.58 ± 0.19[b]	20.25 ± 0.96[b]
2.00 ± 0.45[a]	2.83 ± 0.17[a]	4.00 ± 0[b]	3.67 ± 0.21[b]	22.83 ± 1.05[b]

Histologic Appearance

MACI improved most individual histologic parameters compared with defects implanted with the cell-free collagen membrane or empty defects (Fig. 2). The scoring system has been described in previous publications[32,33], and data are presented in Table IV. Composite tissue healing scores were significantly improved in MACI-implanted defects compared with collagen type-I/III implanted defects, empty defects from implanted joints, and the paired ungrafted empty defects. Several individual parameters including chondrocyte predominance, toluidine blue intensity, and collagen type-II deposition were significantly improved in MACI-implanted defects compared with cell-free membrane and empty defects. Additionally, defect fill and perimeter integration were improved in MACI-implanted defects compared with paired empty defects (Fig. 2). Four of 36 MACI or collagen type-I/III membrane-implanted defects had birefringent residual collagen type-I/III fiber fragments (Fig. 2).

Toluidine Blue Histochemistry

Toluidine blue histochemical staining extended through a significantly greater depth of the cartilage matrix in MACI-implanted defects (Table IV). Improved toluidine scores in

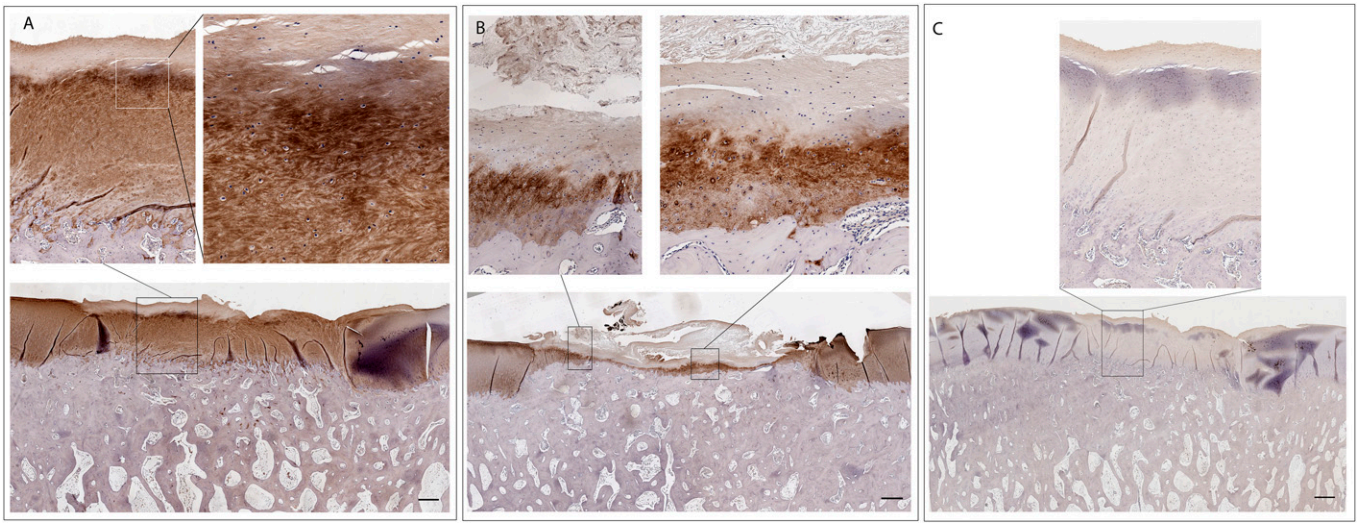

Fig. 4

Figs. 4-A, 4-B, and 4-C Photomicrographs of collagen type-II immunohistochemical reaction. Hematoxylin counterstain. Bar = 1 mm. **Fig. 4-A** MACI-implanted defect 53 weeks after repair, showing extensive collagen formation throughout the defect. Inset (×2.5) (boxed) shows center of the healing defect (left), and higher power magnification (×10) shows the intense collagen type-II formation in the middle zone of the healed defect (right). (Link to the digital whole-slide image: Collagen Type-II Immunohistochemical Reaction in MACI-Implanted Defect.) **Fig. 4-B** Ungrafted empty control defect 53 weeks after repair in joint where the other defect (**Fig. 4-A**) was implanted with MACI, showing a narrow zone of collagen type-II formation adjacent to the subchondral bone attachment. Inset (×2.5) (boxed) shows left side of the healing defect with fibrillar surface cartilage and separation into layers (left). Right inset (×10) shows a narrow zone of fibrocartilage in the defect base with fibrous tissue predominating in the middle and surface layers. (Link to the digital whole-slide image: Collagen Type-II Immunohistochemical Reaction in a Control Defect.) **Fig. 4-C** Photomicrograph of negative procedural control for collagen type-II immunohistochemical reaction of MACI-implanted defect 53 weeks after repair. Inset is ×2.5 magnification. Nonimmune serum was substituted for mouse anticollagen type-II antibody. Serial slide to **Figure 4-A**. (Link to the digital whole-slide image: Negative Procedural Control for Collagen Type-II Immunohistochemical Reaction.)

