Systematic Review

Inlay Scaffold Augmentation of Rotator Cuff Repairs Enhances Histologic Resemblance to Native Enthesis in Animal Studies: A Systematic Review

Juan Bernardo Villarreal-Espinosa, M.D., Rodrigo Saad Berreta, B.A., Stephanie A. Boden, M.D., Zeeshan A. Khan, B.A., Andrew J. Carter, Ph.D., Brian J. Cole, M.D., M.B.A., and Nikhil N. Verma, M.D.

Purpose: To investigate the outcomes of inlay positioned scaffolds for rotator cuff healing and regeneration of the native enthesis after augmentation of rotator cuff tendon repairs in preclinical studies. **Methods:** A literature search was performed using the PubMed, Embase, and Cumulative Index to Nursing and Allied Health Literature databases according to Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. Preclinical studies reporting on outcomes after inlay tendon augmentation in rotator cuff repair were included. Preclinical study quality was assessed using an adapted version of the Gold Standard Publication Checklist for animal studies. The level of evidence was defined based on the inclusion of clinical analyses (grade A), biomechanical analyses (grade B), biochemical analyses (grade C), semiquantitative analyses (grade D), and qualitative histologic analyses (grade E). **Results:** Thirteen preclinical studies met the inclusion criteria. Quality assessment scores ranged from 4 to 8 points, and level-of-evidence grades ranged from B to E. Sheep/ewes were the main animal rotator cuff tear model used (n = 7). Demineralized bone matrix or demineralized cortical bone was the most commonly investigated scaffold (n = 6). Most of the preclinical evidence (n = 10) showed qualitative or quantitative differences regarding histologic, biomechanical, and biochemical outcomes in favor of interpositional scaffold augmentation of cuff repairs in comparison to controls. **Conclusions:** Inlay scaffold positioning in preclinical studies has been shown to enhance the healing biology of the enthesis while providing histologic similarities to its native 4-zone configuration. **Clinical Relevance:** Although onlay positioned grafts and scaffolds have shown mixed results in preclinical and early clinical studies, inlay scaffolds may provide enhanced healing and structural support in comparison owing to the ability to integrate with the bone-tendon interface.

The rotator cuff tendons work synergistically to provide stability to the humeral head and facilitate shoulder strength and range of motion.[1-4] Rotator cuff tears have an estimated prevalence of 20% in the general population, with an increasing prevalence in each decade of life after age 50 years.[1,4] Thereby, symptomatic rotator cuff tears represent a major cause of shoulder pain and functional impairment.[1,4] Alterations in glenohumeral biomechanics after rotator cuff tears eventually result in secondary cartilage degeneration and arthritis, also known as "rotator cuff tear arthropathy."[1,4] A delay in progression to cuff tear arthropathy has been shown after restoration of normal shoulder joint kinematics, thereby highlighting the importance of proper rotator cuff tear management.[1,4,5]

The armamentarium for the management of rotator cuff tears prior to the development of cuff tear arthropathy is dependent on various factors, including tear extent and retraction, as well as tissue quality.[6,7] Rotator cuff repairs are commonly performed for mechanically repairable tears. Although shoulder function and pain can be reliably restored through successful surgical repairs, rates of postoperative structural failures of up to 50% have been reported in long-term studies and may be largely attributable to biological insufficiency.[8] Newer augmentation methods, including scaffolds and patches, have been developed in attempts to decrease rates of tendon rerupture after rotator cuff

From the Department of Orthopaedics, Rush University Medical Center, Chicago, Illinois, U.S.A. (J.B.V-E., R.S.B., S.A.B., B.J.C., N.N.V.); Rush Medical College, Chicago, Illinois, U.S.A. (Z.A.K.); and Prince of Wales Clinical School, UNSW Medicine at the University of New South Wales, Sydney, Australia (A.J.C.).

Received April 1, 2024; accepted June 25, 2024.

Address correspondence to Nikhil N. Verma, M.D., Department of Orthopaedics, Rush University Medical Center, 1620 W Harrison St, Chicago IL 60612, U.S.A. E-mail: Nikhil.verma@rushortho.com

0749-8063/24500/$36.00
https://doi.org/10.1016/j.arthro.2024.06.048

repair.[1,5,9-11] However, most repairs are positioned on top of the tendon (onlay configuration) and therefore fail to re-create or enhance the native enthesis at the bone-tendon interface, which is the location at which most repairs have been shown to fail.[1,5,9-11]

The native enthesis of the rotator cuff transitions from tendon to fibrocartilage to mineralized fibrocartilage and subsequently bone, and it is integral to the transmission of loads and consequent functionality of the rotator cuff tendons.[1,12] Current repair augmentation techniques using onlay scaffolds have not been shown to effectively restore native enthesis anatomy.[13] The purpose of this systematic review was to investigate the outcomes of inlay positioned scaffolds for rotator cuff healing and regeneration of the native enthesis after augmentation of rotator cuff tendon repairs in preclinical studies. Our hypothesis was that similar enthesis morphology would be achieved in animal studies as the scaffold would contribute to in situ native enthesis regeneration.

Methods

Search Strategy

Three databases (PubMed, EMBASE, and Cumulative Index to Nursing and Allied Health Literature) were queried from inception to February 2024 by 2 reviewers (J.B.V.E., R.S.B.) in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines.[14] The search was performed using the following strategy and terms: "(demineralized bone matrix or demineralized cortical bone) AND (tissue scaffold or tissue engineering or ligament or tendon)" and "((demineralized bone matrix or demineralized cortical bone) AND (tissue scaffold or tissue engineering or ligament or tendon)) AND (rotator cuff)." The included articles' references were also evaluated for inclusion of potentially relevant studies that were missed by the aforementioned search strategy.

All included studies assessed histologic, biomechanical, biochemical, or other outcomes after inlay (interposition between tendon and bone) scaffold augmentation of rotator cuff tear models in the preclinical setting. Additionally, articles had to be written in English or Spanish to meet the predetermined inclusion criteria. The exclusion criteria included clinical studies; non-inlay positioning of scaffold; non—rotator cuff tear models; case series, systematic reviews, case reports, or cadaveric studies; non-English- or Spanish-language studies; and studies for which no full text was available.

