Original Article

Autologous Peripheral Blood Stem Cells With Adjuvant Hyaluronic Acid Enhance Cartilage Repair After Subchondral Drilling in a Sheep Model

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Purpose: To determine histologically whether intra-articular injections of autologous peripheral blood stem cells (PBSC) and adjuvant hyaluronic acid (HA) after subchondral drilling result in better articular cartilage repair. **Methods:** Fifteen sheep (aged 12-36 months) were included in this study. An 8-mm full-thickness cartilage defect was created. There were 3 groups: group A, which comprised 5 control (subchondral drilling only); group B, which comprised 5 subchondral drilling + HA (3 injections); and group C, which comprised 5 subchondral drilling + PBSC + HA (3 injections). Each injection was given intra-articularly at 7 days apart. Animals were killed humanely at 6 months after operation. The left stifle joint was examined macroscopically as well as histologically (hematoxylin and eosin, safranin-O, collagen type 1 and 2, International Cartilage Regeneration & Joint Preservation Society [ICRS] II scale). **Results:** All the animals survived the duration of the study. Macroscopic evaluation showed the presence of repair tissues in all groups, although the chondral defects were not completely filled with repair cartilage. Greater ICRS II scores indicate better chondrogenesis. Group C (902 ± 229) showed significantly greater ICRS II scores compared with group A (536 ± 81) and group B (563 ± 83), with *P* values of .014 and .016, respectively. However, the scores for group C were lower to those of normal cartilage (1266 ± 35), with a *P* of .014. **Conclusions:** In this sheep model, postoperative intra-articular injections of autologous PBSCs with HA were associated with better histologic cartilage repair after subchondral drilling when compared with HA alone, as assessed using the ICRS II scoring system. **Clinical Relevance:** This large animal study shows that intra-articular injections of autologous PBSC combined with HA are associated with improved histologic features of cartilage repair after subchondral drilling, supporting further investigation of this approach in preclinical models and the findings may have relevance in guiding future treatment strategies for cartilage defects in humans.

Articular cartilage is a specialized avascular tissue that has very limited healing capacity on its own.[1] Full-thickness cartilage defects that penetrate the subchondral bone can mobilize marrow-derived stem cells to initiate repair,[2] which forms the basis of many current cartilage repair techniques.[3,4] Mesenchymal stem cells are most commonly studied,[4] although peripheral blood stem cells (PBSC) have been shown to have

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similar potential for articular hyaline cartilage in clinical settings, including applications in other parts of musculoskeletal system[5-16] and also treatment of osteoarthritis.[17]

Treatment involving stem cells originated in hematology and oncology, using bone marrow aspirate and marrow progenitor cells. To reduce morbidity, cell collection has shifted to mobilization via hormonal stimulation such as granulocyte colony-stimulating factor (G-CSF), followed by apheresis. This approach has been proven to be safe and effective to obtain multipotent stem cells from healthy donors.[18] In vitro studies confirmed this ability of PBSC to differentiate into bone, cartilage, and other musculoskeletal lineages.[19,20] High-molecular-weight hyaluronic acid (HA) plays an important role in the synovial fluid by providing viscoelasticity and reducing chondrocyte apoptosis.[21] Various commercial HA products have been studied for cartilage repair in both partial- and full-thickness defects alongside mesenchymal stem cells, microfracture, and osteochondral transplantation.[22] Results have been encouraging, with reports of enhanced chondrogenic differentiation, reduced joint inflammation, improved proteoglycan content, better defect filling and incorporation, and improved postprocedural joint friction properties.[23-27] In addition, animal studies involving serial intra-articular injections of stem cells combined with HA have documented histologic evidence of hyaline cartilage regeneration.[28-32] Although HA is well known for its lubricating and viscoelastic properties in synovial fluid, in our study its contribution is more likely associated with its role in the cartilage extracellular matrix, where it may support matrix organization and tissue repair.

Despite encouraging clinical results, further mechanistic evidence from controlled preclinical models is needed. Unlike human studies, animal models allow for consistent defect creation, standardized treatment, and full tissue harvesting for histologic analysis. Large animals like sheep, whose knees share anatomical and biomechanical traits with human knees, are well suited for translational cartilage research.[33,34] The feasibility and safety of PBSC harvesting in sheep via apheresis also has been reported,[35,36] supporting its use for evaluating tissue-level outcomes of PBSC therapy.

We wanted to establish a large animal model to further research into the pluripotency of PBSC. Subchondral drilling is performed on cartilage defect to provide access to the bone marrow via blood clot formed by the drilled holes. These blood clots from the drilled holes serve as an autologous scaffold for all the postoperative intra-articular injections of autologous PBSC and promote the recruitment of endogenous stem cells from the marrow.[37] The purpose of this study was to histologically evaluate whether intra-articular injections of autologous PBSC in combination with HA following subchondral drilling results in improved repair of articular cartilage. Our hypothesis was that postoperative intra-articular injections of PBSC in combination with HA after subchondral drilling would result in improved quality of cartilage repair.