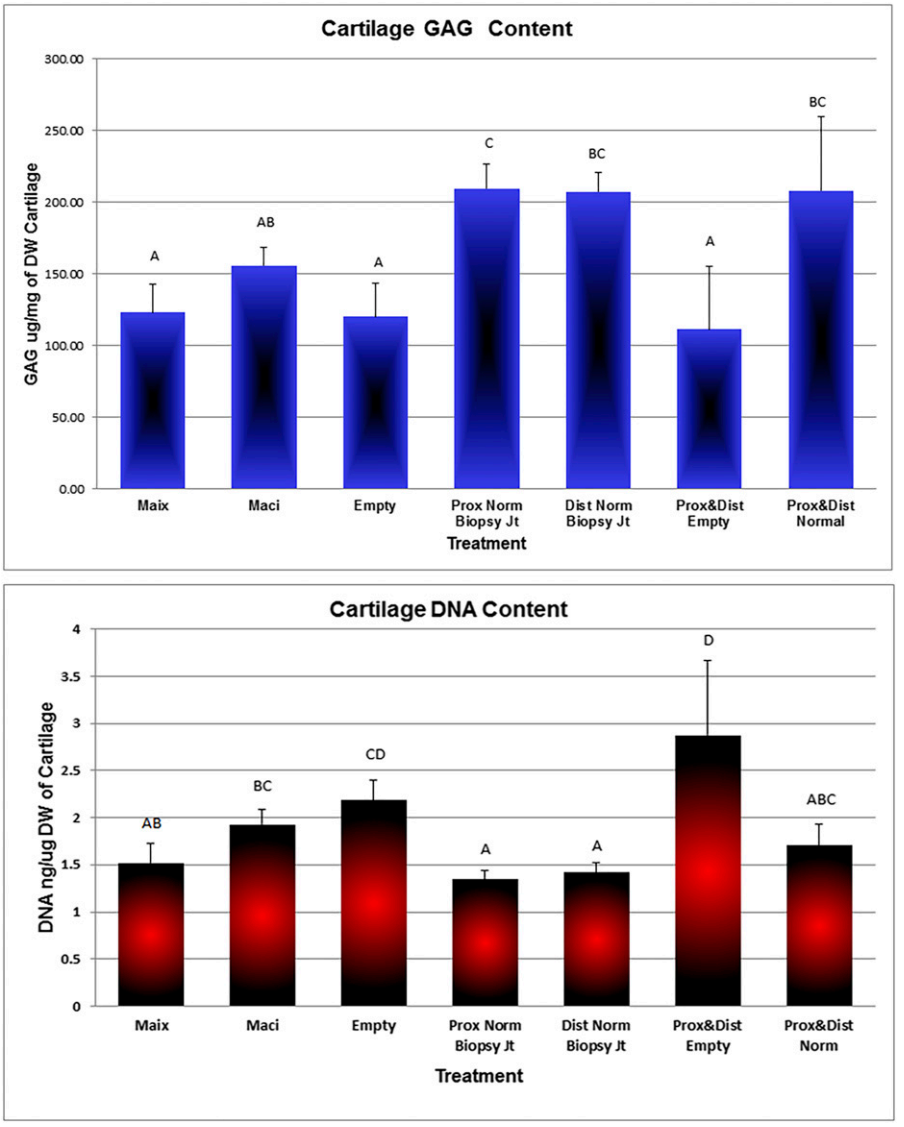

Fig. 5

Charts showing the cartilage GAG (top) and DNA content (bottom) from implanted defects (MACI or cell-free ACI-Maix), empty defects, proximal or distal sites in relatively normal joints with adjacent previous biopsy (Prox Norm Biopsy or Dist Norm Biopsy), a pair of empty defects (Prox&Dist Empty), or proximal and distal sites within the lateral trochlear ridge of completely normal joints (Prox&Dist Normal). The bars and the I-bars indicate the mean and the standard deviation. Significant differences are denoted by different letters above each column ($p < 0.05$). DW = dry weight.