Data Extraction

Abstract, title, and subsequent full-text screening of studies retrieved by the initial search was performed by 2 reviewers (J.B.V.E., R.S.B.) with consideration of the predetermined inclusion criteria. A third reviewer (Z.A.K.) resolved any disagreements, and consensus was established prior to moving forward. Data were extracted and collected in a Microsoft Excel spreadsheet (2007 version; Redmond, WA). Study characteristics of preclinical studies included authors, animal model used, scaffold used, methods used, follow-up length, and findings.

Quality Assessment

Assessment of methodologic and study quality, in addition to the level of evidence of the yielded animal studies, was performed by 2 reviewers (J.B.V.E., R.S.B.) using the Gold Standard Publication Checklist, which has been used and modified by previous in vivo animal studies as shown in Tables 1 and 2.[15-17] The level of evidence was defined based on the inclusion of clinical analyses (grade A), biomechanical analyses (grade B), biochemical analyses (grade C), semiquantitative

Table 1. Assessment Criteria for Methodologic Quality of Animal Studies of Rotator Cuff Tendon-Bone Healing*

Criterion	Scores	Comments
Unit of sample	Unilateral: 1 Bilateral: 0	"Studies with bilateral surgeries may regard limbs as independent samples and assign them to different treatment groups"
Standardization of surgical procedure	Yes: 1 No: 0	"Descriptions about graft harvest, surgical approach, drilling tunnels, graft tensioning, and fixation method are important"
Description of surgical complications	Yes: 1 No: 0	"Details such as wound infection and postoperative morbidity and mortality"
Biomechanical testing	Yes: 1 No: 0	"Mechanical testing is a useful outcome when assessing tendon-bone healing"
Variation (ratio of SD to mean)	<50%: 1 >50%: 0	"Large SD may imply poor precision or large intragroup variations"
Statistical method and control group	Appropriate: 1 Inappropriate: 0	"Appropriate statistical tests were used"
Description of tendon-bone interface	Yes: 1 No: 0	"During histological analysis, sampling description for region of interest is important"
Semiquantitative histologic analysis	Yes, 1 No, 0	"During histological analysis, the use of scoring systems indicates better study quality"

SD, standard deviation.
*Adapted by Hexter et al.[16].

Table 2. Clinical Relevance of Outcome Measures Used in Animal Studies*

Evidence Level	Definition
A	Clinically useful quantitative outcome measures
B	Biomechanical testing as quantitative outcomes
C	Biochemical measurement (i.e., immunohistochemistry) as quantitative outcome measures
D	Semiquantitative histologic and/or imaging assessment
E	Qualitative or nonquantitative histologic and/or imaging assessment

*Adapted by Fu et al.[15] and Hexter et al.[16]

histologic and/or imaging analyses (grade D), and qualitative histologic and/or imaging analyses (grade E).[15] The highest letter grade stood as the level of evidence of each included study irrespective of the inclusion of additional lower-evidence analyses.

Results

Of the 670 screened studies, 13 met the inclusion criteria and were included in this systematic review[18-30] (Fig 1). Ten of the retrieved studies comprised all female animal cohorts,[18-20,22,24-27,29,30] whereas 2 included solely male animals[21,23] and 1 did not

