Effect of a solution of hyaluronic acid–chondroitin sulfate–*N*-acetyl glucosamine on the repair response of cartilage to single-impact load damage

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Objective—To investigate effects of 1% hyaluronic acid–chondroitin sulfate–*N*-acetyl glucosamine (HCNAG) on the damage repair response in equine articular cartilage.

Sample—Articular cartilage from 9 clinically normal adult horses.

Procedures—Full-thickness cartilage disks were harvested from the third metacarpal bone. Cartilage was single-impact loaded (SIL) with 0.175 J at 0.7 m/s and cultured in DMEM plus 1% (vol/vol) HCNAG or fibroblastic growth factor (FGF)-2 (50 ng/mL). Histologic and immunohistochemical techniques were used to identify tissue architecture and apoptotic cells and to immunolocalize type I and II collagen and proliferating nuclear cell antigen (PCNA).

Results—Type II collagen immunoreactivity increased in SIL cartilage, compared with control samples. At days 14 and 28 (day 0 = initiation of culture), control samples had significantly fewer repair cells than did other treatment groups. In control samples and SIL + HCNAG, there was a significant decrease in apoptotic cell number, compared with results for SIL and SIL + FGF-2 samples. At days 14 and 28, there was a significant increase in chondrocytes stained positive for PCNA in the control samples.

Conclusions and Clinical Relevance—1% HCNAG significantly affected apoptotic and repair cell numbers in an SIL damage-repair technique in adult equine articular cartilage. However, HCNAG had no effect on the number of PCNA-positive chondrocytes or on type II collagen immunohistochemical results. The inclusion of 1% HCNAG in lavage solutions administered after arthroscopy may be beneficial to cartilage health by increasing the number of repair cells and decreasing the number of apoptotic cells. (*Am J Vet Res* 2012;73:306–312)

Arthroscopic treatment of joint disease is a standard orthopedic technique with widespread applications, including treatment of soft tissue pathological lesions and cartilage disease.[1] During arthroscopic treatment, SF, the physiologic constituent of the joint space, is washed out of the joint, and on completion of the arthroscopic procedure, the operated joint contains lavage solution but little SF.[2,3] Synovial fluid is vital to the health of a joint. It has a number of functions, including acting as a lubricant between the opposing cartilage surfaces[4] and, via steric hindrance, as a chondroprotective agent.[5] This latter role is primarily attributable to HA.[4]

Abbreviations

CS	Chondroitin sulfate
DMEM	Dulbecco modified Eagle medium
dUTP	Deoxyuridine triphosphate
ECM	Extracellular matrix
FGF	Fibroblastic growth factor
HA	Hyaluronic acid
HCNAG	Hyaluronic acid–chondroitin sulfate–*N*-acetyl glucosamine
NAG	*N*-acetyl glucosamine
PCNA	Proliferating cell nuclear antigen
SF	Synovial fluid
SIL	Single-impact load

Exposure of joint tissues to solutions used for joint lavage can initiate deleterious effects, including a decrease in cartilage proteoglycan synthesis, increases in chondrocyte death, and meniscal cell damage.[6–8] A number of modifications of lavage fluid, including the supplementation of lavage solutions with preparations containing constituents similar to those of SF (0.5% HA),[2,9] have been described to prevent cartilage damage after surgery. The

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addition of HA-containing solutions may have beneficial short-term[9] and long-term[10] clinical effects after arthroscopy.[2] Hyaluronic acid has a number of functions within a joint, including providing viscoelasticity, inhibiting the production of ECM protein–degrading enzymes (including matrix metalloproteinases),[10] enhancing the synthesis of ECM proteins, and suppressing cartilage damage by fibronectin fragments.[11]

In addition to HA having potentially chondroprotective effects within the joint, other molecules play an important role in maintaining joint homeostasis. *N*-acetyl glucosamine is a rate-limiting precursor in HA formation,[12] and NAG has anabolic effects within joints and direct effects on matrix metalloproteinases.[13] Chondroitin sulfate is the most abundant glycosaminoglycan in cartilage and contributes to the viscoelastic properties of SF when introduced into a joint.[14] Chondroitin sulfate has both catabolic and anabolic activity within joints; CS increases the synthesis of HA, glucosamine, and type II collagen and inhibits the action of ECM-degrading enzymes.[15] Thus, the inclusion of CS in a postsurgical lavage solution could potentially help joint tissue integrity.

