Articular Cartilage Regeneration With Autologous Marrow Aspirate and Hyaluronic Acid: An Experimental Study in a Goat Model

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Purpose: The purpose of the study was to determine whether postoperative intra-articular injections of autologous marrow aspirate (MA) and hyaluronic acid (HA) after subchondral drilling resulted in better cartilage repair as assessed histologically by Gill scoring. **Methods:** In a goat model we created a 4-mm full-thickness articular cartilage defect in the stifle joint (equivalent to 1.6 cm in the human knee) and conducted subchondral drilling. The animals were divided into 3 groups: group A (control), no injections; group B (HA), weekly injection of 1 mL of sodium hyaluronate for 3 weeks; and group C (HA + MA), similar to group B but with 2 mL of autologous MA in addition to HA. MA was obtained by bone marrow aspiration, centrifuged, and divided into aliquots for cryopreservation. Fifteen animals were equally divided between the groups and sacrificed 24 weeks after surgery, when the joint was harvested, examined macroscopically and histologically. **Results:** Of the 15 animals, 2 from group A had died of non–surgery-related complications and 1 from group C was excluded because of a joint infection. In group A the repair constituted mainly scar tissue, whereas in group B there was less scar tissue, with small amounts of proteoglycan and type II collagen at the osteochondral junction. In contrast, repair cartilage from group C animals showed almost complete coverage of the defect with evidence of hyaline cartilage regeneration. Histology assessed by Gill scoring was significantly better in group C with 1-way analysis of variance yielding an F statistic of 10.611 with a P value of .004, which was highly significant. **Conclusions:** Postoperative intra-articular injections of autologous MA in combination with HA after subchondral drilling resulted in better cartilage repair as assessed histologically by Gill scoring in a goat model. **Clinical Relevance:** After arthroscopic subchondral drilling, this novel technique may result in better articular cartilage regeneration. **Key Words:** Articular cartilage—Arthroscopic subchondral drilling—Hyaline cartilage—Hyaluronic acid—Mesenchymal stem cells—Microfracture.

Articular cartilage is a specialized avascular tissue composed of chondrocytes embedded in a matrix consisting mainly of type II collagen and glycosaminoglycans such as hyaluronic acid (HA) and chondroitin sulfate. Its main function is to allow smooth articulation of weight-bearing joints and to cushion the underlying bone from transmitted compressive and shear forces involved in joint movement.

Because of its avascular nature, articular cartilage has very limited capacity for repair and partial-thickness defects do not heal spontaneously. Full-thickness injuries that penetrate the subchondral bone can, however, undergo repair through mobilization of marrow-derived stem cells (MSCs) from bone marrow into the blood clot at the site of injury.[1]

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Microfracture surgery takes advantage of this repair mechanism by creating tiny punctures with a bone awl into the subchondral bone through an arthroscopic procedure. Subchondral drilling is a variant of microfracture surgery in which a drill, burr, or Kirschner wire is used to create drill holes into subchondral bone rather than awls.[2] The defect is eventually replaced by a hybrid of fibrocartilage and hyaline-like cartilage rather than the original hyaline cartilage.[3] The replacement tissue is inferior biomechanically because it is composed mainly of type I collagen, which is better at resisting tensile forces rather than compressive forces as found in a typical joint.[4] Furthermore, whereas fibrocartilage does reduce friction when compared with bare bone, it does so to a lesser degree than hyaline cartilage.

Autologous MSCs cultured in vitro have been shown to repair full-thickness defects, with formation of hyaline cartilage and reconstitution of the underlying subchondral bone. In one study, when compared with matured chondrocytes, MSCs were found to produce better results when used for cartilage repair.[5]

Intra-articular injections of HA have been widely used for a number of years in the treatment of osteoarthritis. HA is relatively free of side effects and provides relief of symptoms in some patients. There is also some evidence from animal studies that HA can modify disease activity and protect articular cartilage from further degeneration.[6,7] Some animal studies have suggested that it can improve outcome when used in combination with microfracture surgery.[8] In addition, intra-articular injections of MSCs together with HA have also been used with good results in a goat model of osteoarthritis.[9]

The purpose of the study was to determine whether postoperative intra-articular injections of autologous marrow aspirate (MA) and HA after subchondral drilling resulted in better cartilage repair as assessed histologically by Gill scoring. Our hypothesis was that these intra-articular injections can improve the quality of repair cartilage after subchondral drilling into articular cartilage defects. We believed that separation of MSCs from the aspirate and in vitro expansion was unnecessary, as well as that the MA itself could reproduce all the regenerative effects of MSCs seen in previous studies.

