Histologic Evaluation of Biopsy Specimens Obtained After Rotator Cuff Repair Augmented With a Highly Porous Collagen Implant

Steven P. Arnoczky, D.V.M., Shariff K. Bishai, D.O., M.S., F.A.O.A.O., Brian Schofield, M.D., Scott Sigman, M.D., Brad D. Bushnell, M.D., M.B.A., Jan Pieter Hommen, M.D., and Craig Van Kampen, Ph.D.

Purpose: To histologically evaluate biopsy specimens from patients who previously underwent rotator cuff repair augmented with a highly porous collagen implant. **Methods:** Biopsies of collagen implant/host-tissue constructs were obtained from 7 patients undergoing a second arthroscopic procedure at various time periods (5 weeks to 6 months) after arthroscopic rotator cuff repair augmented with a collagen implant overlay. The biopsy specimens were examined histologically for host-tissue ingrowth, host-tissue maturation, and host-implant biocompatibility. **Results:** At the earliest time period (5 weeks), the biopsy revealed the presence of host cells (fibroblasts) within the interstices of the porous collagen implant. Cells were aligned along the linear orientation of the collagen implant structure, and there was evidence of early collagen formation. The 3-month biopsies showed increased collagen formation, maturation, and organization over the surface of the implant and evidence of the collagen implant. At 6 months, the newly generated tissue had the histologic appearance of a tendon, suggesting functional loading of the new generated host tissue. There was no evidence of any remnants of the collagen implant in the 6-month biopsy. There was no evidence of any inflammatory or foreign body reaction within any of the tissue samples. **Conclusions:** Biopsies of collagen implants retrieved from human rotator cuff repair subjects revealed cellular incorporation, tissue formation and maturation, implant resorption, and biocompatibility. **Clinical Relevance:** The histologic observations from these clinical biopsies support the biocompatibility of this implant and its ability to promote new connective tissue with the histological appearance of tendon over the surface of the native cuff tendon.

Recently, a new paradigm of host-generated tissue augmentation of arthroscopic rotator cuff repair has been introduced.[1-3] In this approach, a highly porous, highly organized, reconstituted collagen implant designed to promote new host tissue regeneration is placed over the bursal surface of partial-thickness rotator cuff injuries[3] or full-thickness cuff repairs.[2] The subsequent generation and functional remodeling of new host tissue has been shown to improve the healing environment of both partial-thickness lesions[3] and full-thickness repairs.[2]

One of the key factors in the ability of a biodegradable implant to promote the generation and maturation of a functionally aligned connective tissue is the degradation profile of the implant.[4] Ideally, the implant should degrade (resorb) at the same rate the host tissue is generated and remodeled.[4] In addition, while an implant should not elicit an adverse inflammatory response, it should allow for the rapid ingrowth of cells and a vascular network and permit a functional orientation, maturation, and remodeling of the newly synthesized tissue in response to the local stresses.[5] The integration of the new tissue with the underlying host tissue is important if this new tissue is to contribute to the strength of the repaired tissue.[1,5]

From the Laboratory for Comparative Orthopaedic Research (S.P.A.) and College of Osteopathic Medicine (S.K.B.), Michigan State University, East Lansing, Michigan; Schofield, Hand, Bright Orthopaedics (B.S.), Sarasota, Florida; Lowell General Hospital (S.S.), North Chelmsford, Massachusetts; Harbin Clinic Orthopaedics and Sports Medicine (B.D.B.), Rome, Georgia; Hommen Orthopaedic Institute (J.P.H.), Miami, Florida; and Rotation Medical, Inc. (C.V.K.), Plymouth, Minnesota, U.S.A.

The authors report the following potential conflicts of interests or sources of funding: S.A. receives support from Rotation Medical. B.D.B. receives support from Rotation Medical, Depuy-Synthes Sports Medicine, and the Arthroscopy Association of North America.

Received February 5, 2016; accepted June 29, 2016.

