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Does Demineralized Bone Matrix Enhance Tendon-to-Bone Healing after Rotator Cuff Repair in a Rabbit Model?

Woo-Yong Lee, MD, Young-Mo Kim, MD, Deuk-Soo Hwang, MD, Hyun-Dae Shin, MD, Yong-Bum Joo, MD, Soo-Min Cha, MD, Kyung-Hee Kim, MD*, Yoo-Sun Jeon, MD, Sun-Yeul Lee, MD†

Department of Orthopedic Surgery, Regional Rheumatoid and Degenerative Arthritis Center, Chungnam National University Hospital, Chungnam National University School of Medicine, Daejeon,
**Department of Pathology, Chungnam National University Hospital, Chungnam National University School of Medicine, Daejeon,*
†Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Chungnam National University School of Medicine, Daejeon, Korea

Background: The purpose of this study was to compare the histologic outcomes of rotator cuff (RC) repair with demineralized bone matrix (DBM) augmentation and those without DBM augmentation and to evaluate the role of DBM for tendon-to-bone (TB) healing in a rabbit model.

Methods: Twenty-six adult male New Zealand white rabbits were randomly allocated to the control group (n = 13) or the DBM group (n = 13). Repair was performed 8 weeks after complete transection of the right supraspinatus tendon of all rabbits. In the control group, RC repair was achieved by a standard transosseous technique. In the DBM group, RC repair was achieved using the same technique, and DBM was interposed between the cuff and bone. After 8 weeks, the RC tendon entheses from all rabbits were processed for gross and histologic examination.

Results: On gross TB healing, 2 of 11 specimens in the control group were unhealed and no specimen was grossly unhealed in the DBM group ($p = 0.421$). In the control group, the tendon midsubstance was disorganized with randomly and loosely arranged collagen fibers and rounded fibroblastic nuclei. The TB interface was predominantly fibrous with small regions of fibrocartilage, especially mineralized fibrocartilage. In the DBM group, the tendon midsubstance appeared normal and comprised densely arranged collagen fibers, with orientated crimped collagen fibers running in the longitudinal direction of the tendon. These fibers were interspersed with elongated fibroblast nuclei. The TB interface consisted of organized collagen fibers with large quantities of fibrocartilage and mineralized fibrocartilage.

Conclusions: The use of DBM for TB interface healing in rabbit experiments showed good results in gross and histologic analysis. However, it is difficult to draw a solid conclusion because the sample size is small. Further evaluation in the *in vivo* setting is necessary to determine clinical recommendations.

Keywords: *Demineralized bone matrix, Rotator cuff, Tear, Repair, Tendon-to-bone healing*

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Correspondence to: Yoo-Sun Jeon, MD
Department of Orthopedic Surgery, Regional Rheumatoid and Degenerative Arthritis Center, Chungnam National University Hospital, 282 Munhwa-ro, Jung-gu, Daejeon 35015, Korea
Tel: +82-42-338-2480, Fax: +82-42-338-2482, E-mail: ooppss@hanmail.net
Co-Correspondence to: Sun-Yeul Lee, MD
Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, 282 Munhwa-ro, Jung-gu, Daejeon 35015, Korea
Tel: +82-42-280-7840, Fax: +82-42-338-2482, E-mail: neoquack@naver.com
Woo-Yong Lee and Young-Mo Kim contributed equally to this work as first authors.

Rotator cuff (RC) tears comprise the majority of shoulder lesions in adult patients. The prevalence of RC tears in the general population is 22.1% and increases with age.[1] Despite this high incidence, RC repair does not always lead to clinically satisfactory outcomes; indeed, the failure rates of RC repair are reportedly 40% to 50%.[2-4] Therefore, RC reattachment to bone following RC repair is a challenging clinical problem. Although numerous repair techniques to reduce the retear rate have been reported, these did not significantly reduce the retear rates during long-term follow-up.[5,6] Also, the development of novel techniques have not been linked to improvement of RC repair.[7] Hence, a new approach to enhancing RC healing is needed.