METHODS

In this pilot study 15 Katjang male goats were used. All goats were aged between 1 and 2 years and weighed between 15 and 25 kg at the time of surgery. The goats were kept in individual cages in a barn and were fed nutrient pellets and hay on a daily basis. Ethical approval for animal research was obtained from the institutional animal care and use committee.

Surgery was done with an aseptic technique with animals under general anesthesia at the local veterinary hospital. Anesthesia was induced with ketamine-xylazine and maintained with halothane under 100% oxygen. Chondral defects were created on the left stifle joint of the goats, which is equivalent to the human knee joint.

A lateral parapatellar arthrotomy was performed to expose the joint, and a 4-mm-diameter full-thickness chondral defect was created in the intercondylar area of the femur by use of a punch specially designed to produce a standard articular defect. This was followed by subchondral drilling to a depth of 5 mm with a 0.6-mm Kirschner wire. Each chondral defect was able to accommodate 9 drill holes. The defect size of 4 mm represents approximately 20% of the condylar width and an area of 12 mm^2, which corresponds proportionally to a 200-mm^2 defect (1.6 $\times$ 1.6 cm) in the human knee by use of the calculation method used by Gill et al.[10] and modified for a circular defect.

The arthrotomy wound was then sutured with No. 3-0 Vicryl (Ethicon, Somerville, NJ). After this, bilateral iliac crest bone marrow aspirations were performed in group C animals with an Islam needle. The goats were allowed to mobilize freely within their cages after surgery.

To prevent clotting and maintain cell viability, citrate phosphate dextrose solution (Terumo, Tokyo, Japan) was added with gentle agitation to the MA. The contents were then placed in a cooler bag and sent to the processing laboratory. The aspirates were centrifuged at 1,900 rpm for 10 minutes at 10°C to deplete red blood cells and plasma. A mean final volume of 4.4 mL (range, 4.0 to 6.0 mL) of MA suspension was obtained. The cell suspensions were then divided into vials and cryopreserved in 10% dimethyl sulfoxide for storage at −196°C. Quantification was done with a cell counter. Before use, the cryopreserved cells were thawed at 37°C and washed in buffer containing acid citrate dextrose (Terumo). The mean total nucleated cell count from the extracted samples was 220 $\times$ 10^6 cells (range, 159 to 438 $\times$ 10^6 cells) (Table 1).

Three experimental groups were studied: group A (control group) was not given any additional treatment; group B (HA group) was given intra-articular injections of 1 mL of HA (Hyalgan [sodium hyaluronate]; Fidia Farmaceutici, Abano Terme, Italy) on a weekly basis for 3 weeks, starting 1 week after sur-

Table 1. *MA Injection Regimen for Group C (HA + MA Group)*

Goat No.	No. of Injections	Total Nucleated Cells Injected ($\times 10^6$)	Gill Score
11	3	438	5
12	2	159	8
13	2	187	10
14	2	159	7
15	2	160	16

gery; and group C (HA + MA group) was given 1 mL of HA together with 2 mL of autologous MA according to the same schedule used in group B. Only 1 of the goats in group C (goat 11) had sufficient MA for all 3 injections, whereas the rest received MA for the first 2 injections only (Table 1).