MACI-implanted defects correlated to the histochemical appearance (Fig. 3). Development of full-thickness cartilage toluidine histochemical reaction was not apparent in MACI or control-implanted cartilage defects. However, the deeper and middle zones of MACI-implanted defects had a preponderance of toluidine blue-stained matrix.

Collagen Type-II Abundance

Collagen type-I content was increased in MACI-implanted defects (Fig. 4), and this predominated in the base and middle zones of the healing cartilage. This was confirmed by collagen type-II scores, which were significantly improved in MACI-implanted defects (Table IV). Repair tissue at the junction with perimeter adjacent cartilage also had increased type-II content. Occasional regions of residual collagen type-I/III fiber in MACI repairs were surrounded by chondrocytes synthesizing abundant collagen type II. Collagen type II in cell-free membrane-implanted defects was not significantly different from empty defects.

Collagen type-I reaction extended uniformly throughout the repair tissue compared with the type-II predominance in the middle and deeper zones of cartilage. Additionally, collagen type-I reaction was evident in adjacent cartilage for several millimeters.

TABLE V Gene Expression of Matrix Aggrecan and Collagen Type II in Healed Defects 53 Weeks After Implantation*

Treatment Group	Aggrecan (copies ×10e3/ng)	Collagen Type II (copies ×10e3/ng)
MACI (n = 24)	34.21 ± 10.94[a]	1,444.68 ± 509.96[a]
Cell-free ACI-Maix (n = 12)	23.81 ± 10.87[a]	808.08 ± 362.41[ab]
Empty (n = 12)	26.75 ± 16.59[a]	196.02 ± 155.12[c]

*The values are given as the mean and the standard deviation. Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them. RNA copy number is presented as copy/ng RNA.

Biochemical Characterization

Cartilage GAG and DNA were analyzed in defect repair tissue and from similar sites in contralateral joints. Defects implanted with MACI had a trend ($p = 0.1$) toward increased cartilage GAG content compared with empty defects (Fig. 5). All defects had less total matrix GAG than contralateral, previously biopsied joints or normal cartilage GAG, although statistically the GAG content of MACI-implanted defects was similar to the contralateral biopsied joint and normal cartilage ($p = 0.13$). DNA content of the defects and cartilage from control joints is presented in Figure 5. MACI-implanted defects were less cellular than empty defects, but generally were more cellular than normal cartilage. This was associated with histologic appearance.

Matrix Gene Expression

Gene expression of aggrecan was similar in grafted and empty ungrafted effects. However, collagen type II in MACI-implanted defects was significantly increased ($p = 0.004$) compared with empty defects, and showed a trend ($p = 0.08$) toward increased levels compared with cell-free collagen type-I/III implanted defects (Table V).

Synovial Membrane Histology

Synovial membrane biopsy specimens obtained at the 12-week second-look assessment and 53-week termination were examined and scored for parameters shown in Table VI. At termination, there were few differences between joints receiving MACI and the various controls (Fig. 6). Complete results of synovial histologic analysis are provided in the Appendix.

Lymph Node, Organ, and Brain Pathology

There were no gross or histologically abnormal findings in draining lymph nodes, abdominal and thoracic organs, or brain samples.

TABLE VI Synovial Membrane Histologic Scores at Necropsy Harvest 53 Weeks After Implantation*

Joint Description	Villous Architecture *(0-3 points)*	Subintimal Fibrosis *(0-3 points)*	Intimal Layer Thickening *(0-3 points)*	Vascularity *(0-3 points)*	Inflammatory Cell Infiltrate *(0-3 points)*	Total *(0-15 points)*
MACI + Maix joint—group 1	0.17 ± 0.17[a]	0.83 ± 0.21[a]	0.67 ± 0.22[a]	0.42 ± 0.15[a]	0.75 ± 0.18[b]	2.83 ± 65[b]
MACI + empty joint—group 2	0.17 ± 0.17[a]	0.67 ± 0.26[a]	0.42 ± 0.29[a]	0.08 ± 0.08[a]	0.83 ± 0.24[b]	2.17 ± 0.75[ab]
Ungrafted defect joint—group 3	0.33 ± 0.33[a]	0.00 ± 0.00[a]	0.67 ± 0.67[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.00 ± 0.58[ab]
Paired biopsy joint—group 1	0.00 ± 0.00[a]	0.08 ± 0.08[a]	0.08 ± 0.08[a]	0.17 ± 0.20[a]	0.08 ± 0.11[a]	0.42 ± 0.15[a]
Paired biopsy joint—group 2	0.17 ± 0.17[a]	0.58 ± 0.23[a]	0.50 ± 0.20[a]	0.17 ± 0.11[a]	0.25 ± 0.13[a]	1.67 ± 0.45[ab]
Normal joint—group 3	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

*The values are given as the mean score and the standard deviation. Joint description refers to implants in the 2 cartilage defect sites within the joint. Lower scores indicate more normal findings, with a total minimum of 0 and a maximum of 15. Values with the same superscript letters (i.e., a and a) have no significant difference; however, values with different letter designations (i.e., a and b) indicate that there is a significant difference ($p < 0.05$) between them.