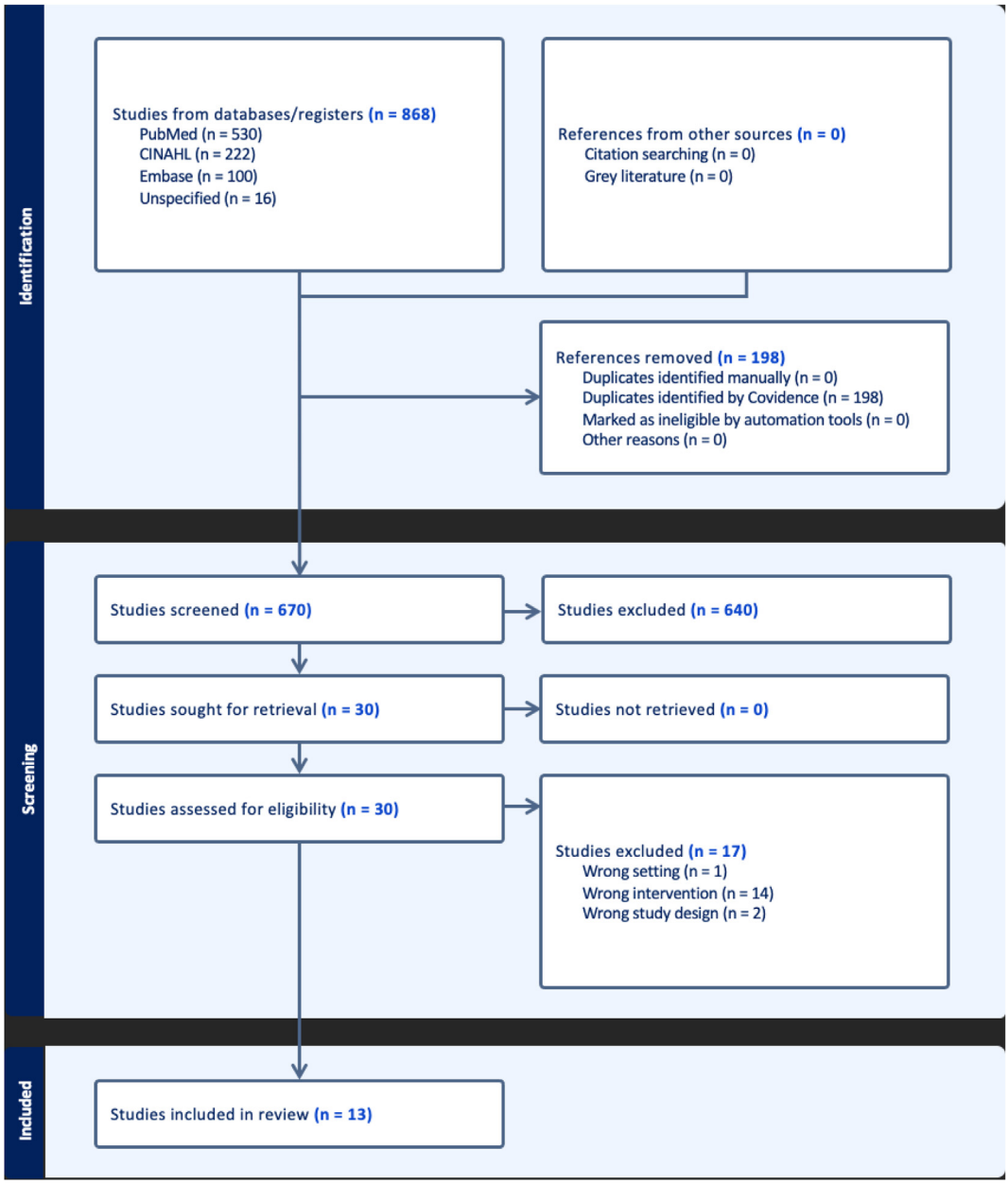

Fig 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowchart. (CINAHL, Cumulative Index to Nursing and Allied Health Literature.)

differentiate between sexes.[28] Demineralized bone matrix (DBM) or demineralized cortical/cancellous bone (DCB) was the most consistently used scaffold in the preclinical setting (6 studies),[19,21,23,28-30] followed by synthetic scaffolds, including poly-lactide-co-glycoside (PLGA) anchors[20] (1 study), biphasic allografts[18] (1 study), bipolar poly-L-lactic acid (PLLA) scaffold[24] (1 study), biphasic polyglycolic acid (PGA)/poly-L-lactide-co-caprolactone (PLCL) scaffold[27] (1 study), tissue-engineered scaffold[25] (1 study), and type I collagen scaffold[22,26] (2 studies). The most important findings of the preclinical studies are summarized in Table 3.

The median follow-up of in vivo studies was 12 weeks (range, 3-26 weeks). Eight investigations used an acute rotator cuff tear model,[19-22,24-27] whereas 5 simulated a chronic rotator cuff tear by detaching the rotator cuff and then repairing it weeks apart in a 2-stage fashion.[18,23,28-30] Additionally, sheep/ewes were the most frequent animal model (7 studies),[18-20,22,25-27] followed by rabbits (3 studies),[21,23,24] rats (2 studies),[29,30] and dogs (1 study).[28]

Quality Assessment

The highest level-of-evidence grade given was B, categorizing 8 of the yielded studies.[20-22,24-28] Within these 8 reports, quality scores ranged from 6 to 8 points. In addition, 4 investigations were given a grade D evidence level while having quality scores of 6 or 7 points.[18,23,29,30] Finally, 1 study was found to have solely a grade E evidence level because it only reported histologic findings in a qualitative manner.[19] This study was given a quality score of 4 points. To summarize these findings, quality scores of animal investigations ranged from 4 to 8 points, whereas level-of-evidence grades ranged from B to E, with no study reaching grade A.

Measured Outcomes and Determination of Positive and/or Negative Findings

Findings were considered enhanced or positive if they were regarded as superior to controls or baseline in a semiquantitative or qualitative manner for animal studies as reported in previous investigations.[15,16] Six studies were regarded as reporting completely positive findings among their reported outcomes,[19-23,28] whereas 4 in vivo investigations were categorized as reporting mixed findings[24-27] and 3 reported no positive findings within their measured outcomes.[18,29,30]

DBM or DCB Scaffolds

Two of the included in vivo studies incorporated biomechanical data (level-of-evidence grade B),[21,28] with both investigations reporting positive biomechanical differences, including significantly higher load to failure compared with controls. Moreover, biochemical evaluation (level-of-evidence grade C) was included in only one of the DBM/DCB studies, with positive immunohistochemical findings in the experimental group including higher bone volume, recruitment of stromal cells, and expression of bone morphogenetic protein 2.[21] Furthermore, 5 preclinical investigations conducted semiquantitative histologic analysis (level-of-evidence grade D) of the repaired tendons,[21,23,28-30] with 3 of 5 studies observing positive histologic differences, evidence of enthesis regeneration, and/or higher histologic grades while using scoring systems in contrast to controls.[21,23,28]

One of the 6 included DBM/DCB scaffold preclinical studies incorporated qualitative histologic data (level-of-evidence grade E) in its analysis.[19] This study found evidence of a normal tendon-bone interface in the DCB cohort, which was not present in the control group.

Synthetic Scaffolds

Four of the included synthetic scaffolds (anchor with PLGA graft, bipolar PLLA membrane, tissue-engineered construct, and biphasic PGA/PLCL) performed biomechanical analyses (level-of-evidence grade B) of the repairs,[20,24,25,27] with 2 revealing biomechanical superiority regarding controls, such as increased ultimate failure load and construct stiffness.[20,24] However, only 2 investigations using suture anchors with PLGA[20] and a tissue-engineered graft[25] incorporated biochemical analyses (level-of-evidence grade C) while presenting differing results. Novakova et al.[25] did not find any significant differences in fiber composition or cross-sectional area between cohorts, whereas Easley et al.[20] found enhanced immunohistochemical evidence in their experimental arm while using suture anchors with a PLGA scaffold.

Regarding semiquantitative histologic analyses (level-of-evidence grade D), 4 animal studies (anchor with PLGA graft, bipolar PLLA membrane, tissue-engineered graft, and biphasic PGA/PLCL) reported enhanced objective histologic findings using predefined grading criteria assessing for enthesis and tissue maturation on comparison of experimental and control cohorts.[20,24,25,27] One additional synthetic scaffold investigation (biphasic allograft) found no difference in enthesis maturation or histologic scores between treatment arms.[18] Additionally, Romeo et al.[27] reported a positive native enthesis resemblance in the experimental group as part of their qualitative histologic analysis (level-of-evidence grade E).