The animals were sacrificed at 24 weeks after surgery, during which the distal femur was transected at the supracondylar level for macroscopic evaluation and photographed. The specimens were preserved in 10% formalin, decalcified, and embedded in paraffin for staining with H&E and safranin O, and they underwent immunostaining for type I collagen (rabbit polyclonal antibody; Gene Tex, San Antonio, TX) and type II collagen (mouse monoclonal antibody; Thermo Fisher Scientific, Fremont, CA). The results were then evaluated by 2 independent blinded histopathologists by use of a semiquantitative histologic grading scale used by Gill et al.[10] in a study of chondral defect healing after microfracture. This scale yields a score from 0 to 27, with low scores indicating good repair tissue that approximates normal hyaline articular cartilage.

For one of the experimental groups to show better cartilage repair over the other groups, this group would need to have a significantly lower Gill score as compared with the others. In this study we were attempting to detect a difference in Gill score of at least 4 points. The average standard deviation (SD) in Gill scores at the 12-week mark between the medial and lateral femoral condyles based on the original study was 2.0 points.[10]

On the basis of the null hypothesis that there is no difference in Gill scores between the groups, power analysis with the program G*Power 3[11] for a 1-way analysis of variance (ANOVA) showed that for a 5% type I error rate (α level, .05) and 20% type II error rate (80% power) with an ability to detect a difference of 4 points among the 3 groups (Cohen *f*, 0.943; SD, 2.0; groups of equal size), a minimum sample size of 5 subjects in each group was needed.[12] Normality of data was tested by use of the Kolmogorov-Smirnov test, whereas equality of variances was tested with the Levene test.

Gill scores were also analyzed according to category by use of 1-way ANOVA. Equality of variances was tested with the Levene test, and correction of the ANOVA *P* value for multiple comparisons was done with an appropriate test. If variances were equal in all groups, then the Tukey HSD (Honestly Significant Difference) test would have been used. In this case the Tamhane T2 test was used instead because variances were unequal.

All statistical computations were performed with SPSS software for Windows, version 13.0 (SPSS, Chicago, IL), and conducted at a 5% level of significance unless stated otherwise.

RESULTS

Results are presented for the 15 study animals. Two of five animals from group A died unexpectedly: the first animal died of pneumonia within a month after surgery, whereas the second animal died of peritonitis caused by a ruptured bladder after a fight. One animal in group C (goat 15) had a wound infection with purulent discharge. This was treated with 2 courses of intramuscular benzathine-procaine penicillin, 300,000 IU/10 kg per day. In 1 animal in group B a small effusion of the operated joint developed, which resolved spontaneously without treatment after 2 months.

A box plot of Gill scores by grouping showed a single outlier (goat 15) in group C, which corresponded with the animal that had a wound infection (Fig 1). In contrast, the animal in group B with an effusion had a Gill score that was not significantly different from the Gill scores of the other animals in that group. Once the outlier (goat 15) was excluded from analysis, the mean of group C was found to be much lower than that of groups A and B, with an average SD of 1.98 (Table 2). A breakdown of Gill scores for each animal is presented in Table 3.

One-way ANOVA was performed, yielding an *F* statistic of 10.611 with a *P* value of .004, which is highly significant. Normality of distribution was checked by use of the Kolmogorov-Smirnov test, which was nonsignificant in all 3 groups (group A, $Z = 0.667$, $P = .766$; group B, $Z = 0.539$, $P = .933$; and group C, $Z = 0.310$, $P > .999$), suggesting that the groups were normally distributed. The Levene test of homogeneity of variances was not significant ($F = 0.596$, $P = .571$), implying that group variances were similar enough that this test was

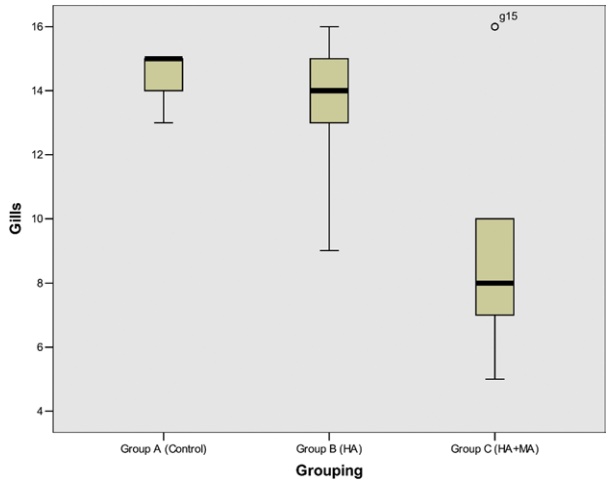

FIGURE 1. Box plot of Gill score by grouping showing single outlier (goat 15) in group C.

appropriately used. Because the number of subjects was relatively small, the results were rechecked by performing a nonparametric Kruskal-Wallis 1-way ANOVA. This yielded a χ^2 of 6.685 with a P value of .035, which is still significant.