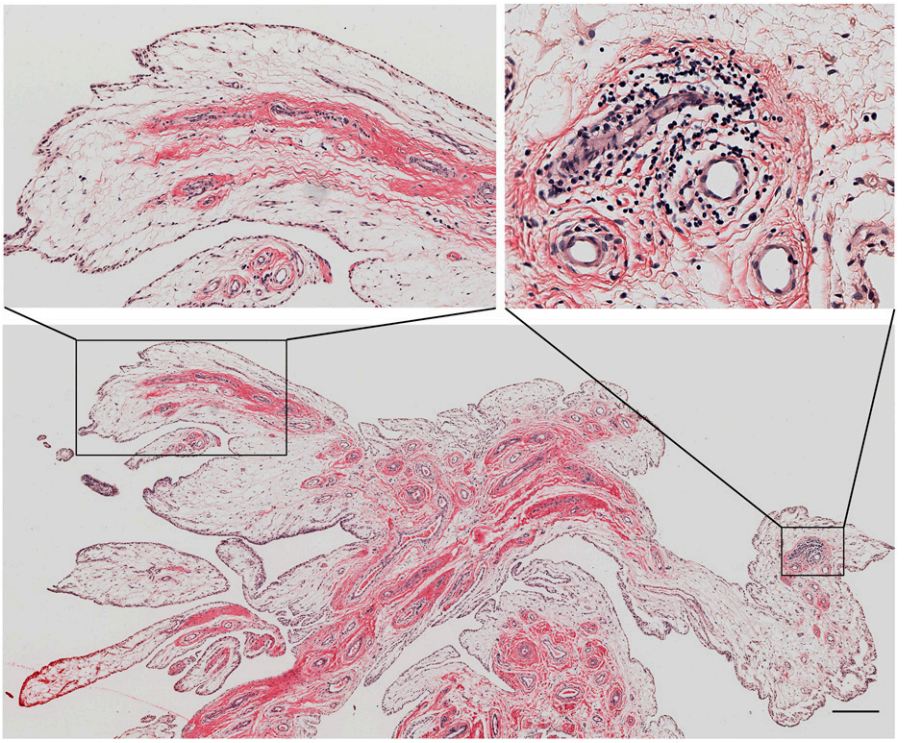

Fig. 6

Synovial membrane showing more typical mild reaction to surgery and MACI implant with mild villous fibrosis (left inset; ×2.5) and mild lymphocytic accumulation (right inset; ×5). Joint was implanted with MACI and cell-free ACI-Maix 53 weeks prior to assessment. Hematoxylin and eosin stain. Bar = 200 μm. (Link to the digital whole-slide image: Synovial Membrane Reaction to MACI Implant.)

Discussion

The MACI implant improved the quality of cartilage repair compared with defects implanted with acellular collagen membrane or spontaneously healed defects. Markers of hyaline cartilage were consistently evident 53 weeks after the MACI implant, including chondrocyte predominance, extensive toluidine blue-stained matrix, and collagen type-II abundance. The histologic and immunohistologic data were supported by increased collagen type-II gene expression, and a trend toward increased GAG content. The combination of unblinded arthroscopic second-look data, gross healing scores, histologic indices, and biochemical analyses indicated MACI-treated cartilage defects were superior to acellular collagen membrane implanted regions or to cartilage defects that healed spontaneously. A previous short-term study in horses verified that MACI improved cartilage healing compared with empty defects, but lacked data describing the response to the carrier membrane alone[32]. Both studies are limited by a lack of direct comparison with other third-generation ACI techniques, microfracture, or osteochondral allograft implantation in the same experimental model.