Collagen Scaffolds

Hee et al.[22] and Rodeo et al.[26] both used scaffolds composed of type I collagen as their augmentation strategies. On assessment of biomechanical properties (level-of-evidence grade B), one investigation reported that a higher load to failure was observed in the

Table 3. Summary of Preclinical Studies

Study	Animal Model	RC Tear Model	Scaffold Used	Groups	Follow-Up	Methods	Findings	Enhanced Findings vs Controls*	Quality Score	Evidence Level
Dickerson et al.,[19] 2013	3 ewes	Acute supraspinatus/ infraspinatus tear model	DCB/allogeneic/ transitional or demineralization gradient	DCB (n = 6) and control (n = 6)	16 wk	Histology of tendon repairs	Evidence of normal tendon-bone interface only in experimental samples	Yes	4	E
He et al.,[21] 2021	24 New Zealand white male rabbits	Acute infraspinatus tear model	DCB/allograft/ transitional or demineralization gradient	Control (n = 6), DCB (n = 6), hDCB (n = 6), and hDCB-ECM (bone, pDCB, DCB-ECM, TDSC-derived ECM) (n = 6)	12 wk	(1) MicroCT and IHC staining (2) Biomechanical analysis (3) Histology of tendon repairs	(1) Significantly higher bone volume, recruitment of stromal cells, and presence of BMP-2 at bony trough (indicative of new bone formation) in hDCB-ECM and hDCB groups (2) hDCB-ECM, hDCB, and DCB showed significantly greater ultimate tensile stress (3) New fibrocartilage tissues found at tendon-bone interface in DCB, hDCB, and hDCB-ECM groups, with significantly larger metachromasia area in hDCB-ECM group	(1) Yes (2) Yes (3) Yes	7	B, C, and D
Lee et al.,[23] 2021	26 New Zealand white male rabbits	Chronic supraspinatus tear model	DBM	Control (n = 13) and DBM (n = 13)	8 wk after RC repair surgery	Histologic of tendon repairs	Significant difference in histologic morphology favoring DBM group	Yes	7	D

(continued)

Table 3. Continued

Study	Animal Model	RC Tear Model	Scaffold Used	Groups	Follow-Up	Methods	Findings	Enhanced Findings vs Controls*	Quality Score	Evidence Level
Smith et al.,[28] 2018	10 purpose-bred dogs	Chronic supraspinatus tear model	DBM sponge soaked in LP-PRP	Control (n = 10) and DBM-PRP (n = 10)	12 wk after RC repair surgery	(1) MRI (2) Biomechanical analysis (3) Histology of tendon repairs	(1) DBM-PRP group rated significantly higher on assessment of proximal humerus, tendon-bone junction, and tendon/muscle status (2) Ultimate failure load, stiffness, and load for 5- to 15 mm of displacement significantly greater in DBM-PRP group (3) Significantly higher rating for DBM-PRP group pertaining to bone-tendon junction	(1) Yes (2) Yes (3) Yes	7	B and D
Thangarajah et al.,[29] 2017	18 female Wistar rats	Chronic supraspinatus tear model	DBM from rat tibia/allogeneic	Control (n = 6), acellular dermal allograft (n = 6), and DBM (n = 6)	3 wk after RC repair surgery	(1) pqCT for assessment of BMD (2) Histology of tendon repairs	(1) No superiority of BMD of augmented repairs (2) No significant differences found in enthesis maturation and tendon degeneration scores	(1) No (2) No	6	D
Thangarajah et al.,[30] 2018	18 female Wistar rats	Chronic supraspinatus tear model	DBM + MSCs from rat tibia/allogeneic	Control + MSCs (n = 6), acellular dermal allograft + MSCs (n = 6), and DBM + MSCs (n = 6)	3 wk after RC repair surgery	(1) pqCT for assessment of BMD (2) Histology of tendon repairs	(1) Bone mineral density not significantly higher in group receiving DBM + MSCs (2) No significant differences found in enthesis maturation and tendon degeneration scores	(1) No (2) No	6	D

(continued)

Table 3. Continued

Study	Animal Model	RC Tear Model	Scaffold Used	Groups	Follow-Up	Methods	Findings	Enhanced Findings vs Controls*	Quality Score	Evidence Level
Easley et al.,[20] 2020	56 skeletally mature female Columbia cross sheep	Acute infraspinatus tear model	Vented SA with PLGA scaffold	Control (n = 28) and PLGA-augmented SA (n = 28)	7 or 12 wk	(1) Biomechanical analysis (2) Histology of tendon repairs and IHC	(1,2) Significant, positive correlation in experimental group between increased failure loads and tendon-bone integration and type III collagen formation	(1,2) Yes	8	B, C, and D
Hee et al.,[22] 2011	60 skeletally mature Columbia cross ewes	Acute infraspinatus tear model	Type I bovine collagen matrix + rhPDGF-BB	Control (n = 12) and suture + collagen matrix scaffold with 0, 75, 150, and 500 μg of rhPDGF-BB (n = 12, n = 12, n = 12, and n = 12, respectively)	12 wk	(1) Biomechanical analysis (2) Histology of tendon repairs	(1) Collagen matrix scaffold augmented with 75 and 150 μg of rhPDGF-BB resulted in significantly higher load to failure, stiffness, and elongation (2) Bone-tendon interface increased in 75- and 150-μg rhPDGF-BB groups	(1) Yes (2) Yes	8	B and D
Li et al.,[24] 2017	144 mature New Zealand white female rabbits	Acute supraspinatus tear model	BFM of PLLA fibrous membrane (upper layer) and nHA-PLLA fibrous membrane (lower layer), as well as SFM of PLLA	Control (n = 48), SFM (n = 48), and BFM (n = 48)	4, 8, and 12 wk	(1) MicroCT assessing for TMD and BMD (2) Biomechanical analysis (3) Histology of tendon repairs	(1) BFM group TMD values significantly larger; no significant difference in BMD among all groups (2) BFM had highest load to failure, stiffness, and ultimate stress to failure scores (significantly greater) (3) BFM and SFM metachromasia significantly higher than control at 12 wk; both SFM and BFM had significantly higher maturation than control at 12 wk	(1) Partially (2) Yes (3) Yes	7	B and D