Address correspondence to Steven P. Arnoczky, D.V.M., Laboratory for Comparative Orthopaedic Research, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, U.S.A. E-mail: arnoczky@cvm.msu.edu

0749-8063/16105/$36.00

http://dx.doi.org/10.1016/j.arthro.2016.06.047

A preclinical study has documented the natural history and biocompatibility of the aforementioned collagen implant as well as the maturation and functional orientation of the newly induced tissue over time.[1] While clinical studies have demonstrated magnetic resonance imaging (MRI) evidence of new tissue generation in humans,[2,3] the histologic character of this tissue can only be inferred. The ability to obtain and examine clinical material from patients at various times postsurgery provides a unique and valuable opportunity to compare and contrast the histologic picture in humans with that of the preclinical animal studies.[5]

Therefore, the purpose of this study was to histologically evaluate biopsy specimens from patients who previously underwent rotator cuff repair augmented with a highly porous collagen implant. We hypothesized that these human specimens would demonstrate host-tissue ingrowth, host-tissue maturation, and host-implant biocompatibility.

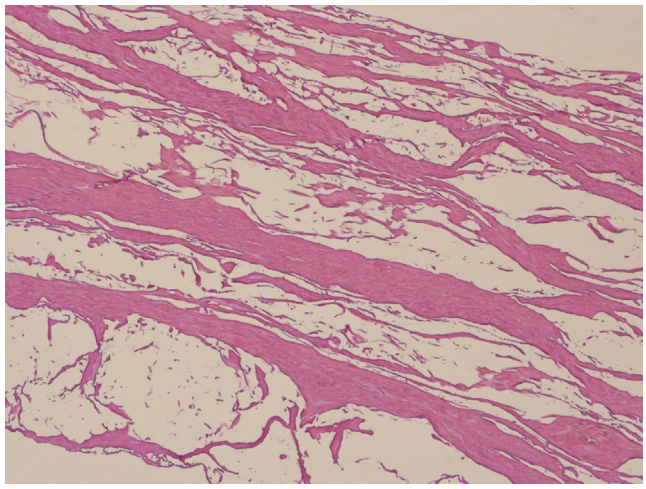

Fig 1. Photomicrograph of the highly porous, highly aligned bovine collagen implant prior to implantation. Hematoxylin and eosin ×100.

Methods

This was a retrospective study of biopsies from 7 patients, treated by 5 surgeons, who underwent rotator cuff procedures using a collagen implant, followed by a second surgery with biopsy, over a 14-month period. Over 350 rotator cuff procedures using this implant have been performed by these 5 surgeons with no adverse events related to the implant at follow-up ranging from 1 day to 20 months.

Biopsies were collected at the occasion of the second procedure, during which the implant/tissue construct was harvested and the location of the biopsy noted. The biopsies ranged from approximately 2 to 4 mm^3 in size. The specimens were fixed in 10% buffered formalin, embedded in paraffin, and 5μ thick sections were cut and stained with hematoxylin and eosin for histologic examination. The same individual (S.P.A.) subjectively evaluated all of the sections for cell/tissue ingrowth, tissue organization and maturation, implant resorption, and biocompatibility.

The index procedure involved surgical treatment of a rotator cuff tear augmented with a highly porous, highly aligned, reconstituted bovine collagen implant (Rotation Medical Inc., Plymouth, MN; Fig 1). After initial arthroscopic repair of the supraspinatus tendon, a collagen implant was placed over the bursal surface of the repaired tendon and secured with custom designed tendon and bone staples.[2,3] Patients were asked to follow each surgeon's rehabilitation protocol for the procedure performed.

The second arthroscopic procedure permitted an opportunity to biopsy the collagen implant along with any new host tissue generated within it and/or on its surface. All patients had provided informed consent for the biopsy during the second surgery. There were no exclusion criteria.

In one case, a staged hemiarthroplasty was planned 6 months after repair of a massive, full-thickness tear in a severely arthritic glenohumeral joint. At that time, gross examination of the repair site revealed that the augmented rotator cuff repair was healed and a layer of new tissue had formed over the surface of the supraspinatus tendon and its humeral footprint. The new tissue was adherent to the underlying cuff tendon. A biopsy of the new tissue generated by the implant was taken from the anterior distal aspect of the supraspinatus tendon.

Results

None of the patients have reported any issues after the second arthroscopic procedure. All patients are at least 4 months (longest 9 months) out from their second procedures.

The reasons for the second procedure, pertinent patient information, and the time post–index surgery and biopsy location are summarized in Table 1.