When performing cartilage repair procedures, it is vital to maintain the viability of native chondral tissue so as to lessen the degree of intrinsic damage and subsequent cell death following debridement. Investigators in 1 study[16] reported that after incision of the chondral surface, there is a wave of cell apoptosis that extends away from the incised edge. Those investigators also found that for solutions between 100 and 600 mOsm, increasing the osmoloarity of solutions (saline [0.9% NaCl] solution and Hartmann's solution) that could potentially be used for joint lavage decreased chondrocyte death following dissection[6] and indicated the mechanism by which adjustments in the fluid environment can modulate cell survival after insult. This has major implications in cartilage repair procedures because perilesional cell death may adversely affect the subsequent integration of graft and host tissue in procedures such as autologous chondrocyte implantation or osteochondral autograft transfer.

To examine the effects of a lavage solution for postarthroscopic administration, it is essential to have a characterized technique for evaluation of cartilage behavior after insult. A study[17] conducted by our laboratory group revealed that following SIL and subsequent culture, mature mammalian cartilage is capable of mounting an intrinsic damage repair response. This response can be modified by the addition of exogenous factors. For example, the response is promoted by FGF-2 and inhibited by bone morphogenic protein-2. This technique can be considered suitable for mimicking postarthroscopic tissue biological processes because the cartilage will have been subjected to dissection and manipulation followed by a period of undisturbed tissue metabolism during medium- to long-term tissue culture.

The objective of the study reported here was to investigate the effects of an HCNAG-containing solution for use as an intraoperative replacement for SF on the intrinsic damage repair response in articular cartilage. We hypothesized that the addition of this fluid containing key elements of SF would alter the intrinsic damage repair response in articular cartilage (specifically, ECM variables, number of repair cells, number of cells undergoing apoptosis, and number of proliferating cells).

Materials and Methods

Samples—Cartilage was obtained from 9 adult horses (range, 5 to 7 years old) that were euthanized for reasons other than orthopedic disease. Disks of articular cartilage (7 mm in diameter) were aseptically harvested by use of a cork borer from the articular surface of the distal aspect of the third metacarpal bone in the metacarpophalangeal joint. The depth of the disks was approximately 2 to 4 mm and included all zones of the cartilage from the articular surface to, but not including, the calcified cartilage and subchondral bone. All explants were immediately placed into sterile PBS solution containing penicillin (200 U/mL) and streptomycin (2.5 and 100 µg/mL) and then were washed 3 times in sterile PBS solution.

SIL—Cartilage was impacted by use of a device as described elsewhere.[17] Samples were impacted with 0.175 J at 0.7 m/s (500 g dropped from a height of 2.5 cm), which corresponded to a nominal stress of approximately 8 MPa.[18] Unimpacted disks served as control samples.

Culture of cartilage disks—After the SIL was performed, explants were cultured for 3, 6, 9, 14, and 28 days in DMEM containing 10% fetal calf serum and antimicrobials (initiation of culture = day 0). Medium was changed every other day. Explants were allocated into 4 groups: unimpacted cartilage cultured in DMEM plus 10% fetal calf serum (control samples), SIL cartilage cultured in DMEM plus 10% fetal calf serum, SIL cartilage cultured in DMEM plus 10% fetal calf serum with 1% (vol/vol) HCNAG[a] (HA sodium salt [50 µg/mL], sodium CS [1 mg/mL], and NAG [1 mg/mL]), and SIL cartilage cultured in DMEM plus 10% fetal calf serum with recombinant human FGF-2[b] (50 ng/mL).

All samples were cultured in quadruplicate for each of the experimental time points. After culture, cartilage disks were placed into 4% formal–saline solution and processed for routine histologic examination or snap-frozen in liquid nitrogen. Paraffin and cryostat sections (thickness, 7 µm) were used in histologic and immunohistochemical analyses.

Histologic analysis—Adjacent sections from each experimental time point were stained with H&E or toluidine blue. Routine light microscopy was used in the evaluation of cartilage architecture, the extent of cartilage damage, and changes in chondrocyte morphology and to estimate proteoglycan content within the ECM of the sample. Proteoglycan loss was assessed by use of a semiquantitative scoring system (0, no proteoglycan loss; 1, mild proteoglycan loss; 2, moderate proteoglycan loss; and 3, marked proteoglycan loss). Proteoglycan loss was assessed for each replicate at each experimental time point, and a mean ± SD score for proteoglycan loss was calculated.