(continued)

Table 3. Continued

Study	Animal Model	RC Tear Model	Scaffold Used	Groups	Follow-Up	Methods	Findings	Enhanced Findings vs Controls*	Quality Score	Evidence Level
Novakova et al.,[25] 2018	23 female black Suffolk sheep	Acute infraspinatus tear model	ETG-RC	ETG-RC (n = 12), suture-only repair (n = 11), and contralateral shoulder (n = 23)	26 wk	(1) Radiographs to assess health of bone and IHC analysis (2) Biomechanical analysis (3) Histology of tendon repairs	(1) ETG-RC group showed no significant difference from respective contralateral tendons (2) No difference in tangent modulus between groups (3) ETG shoulders had significantly greater collagen alignment and ETG-RC repair enthesis was composed of graded zones that resembled native enthesis	(1) No (2) N**o** (3) Yes	7	B, C, and D
Credille et al.,[18] 2023	30 skeletally mature female Rambouillet cross sheep	Chronic infraspinatus tear model	Biphasic allograft	Controls (n = 15) and biphasic allograft (n = 15)	3 wk, 6 wk, and 12 wk	Histology of tendon repairs	No significant differences detected for any histologic characteristic between treatment and control groups	No	6	D
Rodeo et al.,[26] 2007	72 female Rambouillet × Columbia sheep	Acute infraspinatus tear model	1.0 mg of osteoinductive bone protein extract (Growth Factor Mixture [GFM]; Sulzer Biologics, Wheat Ridge, CO) on type I collagen sponge carrier	Sponge carrier with GFM (n = 24), collagen sponge carrier with no growth factors (n = 24), and tendon repair with no implant (n = 24)	6 wk and 12 wk	(1) Radiographs/MRI for assessment of new bone formation (2) Biomechanical analysis (3) Histology of tendon repairs	(1) Imaging showed significantly higher new bone and soft-tissue formation in GFM cohort (2) Ultimate load to failure significantly higher in GFM cohort and collagen control group was significantly stiffer than GFM cohort (3) More robust fibrocartilage formation in tendon-bone gap in GFM cohort but insertion site did not resemble native enthesis	(1) Yes (2) Partially (3) Partially	6	B, D, and E

(continued)

Table 3. Continued

Study	Animal Model	RC Tear Model	Scaffold Used	Groups	Follow-Up	Methods	Findings	Enhanced Findings vs Controls*	Quality Score	Evidence Level
Romeo et al.,[27] 2022	40 female Columbia cross sheep (*Ovis aries*)	Acute infraspinatus tear model	Nanofiber scaffold composed of bioabsorbable biphasic PGA and PLCL polymer	Control (n = 20) and augmentation with nanofiber scaffold device (n = 20)	6 wk and 12 wk	(1) Biomechanical analysis (2) Histology of tendon repairs	(1) Augmented group had higher ultimate failure load, stiffness, Young modulus, and ultimate failure stress between groups (2) Scaffold-treated group displayed insertion that was beginning to be organized similar to "native" enthesis	(1) No (2) Yes	7	B, D, and E

<TAB-FN>BFM, bipolar fibrous membrane; BMD, bone mineral density; BMP-2, bone morphogenetic protein 2; DBM, demineralized bone matrix; DCB, demineralized cancellous/cortical bone; ECM, extracellular matrix; ETG-RC, engineered tissue graft for rotator cuff; hDCB, hierarchically demineralized cortical bone; IHC, immunohistochemistry; LP, leukocyte poor; MicroCT, micro computed tomography; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; nHA-PLLA, nano hydroxyapatite-poly-L-lactic acid; pDCB, partial demineralized cortical bone; PGA, polyglycolic acid; PLCL, poly-L-lactide-co-caprolactone; PLGA, poly-lactide-co-glycoside; PLLA, poly-L-lactic acid; pqCT, peripheral quantitative computed tomography; PRP, platelet-rich plasma; RC, rotator cuff; rhPDGF-BB, recombinant human platelet-derived growth factor; SA, suture anchor; SFM, single fibrous membrane; TDSC, tendon-derived stem cell; TMD, tissue mineral density.

*"Yes" indicates that a statistically significant difference was observed, whereas "no" denotes that no statistically significant difference was observed.

treatment arm[22] whereas the other did not observe differences between groups.[26] Both studies also incorporated semiquantitative histologic analysis (level-of-evidence grade D) and reported enhanced objective histologic findings such as more robust fibrocartilage and bone-tendon interface within the experimental arm. Additionally, only the investigation by Rodeo et al. included qualitative histologic analysis (level-of-evidence grade E) in which a more robust fibrocartilage zone was observed within the treatment arm.

Discussion

In this systematic review, inlay positioned scaffolds were shown to consistently enhance the healing biology of the native enthesis while providing histologic resemblance to its native 4-zone configuration in the preclinical setting. Among the yielded in vivo animal studies, DBM and demineralized cortical bone were the most consistently studied scaffolds showing promising results. Although the included preclinical studies reported histologic advantages for DBM over controls, these results require further validation given the low level of evidence associated with in vivo studies and the lack of robust clinical reports.

Despite advances in surgical techniques for rotator cuff repair, high retear rates remain, which have been associated with worse clinical outcomes, poor patient satisfaction, increased pain, higher rates of arthritis, and worse functional outcomes, highlighting the importance of rotator cuff repair healing.[31] The available preclinical evidence suggests that biological factors such as scaffolds may be vital for the regeneration of a fibrocartilaginous enthesis, which enhances the structural integrity of the repair.[9,10,32-35] Given the potential negative impacts of retear or failure to heal after rotator cuff repair, scaffold or patch augmentation presents an intriguing option as a possible cost-effective adjunct to enhance healing and reduce retear rates.