In 4 out of 7 cases the patients suffered a traumatic event that resulted in various degrees of repair disruption. In 3 of those cases, the repair was revised and another implant was placed over the repair. In one case, the anterolateral corner of the collagen implant was torn and dislodged and loose. This free portion of the implant was removed.

One patient had persistent pain, and the second-look arthroscopy at 3 months revealed that an area of the repair where the implant came detached did not heal. That portion of the repair was revised and the implant reattached over the repair with a single anchor.

One patient developed postsurgical arthrofibrosis, which required a second surgery at 2 months for lysis of the adhesions. At that time the implant appeared well

Table 1. Summary of Patient/Procedure Information Relating to Second-Look Biopsies

Patient No. (Sex/Age)	Original Procedure/ Etiology	Comorbidities/Ancillary Procedures	Time of Biopsy	Location of Biopsy	Reason for Second Look
1 (F/46)	Primary FT-RCR massive/traumatic	Glenohumneral osteoarthritis/ chondroplasty, capsular release, acromioplasty	6 months	Anterior aspect of repair	Staged hemiarthroplasty
2 (F/23)	Primary FT-RCR medium/traumatic	None/acromioplasty, labral debridement	3 months	Anterior aspect of repair	Patient fell and disrupted repair
3 (F/50)	Medium PT (B)/ degenerative	None/acromioplasty	3 months	Anterolateral aspect of repair	Patient's arm was jerked while walking dog on leash
4 (M/51)	Revision FT-RCR medium/degenerative	Hypertension/acromioplasty, biceps tenodesis	5 weeks	Anterolateral aspect of implant at bone attachment	Patient fell and disrupted repair
5 (F/43)	Primary FT-RCR large/ degenerative	None/acromioplasty, biceps tenodesis, labral/chondral debridement	3 months	Posterolateral aspect of repair	Pain; portion of tear not covered by implant was not healing
6 (F/45)	Coverted high-grade PT (B) to FT-RCR small/ degenerative	None/acromioplasty, biceps tenodesis, labral/chondral debridement	2 months	Antero-lateral aspect of repair	Arthrofibrosis
7 (M/55)	Primary FT-RCR medium/degenerative	None/acromioplasty	2 months	Multiple areas of the implant	Patient fell and disrupted repair

B, bursal side; F, female; FT, full-thickness tear; M, male; PT, partial-thickness tear; RCR, rotator cuff repair.

integrated with healthy host tissue and was firmly attached to the underlying tendon.

In the last patient, a staged hemiarthroplasty was performed as described above.

At the earliest time period (5 weeks), the biopsy revealed the presence of host cells (fibroblasts) within the interstices of the porous collagen implant (Fig 2A). The cells were aligned along the linear orientation of the collagen implant structure, and there was evidence of early collagen formation (Fig 2B). There was no indication of any inflammatory or foreign body reaction within the 5-week tissue sample.

Similar to the 5-week sample, the two 8-week biopsies demonstrated host incorporation throughout the implant and evidence of collagen formation on the surface and within the depths of the implant. As in the 5-week samples, there was a linear orientation of new host collagen fibers along the collagen structure of the implant. There was no evidence of any inflammatory or foreign body reaction to the implant in either of the 8-week biopsies.

All of the 3-month biopsies showed increased collagen formation, maturation, and organization over the surface of the implant (Figs 3 and 4). While remnants of the collagen implant were still present in all

