Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 01/15/2019

NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Grant Number: 1R01AR073811-01A1
FAIN: R01AR073811

Principal Investigator(s):
Chunfeng Zhao, MD

Project Title: Tissue Engineered Tendon Complex for Rotator Cuff Repair and Regeneration

Grace, Janice S
Mayo Clinic
Office of Sponsored Projects Administration
200 First Street Southwest
Rochester, MN 559050001

Award e-mailed to: researchadmin@mayo.edu

Period Of Performance:
Budget Period: 01/15/2019 – 12/31/2019
Project Period: 01/15/2019 – 12/31/2023

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $349,800 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to Mayo Clinic in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Arthritis And Musculoskeletal And Skin Diseases of the National Institutes of Health under Award Number R01AR073811. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Yen Thach
Grants Management Officer
NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AR073811-01A1

Award Calculation (U.S. Dollars)

Federal Direct Costs	$220,000
Federal F&A Costs	$129,800
Approved Budget	$349,800
Total Amount of Federal Funds Obligated (Federal Share)	$349,800
TOTAL FEDERAL AWARD AMOUNT	$349,800
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$349,800

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$349,800	$349,800
2	$349,800	$349,800
3	$349,800	$349,800
4	$349,800	$349,800
5	$349,800	$349,800

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Arthritis, Musculoskeletal and Skin Diseases Research
CFDA Number:	93.846
EIN:	1416011702A1
Document Number:	RAR073811A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019	2020	2021	2022	2023
AR	8472466	$349,800	$349,800	$349,800	$349,800	$349,800

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 3 C / **OC**: 414A / **Released**: eRA Commons User Name 01/04/2019
Award Processed: 01/15/2019 12:05:38 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AR073811-01A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AR073811-01A1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AR073811. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AR Special Terms and Conditions – 1R01AR073811-01A1

Clinical Trial Indicator: No

This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

COMPETING FUNDING LEVEL
This award includes funding adjustments in accordance with current NIAMS FY2019 policy.

SHORTENED FIRST YEAR BUDGET
This award includes funds for twelve months of support. The competing budget period is awarded for less than 12 months. Continuation of this award will cycle each year on January 1st.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Steve Austin
Email: Steve.Austin@nih.gov **Phone**: (301) 594-3504 **Fax**: (301) 480-5450

Program Official: Fei Wang
Email: wangf@mail.nih.gov **Phone**: 301-594-5055 **Fax**: 301-480-4543

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AR073811-01A1

INSTITUTION: Mayo Clinic

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
TOTAL FEDERAL DC	$220,000	$220,000	$220,000	$220,000	$220,000
TOTAL FEDERAL F&A	$129,800	$129,800	$129,800	$129,800	$129,800
TOTAL COST	$349,800	$349,800	$349,800	$349,800	$349,800

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	59%	59%	59%	59%	59%
F&A Cost Base 1	$220,000	$220,000	$220,000	$220,000	$220,000
F&A Costs 1	$129,800	$129,800	$129,800	$129,800	$129,800

PI: **Zhao, Chunfeng**	Title: Tissue Engineered Tendon Complex for Rotator Cuff Repair and Regeneration	
Received: 04/03/2018	FOA: PA18-484 Clinical Trial:Not Allowed	Council: 10/2018
Competition ID: FORMS-E	FOA Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 AR073811-01A1	Dual: EB	Accession Number: 4156869
IPF: 4976101	Organization: MAYO CLINIC ROCHESTER	
Former Number:	Department: Orthopedic Surgery	
IRG/SRG: MTE	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Chunfeng Zhao MD	Mayo Clinic	PD/PI
Redacted by agreement	Mayo Clinic	Consultant
	Mayo Clinic	Consultant
	Mayo Clinic	Co-Investigator
	Mayo Clinic	Co-Investigator

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
AR073811

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**

5. APPLICANT INFORMATION **Organizational DUNS*:** 006471700

Legal Name*: Mayo Clinic
Department: Orthopedic Surgery
Division: Orthopedics
Street1*: 200 First Street SW
Street2:
City*: Rochester
County:
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 559050001

Person to be contacted on matters involving this application
Prefix: First Name*: Janice Middle Name: S Last Name*: Grace Suffix:
Position/Title: Institutional Official
Street1*: 200 First Street SW
Street2:
City*: Rochester
County:
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 559050001
Phone Number*: 507-284-4715 Fax Number: 507-284-4288 Email: researchadmin@mayo.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 41-6011702

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Tissue Engineered Tendon Complex for Rotator Cuff Repair and Regeneration

12. PROPOSED PROJECT		**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
Start Date*	Ending Date*	MN-001
09/01/2018	08/31/2023	

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Chunfeng Middle Name: Last Name*: Zhao Suffix: MD

Position/Title: Professor
Organization Name*: Mayo Clinic
Department: Orthopedic Surgery
Division: Orthopedics
Street1*: 200 First Street SW
Street2: RO_GU_01_28BIOM
City*: Rochester
County:
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 559050001
Phone Number*: 507-266-0982 Fax Number: Email*: zhao.chunfeng@mayo.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$1,987,500.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$1,987,500.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Janice Middle Name: S Last Name*: Grace Suffix:

Position/Title*: Institutional Official
Organization Name*: Mayo Clinic
Department: RS-Research Services
Division: RS-Institutional Review Bd
Street1*: 200 First Street SW
Street2:
City*: Rochester
County:
State*: MN: Minnesota
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 559050001
Phone Number*: 507-284-4715 Fax Number: 507-284-4288 Email*: researchadmin@mayo.edu

Signature of Authorized Representative*
Janice.Grace

Date Signed*
04/03/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_ltr_CFZ.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Mayo Clinic
Duns Number:	006471700
Street1*:	200 First Street SW
Street2:	
City*:	Rochester
County:	
State*:	MN: Minnesota
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	559050001
Project/Performance Site Congressional District*:	MN-001

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00187

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_summary_final.pdf
8. Project Narrative*	PROJECT_NARRATIVE_final.pdf
9. Bibliography & References Cited	Bibliography_final.pdf
10.Facilities & Other Resources	Facilities_and_Resources_final.pdf
11.Equipment	Equipment.pdf

PROJECT SUMMARY/ABSTRACT

Rotator cuff tears are common, disabling, and are costly musculoskeletal injuries. Surgical repair is a common treatment, but recurrent tears occur in 20 to 90% of patients, especially in those with large or massive tears. Rotator cuff repair with biomaterial augmentations, including mechanical augmentation to increase the repair strength and biological healing to accelerate tissue regeneration, may reduce postoperative complications. However, the current graft materials used for augmentation are far from satisfactory. Tissue engineering offers the potential to generate functional tissue replacement, however, designing an appropriate scaffold that can has native tendon mechanical properties and provide an optimal environment for cell seeding, growth, and differentiation, especially for tendon enthesis regeneration, has proven challenging. In this application, we propose a novel tissue engineering approach using a sliced tendon fibrocartilage bone composite (TFBC) as a scaffold to seed bone marrow-derived stem cells (BMSC) for rotator cuff repair augmentation. Our preliminary studies indicated that this TFBC can mechanically enhance the rotator cuff repair. The TFBC can be revitalized by seeding BMSCs, which express tenogenesis in native tendon environment under mechanical loading. Our recent in vivo data demonstrated that our engineered TFBC biologically augmented the rotator cuff repair better when compared to repair or TFBC scaffold alone after six weeks post-surgery in our newly developed canine non-weight bearing shoulder model which mimics human shoulder function and activities. Based on our preliminary studies***, the overall goal of the current proposal is to develop and test a functional engineered composite tissue (TFBC) for rotator tear treatment. Our underlying hypothesis is that our engineered TFBC, as an augmentation biomaterial, will improve functional outcomes following rotator cuff repairs compared to the clinically used biomaterial (GraftJacket). We also postulate that TFBC will help to rebuild the tendon enthesis, which has been a great challenge to regenerate in the current approaches, as the TFBC contains a native fibrocartilage zone.*** To test our hypothesis, the following two specific aims are proposed. Specific Aim 1 is to test the TFBC mechanical augmentation properties when the TFBC is used for rotator cuff repair in an in vitro model. Specific Aim 2 is to test engineered TFBC biological augmentation and enthesis regeneration in a canine in vivo model with a long-term followup. We expect two *important outcomes* from this investigation: 1) The TFBC strengthens the mechanical properties of the rotator cuff repair, and 2) the engineered TFBC effectively reduce the rotator repair failure, improve the rotator cuff repair healing, and rebuild a tendon enthesis which has not been successfully regenerate to date. If our hypothesis is supported, we would have developed a novel, effective, and clinically-applicable biomaterial to improve the quality of rotator cuff repairs, which has a *significant impact* on the field of rotator cuff tear, a challenging clinical and research issue.

PROJECT NARRATIVE

This research proposal is to develop a biological material that can be revitalized by seeding stem cells for the surgical treatment of rotator cuff tears, which is the most common cause of shoulder pain and dysfunction. Although surgical repair of rotator cuff tears has a good clinical result for small or partial tears, the treatment of large or massive tears remains difficult. This new method, if successful, would provide surgeons an effective treatment method to improve clinical results.

FACILITIES AND OTHER RESOURCES

Mayo Clinic—Overview

Mayo Clinic is the first and largest integrated not-for-profit group practice in the world. Doctors from every medical specialty work in collaborative teams to care for patients, joined by integrated support systems, and a core philosophy that *the needs of the patient come first*. Over 4,500 physicians and scientists and 57,000 allied health staff work at Mayo Clinic, with main campuses in Rochester, Minnesota; Jacksonville, Florida; and Scottsdale/Phoenix, Arizona; as well as more than 70 smaller hospitals and facilities (the Mayo Clinic Health System) across six states. Collectively, these locations treat more than 1.3 million people each year. The surgical practice at Rochester campus of Mayo Clinic is one of the largest multispecialty practices in the world. In 2015 alone, the number of surgical patients exceeded 58,000. Further, through the Mayo Clinic Care Network, our reach to patients and providers is even broader and more impactful, extending to over 12,000 physicians across 19 states, including Puerto Rico and Mexico. Mayo Clinic is governed by a 33-member Board of Trustees, a majority of which are representatives of the general public. Mayo's activities are overseen by its Board of Governors and their three standing committees: Clinical Practice, Education, and Research.

Mayo Clinic—Research: Mayo Clinic stands among the world's elite of biomedical research institutions. Mayo Clinic's commitment to patients includes maintaining comprehensive and robust research programs that lead to improvements in patient care. Mayo provides an ideal research environment where multidisciplinary teams, reflecting Mayo's broad expertise and unique approach to bench science and direct patient care, develop an infrastructure capable of conducting meaningful patient-oriented research. Indeed, in addition to 332 research faculty, more than 750 physicians are directly engaged in research activities. In total, 3,120 personnel work full-time in research. This spectrum demonstrates the continuum at Mayo that links laboratory, clinical, and translational research—integrated and aligned with Mayo Clinic strategic goals. In this spirit of collaboration, considerable effort and investment has been devoted to cultivating a diverse and dedicated group of investigators.