After traditional rotator cuff repair, biomechanically inferior fibrovascular tissue is formed at the bone-tendon interface, which differs from the native fibrocartilaginous enthesis.[16] Biological tissue scaffolds have gained popularity as an augmentation to reinforce rotator cuff repair by providing structural support at the suture-tendon interface during the early healing stages, as well as in an attempt to improve healing by providing additional collagen to the repair site. Despite the theoretical benefit of biological tissue scaffolds, there is a lack of commercially available biological scaffold products for use in rotator cuff enthesis repair, and most options involve augmentation via onlay of a scaffold or graft over the repaired tendon.[10] The majority of studies, to date, have therefore focused on onlay positioning of scaffolds, with a relative paucity of both preclinical and clinical studies evaluating inlay scaffold augmentation.[10] To exemplify this, whereas only 13 studies met the inclusion criteria for our review, a recent systematic review of onlay grafts and scaffolds included 62 studies (47 preclinical and 15 clinical).[10]

The quality of the tendon-bone repair interface has been shown to most significantly impact the mechanical properties of a repaired rotator cuff tendon.[36] Although onlay grafts and scaffolds have shown mixed results in preclinical and early clinical studies, inlay scaffolds may provide enhanced healing and structural support in comparison owing to the ability to integrate with the bone-tendon interface. Although the goals of this study were not to evaluate clinical studies reporting on inlay positioning of augmentation strategies, to our knowledge, only 2 clinical studies have been published to date. Seetharam et al.[11] published a case series of 33 patients who underwent rotator cuff repair with augmentation using an inlay nanofiber resorbable scaffold. They reported an overall failure rate of 9% (3 of 33 patients), with only 3% of failures (1 of 33 patients) occurring at the tendon-graft interface. They also noted evidence of complete scaffold resorption on magnetic resonance imaging (MRI) and significant improvements in American Shoulder and Elbow Surgeons scores, Simple Shoulder Test scores, and range of motion in the included patients. Both failure rates were lower than what is established in the current literature for onlay biological augmentation (11.8%), although no direct comparison studies exist, so whether this benefit arises exclusively from inlay scaffold augmentation cannot be stated conclusively.[10] Similarly, Krupp et al.[37] presented a cohort study of 71 patients who underwent rotator cuff repair for moderate to large rotator cuff tears with an interpositional scaffold suture anchor; they found retears at the repaired tendon-bone interface in only 3 of 52 patients (5.8%) on 6-month postoperative MRI scans. However, it should be noted that they found an overall retear rate of 30.8% when including tears that occurred medial to the footprint, suggesting that improved healing at the tendon-bone interface may not change overall retear rates.

DBM and DCB have been used to provide biological enhancement of the bone-tendon interface in the knee.[38-41] The increased porosity and extracellular matrix components (mainly type I collagen and growth factors such as bone morphogenetic proteins) in DBM provide osteoinductive properties allowing for regeneration of the native bone-tendon interface via endochondral ossification, which is the same method through which the enthesis is formed in embryos.[19,38,42] In our review, 4 of the 6 preclinical studies using either DBM or DCB reported enhanced histology, biomechanical features, and bone mineral density at the repaired enthesis in comparison to controls.[19,21,23,28] These studies specifically reported the histologic presence of a transitional zone replicating the native enthesis in the experimental groups. The ability

to engender a histologically native enthesis with 4 distinct tissue zones with different cellular compositions, mechanical properties, and functions is an exciting development in tendon repair, with significant implications for enthesis healing and integrity. However, 2 of the included studies using DBM as a scaffold did not corroborate these findings and reported no histologic or bone mineral density benefit of DBM over controls.[29,30] None of the aforementioned clinical studies used a DBM or DCB scaffold, opting instead for PLGA fibers simulating the native tendon collagen orientation[37] and a microporous nanofiber scaffold.[11]

A variety of different inlay positioned scaffolds were used in the remaining included in vivo studies. Two studies used a type I collagen scaffold that was augmented with exogenously produced growth factors, providing a similar mechanism of action to that of DBM/DCB scaffolds.[22,26] Both of these investigations reported biomechanical advantages in the augmented repairs, particularly a higher load to failure and increased fibrocartilage formation. These findings were in line with the DBM/DCB results, in which 4 of 6 studies found a histologic benefit and 2 studies incorporating biomechanical data noted structural advantages for DBM/DCB. Investigations using biphasic[18,24,27] and tissue-engineered[25] scaffolds delivered mixed biomechanical and bone density assessment results. However, the discrepancies in findings may be the result of heterogeneity among scaffold components. Nonetheless, all but one study[18] agreed that each experimental group possessed a greater histologic resemblance to the native enthesis in comparison with their respective controls.

Incorporation of scaffolds into contemporary rotator cuff repair methods via suture anchors in which the scaffold is positioned in an inlay orientation has also shown favorable results in preclinical and early clinical settings.[11,20,37] Easley et al.[20] observed a significant positive correlation between increased failure loads and scaffold integration in a sheep rotator cuff model. In addition, Krupp et al.[37] evaluated clinical results after inlay implant augmentation using an interpositional scaffold suture anchor for moderate to large rotator cuff tears and revealed a 97% rate of anchor survival at 1 year, which correlated with minimal clinically important difference threshold achievement of 80% for the American Shoulder and Elbow Surgeons Standardized Form, Veterans RAND 12-Item Health Survey (VR-12), and visual analog scale score. Moreover, as mentioned earlier, retearing at the repaired footprint was noted in only 3 of 52 patients (5.8%) on 6-month postoperative MRI scans.

Moving forward, any techniques for tendon healing augmentation in rotator cuff repair must be considered in the context of cost, insertion technique, and outcomes. For any technique to be clinically viable, it must be technically feasible for broad application, it must be cost-effective in the setting of our current economic health care environment, and most importantly, it must be correlated with improvements in clinical outcomes. Nevertheless, high-quality preclinical and clinical studies are needed to elucidate the optimal composition and position of biological scaffolds for augmentation in rotator cuff repairs, to determine whether preclinical success translates to improved patient outcomes, and to identify which patients may benefit from rotator cuff repair with augmentation.