Analysis of Gill scores by category showed that safranin O staining and cell morphology were significantly different across groups. However, when corrected by use of the Tamhane T2 test, only safranin O staining remained significant, with group C showing markedly lower scores (Table 4).

Macroscopic Findings

The chondral defects were covered with repair tissue in all groups, without evidence of synovitis or synovial thickening (Fig 2). In group A the defects were covered with semitransparent tissue having recognizable margins but with an irregular surface. A similar appearance was seen in group B goats. In group C the defect coverage was almost complete, and the color of the repair tissue was indistinct from surrounding cartilage. The surfaces were smooth and appeared level with adjacent normal cartilage.

Histologic Findings

Under H&E staining, scar tissue was present in group A, which was characterized by a disordered arrangement of fibroblasts in an edematous stroma (Fig 2). The interface of scar tissue with subchondral bone was marked by the presence of dilated capillaries and venules. In group B scar tissue was less pronounced, and islands of hyaline-like cartilage were seen at the interface with subchondral bone and also adjacent to normal cartilage at the defect margins. Edema was also less marked than in the control group. In group C there was chondrogenesis with evidence of hyaline cartilage formation. The hyaline cartilage also showed features of maturation as evidenced by a linear arrangement of chondrocytes extending from the subchondral bone toward the surface. No edema was seen in this group.

With safranin O staining, proteoglycans were notably absent from the repair tissue in group A. In group B proteoglycans were seen only at the base and sides of the defect in the same distribution as the hyaline-like cartilage. In group C there was marked proteoglycan accumulation in the deeper layers, excluding the perichondrium, which normally does not contain proteoglycans.

Under collagen staining, the scar tissue in group A was found to contain only type I collagen, with an absence of staining for type II collagen. In group B type I collagen staining in the repair tissue was less pronounced, with light staining for type II collagen around the areas of hyaline-like cartilage. In group C type I collagen staining was found only in the perichondrium, whereas the deeper cartilage stained strongly for type II collagen.

In the outlier (goat 15) there was hyaline cartilage at the base of the defect with an irregular cap of fibrous tissue. There was marked edema in the superficial fibrous layer, along with matrix resorption around existing chondrocytes in the repair tissue (Fig 3).

DISCUSSION

In previous studies penetration of the subchondral bone was shown to release the underlying marrow, which initiated repair of the chondral defect with fibrocartilage in a pattern that was observed in group

TABLE 2. *Descriptive Statistics for Gill Scores Across Groups*

Group	No. of Goats	Mean	SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
A (control)	3	14.3	1.15	11.5	17.2
B (HA)	5	13.4	2.70	10.0	16.8
C (HA + MA)	4	7.5	2.08	4.19	10.8

