Does demineralized bone matrix enhance tendon-to-bone healing after rotator cuff repair in a rabbit model?

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Research article

Keywords: demineralized bone matrix, rotator cuff, tear, repair, tendon-to-bone healing

Posted Date: November 7th, 2019

DOI: <https://doi.org/10.21203/rs.2.16781/v1>

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Version of Record: A version of this preprint was published at Clinics in Orthopedic Surgery on January 1st, 2021. See the published version at <https://doi.org/10.4055/cios20099>.

Abstract

Background The purpose of this study was to compare the histologic outcomes after rotator cuff (RC) repair between with demineralized bone matrix (DBM) augmentation and without DBM 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). A chronic RC tear was generated on the right shoulder 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** 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. **Conclusion** DBM augmentation at the RC-to-bone interface enhances TB healing after RC repair.

Background

Rotator cuff (RC) tears comprise the majority of shoulder lesions in adult patients. The prevalence of RC tear 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 40-50%.²⁻⁴ Therefore, RC reattachment to bone following RC repair is a challenging clinical problem. Although numerous repair techniques to reduce the re-tear rate have been reported, these did not significantly reduce the re-tear 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 (PRP), 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 improves TB healing and showed that growth factors such as bone morphogenetic proteins (BMPs) improve the pull-out strength of the repair site.

Demineralized bone matrix (DBM) is an exogenous osteoinductive material that slowly releases BMPs and acts as a scaffold for many cell types.^{13,14} To date, DBM has been used for fracture unions to improve bone to bone healing. Moreover, in animal studies DBM enhances TB healing.⁹ To our knowledge, no study has used a rabbit model to assess the effectiveness of DBM augmentation on TB healing. We hypothesize that DBM augmentation may enhance TB healing after RC repair in a rabbit model.

Methods

Study design and experimental groups

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. The commercially available Dynagraft II DBM in 1 mL syringes (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. All procedures and protocols were approved by the Institutional Animal Care and Use Chungnam National University (Approval number, CNU-00791).

Surgical procedure

The rabbits were fully anesthetized with ketamine, acepromazine, and xylazine. 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 number-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, New Jersey, USA) interrupted sutures. The rabbits received 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 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 (Fig. 2A,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 and bone (Fig. 2C,D). A total of 0.3 mL of the DBM was used per rabbit. Surgical wound closure and postoperative care were performed as the initial operation.

All rabbits tolerated the surgery, with the exceptions of two rabbits in the control group and one rabbit in the DBM group, which developed postoperative deep infections. Therefore, among the 26 rabbits, 3 (two in the control and one 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 assessing the TB healing using 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 70-100 μm , and stained with hematoxylin and eosin. An independent pathologist with experience in musculoskeletal pathology in 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 two sections taken at the insertion site in each rabbit.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) ver. 19.0 (SPSS, Chicago, IL, USA) was used for data analysis. Pearson's chi-squared test for categorical variables was used to compare the control and DBM groups. Differences were considered significant at the 0.05 level.

Results

Gross TB healing

The author and 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 all 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 all DBM group specimens, the interpositional DBM had been completely remodeled. The tendon midsubstance appeared normal, and 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).

Discussion

Healing or regeneration of the musculoskeletal system requires at a minimum cells, morphogenetic signals, and matrices or scaffolds.¹⁵ Numerous biological studies have focused on enhancing bone-to-bone and/or tendon-to-tendon healing.¹⁶ However, there is no consensus on the methods of enhancing TB healing. Recently, new biological solutions to enhance TB healing have been investigated.¹⁴⁻¹⁶ The involvement of GFs, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and BMP in TB healing has been described.¹⁴⁻¹⁶ TB healing occurs through progressive mineralization of tendon through bone growth and subsequent remodeling of tissues at the TB interface by endochondral ossification.^{17,18} DBM contains exogenous proteins and exerts an osteoinductive effect.¹⁹ It also slowly releases BMP and acts as a scaffold for many cell types, suggesting it to have potential for TB healing.^{13,14} Unlike other biologic or synthetic scaffolds based on mammalian materials, DBM has no associated immunogenicity or pathogen transmission.²⁰ DBM is commercially available, approved for clinical use, and easy to use.¹⁵ Furthermore, DBM contains various GFs such as FGF, PDGF, and BMP.^{13,14,21}

This study aimed to determine the effect of DBM on TB healing after RC repair. Our results suggest that DBM augments RC-to-bone healing by increasing woven bone formation and bone remodeling. Sundar et al¹⁰ 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 reattachments. Lobric et al⁹ investigated the effects of DBM on intra-articular TB healing using a rodent model of anterior cruciate ligament reconstruction, and reported that DBM has potential for clinical use to augment TB healing. The findings of the above-mentioned studies are consistent with our results.