Methods

Animals

All animals survived the duration of the study. In this animal study, 15 mixed breed (with varying genetic lines from Malin, Dorset, Siamese Long Tail, and Barbados Blackbelly) male sheep were used. All the sheep were adults aged between 12 and 36 months of age (average age was 21.7 months) and weighed between 36 and 52 kg (average weight was 44.0 kg) before surgery. Each animal was housed individually in a barn to prevent fighting and reduce the risk of injury. They were fed twice daily with freshly cut grass and commercial pellets.

Ethics approval was obtained from the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (reference number: AUP-R014/2022). All procedures were conducted in accordance with the Animal Welfare Act 2015, Institutional Animal Care and Use Committee Policy and Code of Practice for the Care and Use of Animals for Scientific Purposes.

Fifteen sheep were divided into 3 groups comprising 5 sheep in each group, namely, group A (control), group B (HA), and group C (PBSC + HA). All the animals underwent surgery. As control, no injection was administered to group A. PBSC harvesting was only performed in group C. Surgical, harvesting, and injection procedures are described in the following sections. Six months after surgery, all sheep were killed humanely. Left stifle joints were collected and examined macroscopically and histologically.

All animals were clinically healthy at baseline. Sheep were included on the basis of availability, with only minimum weight and age requirements applied. Because of sourcing constraints, no preselection was done. Randomization was not applied in this study. The initial batch of sheep acquired were assigned to group C for apheresis in order to allow adequate recovery time before surgery. This was necessary due to the additional procedure required for stem cell mobilization and collection, which needed to be completed before the surgical intervention. All animals were healthy and maintained under similar housing and handling conditions throughout the study period, and the allocation method was not expected to introduce systematic bias between the groups.

Autologous PBSC Collection

In group C sheep, the collection of PBSC was conducted 1 month before surgery. An injection dose (5 mg/kg) of G-CSF (Neupogen; Amgen, Thousand Oaks, CA) was given to each animal subcutaneously once a day for three consecutive days prior to apheresis. On day 4, autologous PBSC were collected by an automated cell separator (apheresis) via central venous access. A catheter with 11-F dual lumen was flushed with heparinized saline before insertion into the jugular vein using sterile technique. Apheresis was performed using Spectra Optia Apheresis Machine (Caridian BCT, Denver, CO). All collected PBSC were cryopreserved in 10% dimethyl sulfoxide and divided into 2-mL cryovials for storage in liquid nitrogen at −196°C. Hemocytometer was used to obtain viable cell counts and viability, whereby $CD34^{+}CD45^{+}$ cell population was analyzed from flow cytometric analysis. Further details with results and analysis of the cells are available from our recent publication.[35] The mean viable cell counts, viability and $CD34^{+}CD45^{+}$ cell population were found to be $51.69 \pm 29.21 \times 10^{6}$ cells per mL, 99.11% ± 0.42 and 5.77% ± 2.72, respectively.

Surgery

All surgeries were performed under general anesthesia at Universiti Putra Malaysia Veterinary Hospital. Sedation was induced in the sheep using ketamine-diazepam, who were intubated and maintained on isoflurane in oxygen. The surgeries were carried out by 2 surgeons (K-Y.S. and N.H.K.) with extensive clinical backgrounds. One is a veterinary specialist with a doctoral degree in equine surgery and fellowship training in orthopaedics. The other is a consultant orthopaedic surgeon with multiple international fellowships in joint and trauma surgery and experience in regenerative therapy with stem cells.

The left stifle joint was used for the study. Surgery involved a lateral parapatellar approach to dislocate the patella medially to expose the entire trochlear of the stifle joint. An 8-mm diameter full-thickness chondral defect was created in the central trochlear by using a punch and curettage to produce a standard articular defect (Fig 1). Nine drill holes of 6 mm depth were performed for each defect using a specially made 2-mm drill (patent number: US 10,702,289 B2).[8] The wounds were closed in layers and the sheep were awoken from general anesthesia and allowed to mobilize immediately.

The choice of the central trochlear region for defect creation was based on anatomical and functional similarities between the ovine and human knee. Although the stifle joint in sheep bears a greater proportion of weight due to the animal's flexed posture, its accessibility and surface area make it a suitable site for evaluating chondral repair. The central trochlear surface, as used in this study, also mirrors a common site of injury in human knees.

Intra-articular Injections

In group A (control), no injections were given. For the sheep in group B (HA), intra-articular injections of HA (Ostenil; TRB Chemedica AG, Germany) were performed at day of surgery, then 7 and 14 days postoperatively. Two milliliters of HA was injected at each interval. For those in group C (PBSC+HA), intra-articular injections of 2 mL PBSC mixed with 2 mL of HA were given with the same schedule as group B: at day of surgery, then 7 and 14 days postoperatively. In total, 3 intra-articular injections were administered to the sheep in groups B and C. For the 2nd and 3rd postoperative injections, the sheep were intravenously sedated with ketamine-diazepam (at 2 and 0.2 mg/kg respectively) while they remained in their animal cages.