In recent years, growth factors (GFs), platelet-rich plasma, and stem cells have been suggested to enhance tendon-to-bone (TB) healing.[8-10] Rodeo et al.[11,12] reported that increased bone ingrowth between the tendon and bone improved TB healing and showed that GFs such as bone morphogenetic proteins (BMPs) improved the pull-out strength of the repair site.

Demineralized bone matrix (DBM) is an exogenous osteoinductive material, which slowly releases BMPs and acts as a scaffold for many cell types.[13,14] To date, DBM has been used for fracture union to improve bone to bone healing. Moreover, Lovric et al.[9] reported that DBM at the tendon-bone interface promoted healing between the tendon and bone in a rodent anterior cruciate ligament (ACL) model. The size of the rabbit shoulder model allows for many standard surgical techniques to be reproduced, thereby promoting accuracy and reproducibility of injury and repair manipulations.[15] To our knowledge, no study has used a rabbit model to assess the effect of DBM augmentation on TB healing. We hypothesized that DBM augmentation might enhance TB healing after RC repair in a rabbit model.

METHODS

Study Design and Experimental Groups

This study is a controlled laboratory study involving rabbits. Twenty-six adult male New Zealand white rabbits, weighing 3.5 to 4.0 kg, were randomly allocated to the control group (n = 13) or the DBM group (n = 13). A chronic RC tear was generated on the right shoulder of all rabbits. In the experimental group, RC repair was performed by applying DBM, and simple repair was performed in the control group. The commercially available Dynagraft II DBM in 1 mL syringe (IsoTis, Irvine, CA, USA) was used. After 8 weeks, the RC tendon entheses from all rabbits were processed for gross and histologic examination. All of the rabbit experiments were obtained from the Korea BioLink Co., Ltd. (Eumseong, Korea). All procedures and protocols were approved by the Institutional Animal Care and Use Chungnam National University (Approval No. CNU-00791). Coleman et al.[16] compared the 6-week delayed repair group with the 18-week delayed repair group in a sheep RC study because most RC surgery is performed on chronic tears. At 6 weeks after RC injury, the average retraction was 2.6 cm, and the tendon was repairable; however, after 18 weeks, the average retraction was 5.5 cm, and the tendon was irreparable. In our study, repair was performed 8 weeks after the first surgical RC injury to make chronic RC injury.

Surgical Procedure

The rabbits were fully anesthetized with ketamine (50 mg/kg, Ketamine HCl, Huons, Seongnam, Korea), acepromazine Maleate (10 mg, AceproTabs; Vetus, Melville, NY, USA), and xylazine (20 mg/kg, Rompun; Bayer Korea, Seoul, Korea). Under aseptic conditions, a skin incision was made just inferior to the clavicle of the right shoulder. Then, the deltopectoral interval was split and retracted to gain access to the RC (Fig. 1A). The supraspinatus tendon was visualized, and a small Langenbeck retractor was inserted to expose the entire tendon at its insertion on the greater tuberosity of the humerus. The supraspinatus tendon was completely transected with a No. 11 scalpel blade at its insertion to the humerus. A small piece of sterilized plastic wrap was interposed between the cuff and bone to prevent natural TB healing (Fig. 1B). Surgical wounds were closed using 2-0 Nylon (Ethicon-Johnson and Johnson, Somerville, NJ, USA) interrupted sutures. The rabbits received

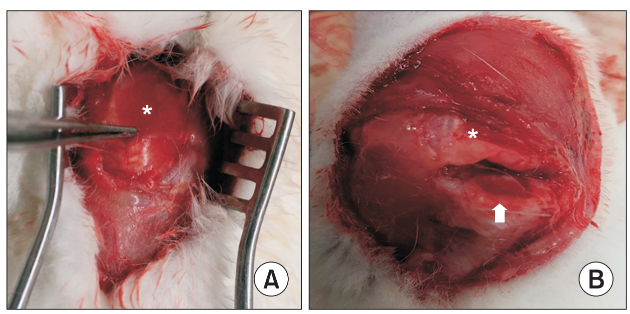

Fig. 1. Adult male New Zealand white rabbits, weighing 3.5 to 4.0 kg, underwent an initial operation. (A) The deltopectoral interval was split and retracted to gain access to the rotator cuff (asterisk). (B) The supraspinatus tendon (asterisk) was completely transected with a No. 11 scalpel blade at its insertion to the humerus. Next, a small piece of sterilized plastic wrap (arrow) was interposed between the cuff and bone to prevent natural tendon-to-bone healing.

analgesics and antibiotics for 7 days after the surgery.