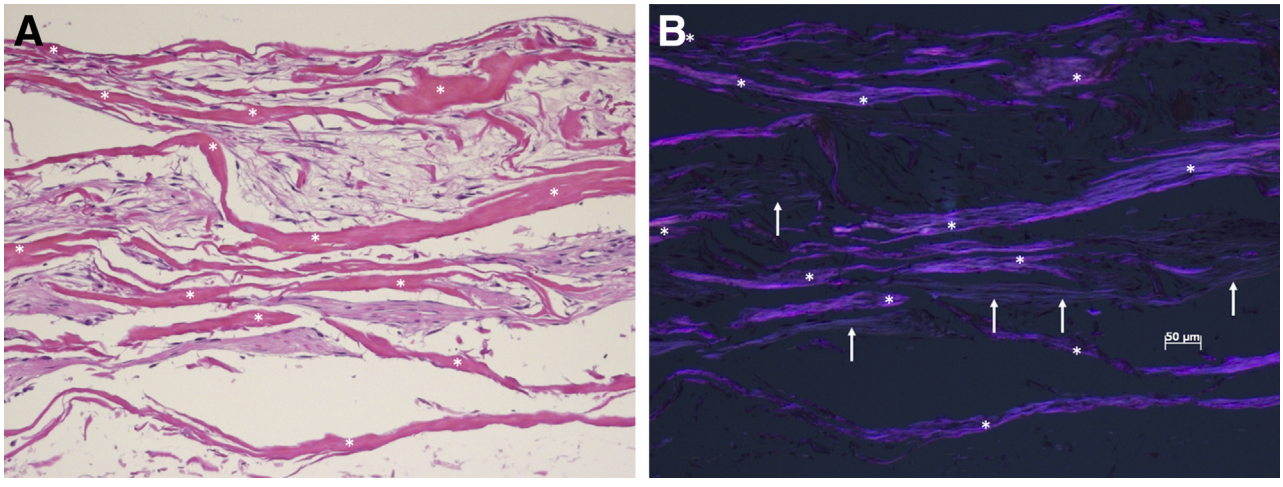

Fig 2. Light (A) and polarized light (B) photomicrographs of a collagen implant illustrating host cell ingrowth and early collagen production and alignment (arrows) at 5 weeks. The collagen implant (darker pink structure) is clearly visible (*). Hematoxylin and eosin ×100.

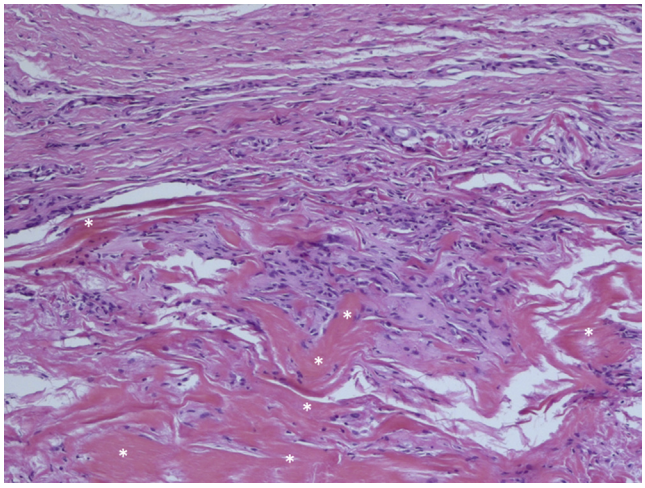

Fig 3. Photomicrograph showing increased collagen formation, maturation, and orientation over the surface of the implant at 3 months. Remnants of the collagen implant are still present (*). Hematoxylin and eosin ×100.

specimens at 3 months, there was evidence of dissolution of the implant by invading fibroblasts (Fig 5). There was no indication of any inflammatory or foreign body reaction related to the implant within any of the 3-month tissue samples.

At 6 months, the newly generated tissue had the histologic appearance of a tendon (dense, regularly oriented connective tissue containing parallel rows of fibroblast within parallel bundles of collagen fibers; Fig 6). The presence of highly oriented collagen fibers in this sample at 6 months suggests functional loading of the new generated host tissue. There was no evidence of any remnants of the collagen implant in the 6-month biopsy, and there was no evidence of any inflammatory or foreign body reaction within the tissue sample.

Discussion

The histological picture of the new tissue resulting from implantation of a highly porous, collagen implant in the biopsy specimens in the current study precisely mirrors the results seen in a preclinical animal study at the same time periods.[1] Rapid ingrowth and linear orientation of host cells was observed by 5 weeks. By 3 months there was significant collagen formation within and on the surface of the implant. This increase in collagen deposition at 3 months was also noted in histological analysis of the preclinical animal study,[1] as well as demonstrated by MRI evidence of an increase thickening of the supraspinatus tendon in 2 clinical studies.[2,3] As in the preclinical study, the collagen on the surface of the implant in the 3 month biopsies was well oriented, suggesting a functional remodeling on the newly formed tissue in response to increasing loads.[1,6]