Quantification of repair cells—The number of repair cells (cells that had migrated from the ECM of

the parent cartilage and were adherent to the surface of the cartilage sample) was quantified by counting the number of repair cells in the entire sample (cells that were visible on the surface of the cartilage) at each experimental time point, as described elsewhere.[17] Values were expressed as the mean ± SD number of repair cells observed.

Immunohistochemical analysis—Immunohistochemical analysis was performed as described elsewhere.[17] Primary antibodies used for the analysis were monoclonal mouse anti-human type I collagen,[c] monoclonal mouse anti-human type II collagen,[c] and monoclonal mouse anti-human PCNA.[d] Horseradish peroxidase–conjugated secondary anti-rabbit and mouse immunoglobulins were used as appropriate, and the color reaction was developed with 0.1% 3′,3-diaminobenzidine tetrachloride–0.01% hydrogen peroxide. Species-specific normal serum was used as a control sample in all experiments. A semiquantitative scoring system, which was modified from that described in another study,[19] was used for immunohistochemical analysis of collagen types I and II. The degree of positive results (ie, staining) for the proteins was evaluated by semiquantitative scoring of staining intensity on a scale of 1 to 4 (1, inconspicuous; 2, mild; 3, moderate; and 4, strong).

Immunohistochemical quantification of PCNA was performed by counting the number of cells with positive results when stained for PCNA (PCNA-positive cells). At each experimental time point, 3 full-thickness microscopic views were obtained by use of a 20X lens, and the number of cells with positive and negative results for PCNA immunoreactivity was recorded. The number of PCNA-positive cells was expressed as a percentage of the total number of cells in the sample. This was repeated in each of the 4 experimental replicates, and a mean ± SD percentage for the proliferation of cells was calculated.

Apoptosis analysis—A terminal deoxynucleotidyl transferase–dUTP nick-end labeling stain was used to detect fragmented DNA (which is a hallmark of cell death) by use of a terminal deoxynucleotidyl transferase fluorescein kit.[e] Sections were mounted by use of a 4′-6-diamidino-2-phenylindole nuclear stain containing mounting medium.[f] Sections were viewed by use of a fluorescent microscope.[g] At each experimental time point, 3 full-thickness microscopic