Eight weeks after the initial operation, the rabbits underwent a second operation for RC repair. Anesthesia and approach to the cuff were performed as in the initial operation. Then, in the control group, bone tunnels on the great tuberosity were made using a sterilized drill bit, and a standardized transosseous repair was performed using No. 2 Ethibond (Ethicon-Johnson and Johnson) (Fig. 2A and B). In the control group, RC repair was achieved by a standard transosseous technique. In the DBM group, RC repair was achieved using the same technique, and DBM was interposed between the RC tendon and bone (Fig. 2C and D). A total of 0.3 mL of the DBM was used per rabbit. Surgical wound closure and postoperative care were performed as in the initial operation. After the first and second surgery, the rabbits were left free in the cage.

All rabbits tolerated the surgery, with the exception of 2 rabbits in the control group and 1 rabbit in the DBM group, which developed postoperative deep infections. Therefore, among the 26 rabbits, 3 (2 in the control group and 1 in the DBM group) were excluded from the study.

Eight weeks after the second operation, the rabbits were euthanized by a lethal dose of sodium pentobarbital, and the supraspinatus muscle, tendon, and proximal part of the humerus were harvested after TB healing was assessed with the naked eye.

Histologic Analysis

Specimens were fixed in 10% buffered formalin for 2 days and embedded in paraffin. The paraffin-embedded sections were cut along the longitudinal direction of the supraspinatus fibers to include the entire length of the supraspinatus tendon. Sections were cut at a thickness of 70 to 100 μm and stained with hematoxylin and eosin. An independent pathologist (KHK) with experience in musculoskeletal pathology at our institution performed a qualitative morphological analysis of the specimens using a light microscope (Zeiss, Hamburg, Germany) and slide-scanning software (Aperio ImageScope; Leica Biosystems, Wetzlar, Germany). Histologic analyses were performed using 2 sections randomly collected at the insertion site in each rabbit. The histological grading system was cited in Street et al.[17] It was based on collagen fiber density, collagen fiber orientation, quality of healing at bone-tendon interface, vascularity, and presence of inflammatory cells (Table 1). These 5 parameter scores were added together and higher scores were considered to indicate greater healing.

Statistical Analysis

IBM SPSS ver. 19.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Pearson's chi-square test and Mann-Whitney test for categorical variables were used to compare the control and DBM groups. Differences were considered significant at the 0.05 level.

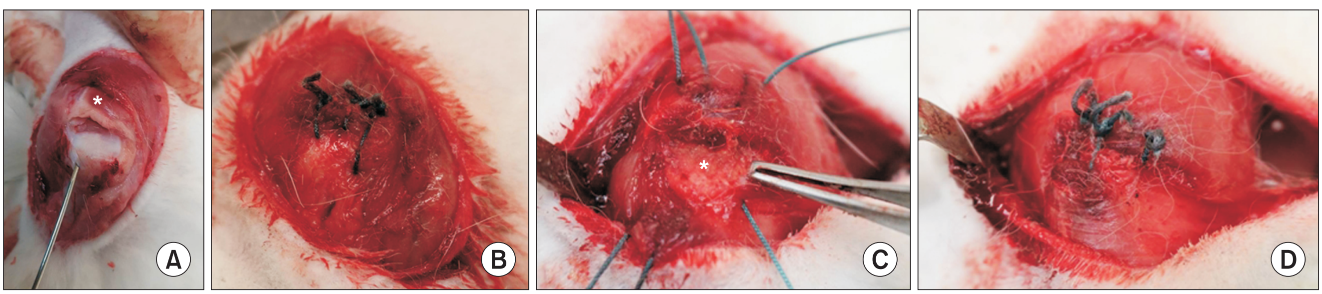

Fig. 2. Eight weeks after the first operation, the rabbits underwent a second operation. (A, B) The ruptured supraspinatus tendon (asterisk) was repaired by a transosseous technique using a sterilized drill bit to make a bone tunnel. (C, D) In the demineralized bone matrix (DBM) group, rotator cuff repair was augmented by interposing DBM (asterisk) between the cuff and bone.