Histology Sample Preparation

All animals were killed humanely 6 months after the surgery. The left stifle joints were harvested. The harvested stifle joint was exposed, evaluated macroscopically, and photographed. A block was then cut out from the previously drilled area. For the histologic evaluation, 5 samples of normal cartilage were harvested from joint of the sheep in group A (Control) to serve as a normal point of reference for comparison with the treatment groups. The specimens were preserved in 10% formalin, decalcified, and embedded in paraffin for tissue blocking. Sectioning of tissues at 4 μm was done. Samples were taken from the middle section of the chondral defect and these were stained with hematoxylin and eosin and 0.5% safranin-O. Positive safranin-O staining was detected as a reddish color reaction.

Immunochemistry was performed to assess the presence of type I collagen (rabbit polyclonal antibody; Gene Tex, San Antonio, TX) and type II collagen (mouse monoclonal antibody; Thermo Fisher Scientific, Fremont, CA). Antigen retrievals were done by overnight heating in the oven at 80°C of the tissue slides in target retrieval solution at pH9. Three percent hydrogen peroxide was used for blocking endogenous peroxidase for 15 minutes. Then, incubation of each slide with primary antibody type I collagen was done in 30 minutes. This was followed by incubation with a secondary antibody (Anti Rabbit, HRP conjugate [Abcam]) for 30 minutes. For type II collagen stain, the aforementioned procedures were repeated with replacement of the antibody of type I collagen to type II collagen. Application of 3,3′-diaminobenzidine gave the brownish color reaction for both type I and type II antibodies.

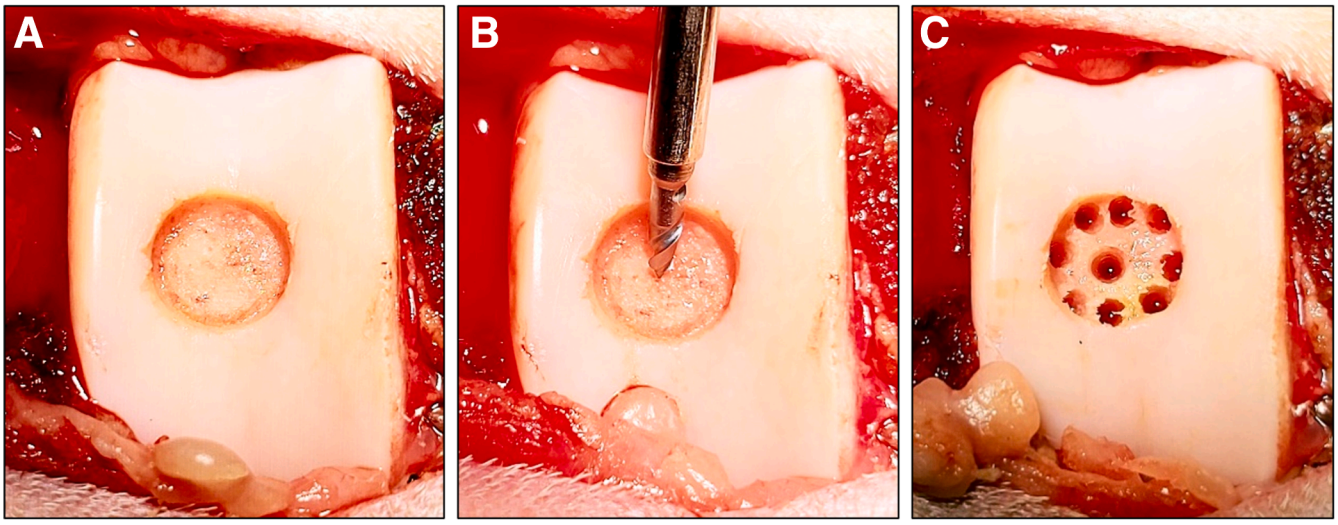

Fig 1. (A) View after creating an 8-mm diameter full-thickness articular cartilage defect in the central trochlear to the left stifle joint of the sheep, equivalent to an area size of 50.2 mm^2. (B) A specially made drill for subchondral drilling with a diameter of 2 mm, drilling to a depth of 6 mm. (C) Each articular cartilage defect accommodated 9 drill holes.

Histologic Evaluation and Grading

The tissue samples were then evaluated by 2 independent-blinded histopathologists. The International Cartilage Regeneration & Joint Preservation Society (ICRS) II grading systems was used in this study. The total score for each sample was calculated by adding up the individual scores of these 14 components. The maximum possible total score is 1,400, and this is only achievable if each of the 14 components has a perfect score of 100.

Histologic scoring was carried out by 2 independent histopathologists who were blinded to the treatment groups. Blinding was not applied during the surgical procedures or treatment administration because of the practical constraints of performing stem cell mobilization and apheresis.

Statistical Analysis

Sample size was calculated using G*Power 3.1.9.4 for 1-way analysis of variance, based on the method[38] and data[39] from published animal studies, in which the total ICRS II score difference was calculated to be at least 400 with average standard deviation of 124.6. The analysis showed that for a 5% type-I error rate (α level 0.05) and 20% type II error rate (80% power) with an ability to detect a difference of 400 scores among the 3 groups (Cohen f = 1.513; standard deviation = 124.6, groups of equal size), a minimum of 3 sheep per group were needed in order to have a study of adequate statistical power, but 5 were included per group instead to accommodate potential withdrawal due to illness or mortality.