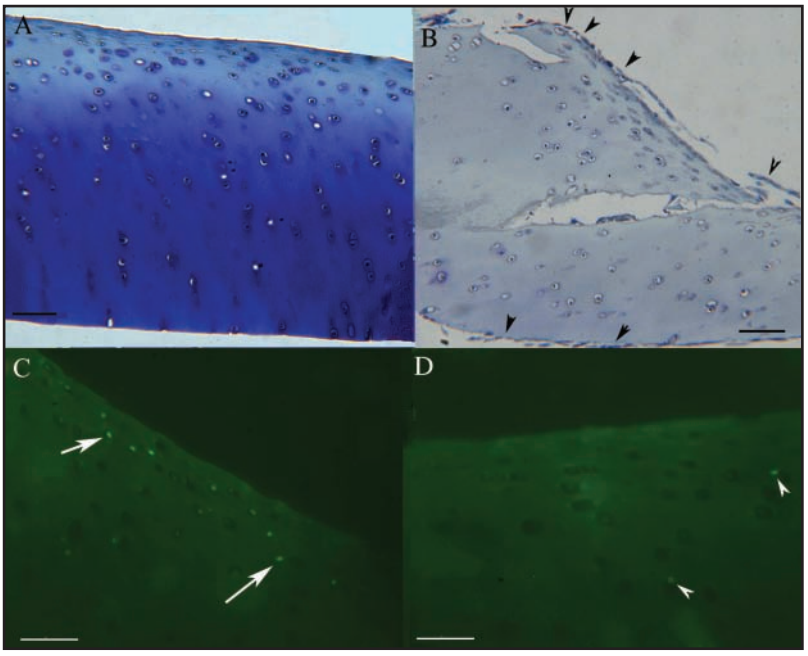

Figure 1—Photomicrographs of sections of equine cartilage stained with toluidine blue at day 28 after initiation of culture (A and B) or stained by use of a terminal deoxynucleotidyl transferase–dUTP nick-end labeling kit to reveal apoptotic cells (C and D). A—Unimpacted control cartilage cultured in DMEM with 10% fetal calf serum. There is a smooth articular surface and no damage to the cartilage. There is a moderate amount of proteoglycan in the section, as indicated by the intensity of the blue stain. No repair cells are evident on the surface of the cartilage. B—Cartilage after SIL and culture in DMEM with 1% HCNAG. In this impacted cartilage, there is cartilage damage (fissures and clefts) consistent with expectations after SIL. There is loss of proteoglycan content in the matrix of the cartilage, as indicated by the reduction in the intensity of the blue stain. Numerous repair cells (black arrowheads) are evident on all surfaces of the cartilage. C—Unimpacted control cartilage cultured in DMEM with 10% fetal calf serum. A number of apoptotic cells are evident as bright green fluorescent cells (white arrows) in the section. D—Cartilage after SIL and culture in DMEM with 1% HCNAG. Notice that there are fewer apoptotic cells (white arrowheads) in this section than in the section in panel C.

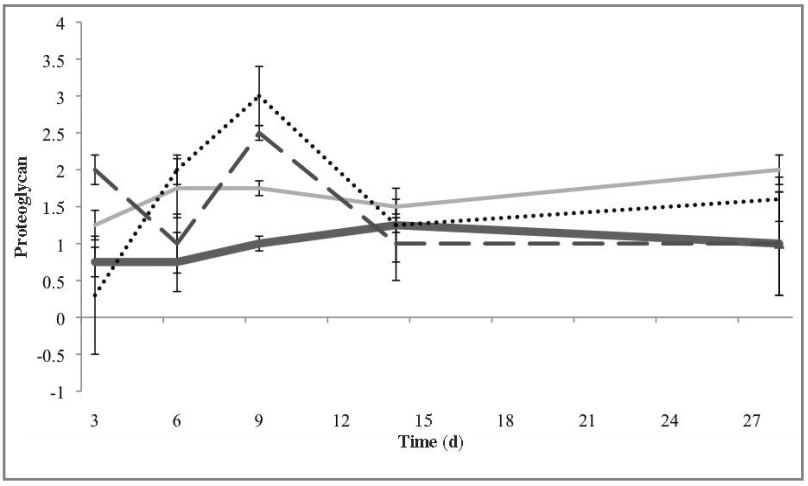

Figure 2—Mean ± SD proteoglycan score for equine cartilage sections obtained from 9 adult horses. The 4 treatment groups were unimpacted cartilage cultured in DMEM with 10% fetal calf serum (control samples [thick gray line]), SIL cartilage cultured in DMEM with 10% fetal calf serum (thin gray line), SIL cartilage cultured in DMEM with 10% fetal calf serum and 1% (vol/vol) HCNAG (HA sodium salt [50 μg/mL], sodium CS [1 mg/mL], and NAG [1 mg/mL; dashed gray line]), and SIL cartilage cultured in DMEM with 10% fetal calf serum and recombinant human FGF-2[b] (50 ng/mL [dotted black line]). Proteoglycan loss was assessed for each of the quadruplicate replicates at each experimental time point by use of a semiquantitative scoring system based on the intensity of staining with toluidine blue (0, no proteoglycan loss; 1, mild proteoglycan loss; 2, moderate proteoglycan loss; and 3, marked proteoglycan loss). First day of culture was designated as day 0.

views were obtained by use of a 20X lens, and the total number of cells and the number of cells with positive results (ie, staining) were counted. This was repeated in each of the 4 experimental replicates, and a mean ± SD percentage for apoptotic cells was calculated.

Statistical analysis—For all variables, a paired Student *t* test was used to detect significant differences between groups. Values were considered significant at $P < 0.05$.

Results

Cartilage damage following SIL—Cartilage had characteristic damage at the articular surfaces after SIL. The damage pattern immediately after SIL was found to be variable across the surface of the impacted cartilage and included mild, intermittent roughening of the surface, isolated 45° fissures, and multiple 90° minifissures (**Figure 1**). Between the damage sites, the cartilage appeared histologically normal. All control samples had a smooth, intact articular surface along the entire length of the explant, with uniform metachromatic staining. This typical pattern seen immediately after SIL did not change during culture.