Table 1. The Histological Grading System for Evaluating Rotator Cuff Healing Outcomes

No.	Collagen fiber density	Collagen fiber orientation	Bone-tendon interface	Vascularity	Inflammation
0	None	None	0%–24% interdigitation	Abundant	Abundant inflammatory cells
1	Low	Disorganised fibers	25%–49% interdigitation	Moderate	Moderate inflammatory cells
2	Medium	Moderate alignment	50%–75% interdigitation	Minimal	Minimal inflammatory cells
3	High	Highly aligned	> 75% interdigitation	No	No inflammatory cells

RESULTS

Gross TB Healing

The authors and the pathologist assessed gross finding of TB healing in each specimen with the naked eye. In the control group, 2 of 11 specimens were unhealed (Fig. 3A). In contrast, no specimen was grossly unhealed in the DBM group (Fig. 3B). However, there was no significant difference between the control and DBM groups ($p = 0.421$).

Histologic Analysis

Significant differences in histological morphology between the groups were identified. In control group specimens, the tendon midsubstance was disorganized with randomly and loosely arranged collagen fibers and rounded fibroblastic nuclei (Fig. 4A). The TB interface was predominantly fibrous with small regions of fibrocartilage, especially mineralized fibrocartilage (Fig. 4B). In DBM group specimens, the interpositional DBM had been remodeled. The tendon midsubstance was organized with densely arranged collagen fibers, with orientated crimped collagen fibers running in the longitudinal direction of the tendon. These fibers were interspersed with elongated fibroblast nuclei (Fig. 4C). The TB interface consisted of organized collagen fibers and large amounts of fibrocartilage and mineralized fibrocartilage (Fig. 4D). In the histological grading system, the total score showed a significant difference ($p < 0.001$) (Table 2). For each score, the DBM group scored higher on average than the control group. But there were no significant differences between control and DBM groups (> 0.05).

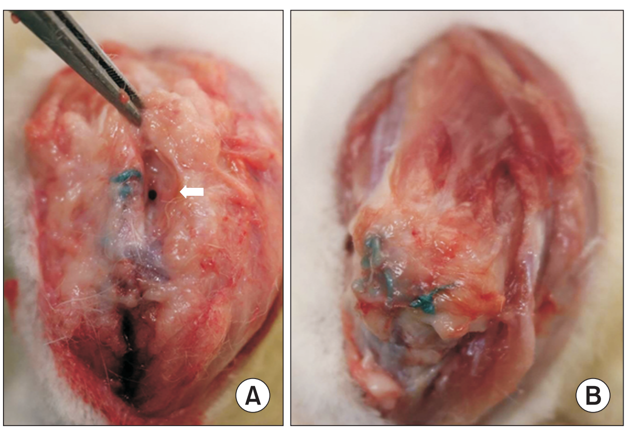

Fig. 3. Eight weeks after the second operation, the rabbits were euthanized using a lethal dose of sodium pentobarbital and underwent a final operation. All authors assessed gross tendon-to-bone healing in each specimen with the naked eye. (A) In the control group, 2 of 11 specimens were unhealed (arrow). (B) No specimen was unhealed grossly in the demineralized bone matrix group.