Research and research training programs are the responsibility of the Executive Dean of Research, Gregory J. Gores, M.D., and the Mayo Clinic Research Committee, representing the three main campuses. Research programs at Mayo reflect the interest and initiative of individual investigators, departmental expertise, and overall strategic research goals of the institution. Presently, there are approximately 200 established laboratory-based research programs funded by more than 4,800 extramural grants and contracts. Across the three main campuses, the research physical infrastructure spans an immense 952,000 square feet.

At Mayo Clinic, support for research has consistently grown. In total, the 2016 budget stood at $710 million, a combination of Mayo reinvestment, extramural funding, and benefactor support. That included $419.8 million in extramural research funding, of which more than $254 million was awarded by the National Institutes of Health (NIH), ranking Mayo in the top 25 for overall NIH research support. Other federal support extends to the Department of Defense, Centers for Disease Control and Prevention, Agency for Health Care Research & Quality, Patient Centered Outcomes Research Institute and others. In 2012, Mayo Clinic and its collaborators were awarded nearly $60 million from the Center for Medicare and Medicaid Innovation (CMMI) to improve health care delivery, a marker of Mayo's innovative approach to improving patient care and outcomes. Internally, Mayo reinvested over $290.5 million of its own revenue in 2016 back into research. In 2016, Mayo's research programs generated 2,937 new protocols; over 11,000 active human research studies; and more than 7,600 research publications and review articles in peer-reviewed journals.

Mayo Clinic is further dedicated to numerous specialty research centers, laboratories, and facilities. They include the Mayo Clinic Cancer Center, a National Cancer Institute-designated comprehensive cancer center since 1973, and Mayo's Center for Clinical Translational Science (CCaTS), one of the largest NIH-funded translational centers in the nation (National Center for Advancing Translational Sciences). Mayo Clinic is also advancing new, cutting-edge medical research through three recently established "transformative centers": the Robert D. And Patricia E. Kern Center for the Science of Health Care Delivery (study of healthcare systems, patient-centered outcomes and population science); the Center for Individualized Medicine (genomic and molecular-based research); and the Center for Regenerative Medicine (dedicated to tissue and organ regeneration and transplant science). In all, Mayo supports more than 30 dedicated centers and programs.

Mayo Clinic is also a founding member of the High Value Healthcare Collaborative, a group of 15 major health systems across the nation that collaborate under the shared core goals of improving health care, reducing

costs, and identifying and disseminating best-practice care models and innovative value-based payment systems to the national provider community.

Biomechanics Laboratory
The Orthopedic Biomechanics Laboratory occupies 5100 sq. ft. of space in the Guggenheim Building. The laboratory is comprised of: 1) Material testing core facility for in vitro analysis of bone and connective tissue mechanical properties; 2) Kinematic testing facility for in vitro assessment of normal and pathologic motion; 3) Computational stress analysis facility; 4) Specimen preparation room; 5) Experimental machine shop for machining or modifying joint implant prototypes, experimental prostheses, internal fixation devices, and testing apparatuses; and 6) Mechanical simulation facility for functional and kinematic assessments of upper extremity, lower extremity, and spine.

Tendon and Soft Tissue Biology (TSTB) Laboratory
The TSTB Laboratory occupies 1,635 sq. ft. of space in the Guggenheim Building next to the Biomechanics Laboratory. The laboratory includes a cell culture facility, biochemistry analysis lab, and mechanical testing lab. The cell culture facility is well equipped with safety cabinets, incubators, high-speed centrifuges, bioreactors, and inverse microscopes for cell and tissue harvesting, culture, and mechanical stimulation. The biochemistry lab contains equipment for cell tracking and staining, frozen tissue sectioning, RT-PCR, Western blot, ELISA, and immunohistochemistry. The mechanical testing lab includes tendon work-of-flexion equipment, several tendon frictional measurement devices, a custom-made microtester, and, MotionMonitor System for joint functional assessment.

The combination of resources within the TSTB Laboratory, Biomechanics Laboratory and adjacent Materials Testing Core greatly enhances our capacity for biological and biomechanical evaluation and provides a multidisciplinary platform for researchers.

Computer Resources within the Biomechanics and Tendon and Soft Tissue Laboratories
The Orthopedic Biomechanics and TSTB Laboratories have various computer systems for use in the collection, reduction, analysis, and visualization of data. The laboratory is equipped with HP computers and all personnel have updated HP computers with enhanced memory and processor capabilities and double 24” monitors. All computers have access to printers and scanners and are connected via Gigabit Ethernet and have access to the Internet. In addition, numerous pieces of equipment are networked including qPCR machines, spectrophotometer etc. Our software packages include word processing, graphics, spreadsheets, a reference manager, statistics, and Oligo-scientific application. Analysis capabilities include finite element analysis and modeling using ABAQUS (Abaqus, Inc.). These packages provide linear and nonlinear stress analysis with capabilities for nonlinear material definition and contact force analysis. Three-dimensional image reconstruction and visualization is performed using Analyze (Biomedical Imaging Resource, Mayo Medical Center). Data collection codes and motor control algorithms are written in LabView (National Instruments). Data analysis code is written in Matlab (MathWorks). Animation of kinematic data is performed using 3D Studio MAX (Autodesk, Inc.). VIMS (Virtual Interactive Musculoskeletal System, John Hopkins) is used for discrete element analysis of rigid bodies. In addition, several locally developed software packages provide kinematic and kinetic analysis, visualization, and joint contact modeling. Three 6-degree-of-freedom magnetic tracking devices (2 Polhemus Libertys, and 1 Ascension Technology Flock of Birds), 1 active marker optoelectric tracking device (Optotrak Certus), and 1 passive marker video tracking device (Motion Analysis, Inc.) provide for real-time kinematic and analog data acquisition. The MotionMonitor software (Innovative Sports Training, Inc) provides data collection, analysis, and animation of data from these systems.

Histology Laboratory
The Histology Laboratory, a unit of Mayo Clinic's Department of Laboratory Medicine and Pathology, processes over 349,200 paraffin-embedded blocks per year and over 901,800 slides per year. Specimens handled in this laboratory include surgical, autopsy, and research material. The function of the Histology Laboratory is to provide accurate diagnosis for patients and excellent services for researchers. Close cooperation exists between clinical services and the Biomechanics Laboratory, thus facilitating referral of patients for functional evaluation studies. Mayo Clinic in Rochester has an integrated, multidisciplinary approach to the study of orthopedic diseases. More than 2,000 orthopedic surgical procedures result in specimens that are processed in the Histology Laboratory in the Department of Laboratory Medicine and

Pathology. Four of the 32 pathologists in the Division of Anatomic Pathology have subspecialty expertise in bone and soft-tissue pathology and work in collaboratively with the members of the Department of Orthopedic Surgery.

Resources—Bone Histomorphometry Laboratory

The Orthopedic Bone Histomorphometry Laboratory covers 1068 sq. ft. and contains 3 laboratory workbenches, two chemical fume hoods, a histology bench-top hood, a darkened microscope room, and storage room for archived specimen blocks and medical reports. In addition, the Histology & Histomorphometry Laboratory has collaborative access to laboratory space (65 sq. ft) across the corridor for the operation of the Exakt™ Cutting Station.

Electron Microscopy Core

The Flow Cytometry Electron Microscopy Core Facility (EMCF) is a research resource laboratory that provides specimen preparation, transmission and scanning electron microscopy, X-ray probe microanalysis, immunogold labeling procedures, and imaging services to investigators from both clinical and basic science laboratories within Mayo Clinic. The facility features three transmission electron microscopes and one scanning electron microscope. In addition to housing state-of-the-art equipment, the facility is staffed by ten experienced technologists specializing in a wide variety of microscopy techniques. The laboratory is dedicated to providing rapid turnaround of high-quality, routine electron microscopy for clinical and research users and to aid in the development of specialized procedures for individual investigators.

Flow Cytometry/Optical Morphology Core

This lab provides instrumentation and expertise in flow cytometry, light microscopy, and image analysis. The flow cytometry area includes both analytical and cell sorting services. The Optical Morphology area includes various light microscopy techniques such as confocal microscopy, ratio image analysis, and total internal reflection fluorescence (TIRF) microscopy. The Flow Cytometry/Optical Morphology Core Facility combines two complementary technologies. The flow cytometry area provides instrumentation, training, and technical support for a wide variety of applications. Seven flow cytometers (BD Biosciences) are currently available in the facility. Five benchtop analytical instruments include a single-laser FACScan, two dual-laser FACSCaliburs with sample loaders, one dual-laser FACSCanto with loader, and a five-laser LSR II flow cytometer with high-throughput sampler for sampling from multiwell plates. Two cell sorters are provided, including a dual-laser FACSVantage SE and a special-order five-laser FACSAria. In addition to having bulk sorting capabilities, both sorters can sort single cells into multiwell plates. The optical morphology area is available for light microscopy and image analysis–related services. Four high-end laser scanning confocal microscopes are provided, including three Zeiss LSM510 microscopes and one LSM5 Live microscope. One of the LSM510 models is equipped with a Meta detector for spectral fingerprinting. An upright Axioplan 2 microscope is available, with a high-resolution digital color camera and associated acquisition software. Two Axiovert 200M inverted microscopes are provided. One is equipped with an Apotome module for optical sectioning. The other is equipped with a DG-4 high-speed optical changer for ratio image acquisition and a module for TIRF microscopy. Stage heaters and incubation chambers are available for live cell work on all of the inverted platforms, including the confocal microscopes. Additional instrumentation in the facility includes a macroscope and an inverted workstation for microinjection. Image analysis is performed on off-line computer workstations. Customized macro programming by shared resource personnel is available for any image analysis application. Shared resource personnel are also available in both areas of the facility to assist with acquisition, training, experimental design, and data analysis.

Department of Comparative Medicine

The mission of the Department of Comparative Medicine is to provide and ensure the research community with the highest quality laboratory animal care and support services, using state-of-the-art equipment, advanced technology, and exceptional personnel. Animal facilities are located in six areas. There are Redacted by agreement animal facilities in the downtown Rochester Mayo Clinic campus: Redacted by agreement Redacted by agreement There is an animal facility in the Redacted by agreement , which is in the Redacted by agreement from the downtown campus. There are also Redacted by agreement Redacted by agreement and at the Redacted by agreement . The

vast majority of animals assigned to investigators are maintained at the Redacted by agreement animal facilities.