TABLE 3. *Breakdown of Gill Scores for All Subjects**

		Group A			Group B					Group C				
	Score	Goat 1	Goat 2	Goat 3	Goat 6	Goat 7	Goat 8	Goat 9	Goat 10	Goat 11	Goat 12	Goat 13	Goat 14	Goat 15
Filling defect relative to surface of original cartilage														
125%	1			1	1	1				1		1	1	
100%	0						0				0			
75%	1	1						1	1					
50%	2		2											
25%	3													3
0%	4													
Integration of repair tissue with surrounding articular cartilage														
Normal	0						0			0		0	0	
Decreased cellularity	1	1	1					1	1		1			1
Gap 1 side	2			2	2									
Gap 2 sides	3					3								
Safranin O staining														
Normal	0									0				
Slightly reduced staining	1										1		1	
Moderately reduced staining	2					2	2					2		
Markedly reduced staining	3			3	3									
No stain	4	4	4					4	4					4
Cell morphology														
Normal	0													
Mostly normal cells with morphology of chondrocytes														
Radial zone columns of 26%-75%	0									0				
Radial zone columns <25%	1													
50% Round cells with morphology of chondrocytes														
Radial zone columns >76%	2										2			
Radial zone columns of 26%-75%	3			3	3	3						3	3	
Radial zone columns <25%	4						4	4						
Mostly spindle cells (fibroblastic)	5	5	5						5					5
Intradefect architecture														
Normal	0	0	0				0			0			0	
1-3 small voids	1			1	1	1		1	1		1	1		1
1-3 large voids	2													
>3 large voids	3													
Clefts with defects	4													
Surface architecture														
Normal	0													
Slight fibrillation	1	1	1	1			1			1			1	1
Moderate fibrillation	2				2	2		2	2		2	2		
Marked fibrillation	3													

TABLE 3. *Continued*

		Group A			Group B					Group C				
	Score	Goat 1	Goat 2	Goat 3	Goat 6	Goat 7	Goat 8	Goat 9	Goat 10	Goat 11	Goat 12	Goat 13	Goat 14	Goat 15
Percentage of replacement of subchondral bone														
If new bone is below original tidemark (100% indicates new bone is level with original tidemark)														
90%-100%	0													
75%-89%	1	1				1					1	1	1	
50%-74%	2								2					
25%-49%	3				3					3				
<25%	4			4										
If new bone is above original tidemark (100% indicates repair cartilage is same thickness as normal, adjacent cartilage)														
90%-100%	0													
75%-89%	1							1						
50%-74%	2		2											
25%-49%	3													
<25%	4													
Tidemark formation														
Complete	0													
75%-99%	1													1
50%-74%	2						2							
25%-49%	3													
<25%	4													
Total score		13	15	15	15	13	9	14	16	5	8	10	7	16
Group mean			14.3				13.4					7.5†		

*Data from reference[10].
†Goat 15 was excluded from the analysis of group mean.

A animals. The reason why fibrous tissue forms instead of hyaline cartilage is not known, but it is likely that the local microenvironment contains paracrine factors that either promote fibrous tissue formation, inhibit cartilage growth, or both.

The addition of HA in group B animals improved the quality of repair tissue by allowing hyaline-like cartilage to form. This suggests that HA modifies the microenvironment in such a way that neutralizes these paracrine factors. The incomplete regeneration could be because of HA being only partially effective or the duration of administration being too short. It appeared that the region adjacent to subchondral bone and the interface with normal cartilage seemed to be most favorable to cartilage formation.

The combination of HA and MA in group C yielded the best results in that the repair tissue was composed of true hyaline cartilage, showing vertical orientation of chondrocyte nests and the presence of type II collagen and proteoglycans in the intermediate and deep cartilage layers, with type I collagen confined to the superficial layer and perichondrium (Fig 2). This suggests that the combination of HA and MA is most effective in neutralizing the paracrine factors, and its effects persist for the duration of the repair process. One of the essential active components in MA is likely the MSC content, given that a previous study in a porcine model showed that cultured autologous MSCs injected intra-articularly together with HA could produce the same results as in the group C animals.[13]

The presence of edema in the repair tissue appears to be proportional to the degree of fibrous scarring and was most marked in group A animals. The dilated capillaries and venules at the base of the defect suggest an inflammatory process that originates in the subchondral bone.