DISCUSSION

This study aimed to determine the effect of DBM on TB healing after RC repair. Our results suggest that DBM augments RC TB healing by increasing organized collagen fiber and mineralized fibrocartilage. Sundar et al.[10] reported that DBM augmentation of the healing patellar TB interface results in earlier mobilization with fewer pullout failures, as well as superior functional and morphological recovery. They concluded that DBM augmentation may improve functional and histological recovery of tendon reattachment. Lovric et al.[9] investigated the effects of DBM on intra-articular TB healing using a rodent model of ACL reconstruction and reported that DBM had potential for clinical use to augment TB healing. In the current study, although it was a rabbit experiment, the use of DBM in the treatment of RC was thought to be helpful for histological healing.

Healing or regeneration of the musculoskeletal system requires minimum cells, morphogenetic signals, and matrices or scaffolds.[18] Numerous biological studies have focused on enhancing bone-to-bone and/or tendon-to-tendon healing.[19] However, there is no consensus on the methods of enhancing TB healing. Recently, new biological solutions to enhance TB healing have been investigated.[14,18,19] The involvement of GFs, such as basic fibroblast growth factor (FGF), vascular endothelial GF, platelet-derived growth factor (PDGF), and BMP in TB healing, has been described.[14,18,19] TB healing occurs through progressive mineralization of the tendon through bone growth and subsequent remodeling of tissues at the TB interface by endochondral ossification.[20,21] DBM contains exogenous proteins and exerts an osteoinductive effect.[22] It also slowly releases BMP and acts as a scaffold for many cell types, suggesting it has potential for TB healing.[13,14] Unlike other biologic or synthetic scaffolds based on mammalian materials, DBM has no associated immunogenicity or pathogen transmission.[23] DBM is commercially available, approved for clinical use, and easy to use.[18] Furthermore, DBM contains various GFs such as FGF, PDGF, and BMP.[13,14,24]

There were also several limitations of our study. First, biomechanical tests were not performed due to a lack of necessary equipment. Second, radiographic evaluations of bone ingrowth by micro-computed tomography were not performed. Third, in histological analysis, bias may have occurred because only two of the insertion sites were randomly selected. Moreover, it is not easy to apply DBM to arthroscopic RC repair. Despite these limitations, this is the first report on the effect of DBM on TB healing after RC repair in a rabbit model.