A considerable amount of collagen implant material was visible in the 3-month human biopsies. This is similar to what was reported in the preclinical animal study.[1] Of interest in the current study was what appeared to be evidence of fibroblastic invasion and breakdown of the collagen construct. Fibroblasts are known to resorb collagen through a proteolytic mechanism.[7] This degradation can occur in response to alterations in extracellular matrix stresses experienced by the cells within the matrix.[8,9] It is possible that the increase in the deposition and alignment of newly synthesized, dense, regularly oriented connective tissue on the surface of the collagen implant seen at 3 months begins to take up more of the local stresses. This change in mechanical environment then triggers a cell-based remodeling of the extracellular matrix, which includes resorption of the implant.[8,9]

Similar to the preclinical animal study, the 6-month clinical biopsy showed no evidence of any residual

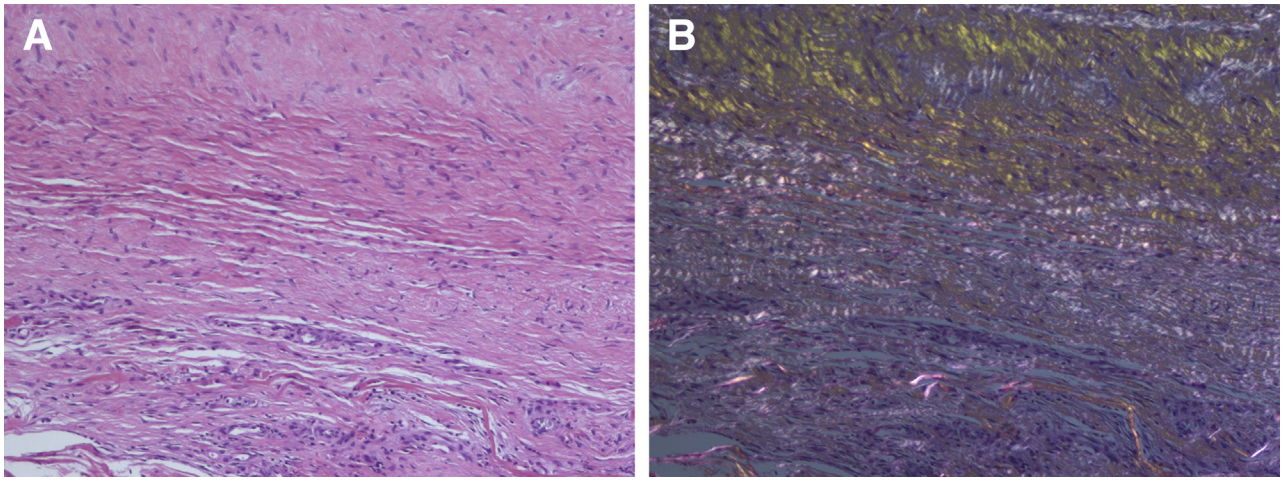

Fig 4. Light (A) and polarized light (B) photomicrographs of the newly regenerated host tissue overlying the implant at 3 months. There is evidence of maturation and functional alignment of the dense, regularly oriented connective tissue. Hematoxylin and eosin ×100.

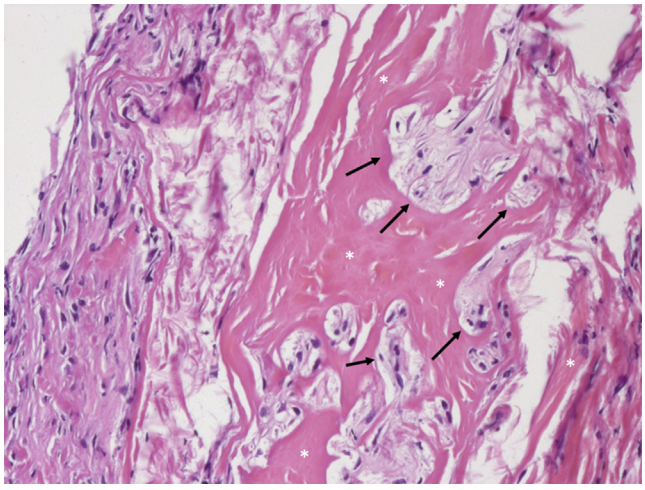

Fig 5. Photomicrograph showing what appears to be dissolution of the collagen implant by invading fibroblasts at 3 months (arrows). Remnants of the collagen implant are still present (*). Hematoxylin and eosin ×100.