The outlier in group C (goat 15) had hyaline cartilage at the base and margins of the defect, which transitioned to fibrous scar tissue with marked edema

Table 4. *Analysis of Gill Scores by Category*

Category	Group	Mean	Levene	ANOVA		Group C Tamhane T2 Test
				F	*P* Value	
Filling defect	A	1.33	0.792	1.421	.291	0.998
	B	0.80				
	C	0.75				
Integration	A	1.33	0.207	2.301	.156	0.251
	B	1.40				
	C	0.25				
Safranin O staining	A	3.67	0.501	9.675	.006	0.039*
	B	3.00				
	C	1.00				
Cell morphology	A	4.33	0.650	4.395	.047	0.217
	B	3.80				
	C	2.00				
Intra-defect architecture	A	0.33	0.422	0.821	.470	0.979
	B	0.80				
	C	0.50				
Surface architecture	A	1.00	0.016	3.000	.100	0.813
	B	1.80				
	C	1.50				
Subchondral bone	A	2.33	0.487	0.516	.614	0.956
	B	1.80				
	C	1.50				

*Significant after adjustment for multiple comparisons.

in the superficial layers (Fig 3). This suggests that the repair process began at the base and margins but was interrupted by the wound infection. The edema in the remainder of the repair tissue was likely due to inflammation from the infection, which influenced the repair process to form fibrous scar tissue instead of hyaline cartilage.

Taken together, these observations indicate that the paracrine factors, which promote fibrous tissue formation, are closely related to the inflammatory process that occurs after mechanical disruption of the chondral plate and that both HA and MA may be able to suppress inflammation locally.

The assumptions of normality and equality of variances for ANOVA were satisfied as shown by the Kolmogorov-Smirnov and Levene tests, respectively. However, it is possible for these tests to produce nonsignificant results when numbers are small, and as a precaution, the nonparametric Kruskal-Wallis 1-way ANOVA was used to verify the results. This test has the advantage of being more resistant to non-normality and non-constant variance when compared with standard ANOVA. However, some of the information from the data set is lost in the process of nonparametric transformation, hence giving a *P* value that is marginally less significant than standard ANOVA.

Analysis of Gill scores by category shows that group C had significantly more safranin O staining for proteoglycans than the other 2 groups. Integration of repair tissue with surrounding articular cartilage and cell morphology were also better in group C, and with larger numbers, these parameters may also become significant (Table 4).

On the basis of the superior quality of repair tissue on histologic examination and the significantly better Gill scores in group C animals, we conclude that our hypothesis—that intra-articular injections with autologous MA and HA can improve the quality of repair cartilage after subchondral drilling for articular cartilage defects—is proven. In addition, this study also showed that the blood clot that forms after subchondral drilling is a suitable natural scaffold for articular cartilage regeneration in combination with HA and MA. This procedure is simpler and more cost-effective when compared with artificial scaffolds currently under investigation.[14]

The nucleated cell count is an indicator of the non–red blood cell content of the MA rather than a true MSC count, which constitutes only a small fraction (0.0002%) of this amount.[15] In addition to the MSC content, the MA suspension contains paracrine factors and support cells that may affect differentiation of stem cells into cartilage.[16]