Most of Mayo Clinic's animal facilities are conventional; however, there are Redacted by agreement and a Redacted by agreement. All facilities include procedural areas, necropsy rooms, and fully equipped survival surgery suites. Animals in all locations are observed on a daily basis. Three board-certified laboratory animal (ACLAM) veterinarians oversee the animal care and use program. Veterinary care, including postoperative care, is provided by the veterinarians with the assistance of six full-time veterinary technicians. Animal husbandry is accomplished by 24 animal care technicians, who report to the facility manager. All animal orders for Mayo Clinic are processed centrally through the Department of Comparative Medicine office on the Redacted by agreement. Health reports and health alerts from all companies providing animals are routinely reviewed by the veterinary staff before an order is placed with a specific vendor. An approved vendor list for animals has been established.

Mayo Information Technology Infrastructure

All staff included in this application have access to Mayo Clinic's secure Intranet via networked desktop and laptop computers. All data are centrally backed up nightly.

- **Other computer facilities:** Mayo Clinic institutional computing resources include 2 IBM zSeries mainframes with access to about 1000Tb of storage. Mid-range Unix servers include more than 200 Sun, HP, or Linux-based machines, accessing 35Tb of shared disk space. Email is managed on approximately 50 Exchange servers. About 650 NT-class servers address dedicated database and general needs, with access to 50Tb of SAN disk storage. User access is managed by a common login system, including 52,000 active accounts in about 100 NT domains. The Rochester primary IDX electronic medical record environment runs on HP Nonstop SQL Guardian servers. All production servers have systematic data backup schedules, performed or delivered on an array of magnetic tape, robotic tape silo, CD-ROM, DVD-ROM and jukebox resources. Data recovery is typically guaranteed on a daily snapshot basis for at least 30 days. Periodic archival snapshots are retained indefinitely. The Department of Health Sciences Research has a suite of approximately 50 Sun Unix servers, which make up the main computing platform of the department. A departmental file server supports more than 1Tb of "home drive" space that can be mounted under Unix or Windows. Several Windows workstations based on P4 or Xeon processors supplement the Sun Unix cluster. Most departmental personnel have desktop Windows or Linux workstations connected by 100Mb links to the network. Network-connected, shared high-volume printers are in close proximity to all personnel, with color and large-paper (11×17) devices available on all floors.

- **Computing resources located in the Research Computing Facility** include 4 Sun database machines (development and production for 2 DBMS systems: Ingres and Sybase), 2 Intel/Linux Web servers, and 3 bioinformatics servers: a Sun Enterprise 3000 server with 4 250-MHz processors, 512 MB of memory, and 180 GB of disk storage for running GCG; a Dell 7150 Linux server with 4 Intel Itanium 1 processors running at 750 MHz with 2 GB of memory and 180 GB of disk for serial bioinformatics applications; and a 22-cpu Linux cluster (10 Racksaver 1200 modules consisting of 2 independent systems, each with an Athlon cpu, 512 MB of memory, and 80 GB of disk: 16 of the systems run at 1.4 GHz, 4 run at 1.2 GHz) for parallel bioinformatics applications. Associated with the Linux cluster are 2 other servers, a master server (Anova Systems Linux computer with 2 1.4-GHz Athlon processors, 2 GB of memory, and 380 GB of disk) and an NFS server (Dell 2650 Linux computer with 2 2.8-GHz Intel Xeon processors, 2 GB of memory, and 1.5 TB of disk).

- **The Mayo-IBM Life Sciences System** is housed on 2 dedicated machines: The primary device is a P690 Unix server with 8 64-bit POWER4+ cpus at 1.7 GHz, 32 Gb memory, and 36 Gb of local disk. A companion development system is a P650 with 2 processors and 16 Gb of memory and 36 Gb of local disk storage. These units share 2 Tb storage consisting of very-high-performance 36-Gb fail-safe disk units and 8 Gb of active cache, arrayed in IBM's Enterprise Storage System configuration.

Division of Biomedical Statistics and Informatics

The division, located on the 7th and 8th floors of the Harwick Building (16,300 square feet) and the first floor of the Kahler building (3,265 square feet), has provided consultation on statistical design and analysis for the clinical and laboratory research staff at Mayo Clinic since this service was introduced by Joseph Berkson (of

"Berkson's bias" fame) in 1932. It is currently staffed by 26 Ph.D. statisticians and 55 statisticians with Master's degrees, whose activities are facilitated by 75 statisticians with Bachelor's degrees ("statistical programmer analysts") and 8 clerical personnel. Also, within the division is a data-entry and management group consisting of a supervisor, five data-entry clerks, and three data librarians who are responsible for the transfer of data from almost any electronic format to our IBM mainframe and UNIX systems for archiving and analysis. A long-term archiving facility retains study data in perpetuity and ensures long-term access. The current location of over 12,000 studies, undertaken since 1966, can be viewed on an online catalogue and retrieved within 24 hours. These efforts are supported by the Statistical Systems Unit of the Department of Information Services, which comprises 18 FTE systems analyst/programmers devoted to the extensive computational and data-handling needs of the statisticians and epidemiologists. The Department of Information Services includes ≈2,000 additional personnel who support Mayo Clinic's information environment. All necessary equipment is available in the Department of Health Sciences Research or through the Mayo Clinic Computer Facility and the Research Computer Facility (see Computer Facilities). The primary data analysis tools in the Division are SAS and S-PLUS. These are augmented with other tools such as StatXact, Data Conversion, R, WinBugs, Matlab, and more than 100 statistical genetics packages such as Solar, Merlin, and SAGE. Familiarity with these packages, particularly the first two, is extensive. The Division of Biomedical Statistics and Informatics has been a user of SAS since its 1972 release and has made significant contributions to the SAS software base, including many of the survival routines. Members of the division have authored all of the survival analysis routines distributed in the S-Plus package, including routines to handle mixed-effects Cox models. The locally written S-Plus recursive partitioning and genetic libraries are also publicly available. Routines developed for internal use include 4 SAS procedures, 7 S-Plus libraries each consisting of numerous functions, and more than 250 SAS macros, including a special macro developed for estimating incidence and prevalence in the Olmsted County population. Programs with this level of distribution require extreme dedication to accuracy, testing, and reliability, with the goal of establishing easy-to-use, reliable, and leading-edge analysis tools.

Office: Each of the investigators has a private office equipped with a networked desktop computer.

EQUIPMENT

The laboratories are well equipped for clinical and basic science research projects. Major equipment/software in the ***Orthopedic Biomechanics Laboratory*** and the ***Tendon & Soft Tissue Research Laboratory*** is briefly described as follows.

1. Three MotionMonitor data collection systems (Innovative Sports Training, Inc.).
2. Optotrak Certus active-marker kinematic data collection system (Northern Digital Inc.).
3. One four-sensor Fastrak electromagnetic kinematic data collection systems (Polhemus).
4. Two 12-sensor Liberty electromagnetic kinematic data collection system (Polhemus)
5. 3-camera Hawk 200 Hz passive marker kinematic data collection system with EVaRT software (Motion Analysis Corp.).
6. Servohydraulic biaxial material testing machine, Model 312 (MTS Systems Corp.)
7. MiniBionix II servohydraulic biaxial material testing machine w/environmental chamber (MTS Systems Corp.)
8. Servohydraulic biaxial material testing machine, Model 1321 (Instron)
9. ELF 3200 electromechanical testing system (Bose Corp.)
10. Custom-fabricated joint stability test machine allowing multi-axial rotation and translation with resultant loads measured by a 6-axis load cell
11. Custom-fabricated pneumatic rail system for customized sports injury testing
12. Custom-fabricated joint range of motion test system
13. Custom-fabricated micro-testing machine using a stepper motor driven linear stage mounted on an Olympus CKX 41 inverted microscope for in vitro tissue strength and viscolelastic assessment
14. Versatile, custom-fabricated tendon actuators using stepper motor driven linear stage
15. Two custom-fabricated friction testing machines used to measure gliding resistance and work of flexion during tendon excursion
16. Hysitron® TI950 Triboindentor (Eden Prairie, MN) for nanomechanical material testing
17. Buehler® EcoMet/AutoMet 250/300 Grinder-Polisher/Power Head (Lake Bluff, IL) for indentation test preparation
18. Two custom-fabricated multi-station joint prosthesis wear testing systems
19. Custom-fabricated dynamic loading test system for dental material testing
20. Custom fabricated multi-axis spine simulator with attached JR3 6-component load cell. Allows pure moments to be applied to multi-segment spines
21. 4-channel DVRT system (Microstrain, Inc.)
22. One Keyence LK-081 and three Epsilon Opto NCDT1302 laser displacement sensors
23. Powermatic 14-inch metal cutting band saw, Bridgeport vertical milling machine model "J," with digital readout, Clausing 12-inch metal turning lathe, and Clausing 15-inch drill press
24. Two C-arm type X-ray systems (MINI6600, OEC Medical Systems, Inc. and BV25, Philips)
25. Abaqus finite element software (Dassault Systemes)
26. ANALYZE medical imaging analysis software (Biomedical Imaging Resource, Mayo Medical Center)
27. Mimics medical image processing software (Materialise)
28. Solidworks Premium Edition Computer Aided Drawing software (Dassault Systemes)
29. LabVIEW software for motor control and data adcquisition (National Instruments)
30. Two Biological Safety Cabinets (model numbers SG400 and SG403, Baker Co.). Used for working with cell cultures while maintaining sterility
31. Custom-fabricated mechanical incubator/actuator system
32. Leica CM1850 Cryostat for cutting thin (from 1 to 60µm) frozen section histological specimens.
33. Waterjacketed CO2 incubator model 3154 (Forma Scientific)
34. Microscopes (model CK40 and CKX41, Olympus)
35. Lightcycler PCR machine (Roche)
36. Mikro tissue dismembrator
37. Mini-PROTEAN 3 Electrophoresis Cell and Mini Trans-Blot Cell (Bio-Rad). Used for Western Blot analysis
38. PowerPac HC Power supply (Bio-Rad)
39. Px2 Thermal Cycler (Thermo)
40. FLUOstar Omega microplate reader (BMG Labtechnologies)
41. Napco 6300 Controlled Environment Incubator (Napco)
42. Access to two -80°C deep freezers
43. One -20ºC Freezer and two refrigerator/freezer combo unit

44. Harvard Apparatus bioreactor
45. Canine Walkway, a custom-designed system to assess canine gait and pedobarograph for functional evaluation of the extremities and digits.
46. IncuCyte® S3 Live-Cell Analysis System: This device can derive deeper and more physiologically relevant information about cell biology with the real-time kinetic data. The IncuCyte automatically acquires and analyzes images around the clock, providing an information-rich analysis that is easy to achieve including cell health and viability assays, cell monitoring and workflow assays, and live cell assays.
47. Digital Image Correlation System (DICS) (ARAMIS 4M, Trilion Quality Systems, Plymouth Meeting, PA): This system is a material, structure, and geometry independent method, that replaces, strain gages, LVDTs, accelerometers, and other contact deformation measurement sensors, and provides experimental data comparable to FEA. Overall this tool is a highly robust, full-field, non-contact optical strain measurement tool that utilizes the highly accurate principals of digital image correlation.