ECM responses—After SIL and subsequent culture, cartilage had a loss of proteoglycan, compared with results for control cartilage (**Figure 2**). In control samples, there was an extremely low amount of proteoglycan loss that was fairly constant throughout the time period (proteoglycan score, 0.8 to 1.1). In SIL cartilage cultured without and with HCNAG, there was no pattern of an increase in loss of proteoglycan over time, compared with results for control samples. The proteoglycan score in SIL cartilage cultured in DMEM alone ranged between 1.25 and 2; the proteoglycan score in SIL cartilage cultured with HCNAG ranged between 2.1 and 2.5. In SIL cartilage cultured with FGF-2, there was a marked loss of proteoglycan until day 9 after initiation of culture (proteoglycan score, 3); however, the proteoglycan score then decreased (1.25 at day 14 and 1.5 at day 28).

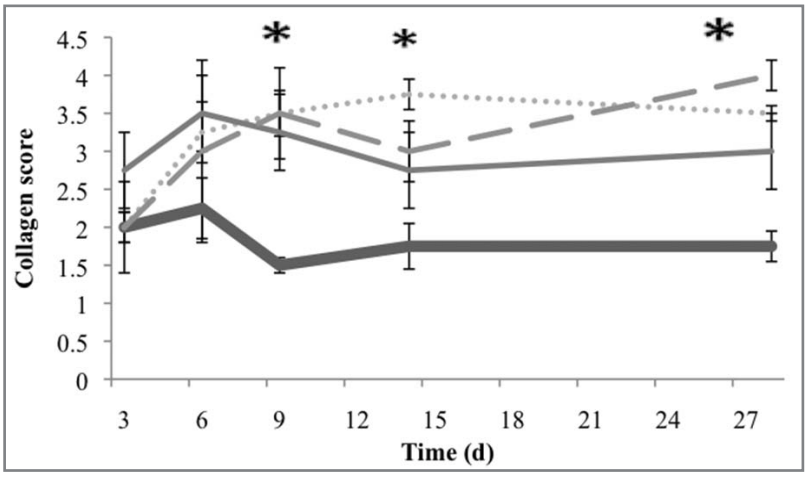

Figure 3—Mean ± SD type II collagen immunostaining within cartilage sections obtained from 9 adult horses. Immunostaining was assessed by use of a semiquantitative scoring system adapted from another study.[19] The degree of positive results (ie, staining) for type II collagen was evaluated by semiquantitative scoring of staining intensity on a scale of 1 to 4 (1, inconspicuous; 2, mild; 3, moderate; and 4, strong). *Within an experimental day, the value for the control sample differs significantly ($P < 0.05$) from the values for the other 3 cartilage samples. *See* Figure 2 for remainder of key.

Type I collagen immunoreactivity was not detected in any of the cartilage samples. However, type II collagen immunoreactivity was detected in all samples. Type II collagen immunoreactivity was evenly distributed throughout the control samples. In all SIL cartilage, there was gross evidence of an increase in type II collagen immunoreactivity. There was a significant reduction in type II collagen immunoreactivity in the control samples, compared with results for the other cartilage samples, at days 9, 14, and 28 (**Figure 3**). There was no difference in the distribution for immunoreactivity between cartilage cultured with HCNAG or FGF-2.

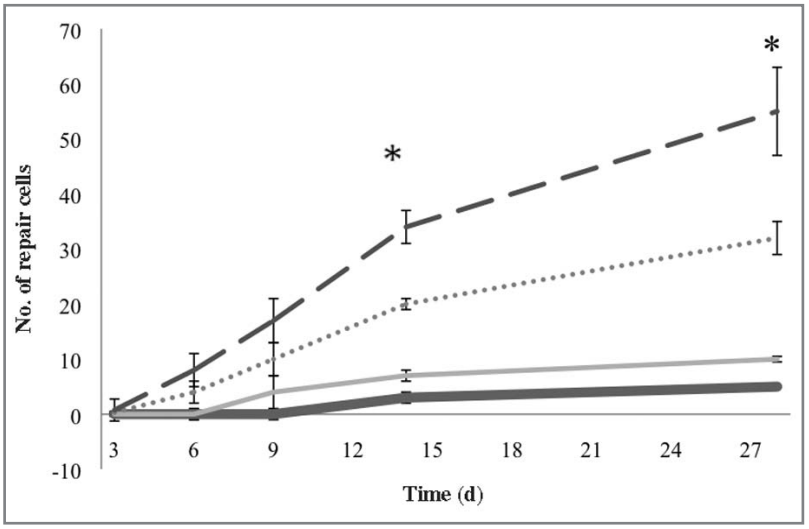

Figure 4—Mean ± SD number of repair cells counted on the surface of cartilage sections obtained from 9 adult horses. *Within an experimental day, values for the SIL cartilage samples cultured with HCNAG or FGF-2 differ significantly ($P < 0.05$) from the values for the control samples and the SIL cartilage cultured with DMEM alone. *See* Figure 2 for remainder of key.