implant material and demonstrated the presence of a dense, regularly oriented connective tissue containing parallel rows of fibroblasts within parallel bundles of collagen fibers, the histologic definition of a tendon.[10]

Although predictions of safety between species (e.g., rat, dog, sheep, monkey, and humans) are generally very good, they may not be perfect.[11] Animal studies are often underpowered to detect rare events, and they are mostly conducted in healthy, quadruped animals.[11] In addition, the local tissue environment (anatomical, biological, and biomechanical) of these animal models may not precisely reflect the indicated use in humans or the specific conditions to which an implant may be subjected.[12,13] Indeed, while some biodegradable devices designed to induce or augment tissue repair and regeneration have shown no ill effects in preclinical animal studies,[14-16] these same devices have been reported to cause significant local reactions in humans, necessitating their removal.[17-20] This reason for this disconnect between the animal and human experience is not clear and underscores the importance of being able to validate the tissue response to these implants in the indicated application(s) in humans.

The opportunity to examine biopsies of a highly porous collagen implant from several clinical cases of rotator cuff repair allowed histologic confirmation of the biocompatibility of the implant, the degree of host tissue generation within and on the surface of the implant, and the natural history of the implant in its designated application. More importantly, these biopsies underscore the safety of this implant in humans as well as its ability to promote new tissue formation in a consistent and predictable manner. These 7 biopsies appear to provide additional support of the natural history of the highly porous collagen implant demonstrated in the preclinical animal study.[1]

Limitations

A potential limitation of this study is that these biopsies can only offer isolated snap-shots of the natural history of this implant in humans. However, since such second-look opportunities are fortunately very rare,[5] the ability to examine biopsies from 7 different individuals which span a 6-month time period of implantation (and were not related to any problem with the implant) represents a unique and valuable assessment perspective.

Conclusions

Biopsies of collagen implants retrieved from human rotator cuff repair subjects revealed cellular

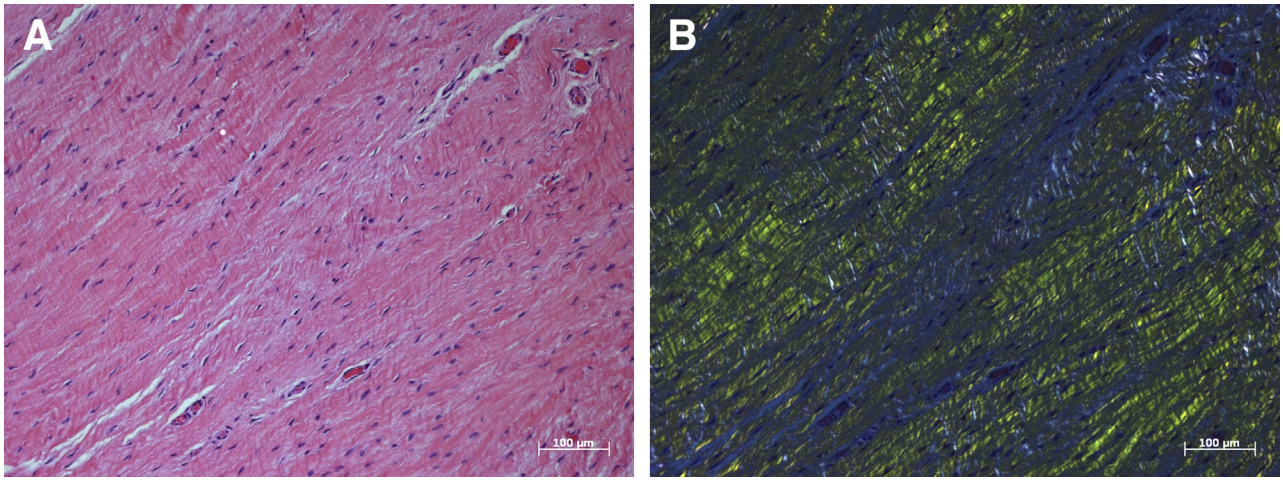

Fig 6. Light (A) and polarized light (B) photomicrographs of the newly regenerated host tissue by the implant at 6 months. This is dense, regularly oriented connective tissue. There was no evidence of any remnants of the collagen implant. Hematoxylin and eosin ×100.

incorporation, tissue formation and maturation, implant resorption, and biocompatibility.