Relevant Equipment in Mayo Clinic Core Facilities for this Proposal:

48. OsteoMeasure systems composed of Nikon eclipse E400 microscopes, Sony 3CCD color video camera (model DXC -970 MD or -390P), digitizing tablet, OsteoMeasure software, and Sony monitor.
49. LSM 510 Confocal Laser Scanning Microscope (Carl Zeiss MicroImaging, Inc, Zeiss, Germany).
50. Confocal Microscope (LSM510, Zeiss, Germany)
51. Hitachi S4700 Field Emission Scanning Electron Microscope (SEM, Hitachi Japan), a completely digital, high resolution SEM for the use of research and analytical samples.
52. Philips Tecnai T12 Transmission on Electron Microscope (TEM) (Philips, Amsterdam, The Netherlands). This microscope is used for diagnostic, research and analytical samples. In addition to the standard transmission mode this instrument has detectors for secondary, backscatter, and x-ray spectrographic imaging and fitted with a 2kx2k digital imaging system.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Chunfeng Middle Name Last Name*: Zhao Suffix: MD

Position/Title*:	Professor
Organization Name*:	Mayo Clinic
Department:	Orthopedic Surgery
Division:	Orthopedics
Street1*:	200 First Street SW
Street2:	RO_GU_01_28BIOM
City*:	Rochester
County:	
State*:	MN: Minnesota
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	559050001

Phone Number*: 507-266-0982 Fax Number:

E-Mail*: zhao.chunfeng@mayo.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: MD Degree Year:

Attach Biographical Sketch*: File Name: Zhao_bio.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-18-484 . Received Date: 2018-04-03T15:57:34.000-04:00

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

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PROFILE - Senior/Key Person

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PROFILE - Senior/Key Person

Redacted by agreement

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Zhao, Chunfeng

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor of Orthopedic and Biomedical Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Tianjin Medical University, Tianjin China	M.D.	06/1982	Medicine
Tianjin Hospital, Tianjin China	Resident	1983-1984	Orthopedic Surgery
Tianjin Renmin Hospital, Tianjin, China	Resident	1984-1988	Orthopedic Surgery
Peking Medical Union Hospital, Beijing, China	Clinical Fellow	1988-1989	Spine Surgery
Orthopedic Biomechanics Laboratory, Mayo Clinic	Research Fellow	1997-2000	Orthopedic Biomechanics

A. Personal Statement

As an orthopedic surgery resident, I assisted with two cases of rotator cuff repair; it was a new surgery in my department at Renmin Hospital, China 35 years ago. However, unsatisfactory results hindered my mentor's ability to continue with the procedure. Although, I have specialized in spine and hand surgery and have done many cases of tendon repair and reconstruction in the upper extremity, rotator cuff repair was a "no man's land" in my practice. This experience, however, provoked my research interest 20 years ago when I started my research career at the Mayo Clinic. While surgical repair of the torn rotator cuff is now a clinical standard treatment due to better understanding of tendon biology and development of surgical tools and skills, high re-tear rate is still problematic. Therefore, improving rotator cuff repair healing and functional recovery is clinically important. During my early research training I learned orthopedic biomechanics, specifically of the upper extremity under the supervision of Dr. Kai-Nan An PhD and tendon biology under Dr. Peter Amadio, MD. I started to develop my own research interests on chemical and tissue engineered tendons to improve functional outcomes after tendon injures over a decade ago. I obtained my first R01 grant Percentile as an independent PI in 2010, which was renewed on the first submission Percentile in 2014 to investigate "Engineered Tendon for Better Outcomes," which focuses on flexor tendon reconstruction, another important clinical issue that has unsatisfactory outcomes. However, rotator cuff repair has remained as part of my research agenda. With the NIH STAR award in 2015, I was able to continue my research along this line and successfully developed a tendon composite material for rotator cuff augmentation. Our preliminary studies are very promising to support our concept that this engineered composite tissue mechanically and biologically enhance the rotator cuff repair and healing better compared to the current used biomaterials in clinic. I have assembled a strong research team including biomechanician, cell biologist, and clinicians who have collaborated with me for many years along this research line. I am very confident that the outcomes from this project will have a significant impact on both musculoskeletal tissue engineering development in basic science and rotator cuff treatment in clinical practice.

B. Positions and Honors

Positions and Employment

1989 – 1994 Consultant Surgeon, Orthopedic Department, Tianjin Renmin Hospital, Nankai University, Tianjin, P.R. China

1994 – 1997 Vice-Chair, Associate Professor in Orthopedic Department, Tianjin Remin Hospital, Nankai University, Tianjin, P.R. China

1997 – 2000 Research Fellow, Orthopedic Biomechanics Laboratory, Mayo Clinic, Rochester, MN

2000 – 2002	Assistant Professor of Orthopedics and Biomedical Engineering, Mayo Clinic Rochester, MN
2000 – 2002	Research Associate, Orthopedic Biomechanical Laboratory, Mayo Clinic Rochester, MN
2002 – 2006	Senior Research Associate, Orthopedic Biomechanics Laboratory, Mayo Clinic Rochester
2006 – 2011	Professional Associate in Research II, Orthopedic Biomechanics Laboratory, Mayo Clinic
2006 – 2013	Associate Professor of Orthopedics and Biomedical Engineering, Mayo Medical School, Rochester, MN
2011 – 2013	Senior Associate Consultant, Orthopedic Biomechanics Laboratory, Mayo Clinic Rochester Rochester, MN
2013 -	Associate Consultant-II, Department of Orthopedic Surgery, Mayo Clinic Rochester, MN
2013 -	Associate Consultant-II, Department of Physiology and Biomedical Engineering, Mayo Clinic Rochester, MN
2013 -	Professor of Biomedical Engineering, Mayo Clinic Rochester, MN
2013 -	Professor of Orthopedics, Mayo Clinic Rochester, MN

Honors

1994	National Scientific Award, (ZDS-Auto Propellant Trephine for Anterior Cervical Fusion), Chinese Scientific Committee
1994	The Excellent Medical Doctor of Tianjin, Authorized by Tianjin Health Bureau
1995	Tianjin Scientific and Technological Star, Authorized by Tianjin Government
1999	Patrick J. Kelly Special Fellows Research Award (Gliding characteristics of flexor tendon repair), Department of Orthopedic Surgery, Mayo Foundation
2004	Poster Exhibit Award: Scientific Content issued by American Society for Surgery of the Hand, 59th Annual Meeting in New York, NY
2004	Best Research Paper (The effect of the gap size on tendon gliding resistance) - International Symposium on Ligaments and Tendons –IV
2014	CORR-ORS Richard A. Brand Award "Engineering Flexor Tendon Repair with Lubricant Cells and Cytokines in a Canine Model" as the First Author - Orthopedic Research Society
2014	J.R. Neff Award - Musculoskeletal Transplant Foundation for the best research proposal with $300.000 research funding

C. Contributions to Science

Flexor Tendon Repair and Rehabilitation

I started my research career in 1997 as a postdoctoral research fellow in the orthopedic biomechanics laboratory at Mayo Clinic with a great research interest in surgical repair and rehabilitation of the flexor tendon injury, which is a historical and modern clinical challenge due to its unique anatomic, structural, biomechanical, and biological characters. Research on this topic has been going on for nearly a century and great improvement has been achieved. However, slow tendon intrinsic healing, postoperative adhesions, and repair rupture are still problematic in current practice. I have conducted many studies, as the first author, to characterize tendon pulley structure, repaired tendon gliding ability with varied surgical techniques and rehabilitation kinematics after flexor tendon injury and repair, which has had a strong impact in this field [(1)]. Based on my training in biomechanics, I developed some novel methodologies to evaluate figure and tendon function[(2)]. I also developed a flexor tendon repair in ex vivo model and conducted a pilot study using cell-based therapy for tendon healing, which elicited a later study that won the CORR-ORS Richard A. Brand Award in 2014[(3)]. More recently, I explored and developed a turkey model for flexor tendon related research, which has been recognized by peer-review experts, as a novel animal model to advance flexor tendon research[(4)]. The study was funded by Mayo Center for Biomedical Discovery.

1. **Zhao C**, Amadio PC, Momose T, Couvreur P, Zobitz ME, An KN. Effect of synergistic wrist motion on adhesion formation after repair of partial flexor digitorum profundus tendon lacerations in a canine model in vivo. Journal of Bone & Joint Surgery - American Volume 84-A:78-84. 2002
2. **Zhao C**, Amadio PC, Berglund L, Zobitz ME, An KN. A new testing device for measuring gliding resistance and work of flexion in a digit. J Biomech 36:295-299. 2003
3. **Zhao C**, Ozasa Y, Reisdorf RL, Thoreson AR, Jay GD, An KN, Amadio PC. CORR(R) ORS Richard A. Brand Award for Outstanding Orthopaedic Research: Engineering flexor tendon repair with lubricant, cells, and cytokines in a canine model. Clin Orthop Relat Res 472:2569-2578. 2014. PMCID: 4117902

4. Kadar A, Thoreson AR, Reisdorf RL, Amadio PC, Moran SL, **Zhao C**. Turkey model for flexor tendon research: in vitro comparison of human, canine, turkey, and chicken tendons. J Surg Res 216:46-55. 2017. PMID: 28807213

Chemical Modification for Tendon Reconstruction

In my research career development, I focus on flexor tendon reconstruction as a way to restore hand function if primary repairs fail or if the injured tendons have large defects that cannot be directly repaired after injury. However, clinical outcomes following flexor tendon reconstruction are poor due to unmatched graft tendons that belong to an extrasynovial tendon, which is different from intrasynovial flexor tendon in surface structure and gliding ability. I, for the first time, used bio-lubricant compounds to modify the extrasynovial tendon to improve extrasynovial tendon gliding ability, thus improving functional restoration after flexor tendon reconstruction in a canine in vivo model[(1)]. Afterward, I developed a clinically relevant large animal model to study flexor tendon reconstruction related research[(2)]. As one of the frontiers in this field, I recently explored, for the first time, the use of chemical modified native synovial fluid as a lubricant substance to improve extrasynovial tendon gliding ability[(3)]. This novel approach has closed the gap from bench to bedside. It has been shown that flexor tendon graft,with native synovial fluid treatment, improved functional outcomes[(4)].