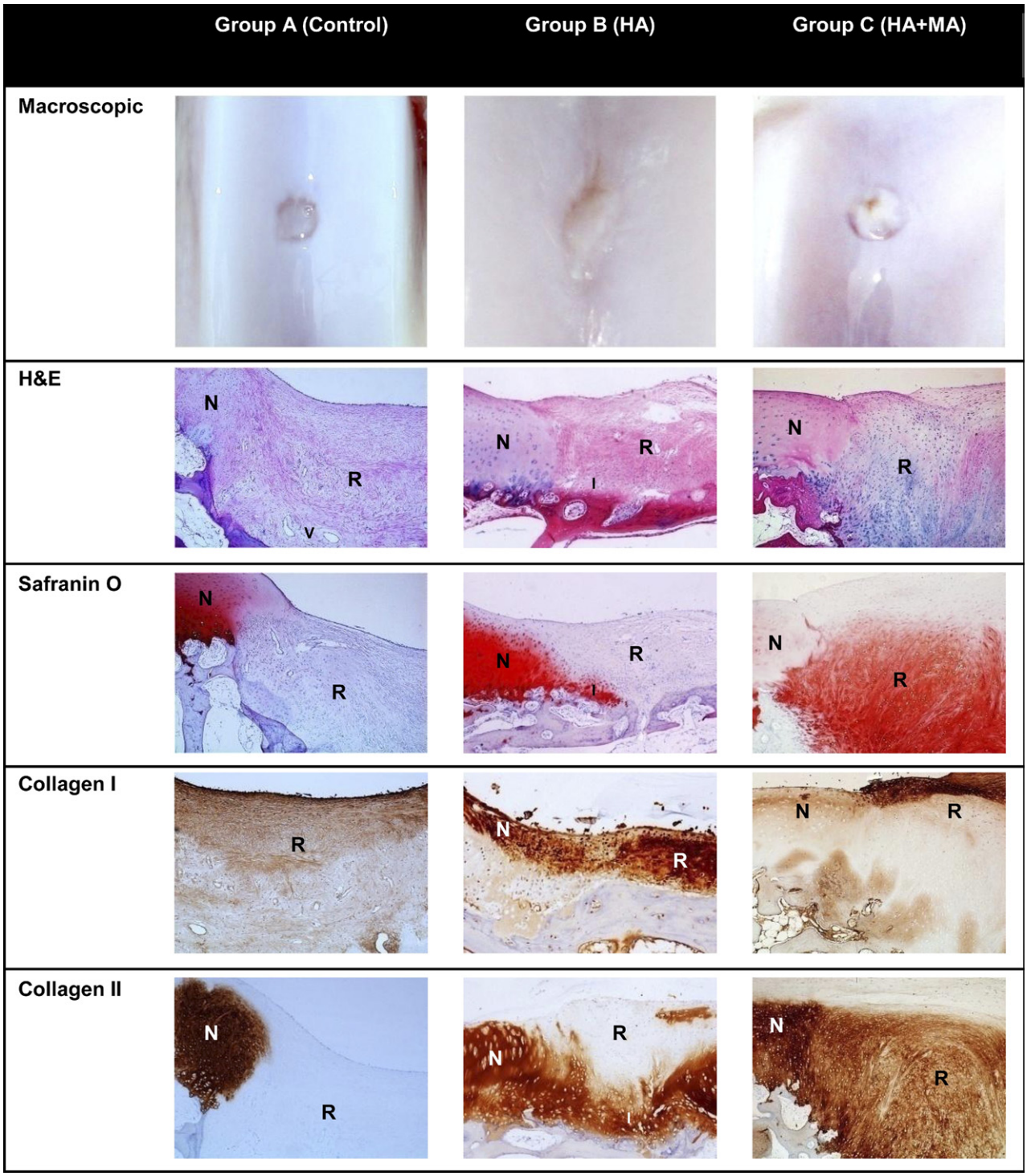

Figure 2. Macroscopic and histologic findings for representative subjects from all 3 groups. (N, normal cartilage; R, repair cartilage; V, dilated capillaries and venules; I, islands of hyaline-like cartilage.)

The injection schedule for HA was based on the manufacturer's recommendations for intra-articular use in the treatment of osteoarthritis.[17] A standard treatment cycle consists of 3 to 5 weekly injections, and in this study we chose to give only 3 weekly injections in groups B and C because the total volume

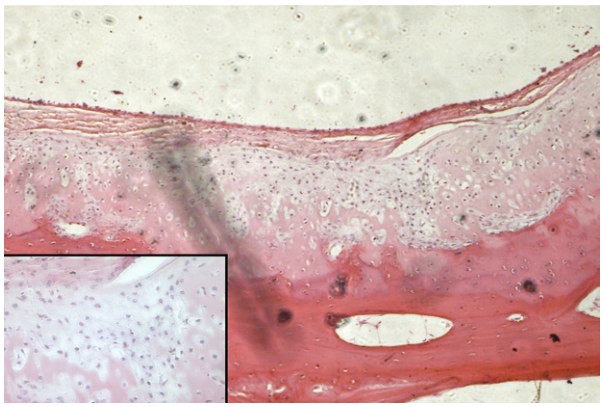

Figure 3. Repair tissue from outlier in group C (goat 15) shown with H&E staining (original magnification ×100). The inset shows tissue edema from the superficial fibrous layer (original magnification ×400).

of MA that could be obtained was limited. The size of the stifle joint also restricted the volume that could be injected at any one time.