This study had the following strengths. Unlike other studies, we used a rabbit model because of the limitations of ovine or rodent models of RC healing. The shoulder of a quadruped animal is a weight-bearing joint, but the human shoulder is not.²² In addition, in rodent models, the portion of the supraspinatus muscle that passes under the acromial arch is muscular, and not tendinous like in human.²³ Moreover, the larger size of the rabbit enhances the accuracy of the surgical procedure. Also, the RC anatomy of the rabbit is similar to that of human, in terms of the relationship of the RC tendon with the anterior bony process.²²

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. With these limitations in mind, this is the first report of the effect of DBM on TB healing after RC repair in a rabbit model.

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 re-tear rate after RC repair is due to inadequate TB integration, not excessive fixation strength.²⁴ 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.¹⁶

Conclusion

DBM augmentation at the RC-to-bone interface enhances TB healing after RC repair. We believe that DBM augmentation could enhance TB healing in humans, which would improve the histological recovery and functional outcomes of tendon reattachment procedures.

List Of Abbreviations

RC: rotator cuff; DBM: demineralized bone matrix; TB: tendon-to-bone; GFs: growth factors; PRP: platelet-rich plasma; BMPs: bone morphogenetic proteins; SPSS: The Statistical Package for the Social Sciences; bFGF: basic fibroblast growth factor; VEGF: vascular endothelial growth factor; PDGF: platelet-derived growth factor

Declarations

Ethics approval and consent to participate

All procedures and protocols were approved by the Institutional Animal Care and Use Chungnam National University (Approval number, CNU-00791).

Consent for publication

Not applicable.

Availability of data and materials

Data and materials not indicated in this manuscript are available from the corresponding author.

Competing interests

The authors have no conflicts of interest relevant to this article.

Funding

The authors declare that they have no funding for the research.

Authors' contributions

YS Jeon and SY Lee designed all of this study. The first draft of the manuscript was written by WY Lee and YM Kim. All authors contributed to interpretation of the results and critical revision of the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

None.

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References

1. Minagawa H, Yamamoto N, Abe H, Fukuda M, Seki N, Kikuchi K, et al. Prevalence of symptomatic and asymptomatic rotator cuff tears in the general population: From mass-screening in one village. *J Orthop* 2013;10(1):8-12. <https://doi.org/10.1016/j.jor.2013.01.008>
2. Elia F, Azoulay V, Lebon J, Faraud A, Bonneville N, Mansat P. Clinical and anatomic results of surgical repair of chronic rotator cuff tears at ten-year minimum follow-up. *Int Orthop* 2017;41(6):1219-26. <https://doi.org/10.1007/s00264-017-3456-8>
3. Heuberger PR, Smolen D, Pauzenberger L, Plachel F, Salem S, Laky B, et al. Longitudinal long-term magnetic resonance imaging and clinical follow-up after single-row arthroscopic rotator cuff repair: clinical superiority of structural tendon integrity. *Am J Sports Med* 2017;45(6):1283-8. <https://doi.org/10.1177/0363546517689873>
4. Barnes LA, Kim HM, Caldwell JM, Buza J, Ahmad CS, Bigliani LU. Satisfaction, function and repair integrity after arthroscopic versus mini-open rotator cuff repair. *Bone Joint J* 2017;99-B(2):245-9. <https://doi.org/10.1302/0301-620X.99B2.BJJ-2016-0055.R1>
5. Kim KC, Shin HD, Lee WY, Han SC. Repair integrity and functional outcome after arthroscopic rotator cuff repair: double-row versus suture-bridge technique. *Am J Sports Med* 2012;40(2):294-9. <https://doi.org/10.1177/0363546511425657>
6. Mascarenhas R, Chalmers PN, Sayegh ET, Bhandari M, Verma NN, Cole BJ, et al. Is double-row rotator cuff repair clinically superior to single-row rotator cuff repair: a systematic review of overlapping