1. **Zhao C**, Sun YL, Amadio PC, Tanaka T, Ettema AM, An KN. Surface treatment of flexor tendon autografts with carbodiimide-derivatized hyaluronic Acid. An in vivo canine model. Journal of Bone & Joint Surgery - American Volume 88:2181-2191. 2006. PMCID3782255
2. **Zhao C**, Sun YL, Ikeda J, Kirk RL, Thoreson AR, Moran SL, An KN, Amadio PC. Improvement of flexor tendon reconstruction with carbodiimide-derivatized hyaluronic acid and gelatin-modified intrasynovial allografts: study of a primary repair failure model. Journal of Bone & Joint Surgery - American Volume 92:2817-2828. 2010. PMID: 21123612.
3. Ikeda J, Sun YL, An KN, Amadio PC, **Zhao C**. Application of carbodiimide derivatized synovial fluid to enhance extrasynovial tendon gliding ability. J Hand Surg Am 36:456-463. 2011. PMCID3625936.
4. Ji X, Reisdorf RL, Thoreson AR, Berglund LR, Moran SL, Jay GD, An KN, Amadio PC, **Zhao C**. Surface Modification with Chemically Modified Synovial Fluid for Flexor Tendon Reconstruction in a Canine Model in Vivo. J Bone Joint Surg Am 97:972-978. 2015. PMCID4469787.

Development of Tissue Engineered Tendon

Tissue engineering is a promising approach for musculoskeletal tissue replacement and regeneration. Typical tissue engineering includes scaffold, cell, and bioactive factors. The scaffold is a key element for the tendon tissue engineering, as tendon function acts as a cable to transfer the force from muscle to bone for joint movement. Therefore, the scaffold needs to be mechanically strong to stand such large loads. Although varied synthesized materials have been developed, it has not achieved satisfaction regarding mechanical properties, appropriate degradation, and biological support for cell seeding. In contrast, the native tendon scaffold could be a good material for tendon tissue engineering, since it has the right mechanical properties, extra cellular matrix, and biological environment. However, repopulating cells into a native decellularized tendon to promote tendon regeneration is quite challenging because the tendon is a high dense connective tissue. I have developed several techniques for engineered tendon including a composite of multilayers tendon slice (COMTS)[(1)] and multi-slit technologies for tendon tissue engineering[(2)]. I also investigated the biological[(3)] or mechanical[(4)] stimulation for tenogenesis. I have made a great contribution to the field of tendon/ligament tissue engineering.

1. Omae H, Sun YL, An KN, Amadio PC, **Zhao C.** Engineered tendon with decellularized xenotendon slices and bone marrow stromal cells: an in vivo animal study. J Tissue Eng Regen Med 6:238-244. 2012. PMCID3617585.
2. Ozasa Y, Amadio PC, Thoreson AR, An KN, **Zhao C.** Repopulation of intrasynovial flexor tendon allograft with bone marrow stromal cells: an ex vivo model. Tissue Eng Part A 20(3-4): 566-74. 2014. PMCID3926143.
3. Ozasa Y, Gingery A, Thoreson AR, An KN, **Zhao C,** Amadio PC. A comparative study of the effects of growth and differentiation factor 5 on muscle-derived stem cells and bone marrow stromal cells in an in vitro tendon healing model. J Hand Surg Am 39(9): 1706-13. 2014. PMCID4146663.
4. Wu JH, Thoreson AR, Gingery A, An KN, Moran SL, Amadio PC, **Zhao C.** The revitalisation of flexor tendon allografts with bone marrow stromal cells and mechanical stimulation: An ex vivo model revitalising flexor tendon allografts. Bone Joint Res 6(3): 179-85. 2017. PMCID5376656.

Rotator Cuff Repair and Repair and Regeneration

Rotator cuff tear is a common musculoskeletal disorder. Rotator cuff repairs, through either open or arthroscopic approach, have been effective and satisfactory in reducing pain and restoring shoulder function for small or partial tears. However, successful repair of large or massive tears, which comprise the majority of rotator cuff tears, continues to present a significant clinical challenge. The re-tear following repair ranges from 20% to 90%. Recently, augmentation with grafting materials to strengthen rotator cuff repair has become a common and attractive strategy to reduce the re-tear rate. However, materials that are currently available do not meet the criteria, including mechanical reinforcement, biological augmentation, and enthesis regeneration. As a clinician-scientist, I am always interested in clinically-translational research topics in this field. I developed a tissue engineering approach for rotator cuff augmentation with tendon disassembling, cell-seeding, and reassembling technology to vitalize the native tendon for rotator cuff regeneration[(1)]. This engineered tendon has been tested for rotator cuff augmentation using a rat in vivo model and found that it significantly increased tendon healing[(2)]. More recently, I have developed tendon-fibrocartilage-bone complex (TFBC) for rotator cuff augmentation, which provide a native tendon enthesis for regeneration, and using an in vitro model, we have proven the TFBC significantly increase the repair strength[(3)]. Although the rat model has been widely used for rotator cuff research, its robust healing capacity, and small scale, make this model less attractive to study surgical technique-dependent related research, especially with material augmentation. Pre-clinical large animal models, such as canine, sheep, goat, etc., have also been used for clinical translational research of rotator cuff repair. However, a high repair rupture rate has been a challenge due to natural weight-bearing of quadrupeds. Based on our successful experience using the canine model for flexor tendon research, I have developed a radial nerve denervation model for rotator cuff repair[(4)]. This novel animal model not only simulates human shoulder (non-weight bearing condition) but also provides a more comfortable handling process for the animals, improves ease of postoperative animal care, and effectively reduces the re-tear rate following rotator cuff repair in large animal models.

1. Qin TW, Sun YL, Thoreson AR, Steinmann SP, Amadio PC, An KN, **Zhao C**. Effect of mechanical stimulation on bone marrow stromal cell-seeded tendon slice constructs: a potential engineered tendon patch for rotator cuff repair. Biomaterials 51:43-50. 2015. PMID: 25770996.
2. Omi R, Gingery A, Steinmann SP, Amadio PC, An KN, **Zhao C.** Rotator cuff repair augmentation in a rat model that combines a multilayer xenograft tendon scaffold with bone marrow stromal cells. J Shoulder Elbow Surg 25:469-477. 2016. PMC5175472.
3. Ji X, Chen Q, Thoreson AR, Qu J, An KN, Amadio PC, Steinmann SP, **Zhao C**. Rotator cuff repair with a tendon-fibrocartilage-bone composite bridging patch. Clin Biomech (Bristol, Avon) 30:976-980. 2015. PMC4631669.
4. Ji X, Bao N, An KN, Amadio PC, Steinmann SP**, Zhao C.** A Canine Non-Weight-Bearing Model with Radial Neurectomy for Rotator Cuff Repair. PLoS One 10:e0130576. 2015. PMC4479444.

Carpal Tunnel Syndrome and Related Research

Carpal tunnel syndrome (CTS) is one of the most common neuropathies, which affects millions of people. However, the etiology for CTS is unknown. As a research fellow 21 years ago, I started to explore the subsynovial connective tissue (SSCT) mechanical and biological behaviors, which I believed might be associated with CTS when I performed a hand flexor tendon research project. My curiosity propelled some exciting preliminary data, which resulted in a R01 grant in 2000, as a co-Principal Investigator and continued funding for over a decade. Our contributions to this field include the role of the SSCT in CTS initiation, progression, and as a possible target for interventions[(1)]. Furthermore, we have successfully developed the first clinically relevant CTS animal model[(2)]. We have also contributed the use of imaging technology to study SSCT kinematics and carpal tunnel pressure[(3,4)] for the possibilities of early CTS diagnosis and prognosis, which branched another R01 and R21 grant related to CTS. Currently, we are working on establishing a CTS biomarker and intervention using our established animal, as compared with CTS patients. This body of work has made a great contribution to understanding of CTS.

1. Ettema AM, Amadio PC, **Zhao C**, Wold LE, An KN. A histological and immunohistochemical study of the subsynovial connective tissue in idiopathic carpal tunnel syndrome. Journal of Bone & Joint Surgery - American Volume 86-A:1458-1466. 2004. PMID: 15252093.
2. Yoshii Y, **Zhao C**, Schmelzer JD, Low PA, An KN, Amadio PC. The effects of hypertonic dextrose injection on connective tissue and nerve conduction through the rabbit carpal tunnel. Archives of Physical Medicine & Rehabilitation 90:333-339. 2009. PMID: 15252093.

3. Wang Y, Qiang B, Zhang X, Greenleaf JF, An KN, Amadio PC, **Zhao C**. A non-invasive technique for estimating carpal tunnel pressure by measuring shear wave speed in tendon: a feasibility study. J Biomech 45:2927-2930. 2012. PMID: 23031416.
4. Kubo K, Zhou B, Cheng YS, Yang TH, Qiang B, An KN, Moran SL, Amadio PC, Zhang X, **Zhao C**. Ultrasound elastography for carpal tunnel pressure measurement: A cadaveric validation study. J Orthop Res 36:477-483. 2018. PMID: 28731271.

Full list of my published work
http://www.ncbi.nlm.nih.gov/pubmed/?term=chunfeng+zhao

D. Additional Information: Research Support and/or Scholastic Performance
Research Support
Ongoing Research Support

NIH/NIAMS R01 AR 57745 Zhao (PI) 09/01/14-08/31/19
Engineering Tendon Grafts for Better Outcomes
This research proposal is to develop a novel method for engineering tendon surfaces to improve the outcomes of tendon reconstruction leading towards restoration of normal hand function.

NIH/NIAMS R21 AR 67421 Zhao (PI) 07/15/15-07/14/18
Novel non-invasive measurement of carpal tunnel pressure
This research proposal is to develop a noninvasive imaging technology for carpal tunnel syndrome detection using ultrasound elastography.

Completed Research Support
NIH/NIAMS STAR Program Zhao (PI) 09/01/15-08/31/17
Engineering Tendon Grafts for Rotator Cuff Repair and Regeneration
This research proposal is to develop a novel method for engineering tendon to augment rotator cuff repair using canine model.

Private Source Zhao (PI) 02/01/14-01/31/18
Synovialization and Revitalization of Tendon Allograft for Flexor Tendon Reconstruction
The overall goal of this project is to develop a clinically-applicable engineered tendon allograft that could become an off-the-shelf, functionally superior alternative to the conventional tendon autograft.

NIH/NIAMS R01 AR 57745 Zhao (PI) 04/07/10-08/31/14
Engineering Tendon Grafts for Better Outcomes
This research proposal is to develop a novel method for engineering tendon surfaces to improve the outcomes of tendon reconstruction leading towards restoration of normal hand function.

NIH/NIAMS R03 AR49407 Zhao (PI) 09/01/04-08/31/07
Cd-HA Gelatin in Flexor Tendon Transplantation
Surface modification with chemical binding HA improves extrasynovial tendon graft in canine model.

Private Source Zhao (PI) 2/01/2003-2006
Modification of Extrasynovial Tendon by Carbodiimide Derivatized Hyaluronic Acid for Tendon Graft
This study was to evaluate the frictional force in vitro and postoperative outcomes in vivo of the tendon graft with surface modification.