Number of repair cells—After SIL and subsequent culture, repair cells were detected in all cartilage samples. At day 3 after initiation of culture, few cells were seen in any samples; however, the number of repair cells then increased with time in all samples. At days 14 and 28, there were significantly more repair cells in the SIL cartilage cultured with HCNAG or FGF-2 than in the control samples or the SIL cartilage cultured in DMEM alone (**Figure 4**). At day 14, there was a mean of 33 repair cells in the SIL cartilage cultured with HCNAG, compared with 20 repair cells in the SIL cartilage cultured with FGF-2, 8 repair cells in the SIL cartilage cultured in DMEM

alone, and 3 repair cells in the control samples. At day 28, there was a mean of 55 repair cells in the SIL cartilage cultured with HCNAG, compared with 30 repair cells in the SIL cartilage cultured with FGF-2, 10 repair cells in the SIL cartilage cultured in DMEM alone, and 7 repair cells in the control samples.

Number of apoptotic cells—After SIL, and subsequent culture, apoptotic cells were detected in all cartilage samples (**Figure 5**). In control samples and in SIL cartilage cultured with HCNAG, there was a significant decrease in the mean number of apoptotic cells, which was evident in SIL cartilage cultured in DMEM alone and SIL cartilage cultured with FGF-2. Between days 3 and 28, 40% to 50% of the chondrocytes were apoptotic in the SIL cartilage cultured with FGF-2, and 47% to 53% of the chondrocytes were apoptotic in the SIL cartilage cultured in DMEM alone. In contrast, in SIL cartilage cultured with HCNAG, only 20% to 38% of chondrocytes were apoptotic during the experimental period.

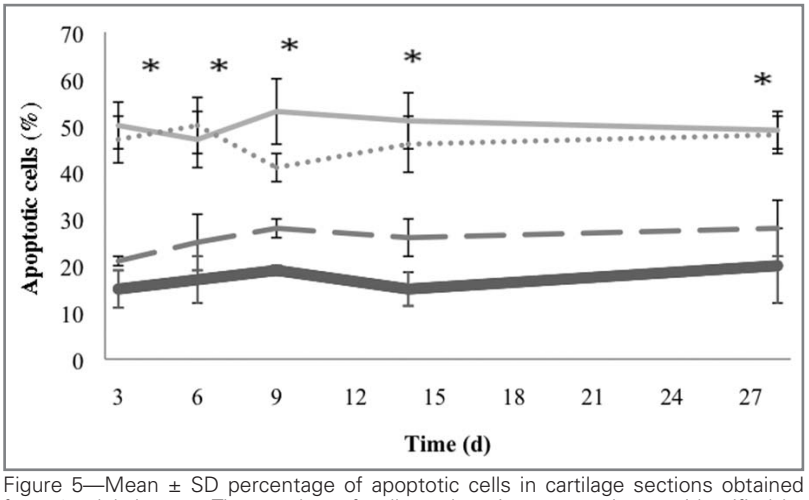

Figure 5—Mean ± SD percentage of apoptotic cells in cartilage sections obtained from 9 adult horses. The number of cells undergoing apoptosis was identified by use of terminal deoxynucleotidyl transferase–dUTP nick-end labeling staining within cartilage sections. *Within an experimental day, the value for SIL cartilage cultured with HCNAG differs significantly ($P < 0.05$) from values for SIL cartilage cultured with DMEM alone and SIL cartilage cultured with FGF-2. *See* Figure 2 for remainder of key.