- meta analyses. *Arthroscopy* 2014;30(9):1156-65. <https://doi.org/10.1016/j.arthro.2014.03.015>
7. McElvany MD, McGoldrick E, Gee AO, Neradilek MB, Matsen FA 3rd. Rotator cuff repair: published evidence on factors associated with repair integrity and clinical outcome. *Am J Sports Med* 2015;43(2):491-500. <https://doi.org/10.1177/0363546514529644>
 8. Beitzel K, Solovyova O, Cote MP, Apostolakos J, Russell RP, McCarthy MB, et al. The future role of mesenchymal stem cells in the management of shoulder disorders. *Arthroscopy* 2013;29(10):1702-11. <https://doi.org/10.1016/j.arthro.2013.06.014>
 9. Lovric V, Chen D, Yu Y, Oliver RA, Genin F, Walsh WR. Effects of demineralized bone matrix on tendon bone healing in an intra-articular rodent model. *Am J Sports Med* 2012;40(10):2365-74. <https://doi.org/10.1177/0363546512457648>
 10. Sundar S, Pendegrass CJ, Blunn GW. Tendon bone healing can be enhanced by demineralized bone matrix: a functional and histological study. *J Biomed Mater Res B Appl Biomater* 2009;88(1):115-22. <https://doi.org/10.1002/jbm.b.31157>
 11. Rodeo SA, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF. Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. *J Bone Joint Surg Am* 1993;75(12):1795-803. <https://doi.org/10.2106/00004623-199312000-00009>
 12. Rodeo SA, Suzuki K, Deng XH, Wozney J, Warren RF. Use of recombinant human bone morphogenetic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med* 1999;27(4):476-88. <https://doi.org/10.1177/03635465990270041201>
 13. Peel SA, Hu ZM, Clokie CM. In search of the ideal bone morphogenetic protein delivery system: in vitro studies on demineralized bone matrix, purified, and recombinant bone morphogenetic protein. *J Craniofac Surg* 2003;14(3):284-91. <https://doi.org/10.1097/00001665-200305000-00005>
 14. Pietrzak WS, Dow M, Gomez J, Soulvie M, Tsiagalis G. The in vitro elution of BMP-7 from demineralized bone matrix. *Cell Tissue Bank* 2012;13(4):653-61. <https://doi.org/10.1007/s10561-011-9286-9>
 15. Evans CH. Advances in regenerative orthopedics. *Mayo Clin Proc* 2013;88(11):1323-39. <https://doi.org/10.1016/j.mayocp.2013.04.027>
 16. Lorbach O, Baums MH, Kostuj T, Pauly S, Scheibel M, Carr A, et al. Advances in biology and mechanics of rotator cuff repair. *Knee Surg Sports Traumatol Arthrosc* 2015;23(2):530-41. <https://doi.org/10.1007/s00167-014-3487-2>
 17. Liu SH, Panossian V, al-Shaikh R, Tomin E, Shepherd E, Finerman GA, et al. Morphology and matrix composition during early tendon to bone healing. *Clin Orthop Relat Res* 1997;339:253-60. <https://doi.org/10.1097/00003086-199706000-00034>
 18. Thomopoulos S, Genin GM, Galatz LM. The development and morphogenesis of the tendon-to-bone insertion: what development can teach us about healing. *J Musculoskelet Neuronal Interact* 2010;10(1):35-45.
 19. Van de Putte KA, Urist MR. Osteogenesis in the interior of intramuscular implants of decalcified bone matrix. *Clin Orthop Relat Res* 1965;43:257-70. <https://doi.org/10.1097/00003086-196500430-00026>

20. Chen J, Xu J, Wang A, Zheng M. Scaffolds for tendon and ligament repair: review of the efficacy of commercial products. *Expert Rev Med Devices* 2009;6(1):61-73.
<https://doi.org/10.1586/17434440.6.1.61>
21. Zhou S, Yates KE, Eid K, Glowacki J. Demineralized bone promotes chondrocyte or osteoblast differentiation of human marrow stromal cells cultured in collagen sponges. *Cell Tissue Bank* 2005;6(1):33-44. <https://doi.org/10.1007/s10561-005-4253-y>
22. Grumet RC, Hadley S, Diltz MV, Lee TQ, Gupta R. Development of a new model for rotator cuff pathology: the rabbit subscapularis muscle. *Acta Orthop* 2009;80(1):97-103.
<https://doi.org/10.1080/17453670902807425>
23. Schneeberger AG, Nyffeler RW, Gerber C. Structural changes of the rotator cuff caused by experimental subacromial impingement in the rat. *J Shoulder Elbow Surg* 1998;7(4):375-80.
24. Edelstein L, Thomas SJ, Soslowsky LJ. Rotator cuff tears: what have we learned from animal models? *J Musculoskelet Neuronal Interact* 2011;11(2):150-62.

Figures

Figure 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 number-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.

Figure 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.

Figure 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 DBM group.

Figure 4

Supplementary Files

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