Limitations

This study should be considered in the context of certain limitations. As a systematic review, this study has the inherent biases of the included publications. The included literature consisted of in vivo animal studies, which yielded a low level of evidence, thereby limiting the interpretation of results. Additionally, scaffold composition and animal models varied throughout the included studies, and the translation between animal data and human applicability remains a challenge. The current literature regarding scaffold augmentation of rotator cuff tendon repair would benefit from direct comparisons between scaffold types—or homogeneously composed scaffolds and animal models—to better assess the true benefit of the intervention. Moreover, a portion of the included studies reported using biological interventions in addition to inlay scaffold placement to enhance tendon-bone healing,[22,26,28,30] which adds to the encountered heterogeneity between the scaffolds used across the yielded studies.

Conclusions

Inlay scaffold positioning in preclinical studies has been shown to enhance the healing biology of the enthesis while providing histologic similarities to its native 4-zone configuration.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A.J.C. has a consulting agreement with Tetrous; owns equity or stocks in Tetrous; and reports a consulting or advisory relationship ConMed. B.J.C. receives funding grants from B Braun Medical, Arthrex, JRF Ortho, and National Institutes of Health; reports board membership with *American Journal of Sports Medicine*, Arthroscopy Association of North America, and *Journal of American Academy of Orthopaedic Surgeons*; reports a consulting or advisory relationship with Arthrex, Elsevier, and *Operative Techniques in Sports Medicine*; and owns equity or stocks in Bandgrip and OSSIO. N.N.V. reports board membership with American Orthopaedic Society for

Sports Medicine, American Shoulder and Elbow Surgeons, Arthroscopy Association of North America, and Slack; receives funding grants from American Shoulder and Elbow Surgeons, Breg, Ossur Americas, and Stryker; reports a relationship with Smith & Nephew; and reports a consulting or advisory relationship with Stryker. All other authors (J.B.V-E., R.S.B., S.A.B., Z.A.K.) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Bedi A, Bishop J, Keener J, et al. Rotator cuff tears. *Nat Rev Dis Primers* 2024;10:8. doi:10.1038/s41572-024-00492-3
2. Dang A, Davies M. Rotator cuff disease: Treatment options and considerations. *Sports Med Arthrosc Rev* 2018;26:129-133. doi:10.1097/JSA.0000000000000207
3. Greenspoon JA, Petri M, Warth RJ, Millett PJ. Massive rotator cuff tears: Pathomechanics, current treatment options, and clinical outcomes. *J Shoulder Elbow Surg* 2015;24:1493-1505. doi:10.1016/j.jse.2015.04.005
4. Rugg CM, Gallo RA, Craig EV, Feeley BT. The pathogenesis and management of cuff tear arthropathy. *J Shoulder Elbow Surg* 2018;27:2271-2283. doi:10.1016/j.jse.2018.07.020
5. Thorsness R, Romeo A. Massive rotator cuff tears: Trends in surgical management. *Orthopedics* 2016;39:145-151. doi:10.3928/01477447-20160503-07
6. Di Benedetto P, Mancuso F, Tosolini L, Buttironi MM, Beltrame A, Causero A. Treatment options for massive rotator cuff tears: A narrative review. *Acta Biomed* 2021;92:e2021026. doi:10.23750/abm.v92iS3.11766 (suppl 3)
7. Sambandam SN, Khanna V, Gul A, Mounasamy V. Rotator cuff tears: An evidence based approach. *World J Orthop* 2015;6:902-918. doi:10.5312/wjo.v6.i11.902
8. Plachel F, Siegert P, Rüttershoff K, et al. Long-term results of arthroscopic rotator cuff repair: A follow-up study comparing single-row versus double-row fixation techniques. *Am J Sports Med* 2020;48:1568-1574. doi:10.1177/0363546520919120
9. Derwin KA, Galatz LM, Ratcliffe A, Thomopoulos S. Enthesis repair: Challenges and opportunities for effective tendon-to-bone healing. *J Bone Joint Surg Am* 2018;100:e109. doi:10.2106/JBJS.18.00200
10. Mandalia K, Mousad A, Welborn B, et al. Scaffold- and graft-based biological augmentation of rotator cuff repair: An updated systematic review and meta-analysis of preclinical and clinical studies for 2010-2022. *J Shoulder Elbow Surg* 2023;32:1784-1800. doi:10.1016/j.jse.2023.03.031
11. Seetharam A, Abad J, Baessler A, Badman BL. Use of a nanofiber resorbable scaffold during rotator cuff repair: Surgical technique and results after repair of small- to medium-sized tears. *Orthop J Sports Med* 2022;10:23259671221094848. doi:10.1177/23259671221094848
12. 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;10:35-45.
13. Moser HL, Abraham AC, Howell K, et al. Cell lineage tracing and functional assessment of supraspinatus tendon healing in an acute repair murine model. *J Orthop Res* 2021;39:1789-1799. doi:10.1002/jor.24769
14. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ* 2009;339:b2700. doi:10.1136/bmj.b2700
15. Fu SC, Cheuk YC, Yung SH, Rolf CG, Chan KM. Systematic review of biological modulation of healing in anterior cruciate ligament reconstruction. *Orthop J Sports Med* 2014;2:2325967114526687. doi:10.1177/2325967114526687
16. Hexter AT, Pendegrass C, Haddad F, Blunn G. Demineralized bone matrix to augment tendon-bone healing: A systematic review. *Orthop J Sports Med* 2017;5:2325967117734517. doi:10.1177/2325967117734517
17. Hooijmans CR, Leenaars M, Ritskes-Hoitinga M. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the three Rs, and to make systematic reviews more feasible. *Altern Lab Anim* 2010;38:167-182. doi:10.1177/026119291003800208
18. Credille KT, Wang ZRC, Horner NS, et al. Biphasic interpositional allograft for rotator cuff repair augmentation is safe in an ovine model. *Arthroscopy* 2023;39:1983-1997. doi:10.1016/j.arthro.2023.03.018
19. Dickerson DA, Misk TN, Van Sickle DC, Breur GJ, Nauman EA. In vitro and in vivo evaluation of orthopedic interface repair using a tissue scaffold with a continuous hard tissue-soft tissue transition. *J Orthop Surg Res* 2013;8:18. doi:10.1186/1749-799X-8-18
20. Easley J, Puttlitz C, Hackett E, et al. A prospective study comparing tendon-to-bone interface healing using an interposition bioresorbable scaffold with a vented anchor for primary rotator cuff repair in sheep. *J Shoulder Elbow Surg* 2020;29:157-166. doi:10.1016/j.jse.2019.05.024
21. He SK, Ning LJ, Yao X, et al. Hierarchically demineralized cortical bone combined with stem cell-derived extracellular matrix for regeneration of the tendon-bone interface. *Am J Sports Med* 2021;49:1323-1332. doi:10.1177/0363546521994511
22. Hee CK, Dines JS, Dines DM, et al. Augmentation of a rotator cuff suture repair using rhPDGF-BB and a type I bovine collagen matrix in an ovine model. *Am J Sports Med* 2011;39:1630-1639. doi:10.1177/0363546511404942
23. Lee WY, Kim YM, Hwang DS, et al. Does demineralized bone matrix enhance tendon-to-bone healing after rotator cuff repair in a rabbit model? *Clin Orthop Surg* 2021;13:216-222. doi:10.4055/cios20099
24. Li X, Cheng R, Sun Z, et al. Flexible bipolar nanofibrous membranes for improving gradient microstructure in tendon-to-bone healing. *Acta Biomater* 2017;61:204-216. doi:10.1016/j.actbio.2017.07.044
25. Novakova SS, Mahalingam VD, Florida SE, et al. Tissue-engineered tendon constructs for rotator cuff repair in sheep. *J Orthop Res* 2018;36:289-299. doi:10.1002/jor.23642