Statistical analysis of ICRS II scores was performed using SPSS, version 26 (IBM, Armonk, NY). Outliers were identified using box plots. Normality of data was evaluated with the Shapiro-Wilk test, and the equality of variances was tested using the Levene test. As the result of unequal variances, the nonparametric Kruskal-Wallis test was used to compare the ICRS II scores among the 3 treatment groups and the normal cartilage samples included for reference. Subsequent post-hoc comparisons were analyzed using the Mann-Whitney U test. Comparative analysis was 2-tailed, and the level of statistical significance was $P < .05$. Inter-rater reliability between the 2 histopathologists was assessed using the intraclass correlation coefficient (ICC), applying a 2-way mixed-effects model for absolute agreement, denoted as ICC(3,2).

Results

ICRS II Scores

Two outliers (A2 & NC3) were identified via box plot analysis and excluded from further analysis. Inter-rater reliability yielded an ICC of 0.97 (95% confidence interval 0.92-0.99, $P < .001$), indicating excellent agreement between the 2 histopathologists in their ICRS II scoring. A detailed breakdown of scores is presented in Table 1. The Kruskal-Wallis test on the ICRS II scores showed a significant difference among the 3 treatment groups and the normal cartilage reference samples, with a test statistic of H = 13.982 and 3 degrees of freedom ($P = .003$), indicating that at least one group differed significantly. Post-hoc comparisons using the Mann-Whitney U test showed that group C (PBSC+HA) had significantly greater ICRS II scores (902 ± 229; mean ± standard deviation) compared with group A (536 ± 81, $P = .014$) and

Table 1. Breakdown of ICRS II Scores for All Animals

Category	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	NC1	NC2	NC3	NC4	NC5
Tissue morphology	40	80	60	50	50	15	30	30	65	40	50	80	40	20	65	95	90	70	90	100
Matrix staining	0	45	0	0	20	0	0	0	0	0	5	93	0	20	0	55	60	60	60	60
Cell morphology	10	85	0	60	10	0	30	10	30	20	25	75	50	65	18	100	100	80	90	100
Chondrocyte clustering	55	85	90	80	70	45	65	65	65	50	100	90	50	10	53	5	80	70	70	80
Surface architecture	60	40	40	40	60	25	75	60	65	60	70	100	60	95	70	100	100	90	100	100
Basal integration	90	30	60	40	50	40	60	50	70	70	90	95	75	100	80	100	90	80	90	90
Tidemark formation	0	10	0	0	0	5	20	0	10	10	10	68	15	80	33	85	90	80	80	90
Subchondral bone abnormalities / marrow fibrosis	85	10	10	35	45	80	30	50	60	90	0	100	75	100	95	95	100	90	100	100
Inflammation	100	95	93	100	100	95	100	100	100	90	100	100	100	100	95	100	100	90	100	100
Abnormal calcification / ossification	50	40	40	50	50	95	50	50	50	85	70	100	40	100	75	100	90	90	90	90
Vascularization (within repaired tissue)	45	90	10	45	20	55	30	20	45	10	45	75	65	95	50	100	100	80	100	100
Surface assessment	50	70	20	50	30	10	30	20	60	25	50	95	60	90	90	100	90	80	90	100
Mid-/deep zone assessment	0	40	0	10	0	15	10	10	20	45	30	80	55	100	75	100	100	90	90	100
Overall assessment	20	50	8	35	10	20	10	10	25	40	15	55	50	93	45	100	90	80	90	100
Total	605	770	430	595	515	500	540	475	665	635	660	1,205	735	1,068	843	1,235	1,280	1,130	1,240	1,310
Mean per group	536 ± 81 (A2 excluded)							563 ± 83					902 ± 229				1266 ± 35 (NC3 excluded)			

NOTE. Mean per Group are presented as mean ± standard deviation. Note: A2 & NC3, being outliers, were excluded from the average calculations.
A, group A (Control); B, group B (HA); C, group C (PBSC+HA); HA, hyaluronic acid; ICRS, International Cartilage Repair Society II; NC, normal cartilage; PBSC, peripheral blood stem cell.

group B (563 ± 83, $P = .016$). However, when compared with normal cartilage (1266 ± 35), group C ICRS II scores were significantly lower ($P = .014$), which is expected, given that native cartilage represents the ideal benchmark for histologic evaluation of cartilage repair. Achieving full restoration comparable with healthy cartilage remains a challenge in this sheep model. Nevertheless, group C ICRS II scores were notably greater than those of groups A and B. This suggests that group C produced repair tissue that more closely resembles hyaline cartilage compared to groups A and B.

Histologic Findings

A compilation of specimens with the greatest and lowest ICRS II scores from each group was presented in Figure 2. A normal cartilage sample with histologic features was also included in this figure.