The goat was chosen as a study model because, being a larger animal, it was technically possible to obtain bone MAs for autologous injection. In addition, the articular cartilage is of reasonable thickness, the stifle joint is relatively large, and the animals are easy to care for.

The goat stifle joint consists of the patellofemoral and femorotibial articulations, of which the patellofemoral joint is the primary weight-bearing surface because goats walk with the joint partially flexed. The femorotibial articulation is therefore less well developed as compared with the human knee joint. The intercondylar area was used rather than the condyles because it was easier to gain access during surgery and it has a larger surface area to create a chondral defect. In humans the intercondylar region represents a common area of chondral injury in those with patellofemoral degenerative changes.

Previous work in an equine model showed that microfracture repair tissue consisted of a mixture of fibrocartilage and hyaline-like cartilage. Clinical functionality and repair tissue volume were improved along with some expression of type II collagen, but there was very little proteoglycan content.[18] In contrast, group C animals from this study showed abundant proteoglycan production. Although similar results were obtained in a porcine model using cultured MSCs from bone marrow,[13] we showed that in vitro cell expansion is unnecessary and that MA can reproduce all the observed regenerative effects of MSCs in articular cartilage.

Although a quantitative comparison with the original study by Gill et al.[10] was not possible because the articular defects were in a different location and the animals were subjected to continuous passive motion therapy after microfracture, certain qualitative observations could still be made. The mean Gill score for that study was 10.75, which was 3.25 points worse than the mean score for group C animals in our study. The best results obtained only showed hyaline-like cartilage,[10] whereas true hyaline cartilage was observed in our study.

The main limitation of this study was that numbers were small, and this was exacerbated by the deaths of 2 goats in group A and exclusion of 1 outlier from group C. However, the reduced number in group A was mitigated by the fact that histologic changes in standard microfracture surgery are well documented, and repair tissue from the remaining subjects in that group was similar to that described in a previous publication.[13] Another mitigating factor was that the difference in Gill scores between groups was large enough that it was possible to show a statistically significant result even with relatively small numbers.

Another weakness in the study was that radiographic documentation of skeletal maturity in the study animals was not done. Given that goats generally achieve skeletal maturity at between 2 and 3 years of age, it was likely that these goats were skeletally immature, and hence their cartilage was better able to undergo repair than that in adult animals.[19]

Staining for lubricin and type X collagen could have been done along with testing for biomechanical properties, because these would conclusively show that the repair tissue in group C was indeed high-quality hyaline cartilage. It was unlikely, however, that the repair tissue in group C was lubricin deficient because there was no synovial thickening or deterioration of superficial zone chondrocytes typical of lubricin deficiency.[20] Although type X collagen was not looked for in this study, it was also unlikely that the repair tissue in group C was undergoing endochondral ossification because there was no evidence of chondrocyte hypertrophy or vascular invasion that typifies this process.[21]

In hindsight, it would have been better to choose a more widely used scoring system, such as the scale of O'Driscoll et al.[22] or Pineda et al.,[23] to allow for comparison between studies. However, this was not a critical problem because the difference in histology between groups was so marked that any reasonable scoring system would have shown similar results.

Continuous passive motion and avoidance of weight bearing postoperatively are essential components of

microfracture surgery.[2] This was difficult to reproduce in our study animals, although as quadrupeds, the goats would naturally offload the operated joint after surgery until the pain subsides.

Finally, these results should be validated by repeating the study in skeletally mature animals, including a group with MA injections alone, and with biomechanical testing of the repair cartilage. In addition, more animals could be included in the intervention group (HA + MA) for sacrifice at 1, 2, and 4 months after surgery to show the temporal progression of the repair process. We estimate that a minimum of 10 animals in each of the 4 groups should suffice, allowing for 20% attrition.

CONCLUSIONS

Taking into account the study limitations, we conclude that postoperative intra-articular injections of autologous MA in combination with HA after subchondral drilling resulted in better cartilage repair as assessed histologically by Gill scoring in a goat model.

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