NIH/NIAMS R01 AR 44391-12 Amadio (PI) 02/01/09-01/31/15
Repair and Rehabilitation of Flexor Tendon Injury
The overall goal of this project is to improve the results of tendon repair through the development of therapies that affect the tendon gliding surface. We propose to investigate methods to preserve the beneficial effects of lubricin, while improving intrinsic tendon healing through a tissue engineering approach.
Role: Co-Investigator

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Withheld pursuant to exemption

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of the Freedom of Information and Privacy Act

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Budget Period: 1

Start Date: 09/01/2018 End Date: 08/31/2019

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	59.00	250,000.00	147,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim and Narendra Gandhi, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2017

Total Indirect (F&A) Costs: 147,500.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 397,500.00

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PHS 398 Modular Budget

Budget Period: 2			
Start Date: 09/01/2019 End Date: 08/31/2020			
A. Direct Costs			Funds Requested ($)
		Direct Cost less Consortium Indirect (F&A)*	250,000.00
		Consortium Indirect (F&A)	0.00
		Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	59.00	250,000.00	147,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim and Narendra Gandhi, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2017

Total Indirect (F&A) Costs: 147,500.00

C. Total Direct and Indirect (F&A) Costs (A + B)	Funds Requested ($)	397,500.00

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PHS 398 Modular Budget

Budget Period: 3			
Start Date: 09/01/2020 End Date: 08/31/2021			

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	59.00	250,000.00	147,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim and Narendra Gandhi, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2017

Total Indirect (F&A) Costs: 147,500.00

C. Total Direct and Indirect (F&A) Costs (A + B) Funds Requested ($): 397,500.00

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PHS 398 Modular Budget

Budget Period: 4			
Start Date: 09/01/2021 End Date: 08/31/2022			

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	59.00	250,000.00	147,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim and Narendra Gandhi, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2017

Total Indirect (F&A) Costs: 147,500.00

C. Total Direct and Indirect (F&A) Costs (A + B) Funds Requested ($): 397,500.00

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PHS 398 Modular Budget

Budget Period: 5			
Start Date: 09/01/2022 End Date: 08/31/2023			
A. Direct Costs			Funds Requested ($)
		Direct Cost less Consortium Indirect (F&A)*	250,000.00
		Consortium Indirect (F&A)	0.00
		Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	59.00	250,000.00	147,500.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim and Narendra Gandhi, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2017

Total Indirect (F&A) Costs: 147,500.00

C. Total Direct and Indirect (F&A) Costs (A + B)	Funds Requested ($)	397,500.00

Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	1,250,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	0.00
Section A, Total Direct Costs for Entire Project Period ($)	1,250,000.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	737,500.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	1,987,500.00

2. Budget Justifications

Personnel Justification	Personnel_Justification_smp.pdf
Consortium Justification	
Additional Narrative Justification	

Tracking Number: GRANT12600641
Funding Opportunity Number: PA-18-484. Received Date: 2018-04-03T15:57:34.000-04:00

PERSONNEL JUSTIFICATION

Chunfeng Zhao, M.D. EFFORT, Principal Investigator, is clinician-scientist with expertise in tendon/ligament related research, who will assume overall responsibility for the project, including TFBC engineered tendon development, TFBC fabrication, assessments, and data analysis in Aim 1. He will perform the animal surgeries and mechanical and biological testing in Aim 2. He will be responsible for manuscript preparation and preparation of the progress reports. Dr. Zhao will also be responsible for the day-to-day management of the project. Dr. Zhao is an orthopedic surgeon who has studied tendon biology and healing for the past 20 years. He has led the team to explore this novel technique for over 6 years, and published many papers regarding this topic.

Redacted by agreement EFFORT co-investigator, is an Assistant Professor in Cell Biology at Mayo Clinic Rochester, with expertise in cell signaling and gene manipulation for musculoskeletal regenerative medicine. Her contributions will greatly strengthen the study by assisting with cell biology work, analysis, and results interpretation. She has worked with the PI the past few years in tendon related research.

Redacted by agreement EFFORT, co-Investigator, is an orthopedic surgeon specialized in upper extremity, especially in shoulder surgery at Mayo Clinic Rochester with more than 20 years in clinical practice. He also has the experience and skill using canine model for rotator cuff repair related research. Redacted by agreement will assist with study design, surgery, data interpretation, and manuscript preparation. He has worked with Dr. Zhao for the past 10 years in the rotator cuff field.

Redacted by agreement EFFORT, consultant, is an emeritus professor and has extensive expertise in orthopedic biomechanics. He has played a key role in TFBC development, especially in biomechanics aspect. He will serve as a collaborator for this project and will participate in data interpretation, manuscript preparation and experimental design. Redacted by agreement has over 40 years of experience in biomechanical testing and analysis, especially in the upper extremity. He has worked closely with Dr. Zhao for the past 20 years.

Redacted by agreement EFFORT, consultant, is a hand surgeon at Mayo Clinic Rochester with more than 30 years experience studying tendon healing. Redacted by agreement will assist with study design, data interpretation, and manuscript preparation. He has worked closely with Dr. Zhao for the past 20 years.

Redacted by agreement EFFORT, Veterinary Technologist, will assist Dr. Zhao with specimen (tendon, shoulder, and bone marrow) harvesting. She will be assisting Dr. Zhao with rotator cuff repair surgeries during the in vivo study and will be in charge of animal postoperative care. She has worked closely with Dr. Zhao for the past 9 years.

Research Fellow [Effort= 6.0 person calendar months], To Be Named. The research fellow will assist Dr. Zhao in all experiments, including engineered tendon fabrication, assessments, and data analysis in Aim 1 and preparation of engineered tendons for surgical repair of rotator cuff in vivo, assistance of surgeries, and post analyses in biomechanics and biology in Aim 2.

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	INTRODUCTIO_final.pdf
Research Plan Section	
2. Specific Aims	Aim_final.pdf
3. Research Strategy*	Strategy_final.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animal_final.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	Combined_PCA_and_KNA_LOS.pdf
10. Resource Sharing Plan(s)	
11. Authentication of Key Biological and/or Chemical Resources	Authentication_FINAL.pdf
Appendix	
12. Appendix	

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Withheld pursuant to exemption

Evaluative Info

of the Freedom of Information and Privacy Act

SPECIFIC AIMS

A rotator cuff tear is one of the most common traumatic and degenerative tendon injuries resulting in over 4.5 million physician visits in the US alone. Functional restoration of rotator cuff defects usually requires surgical repair, estimated at 300,000 cased in the US annually. However, postoperative retear of repaired tendons ranges from 20% in small to medium tears to over 90% in large and massive tears. Recently, augmentation with grafting materials to strengthen a reparable tear or to bridge an unrepairable defect has become a common and attractive strategy to reduce the retear rate, especially for large or massive tears. The ideal augmentation should meet two basic criteria, including mechanical reinforcement of the repair, so that the repaired rotator cuff can withstand early shoulder mobilization without rupture and biological augmentation to accelerate healing and tissue regeneration. Current graft materials, however, have encountered great challenges in achieving these goals. Although many scaffold materials have been designed and studied, including biodegradable synthetic polymers and decellularized allologous or xenologous tissues, none have proven fully satisfactory in terms of mechanical support, cell adhesion, cell survival, function, biocompatibility, and immunogenicity. Furthermore, the transitional zone (fibrocartilage) at the rotator cuff enthesis, which is the most critical and unique structure to distribute stress concentration at the interface between flexible tendon and solid bone, has not been successfully rebuilt following rotator cuff repair.

To meet these challenges, we have developed a composite of multilayer tendon slices (COMTS), revitalized by seeding bone marrow-derived stem cells (BMSCs) within the slices, and then reassembled to an engineered tendon. This engineered COMTS has accelerated repair healing when it was used to augment rotator cuff repair in a rat model. More recently, with NIH STAR award support*, we used the COMTS concept to further develop an engineered tendon with layered tendon-fibrocartilage-bone composite (TFBC) from patellar-tibia unit. Our preliminary data have demonstrated that the TFBC not only mechanically reinforced rotator repair strength but also biologically augmented healing from our both ex and in vivo canine studies. As this unique engineered TFBC contains the native transitional fibrocartilaginous zone, it can potentially rebuild a tendon enthesis. The TFBC is also able to transform tissue healing interfaces from heterogeneous tissues (tendon to bone), a difficult healing interface, into the homogeneous tissues (tendon to tendon and bone to bone), thus improving healing qualities. **The overall goal of this proposal is to develop an engineered TFBC that substantially increases mechanical, biological, and enthesis-regenerative properties, thus improving clinical outcomes after rotator cuff repair.**

Specific Aim 1: Define TFBC mechanical augmentations for rotator cuff repair in canine in vitro model
Our recent preliminary data have shown that TFBC obtained from patellar-tibia composite has the similar histological structure and Young's modulus but stronger ultimate breaking strength compared to a normal rotator cuff unit. This information provides a well-justified rationale that TFBC is a good biomaterial for rotator cuff augmentation. In this aim, we will examine the mechanical effects of the TFBC on augmentation of a rotator cuff repair in an in vitro model using the double-row technique repair, which is often used in an arthroscopic approach. We hypothesize that using TFBC to augment rotator cuff repair will increase the repair strength compared to the repair alone or the commonly used GraftJacket augmentation in clinic.

Specific Aim 2: Test engineered TFBC biological augmentation and enthesis regeneration of rotator cuff repair in canine in vivo model
Although our proof of concept of the TFBC on rotator cuff augmentation has been verified in pilot studies, it needs to be further validated in a pre-clinical large animal model, especially in a long-term followup for functional outcome evaluations. In this aim, we will directly use the engineered TFBC that we have developed and test it with our recently established novel non-weight bearing shoulder canine model for rotator cuff repair augmentation. We hypothesize that the rotator cuff repair with the cell-seeded engineered TFBC augmentation will biologically enhance healing, promote enthesis regeneration, and decrease the retear following rotator cuff repair compared to the TFBC scaffold alone and a clinically used dermal patch (GrafJacket) augmentation. We will also test if a mechanical stimulation of the engineered TFBC could further accelerate rotator cuff healing.

If our aims are achieved, we will have developed a novel and translatable technology to improve clinical outcomes for rotator cuff repair by using an engineered allogenic composite tissue revitalized with autologous cells to enhance mechanical and biological ability for healing and regeneration. This biomaterial also contains the native tendon enthesis that can rebuild this unique structure, which remains a significant ongoing challenge for rotator cuff repair. Together, our proposed projects will have a broad impact on the field of musculoskeletal tissue engineering and a significantly specific impact on the treatment of rotator cuff tear.