References

1. Van Kampen C, Arnoczky S, Parks P, et al. Tissue-engineered augmentation of a rotator cuff tendon using a reconstituted collagen scaffold: a histological evaluation in sheep. *Muscles Ligaments Tendons J* 2013;3:229-235.
2. Bokor DJ, Sonnabend D, Deady L, et al. Preliminary investigation of a biological augmentation of rotator cuff repairs using a collagen implant: a 2-year MRI follow-up. *Muscles Ligaments Tendons J* 2015;5:144-150.
3. Bokor DJ, Sonnabend D, Deady L, et al. Evidence of healing of partial-thickness rotator cuff tears following arthroscopic augmentation with a collagen implant: a 2-year MRI follow-up. *Muscles Ligaments Tendons J* 2016;6:16-25.
4. Navarro M, Michiardi A, Castano O, Planell JA. Biomaterials in orthopaedics. *J R Soc Interface* 2008;5:1137-1158.
5. Snyder SJ, Arnoczky SP, Bond JL, Dopirak R. Histological evaluation of a biopsy specimen obtained 3-month after rotator cuff augmentation with GraftJacket Matrix. *Arthroscopy* 2009;25:329-333.
6. Via AG, De Cupis M, Spoliti M, Oliva F. Clinical and biological aspects of rotator cuff tears. *Muscles Ligaments Tendons J* 2013;3:70-79.
7. Garant PR. Collagen resorption by fibroblasts. *J Periodontol* 1976;47:380-390.
8. Huang C, Yannas IV. Mechanochemical studies of enzymatic degradation of insoluble collagen fibers. *J Biomed Mater Res Symp* 1977;8:137-154.
9. Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. *Nat Rev Mol Cell Biol* 2014;15:802-812.
10. Ross MH, Pawlina W. *Histology: a text and atlas*, Ed 5. Baltimore: Lippincott Williams & Wilkins, 2006;178-179.
11. Cavagnaro J, Silva Lima B. Regulatory acceptance of animal models of disease to support clinical trials of medicines and advanced therapy medicinal products. *Eur J Pharmacol* 2015;759:51-62.
12. Arnoczky SP, Wilson JW. Experimental surgery of the skeletal system. In: Gay WI, Heavner JE, eds. *Methods of animal experimentation. Vol. VII. Research surgery and care of the research animal. Part B: Surgical approaches to the organ systems*. New York: Academic Press, 1986;67-108.
13. Arnoczky SP. Animal models for knee ligament research. In: Daniels D, Akeson WH, O'Connor JJ, eds. *The knee ligaments: structure, function, injury, and repair*. New York: Raven Press, 1990;401-417.
14. Dejardin LM, Arnoczky SP, Ewers BJ, Haut RC, Clarke RB. Tissue-engineered rotator cuff tendon using porcine small intestine submucosa. Histologic and mechanical evaluation in dogs. *Am J Sports Med* 2001;29:175-184.
15. Schlegel TF, Hawkins RJ, Lewis CW, Motta T, Turner AS. The effects of augmentation with swine small intestine submucosa on tendon healing under tension: histologic and mechanical evaluations in sheep. *Am J Sports Med* 2006;34:275-280.
16. Dahhan P, Benazet JP, Rey P, Leandri J. Use of carbon fibers to repair the anterior cruciate ligament of the knee. *J Chir* 1981;118:275-280.
17. Walton JR, Bowman NK, Khatib Y, Linklater J, Murrell GA. Restore orthobiologoic implant: not recommended for augmentation of rotator cuff repairs. *J Bone Joint Surg Am* 2007;89:786-791.
18. Phipatanakul WP, Petersen SA. Porcine small intestine submucosa xenograft augmentation in repair of massive rotator cuff tears. *Am J Orthop* 2009;38:572-575.
19. Rushton N, Dandy DJ, Naylor CP. The clinical, arthroscopic, and histologic findings after replacement of the anterior cruciate ligament with carbon-fibre. *J Bone Joint Surg Br* 1983;65:308-309.
20. DeCarbo WT, Feldner BM, Hyer CF. Inflammatory reaction to implanted equine pericardium xenograft. *J Foot Ankle Surg* 2010;49:155-158.