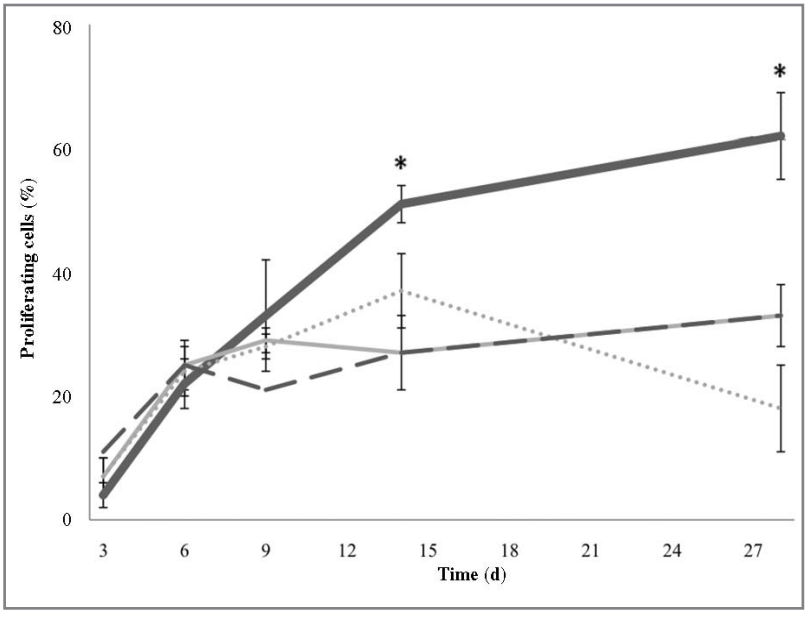

Figure 6—Mean ± SD percentage of cells with positive results for PCNA in cartilage sections obtained from 9 adult horses. *Within an experimental day, the value for the control sample differs significantly ($P < 0.05$) from the values for the other 3 cartilage samples. *See* Figure 2 for remainder of key.

Number of proliferating cells—After SIL and subsequent culture, cells with immunoreactivity for PCNA were detected in all cartilage samples. At day 3, there were few (< 10%) proliferating cells in any cartilage samples; however, the number of PCNA-positive cells then increased with time until day 9. At days 14 and 28, there were significant increases in the number of PCNA-positive chondrocytes in the control samples, compared with the number in each of the other cartilage samples (**Figure 6**). At day 14, there was a mean of 51% PCNA-positive chondrocytes in the control samples, compared with the highest mean of 33% in the other cartilage samples. At day 28, there was a mean of 58% PCNA-positive chondrocytes in the control samples, compared with the highest mean of 40% in the other cartilage samples.

Discussion

The results of the present study provide evidence that the addition of an HCNAG solution to the culture medium for cartilage following SIL may be beneficial to the recovery of cartilage after insult. In this study, HCNAG was used at a concentration of 1%, which yielded a final concentration of 50 µg of HA/mL, 1,000 µg of CS/mL, and 1,000 µg of NAG/mL. These concentrations are within the range described as being efficacious in vitro when the components of HCNAG are used separately.[20,21]

During these experiments, there were effects on ECM components. During culture, there was a steady loss of proteoglycan from all cartilage samples, including control cartilage, as assessed semiquantitatively on the basis of staining with toluidine blue. This leaching of proteoglycan from cartilage during culture has been reported previously[18] and is believed to be attributable to cartilage damage during dissection and tissue handling as well as impact loading.[22] In the study reported here, proteoglycan leaching was not affected by the addition of HCNAG to the culture medium.

The mechanism of action of the individual components of HCNAG on proteoglycan metabolism in cartilage explants and chondrocyte cultures is not fully understood. Hyaluronic acid has been found to promote proteoglycan syn-

thesis in 1 study[23] but was unable to prevent corticosteroid-induced proteoglycan release into the medium in another study.[24] Both HA and CS inhibit aggrecan gene expression in some experimental systems.[25] The effects of these macromolecules on proteoglycan metabolism are complicated, but the results of the study reported here do not indicate that HCNAG has an overall effect on proteoglycan metabolism as assessed grossly and do not support our hypothesis (ie, that HCNAG would affect the damage repair response in cartilage).

Type I collagen immunoreactivity was not detected in any of the cartilage samples, which indicated that no fibrocartilage was being produced in response to cartilage damage and repair. Type II collagen was detected in all of the cartilage samples, with an increased intensity of immunoreactivity detected in the SIL cartilage cultured in DMEM alone, SIL cartilage cultured with FGF-2, and SIL cartilage cultured with HCNAG at days 9, 14, and 28, compared with immunoreactivity for the control samples. Although immunoreactivity intensity is, at best, only a semiquantitative assessment of protein content in the target tissue, it does provide limited evidence that there may be an increase in collagen production and reduction in collagen degradation following SIL and subsequent culture. Such an increase in collagen type II immunoreactivity may reflect a response by the cartilage to repair the damaged matrix after SIL. When cultured in medium that contained HCNAG, this increase in collagen within the ECM of the cartilage remains supported. This finding does not support our hypothesis because HCNAG did not alter the response of the cartilage, compared with the results for SIL and culture alone. An increase in type II collagen mRNA expression attributable to CS and NAG has been described in chondrocyte culture[25]; further studies are required to identify the mechanism by which HCNAG maintains collagen type II immunoreactivity in samples after SIL.