* https://www.niams.nih.gov/newsroom/niams-awards-supplements-advance-research

RESEARCH STRATEGY

A. SIGNIFICANCE

General Information: The rotator cuff includes four muscles and tendons that surround the shoulder joint capsule. It plays an important role in stabilizing the glenohumeral joint and provides strength during shoulder motion. Rotator cuff tear affects 30-50% of people older than 50 years and is the most common cause of shoulder pain and dysfunction in all age groups[1-4]. There are more than 4.5 million rotator cuff-related physician visits and approximately 300,000 ambulatory surgical repairs with a cost of $26K-50K per procedure and return-to-work time of 7-11 months[5,6]. Rotator cuff repairs, through either open or arthroscopic approach, have been effective and satisfactory reducing pain and restoring shoulder function for small or partial tears[8]. However, successful repair of large or massive tears (≥3cm), which comprise the majority of rotator cuff tears, continues to present a significant clinical challenge[8-11]. Closure of a large tear creates tremendous tension on the repair site leading to a high risk of failure (retear). Repair ruptures have been reported in 20% to 90%, depending upon the specific procedure or clinical scenario[12-16]. Although incomplete closure decreases repair tension, a gap between tendon and bone not only delays healing but also significantly diminishes the quality of healing. ***Improvement of clinical outcomes for rotator cuff repair remains a significant clinical concern.***

Augmentation: Rotator cuff repair with graft augmentation has been recognized as an effective surgical procedure enhancing a reparable tear or bridging an irreparable defect for the large or massive tears[17-21]. In 2010 in the USA alone, 30% of the total of large or massive rotator cuff repairs (≥3 cm) were used with some kind of augmentation[22]. Repair augmentation has two purposes. The first is to mechanically strengthen the repair immediately allowing time for healing to take place, especially for a high tension repair. The second is to enhance biological healing by increasing tissue volume and contact area. Currently, the most common graft substitutes for augmentation include dermal allografts or xenografts[23-25], small intestinal submucosa xenografts[26,27], and synthetic materials[28,29]. Unfortunately, published results suggest that these products offer few advantages when compared to repair alone, with high rates of adverse effects[30,31], including aseptic inflammatory reactions, especially for xenografts[32-34]; weakening of the repair[35,36]; and delayed graft healing[37-40]. The other challenge is that the regeneration of tendon enthesis still remains unsolved[41-43]. Finally, the current available augmentation materials are primarily derived from acellular tissues, which require a long time to revitalize leading to delayed healing to host tissues[44,45]. Thus, while repair with a graft augmentation is the procedure of choice for enhancement of rotator cuff repair, ***the graft materials currently available are inadequate to provide sufficient mechanical support, to promote sound healing for large rotator cuff tears, to overcome tendon-bone healing deficiency, and fail to regenerate the fibrocartilage enthesis.***

Tissue Engineering: Tissue engineering offers the potential to generate more functional tissue replacements by supplying a cell source, optimizing the interactions among cells, enhancing the extracellular matrix, and providing appropriate bio-environments for cell differentiation[46-49]. The scaffold is crucial for providing a basic functional structure, as it must not only meet the mechanical properties that is similar to the tendon and enthesis tissues for the functional performance, but must also possess the biological properties that support cells growth, migration, and differentiation[50-52]. Biodegradable synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), have been well studied as scaffolds for bone, cartilage, tendon, and ligament regeneration[53-55]. However, regulation of the polymer surface for cell attachment, porosity for cell survival, mechanical properties for functional performance, and degradation for new tissue regeneration remain challenging[56,57]. The particles released during polymer degradation can result in a highly acidic environment, resulting in an adverse effect on cellular function and tissue remodeling[58-60]. While scaffolds using decellularized native tendon demonstrate some advantages with regard to mechanical properties, tissue structure, and biocompatibility, one major disadvantage is that cell seeding is limited to the tendon surface[61,62]. Lack of cells within the graft may delay the graft healing and regeneration. Tendon scaffold treated with strong chemical detergent increased the porosity and improved cell penetration, but decreased mechanical properties, destroyed extracellular matrix, and induced toxicity due to residual chemicals affecting cell seeding and survival[63-65]. ***Therefore, the development of an engineered tendon that possesses native tendon mechanical and biological properties, supporting cell proliferation, migration, and differentiation, and regenerating enthesis could lead to a breakthrough for rotator cuff repair.***

Animal Model: A variety of animal models has been extensively studied to investigate rotator cuff tear including mouse, rat, rabbit, sheep, dog, bovine, and monkey with either acute laceration or chronic injuries[33,66-69]. However, no rotator cuff tear in an animal model is identical to the human rotator cuff tear, and each model has advantages and disadvantages[70,71]. Determining the appropriate animal model for rotator cuff research is dependent upon the context of the scientific or clinical questions that need to be addressed. A few

critical issues need to be considered when choosing an animal model in terms of interpreting human conditions: **1)** lack of spontaneous tendon-bone healing; **2)** large tendon size allowed for repair techniques similar to those used in human; and **3)** good consistency and reproducibility of the injury creation and surgical technique. Certainly, other concerns such as cost, animal care, ethical concerns, etc., are also important. Although the rat model is the most common animal used for rotator cuff research due to its anatomic similarity to humans and low cost, surgical repair of such small tendon, especially with graft augmentation, can be challenging to achieve consistency and reproducibility[72]. Furthermore, the rotator cuff tendon heals robustly and faster in rats than in humans[73,74]. Canine is a common large animal model for rotator cuff research, especially for the topics related to surgical technique, augmentation, or tendon-bone healing[30,33]. However, high tensile stress applied to the repair site due to large body weight bearing results in failure of the repair by retear. Some have reported that canine acute full-width tendon repairs fail universally within the first postoperative days, regardless of suture type, suture configuration, or postoperative protocol[75]. ***Therefore an appropriate large animal model is critical for translational research and needs to be developed to study any interventional strategies to improve clinical outcomes, including augmentation for rotator cuff repair.***

Significance: Given the frequency and high cost of rotator cuff injuries, as well as the relatively poor results of surgical intervention in large or massive tears, development of innovative and effective strategies for rotator cuff repairs will have a significant impact on clinical practice. Although repair with patch augmentation has been recently investigated in both experimental and clinical settings, limited success has been achieved to satisfy the following fundamental needs: **1)** mechanical strengthening, **2)** biological enhancement, and **3)** enthesis regeneration. In this grant application, we propose to develop a native decellularized tendon-fibrocartilage-bone composite (TFBC) scaffold revitalized with BMSC sheets to achieve these three critical requirements. With the support from the NIH/NIAMS STAR (Supplements to Advance Research) award in 2015 (one of three recipients awarded to expend current R01 research project into a broader multi-faceted research program), we have conducted several preliminary studies for the proof of this concept and have also successfully established a novel canine non-weight bearing shoulder model to mimic the human shoulder. This canine model has been extensively used for flexor tendon research by our group[76,77]. Our preliminary data demonstrated that this animal model is a good model to study rotator cuff repair[78]. ***We believe that this novel engineered tendon composite will provide a breakthrough to improve the functional outcomes of rotator cuff repair, especially for the most challenging large or massive defects, resulting in a significant impact on clinical translation.***

B. INNOVATION

Tissue engineering approach for rotator cuff repair and regeneration has been studied. However, the current proposal brings a new concept and introduces the following innovations and advantages over the existing techniques.

1. The TFBC provides the layered construct for cell-sheets seeding, proliferation, and migration without altering tendon mechanical properties. Native tendon extracellular matrix promotes BMSC tenogenesis and tendon healing.
2. The TFBC scaffold has native tendon-fibrocartilage-bone structure, mechanical properties, and organization that match those of normal rotator cuff enthesis. The engineered TFBC can be directly transplanted, thus rebuilding a tendon enthesis, the most important structure in mechanical and biological function of the rotator cuff.
3. The TFBC converts healing interface from a heterogeneous tendon to bone interface (difficult to heal) into a homogeneous tissue healing interface (tendon to tendon and bone to bone), while maintaining a functional fibrocartilage zone.
4. Large contact area between sandwiched TFBC and rotator cuff tendon increases the tendon-to-tendon healing ability, which forms a tendon complex with bone and fibrocartilage at the end. In this way, rotator cuff repair converts a “tendon avulsion” like repair, which capitalizes on robust fracture healing mechanism (**Fig 1**).
5. The TFBC can be simply fabricated, manipulated, packed, sterilized, and stored shelf-ready for clinical use based on our preliminary studies.

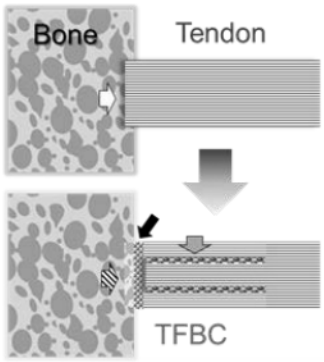

Fig 1. Tendon to bone healing is difficult (white arrow). TFBC convers tendon-bone interface into bone-bone (stripe arrow) and tendon-tendon (grey arrow) healing interface. It also contains fibrocartilage zone (black arrow) for enthesis formation.

6. A novel non-weight bearing limb canine model, which mimics human upper extremity condition will be, for the first time, used for a rotator cuff injury and repair model. Radial nerve denervation at upper arm results in paralyzing all extensor muscles in the forearm. The elbow and wrist is not able to extend to bear weight. We have successfully used this model in the flexor tendon research for non-weight bearing for two decades[76,77]. As

the denervation is below the shoulder, it does not affect rotator cuff muscle activities and rotator cuff healing. We believe our model provides a more comfortable animal handling process compared to a spica cast[79,80], improves ease of postoperative animal care, and effectively reduces the retear rate following rotator cuff repair in a canine model, which is currently very challenging. Furthermore, this model indeed mimics a human shoulder, a non-weight-bearing activities and function.

From our abundant preliminary data, we believe that the abovementioned novelties are realistic, feasible, achievable, and most importantly superior to the current augmentation technologies.

C. APPROACH

C.1. Specific Aim 1: Define TFBC mechanical augmentations in canine in vitro model

C.1.a. Rational and Hypothesis

The fibrocartilage zone in the tendon enthesis has been generally recognized as a transitional structure that reduces the stress concentration when a tensile force transmits from flexible tendon to rigid bone. This structure has been well studied in biomechanical, structural, biological aspects[81-84]. Recently, we have compared the TFBC harvested from patella-tendon-tibia complex with the infraspinatus-tendon-humerus complex and found that the TFBC has similar mechanical and structural properties. Although our preliminary data have shown that the rotator cuff repair in this TFBC augmentation significantly increased the failure strength and stiffness[85], the repair technique used in this study was a Mason-Allan repair, which can only be used when performing an open approach. Recently, the arthroscopic repair has become more common in clinical practice[86]. Double-row repair is the most common repair technique in the arthroscopic approach. In this specific aim, we will mechanically test the TFBC augmentation compared to the commercially available augmentation material, GrafJacket, using a double-row repair technique. Although the double-row technique is different from Mason-Alan in surgical configuration, the basic principle is similar. Therefore, we predict the TFBC could augment double-row repair mechanically.