Macroscopic evaluation revealed the presence of repair tissue at the chondral defect sites in all groups (Figure 3). However, the extent of surface coverage and quality of tissue differed among them. In groups A and B the repair tissue appeared irregular and fibrous, with incomplete filling of the defects (Figure 2). Group C showed more extensive defect coverage with a smoother surface, suggestive of better tissues on the repaired cartilage.

Histologically, the regenerated tissue in group C more closely resembled hyaline cartilage. As shown in Figure 2, safranin-O staining was more prominent in group C samples, indicating higher proteoglycan content. In contrast, groups A and B showed minimal to absent safranin-O staining, consistent with fibrocartilage formation. Collagen staining revealed that group C exhibited stronger collagen type II expression in the mid-to-deep zones of the regenerated tissue, with less collagen type I staining compared with groups A and B.

Specimens from group C also showed better basal integration, improved zonal architecture, and round chondrocyte morphology arranged in clusters, all of which are features of native cartilage. Specimen C2, which had one of the greatest ICRS II scores, showed staining characteristics and tissue morphology that closely matched those of normal cartilage. All groups lacked signs of inflammation, but only group C showed early tidemark formation in all the samples, suggesting active subchondral bone plate remodeling. Tidemark formation was observed in 1 of 5 sheep (20%) in group A, 4 of 5 sheep (80%) in group B, and all 5 of 5 sheep (100%) in group C. The corresponding mean scores were 2 in group A, 10 in group B, 38 in group C, and 84 in the normal cartilage group (calculated from the individual data shown in Table 1). Although group C showed a greater extent of tidemark formation compared to groups A and B, the average score was still