The addition of HCNAG to the culture medium had a significant effect on the numbers of apoptotic cells and repair cells (which supported our hypothesis) but had no effect on the number of PCNA-positive chondrocytes (which did not support our hypothesis). Apoptosis is seen in osteoarthritic cartilage from clinical patients and numerous animals with experimentally induced conditions and is believed to play a key role in the development of disease.[26] Thus, any substance that prevents apoptosis in cartilage after an insult is potentially useful in controlling the development of osteoarthritis. Two of the 3 components of HCNAG, HA and CS, can inhibit apoptosis. Hyaluronic acid can reduce apoptosis in animals with experimentally induced osteoarthritis,[27–29] with investigators in some studies[28,30] suggesting that HA exerts this effect via nitrous oxide metabolism, although this remains unproven.[31] Chondroitin sulfate can also reduce apoptosis.[32,33] Chondroitin sulfate may exert its antiapoptotic effects via the intrinsic pathway of apoptosis (mitochondrial pathway) because in skin fibroblasts submitted to oxidative stress, CS reduced both caspase-9 activity (active in the intrinsic pathway) and caspase-3 activity (active in the common pathway of apoptosis).[33]

The addition of HCNAG to the culture medium had a significant effect on the number of repair cells on the surface of the cartilage. As indicated in another study,[17] the repair cells described in the study reported here must, by experimental design, have been of cartilage origin because no synovial tissue or underlying bone was included in the harvested explants. In the present study, we found evidence that culture with HCNAG was capable of causing the appearance of repair cells on the surface of the cartilage to an extent similar to that induced by culture with FGF-2 (50 ng/mL), which has been reported to be a potent stimulator of repair cells.[17] However, the mechanism by which these substances achieve this increase in the number of repair cells is not clear. In cartilage cultured with HCNAG, there was no increase in the number of PCNA-positive chondrocytes, compared with results for SIL cartilage cultured in DMEM alone, and all SIL cartilage had a significant decrease in the number of PCNA-positive chondrocytes, compared with results for the control samples, which indicated that cartilage damage after SIL inhibits chondrocyte proliferation. However, HCNAG appears to reduce apoptosis; thus, if fewer cells are becoming apoptotic, more cells are potentially available to become repair cells. In addition, HCNAG may have an effect on the number of repair cells via another mechanism. For cells to reach the damaged articular surface, they must migrate from their original position in cartilage to the articular surface. Of the components of HCNAG, HA promotes chondrocyte migration in vitro,[34] whereas CS is recognized as an inhibitor of cell migration in cartilage[35] and other tissues.[36]

Results of the present study supported our hypothesis that HCNAG will have an effect, only in part, on the cartilage damage repair response. The addition of HCNAG to the culture medium did not affect the proteoglycan content of the cartilage, type II collagen immunoreactivity, number of repair cells, or number of proliferating chondrocytes, compared with results for SIL alone. However, the addition of HCNAG to the culture medium significantly reduced the number of apoptotic chondrocytes following SIL and subsequent culture. It can be concluded that the addition of HCNAG to the culture medium does not cause deleterious effects to cartilage during the cartilage damage repair response for up to 28 days and that HCNAG has a beneficial effect on cartilage by reducing chondrocyte apoptosis. To determine whether these observations would have an impact on the resultant cartilage integrity after arthroscopic surgery, further in vivo studies are required.

a. Polyglycan, ArthroDynamic Technologies Inc, Versailles, Ky.
b. Sigma-Aldrich Co Ltd, Gillingham, Dorset, England.
c. MP Biomedicals, Solon, Ohio.
d. Dako North America Inc, Carpinteria, Calif.
e. TACS TdT fluoroscein kit, Trevigen Inc, Gaithersburg, Md.
f. DAPi nuclear stain containing mounting medium, Dako North America Inc, Carpinteria, Calif.
g. Leica Camera AG, Solms, Germany.

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