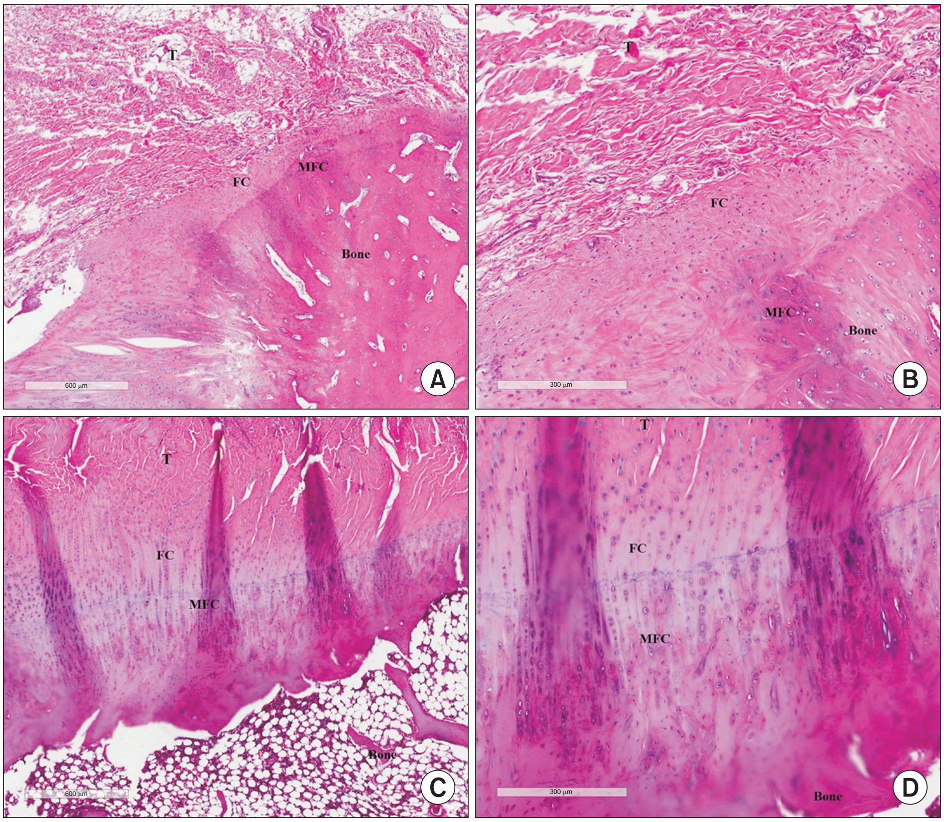

Fig. 4. Sections of specimens were cut at a thickness of 70–100 μm and stained with H&E. (A) Photomicrograph of a control group tendon showing poor organization, loosely arranged collagen fibers, and the absence of a characteristic crimp pattern. Bar = 600 μm. (B) Photomicrograph of a control group tendon showing a predominantly fibrous tendon-to-bone interface with small regions of fibrocartilage, especially mineralized fibrocartilage. Bar = 300 μm. (C) Photomicrograph of a demineralized bone matrix (DBM) group tendon showing a high level of organization, densely arranged collagen fibers, and crimped collagen fibers with elongated fibroblast nuclei. Bar = 600 μm. (D) Photomicrograph of a DBM group tendon showing that the tendon-to-bone interface comprises organized collagen fibers with large quantities of fibrocartilage and mineralized fibrocartilage. Bar = 300 μm. T: supraspinatus tendon, FC: fibrocartilage, MFC: mineralized fibrocartilage.

Table 2. Comparison of the Histological Grading System between Two Groups According to the Criteria Given in Table 1

Variable	Control	DBM	*p*-value*
Collagen fiber density	1.00 ± 0.63	1.58 ± 0.51	0.051
Collagen fiber orientation	0.91 ± 0.54	1.25 ± 0.62	0.260
Bone-tendon interface	0.82 ± 0.75	1.08 ± 0.51	0.379
Vascularity	0.82 ± 0.40	0.92 ± 0.67	0.786
Inflammation	0.82 ± 0.40	1.00 ± 0.74	0.608
Total	5.36 ± 1.03	7.83 ± 1.40	< 0.001

Values are presented as mean ± standard deviation.
DBM: demineralized bone matrix.
*Results of nonparametric test (Mann-Whitney *U*-test); $p < 0.05$ denotes statistical significance.

High fixation strength, mechanical stability, and biological healing of the TB interface are the main goals of RC repair surgery. Improvements in arthroscopic instruments and suture anchors have enabled development of stronger constructs with multiple suture configurations. However, a recent meta-analysis indicated that the development of novel surgical techniques was not related to the improvement of clinical and anatomical results from 1980 to 2012.[7] Indeed, the high retear rate after RC repair is due to inadequate TB integration, not excessive fixation strength.[25] Therefore, advances in the understanding of RC biology and biomechanics, as well as improvements in surgical techniques, have led to the development of new strategies to enhance TB interface healing.[19]

In conclusion, the use of DBM in the repair of chronic RC tears may help healing, but there was no statistical significance in gross and histologic grade in the rabbit experiment. Moreover, it was difficult to draw a solid conclusion because the sample size was too small. The use of DBM in RC repair may be considered, and further studies addressing the limitations will be needed.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Woo-Yong Lee https://orcid.org/0000-0001-8706-6026
Young-Mo Kim https://orcid.org/0000-0002-6969-4499
Deuk-Soo Hwang https://orcid.org/0000-0003-1009-3784
Hyun-Dae Shin https://orcid.org/0000-0003-4290-1125
Yong-Bum Joo https://orcid.org/0000-0003-4130-7431
Soo-Min Cha https://orcid.org/0000-0003-1663-406X
Kyung-Hee Kim https://orcid.org/0000-0003-0214-0296
Yoo-Sun Jeon https://orcid.org/0000-0003-4391-0349
Sun-Yeul Lee https://orcid.org/0000-0002-6124-5138

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