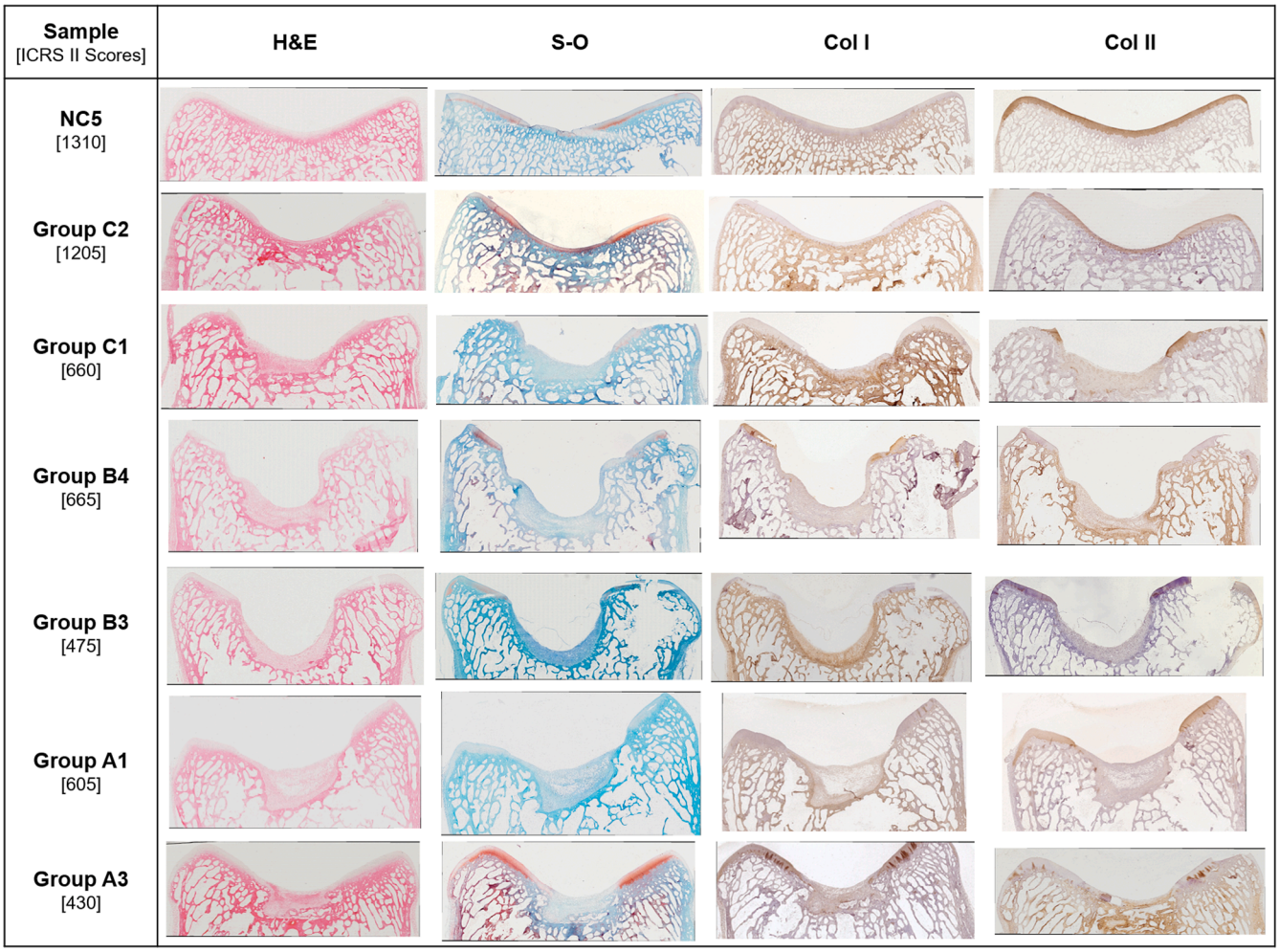

Fig 2. Histologic images showing specimens with the highest and lowest ICRS II (International Cartilage Repair Society II) scores from each group, alongside a normal cartilage specimen. The tissues are stained with hematoxylin and eosin (H&E), safranin-O (S-O), collagen type I (Col I) and collagen type II (Col II). Group A (drilling only) samples shown are A1 (score 605) and A3 (score 430). Group B (HA) is represented by B4 (score 665) and B3 (score 475). Group C (PBSC + HA) includes C2 (score 1,205) and C1 (score 660). The highest normal cartilage specimen (NC5) has a score of 1,310.

lower than that of normal cartilage. This may reflect partial, but not complete, restoration of the tidemark in group C.

Discussion

The results showed that this combination treatment (group C) led to improved histologic outcomes compared with subchondral drilling alone (group A) or drilling with HA only (group B).

The histologic findings in group C indicates a more advanced stage of cartilage repair, with features more closely resembling native hyaline cartilage. The presence of a proteoglycan-rich matrix and stronger type II collagen expression in the mid-to-deep zones reflects better matrix organization and tissue quality. These features are typically absent in fibrocartilage, suggesting that the addition of PBSC may support a repair environment conducive to more hyaline-like tissue formation. Cellular morphology supported this interpretation. Repair tissues in groups A and B were largely populated by fibroblast-like spindle-shaped cells, a feature consistent with fibrous healing (Fig 4). In contrast, group C exhibited rounded chondrocyte-like cells arranged in small clusters, indicative of active cartilage remodeling. This difference may be attributed to the biological activity of PBSC in promoting chondrogenic differentiation or paracrine signaling that supports native cartilage phenotype maintenance. Our results are similar to those reported in a publication using bone marrow aspirate in a goat model where bone marrow aspirate combined with HA led to near-complete hyaline cartilage regeneration after subchondral drilling.[38] Similarly, a clinical study in the knee joint have shown improved outcomes in knee