The MACI implant improved on the original ACI technique[13]. The collagen membrane stabilizes chondrocytes in situ and avoids periosteum, which has inherent vasculogenic and hypertrophic pannus potential. In addition, the composite is glued in place, which abrogates the need for sutures to secure the graft to surrounding cartilage. Combined with the improved chondrogenic potential of cells secured in the base of the defect, the MACI implant provides innovations that potentially improve the quality of cartilage repair. The ACI-Maix collagen membrane is a lyophilized processed porcine collagen type-I/III porous membrane[14,15,37-40]. The membrane is a coarse fibrillar scaffold that has a dense smooth surface and a rough porous surface[38,39,41-43]. Chondrocytes are loaded to the rough surface, using chondrocyte densities of 0.5 to 2×10^6 cells/cm^2.

Immunologic reaction to the porcine membrane was assessed at the cartilage, bone, and synovial membrane levels. The MACI implant and acellular collagen membrane induced minor residual perivascular lymphocytic accumulation in the synovial membrane sections obtained a year after implantation. However, other synovial features showed no abnormal reaction, including intimal cell layer thickness, vascularity, and fibrosis, which were similar in implanted joints and normal joints from the contralateral limb. The persistence of collagen membrane fragments in the repair tissue at 53 weeks was minimal, with short fibrils evident in only 4 of the 36 defects implanted with either MACI or acellular collagen membrane. This compares favorably with the sheets of collagen membrane evident in 6-month studies[32].

Use of the MACI in focal cartilage injury in humans has improved functional outcome[16,27,44-47]. The clinical success of MACI, combined with the results of this long-term study in a large animal model, supports the continued use of MACI for cartilage repair. These data build on previous studies in the horse that were limited by small numbers and short-term

assessment. The MACI implant provides technical improvements through the use of a self-contained chondrocyte-laden membrane that can be shaped to fit the target defect and is secured with fibrin adhesive[13,47]. The reduced surgery time and reduced chance of chondrocyte loss from the implanted defect may further reduce costs and improve outcome. Evaluation of the MACI implant in this animal model revealed a chondrocyte and collagen type-II rich matrix throughout the middle and deep zones. Additional discussion on the biochemical characterization of the repair cartilage is included in the Appendix. Justification and limitations for using the equine model are provided in the Appendix. Some residual fibrocartilage at the surface was common. This may have reflected the distribution of chondrocytes in the collagen membrane, where the rough surface was populated and then secured in the cartilage defect against the debrided subchondral bone. Given this orientation, there were potential chondrocyte sparse regions toward the smooth upper membrane surface. This represents an obvious target for further improvement in the manufacture of the construct.

Chondrocyte predominance was increased in MACI-implanted defects, which correlated with immunohistologically apparent regions of abundant collagen type-II deposition. This was also evident in histologic samples from pilot horses in a previous study[32]; however, better cartilage maturity was apparent in this longer-term study, with improved chondrocyte predominance, collagen type-II distribution, and total healing scores compared with scores at 6 months. Improved cartilage quality in these experimental defects confirms clinical and biopsy information from MACI-implanted defects in the knee[16,22,23]. Semiquantitative scores of chondrocyte predominance in MACI-implanted defects, compared with acellular collagen membrane-implanted and empty defects, suggest that the improved chondrocyte abundance was a result of the MACI implant. Implantation of acellular collagen membrane alone did not improve any of the individual histologic parameters compared with empty and paired empty defects. The primary role of the collagen carrier membrane in the MACI implant seems to be as a supportive scaffold and transport vehicle for chondrocyte implantation, which provides a chondrocyte population that can then proliferate in the interface between debrided bone and the MACI implant. Previous studies have indicated that chondrocytes readily migrate into the interface region[30] and contribute to the cartilage-healing process in the depths of the defect. Direct evidence of cell survival by fluorescent or genetic labeling[48,49] was not done here, to better mimic clinical MACI preparation, but would have better defined chondrocyte survival and contribution to repair. Additionally, sequential MRI of these defects would have added information on the progressive change in cartilage maturity and collagen orientation, but these large joints could not be accommodated in commercial MRI units without disarticulating the limb.

In conclusion, the MACI implant resulted in improved cartilage repair, with an abundant chondrocyte population, toluidine-blue reactive proteoglycan-rich cartilage, and collagen type-II-abundant matrix, compared with acellular collagen membrane-treated and empty defects. These parameters are consistent with the positive clinical outcomes described with the application of the MACI implant in the human knee, and partly define the mechanisms for improved repair in clinical patients.

Appendix

eA An Appendix showing descriptions of the surgical procedures, histologic and gene expression methods, statistical analysis, results of synovial fluid PGE2 formation, and results of synovial membrane histologic analysis and scoring, as well as discussions of the matrix constituent response and the equine model are available with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJS/E422). ■

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