26. Rodeo SA, Potter HG, Kawamura S, Turner AS, Kim HJ, Atkinson BL. Biologic augmentation of rotator cuff tendon-healing with use of a mixture of osteoinductive growth factors. *J Bone Joint Surg Am* 2007;89:2485-2497. doi:10.2106/JBJS.C.01627
27. Romeo A, Easley J, Regan D, et al. Rotator cuff repair using a bioresorbable nanofiber interposition scaffold: A biomechanical and histologic analysis in sheep. *J Shoulder Elbow Surg* 2022;31:402-412. doi:10.1016/j.jse.2021.07.018
28. Smith MJ, Pfeiffer FM, Cook CR, Kuroki K, Cook JL. Rotator cuff healing using demineralized cancellous bone matrix sponge interposition compared to standard repair in a preclinical canine model. *J Orthop Res* 2018;36:906-912. doi:10.1002/jor.23680
29. Thangarajah T, Henshaw F, Sanghani-Kerai A, Lambert SM, Blunn GW, Pendegrass CJ. The effectiveness of demineralized cortical bone matrix in a chronic rotator cuff tear model. *J Shoulder Elbow Surg* 2017;26:619-626. doi:10.1016/j.jse.2017.01.003
30. Thangarajah T, Sanghani-Kerai A, Henshaw F, Lambert SM, Pendegrass CJ, Blunn GW. Application of a demineralized cortical bone matrix and bone marrow-derived mesenchymal stem cells in a model of chronic rotator cuff degeneration. *Am J Sports Med* 2018;46:98-108. doi:10.1177/0363546517727512
31. Randelli PS, Menon A, Nocerino E, et al. Long-term results of arthroscopic rotator cuff repair: Initial tear size matters: A prospective study on clinical and radiological results at a minimum follow-up of 10 years. *Am J Sports Med* 2019;47:2659-2669. doi:10.1177/0363546519865529
32. Carpenter JE, Thomopoulos S, Flanagan CL, DeBano CM, Soslowsky LJ. Rotator cuff defect healing: A biomechanical and histologic analysis in an animal model. *J Shoulder Elbow Surg* 1998;7:599-605. doi:10.1016/s1058-2746(98)90007-6
33. Gulotta LV, Kovacevic D, Ehteshami JR, Dagher E, Packer JD, Rodeo SA. Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair model. *Am J Sports Med* 2009;37:2126-2133. doi:10.1177/0363546509339582
34. Isaac C, Gharaibeh B, Witt M, Wright VJ, Huard J. Biologic approaches to enhance rotator cuff healing after injury. *J Shoulder Elbow Surg* 2012;21:181-190. doi:10.1016/j.jse.2011.10.004
35. Quigley R, Verma N, Evuarherhe A, Cole BJ. Rotator cuff repair with graft augmentation improves function, decreases revisions, and is cost-effective. *Arthroscopy* 2022;38:2166-2174. doi:10.1016/j.arthro.2022.01.011
36. Aurora A, McCarron JA, van den Bogert AJ, Gatica JE, Iannotti JP, Derwin KA. The biomechanical role of scaffolds in augmented rotator cuff tendon repairs. *J Shoulder Elbow Surg* 2012;21:1064-1071. doi:10.1016/j.jse.2011.05.014
37. Krupp R, Frankle M, Nyland J, et al. Interpositional scaffold anchor rotator cuff footprint tear repair: Excellent survival, healing, and early outcomes. *Knee Surg Sports Traumatol Arthrosc* 2023;31:2670-2680. doi:10.1007/s00167-023-07383-2
38. Elnikety S, Pendegrass CJ, de Godoy RF, Holden C, Blunn GW. Augmentation and repair of tendons using demineralised cortical bone. *BMC Musculoskelet Disord* 2016;17:483. doi:10.1186/s12891-016-1323-1
39. Jackson DW, Simon TM, Lowery W, Gendler E. Biologic remodeling after anterior cruciate ligament reconstruction using a collagen matrix derived from demineralized bone. An experimental study in the goat model. *Am J Sports Med* 1996;24:405-414. doi:10.1177/036354659602400403
40. 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;40:2365-2374. doi:10.1177/0363546512457648
41. 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;88:115-122. doi:10.1002/jbm.b.31157
42. Gruskin E, Doll BA, Futrell FW, Schmitz JP, Hollinger JO. Demineralized bone matrix in bone repair: History and use. *Adv Drug Deliv Rev* 2012;64:1063-1077. doi:10.1016/j.addr.2012.06.008