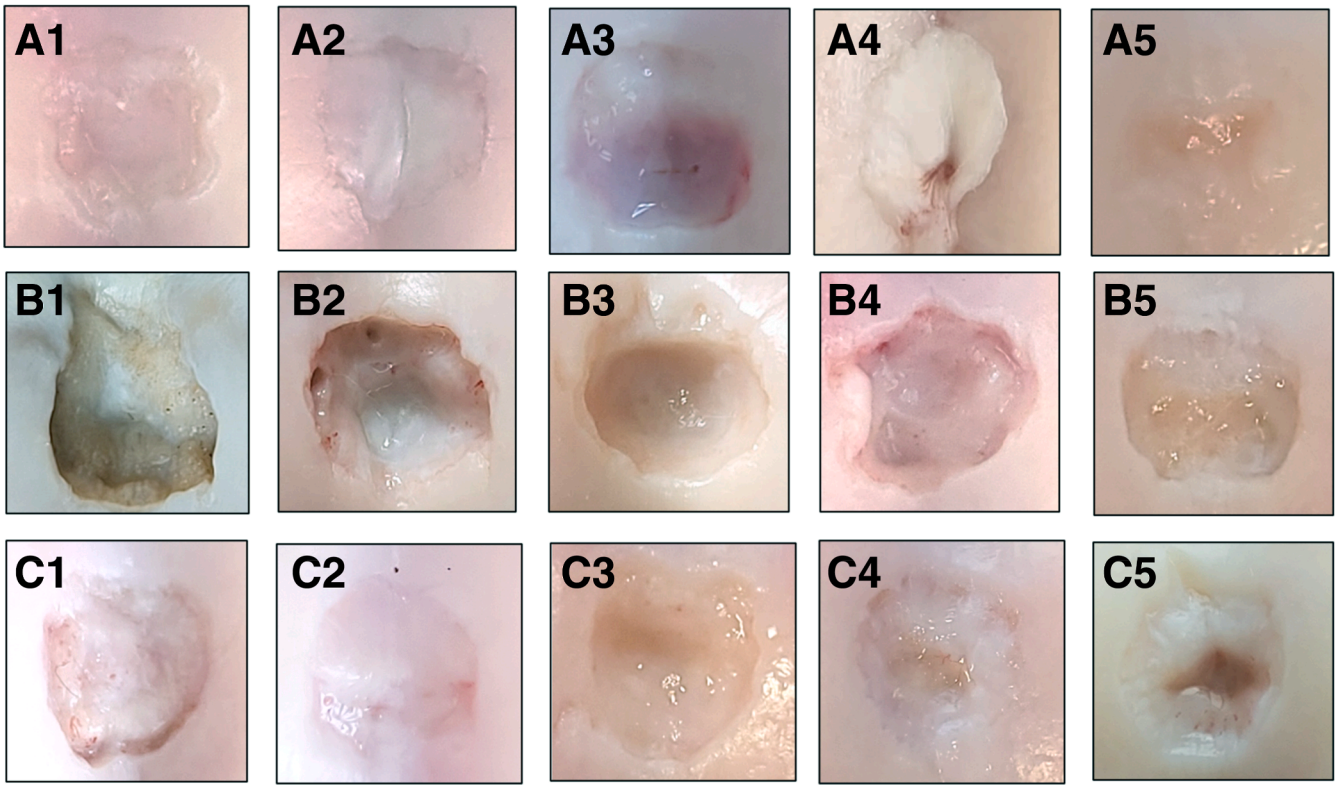

Fig 3. Macroscopic images of the defect sites at 6 months postoperation from all the sheep, showing repair cartilage at the drill site. A1 to A5 are samples from group A (control), B1 to B5 are from group B (HA), and C1 to C5 are from group C (PBSC + HA). Better surface coverage and more complete repair are seen in group C specimens compared to groups A and B. (HA, hyaluronic acid; PBSC, peripheral blood stem cell.)

cartilage repair using PBSC with HA, both histologically and radiologically.[6,8,37] In this animal model it is observed that the repaired cartilage tissues in group C sheep exhibited proteoglycan presence and type II collagen staining reflecting findings reported from these published studies. The repaired cartilage tissues have the closest resemblance to normal cartilage. Despite a statistically significant difference between group C and normal cartilage ICRS II scores ($P = .014$), the average score achieved by group C reached approximately 71% of normal cartilage values of normal cartilage. This finding reveals encouraging early signs of cartilage maturation, pointing to the onset of longer-term repair process that had not yet reached completion within the 6-month study period.

Previous studies evaluating HA as a monotherapy after marrow stimulation have produced mixed results.[23-27] Although HA offers anti-inflammatory and viscoelastic properties, our findings confirm that HA alone does not substantially improve the histologic quality of the repair tissue. Group B showed some degree of surface improvement, but lacked the matrix integrity, cell morphology, and type II collagen content seen in Group C. The data reinforce that although HA may provide a supportive environment, it plays an insufficient role in cartilage repair unless combined with an active regenerative agent such as PBSC.

The rationale for this approach lies in combining subchondral drilling, which releases endogenous marrow-derived cells, with exogenous PBSC and HA to enrich the local environment. PBSC, mobilized via G-CSF and harvested through apheresis, contain a population of CD34+ progenitor cells shown to differentiate into cartilage and bone.[19,20] These injected cells may augment repair by homing to the injury site and contributing directly or indirectly to tissue regeneration through paracrine effects.[31] Subchondral drilling itself is a foundational component of marrow stimulation techniques, designed to penetrate the subchondral bone plate and facilitate the migration of marrow-derived cells into the cartilage defect. The access channels created allow endogenous stem cells to populate the site, initiate clot formation, and support the early phases of tissue repair. In our model, subchondral drilling alone (group A) generated fibrous repair tissue with limited structural integrity, whereas the addition of HA (group B) provided modest benefit. Notably, the combination with PBSC (group C) produced repair tissue with characteristics more closely aligned to hyaline cartilage.

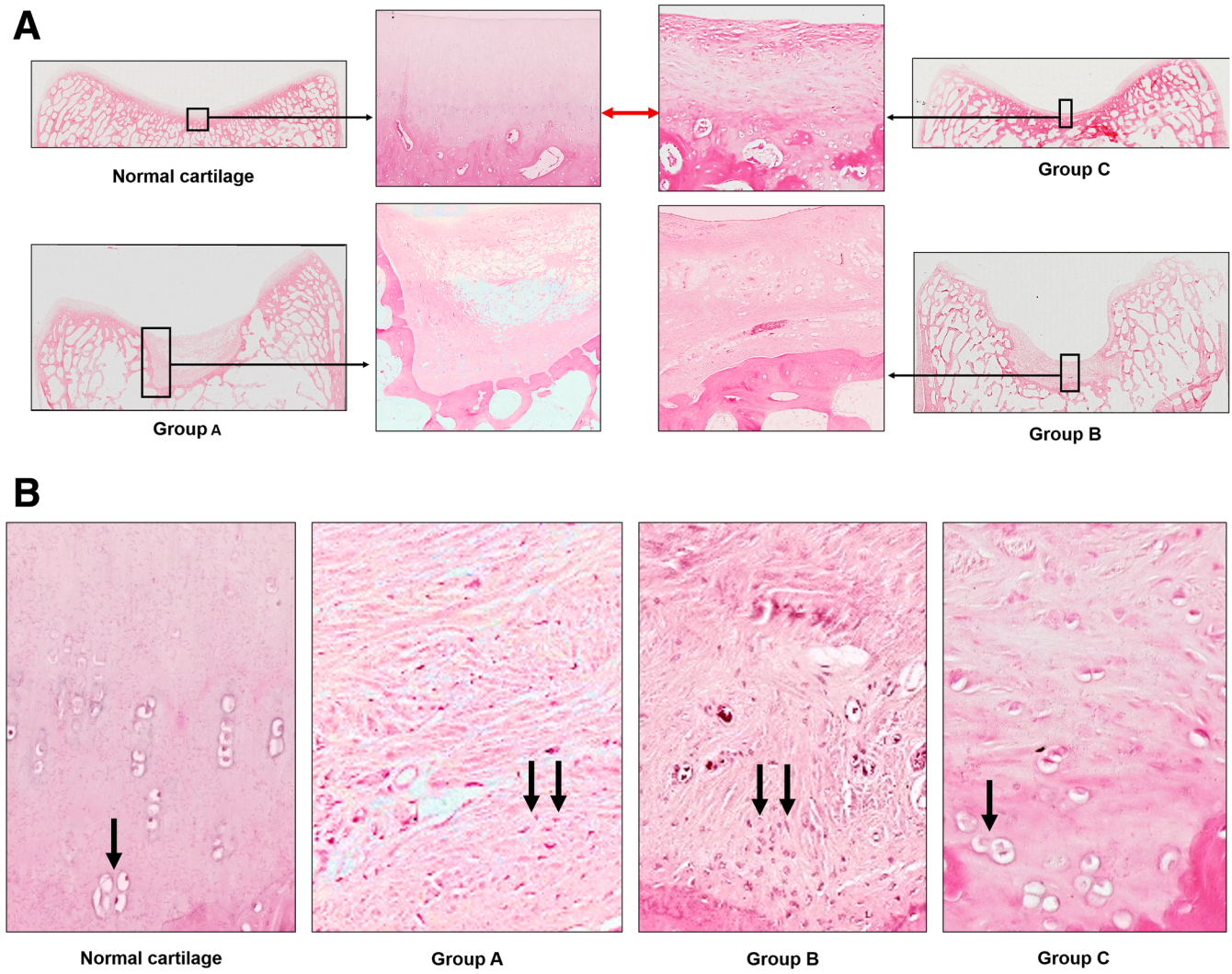

Fig 4. Hematoxylin and eosin–stained images showing overall tissue morphology and tidemark formation (red arrow) observed in normal cartilage and Group C samples only (A) and cellular morphology, with round chondrocyte-like cells and clustering (single black arrow) seen in normal cartilage and Group C, in contrast to spindle-shaped cells (double black arrows) observed in Groups A and B (B).

Tidemark assessment is an important criterion for assessing cartilage repair, as this is the interface between the calcified and noncalcified cartilage layers. Subchondral drilling, which is part of a marrow stimulation procedure, penetrates the subchondral bone plate to allow access to the underlying bone marrow. It is therefore important to assess the regeneration of this subchondral bone plate layer during the repair process.[10] Early tidemark formation observed in group C samples may signal the beginning of osteochondral interface remodeling, which is a critical step in the regeneration of structurally sound articular cartilage. Although this feature was not seen in groups A and B, the partial tidemark regeneration in group C suggests a potential for deeper integration and longer-term stability of the repair tissue. This finding aligns with previous reports where more advanced cartilage remodeling was seen in treatments incorporating stem cells.

We included 2 additional stains (collagen I and II) for the tissue samples to provide further analysis of the quality of the regenerated cartilage. Fibrocartilage typically rich in type I collagen and lacks type II collagen. In contrast, hyaline cartilage contains a predominance of type II collagen and minimal type I collagen. Group C showed greater expression of type II collagen, which aligns with the histologic scoring criteria and supports the classification of the repair tissue as hyaline-like. This staining profile further reinforces the conclusion that PBSC+HA contributes to higher-quality cartilage repair compared to the other treatment modalities. These staining results aligned

with the ICRS II evaluation under criteria of "surface/superficial," "mid/deep zone," and "overall assessment," which favored group C, indicating superior cartilage repair.

Although the findings in group C were encouraging, incomplete defect filling was still observed in some samples. This may be related to the relatively short duration of the study. This may be attributed to the relatively short 6-month study duration. Repair and regeneration of articular cartilage appeared to initiate from the periphery and progress toward the center, which could suggest that longer follow-up is needed to observe complete defect filling. These findings suggest that prolonged postoperative stimulation with PBSC and HA may further enhance cartilage regeneration and maturation in large animal models.

Limitations

This study had several limitations. First, sample sizes in each group were small and potential confounding variables were not accounted for. We acknowledge this as a limitation, but the number was determined on the basis of statistically justified calculations, and we intentionally avoided using a larger cohort in order to minimize unnecessary animal sacrifice, in accordance with ethical research principles. Second, the natural variation in breed and body size among the animals may have affected the results. The 6-month follow-up period also may have been too short to observe the full course of cartilage healing or the development of longer-term degenerative changes. In addition, this study did not include a group that received PBSC injections without HA, so the specific effect of PBSC on cartilage repair cannot be separated from the combined treatment.

Conclusions

In this sheep model, postoperative intra-articular injections of autologous PBSC with HA were associated with better histologic cartilage regeneration after subchondral drilling, when compared with HA alone, as assessed using the ICRS II scoring system.

Disclosures

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