C.1.b. Supporting Preliminary Studies

During the past few years, we have conducted many studies that focused on the rotator cuff research including imaging[87-89], surgical techniques[33,90-93], material development[61,94], tissue engineering[78,95,96], and animal model development[78]. Below are published and unpublished studies that present preliminary data related to this Aim.

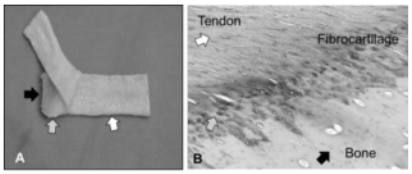

Fig 2. Layered TFBC (**A**) and Safranin O staining (**B**) with three regions, tendon (white arrow), fibrocartilage (grey arrow), and bone (black arrow).

C.1.b.1. Rotator cuff repair with a tendon–fibrocartilage–bone composite bridging patch[85]

In this study, we developed a novel tendon-fibrocartilage-bone composite (TFBC) using decellularized patellar-tibial complex for the rotator cuff repair augmentation. The TFBC was sliced into two slices (**Fig 2A**) with the fibrocartilage region preserved (**Fig 2B**). We compared the mechanical performance of an infraspinatus tendon repaired with a TFBC patch augmentation vs the traditional Mason–Allen repair in an in vitro canine model (**Fig 3**). The ultimate tensile load and stiffness were significantly higher in the TFBC patch group than the control. More interestingly, the repair stiffness displayed different distribution in the different zones in which the distal TFBC showed more than two-folds higher increases in stiffness as compared to repair without augmentation (**Fig 4B**). This demonstrated that TFBC augmentation could help to restore the native rotator cuff mechanical properties. Furthermore, the TFBC transforms the a tendon-to-bone healing interface (dissimilar tissues) into a bone-to-bone and tendon-to-tendon interfaces (similar tissues), which may improve healing quality and reduce retear rate[85].

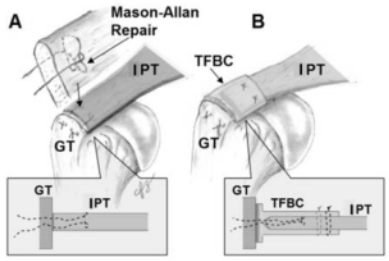

Fig 3. A: Repair alone and **B:** Repair with TFBC augmentation.

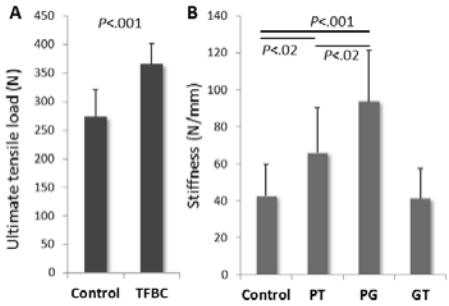

Fig 4. A: Ultimate strength. **B:** Overall and reginal stiffness. PT: entire TFBC construct; GT: TFBC-greater tuberosity repair site; PG: TFBC-infraspinatus tendon repair site.

C.1.b.2. Comparison of TFBC and infraspinatus tendon in mechanical properties and structure

It is important to understand if the TFBC harvested from patellar-tibia composite would have similar mechanical properties and structure to the normal infraspinatus tendon composite (ISTC) that needs to be rebuilt. In this

study, fresh TFBC (n=10) from the knees and ISTC (n=10) from the shoulders were obtained for the dogs (weighted about 20 kg from both sexes) that were sacrificed from other studies. The specimens were mounted on a servohydraulic test machine (858 MiniBionix II; MTS Systems Corp, Eden Prairie, MN) and a Digital Image Correlation System (DICS) (ARAMIS 4M, Trilion Quality Systems, Plymouth Meeting, PA) was used to accurately measure the strain during mechanical testing (**Fig 5**). TFBC and ISTC (n=3) were fixed in 4% paraformaldehyde, decalcified with Decalcifying Solution B (Protocol, Fisher HealthCareTM), dehydrated and paraffin embedded. Longitudinal sections (5 µm thickness) were stained with hematoxylin and eosin (H&E, Sigma), Safranin O/Fast green and Picrosirius red for morphological characteristics, proteoglycan assessment and collagen fiber orientation, respectively. We found that the Young's modulus was similar in both composites, but the failure strength of the TFBC was significantly higher than the ISTC group (p<0.001) (**Fig 6**). The failure mode of the ISTC group broke at the tendon/bone conjunction, while the TFBC failed at the tendon/clamp interface. These mechanical results indicate that the TFBC is stronger than ISTC and mechanically appropriate for the ISTC reconstruction. Histological evaluations of the TFBC and ISTC demonstrated the similar structure in tendon, fibrocartilage transitional zone, and bone regions (**Fig 7**).

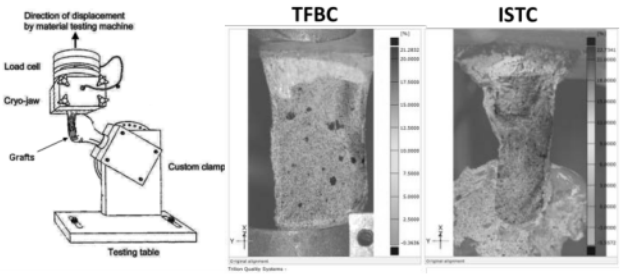

Fig 5. Mechanical testing with DICS system

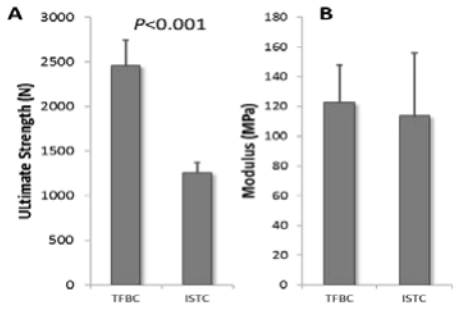

Fig 6. **A:** Ultimate failure strength and **B:** Young's modulus of the TFBC and ISTC

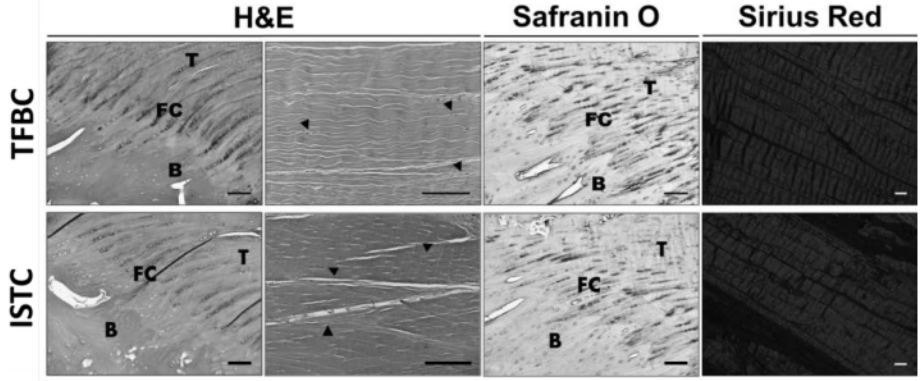

Fig 7. Histomorphological comparisons of TFBC and native infraspinatus tendon composite (ISTC). Safranin O stains the proteoglycans in the fibrocartilage orange to red. Picrosirius red-stained sections shown are under polarized light. The arrows indicate tenocytes. (B: bone; FC: fibrocartilage; T: tendon) Scale bar: 100µm

C.1.c. Rigorous Study Design

In this study, we will test mechanical augmentation of the TFBC. The canine specimens will be used for this study as we have collected enough shoulders and knees that need for this study from our and others previous studies that used canine model with intact shoulders and knees. We did not consider using human cadaveric model is because a large percentage of human cadavers may have rotator cuff problems[97,98], which create a great variability. A total 30 shoulders and 10 knees from the dogs of about age of 1 year old and weight of 20kg in both sexes will be used for this experiment. TFBCs (n=10) will be prepared based on our established procedures (**C.1.b.1.**). Thirty shoulders will be randomly assigned into three groups. 1) Double-row repair alone (n=10), 2) repair with GrafJacket augmentation (n=10), and 3) repair with TFBC augmentation (n=10). Following repairs, the specimens will be mechanically tested with the following protocols.

C.1.d. Methods

C.1.d.1. TFBC Fabrication

TFBCs will be prepared as previously described[85]. Briefly, canine patellar tendons with its intact bony attachment at tibia will be trimmed to roughly 12 × 40 mm in size and then cut horizontally into two layers with #15 surgical blades. The tibial bony portion will be trimmed in to a 5-mm in width. Trimming a small bony segment is for a potential possibility that the TFBC can be introduced through an arthroscopic portal. Rotator cuff augmentation with graft materials has been successfully completed through the arthroscopic approach[22,99]. A special arthroscopic portal could be designed if the TFBC could be translated to clinical practice. The patellar tendons will be cut horizontally into 2 layers to sandwich the lacerated infraspinatus tendon (**Fig 8**). The TFBCs will be immersed in liquid nitrogen for 2 minutes and then thawed in saline solution at 37 °C for 10 minutes. This procedure will be repeated five times to devitalize the tendon. The TFBCs will be incubated in nuclease solution (RNase: 100 µg/mL and

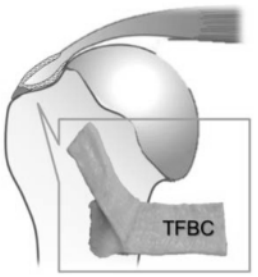

Fig 8. TFBC augment

DNase: 150 IU/mL, Roche Diagnostic) for 12 hours at 37°C to decellularize tendon. The repetitive freeze/thaw combined with nuclease treatment for 12 hours can efficiently remove cellular and nuclear materials[90,96]. Finally, all composites will be lyophilized in a freeze dryer (Millrock Technology Inc) and packed in polyethylene bags for ETO gas sterilization. These procedures did not alter the tendon mechanical properties[100,101].

C.1.d.2. Mechanical Evaluation of TFBC Augmentation in an In Vitro Model

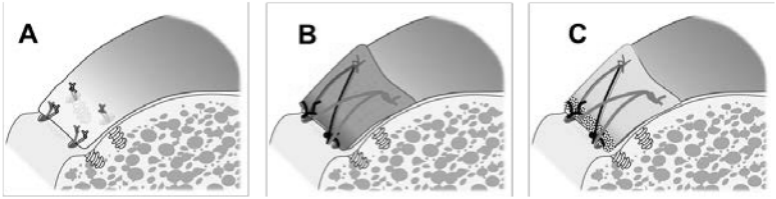

Fig 9. Repair alone (**A**), GraftJacket (**B**) & TFBC (**C**) augmentations

Canine shoulders will be dissected with preservation of infraspinatus muscle and tendon and humerus intact. Rotator cuff tears will be consistently created by sharply transected at the infraspinatus tendon at its insertion site. The prepared shoulders will be randomly assigned to 3 groups (**Fig 9**), with 10 specimens in each group. Double-row repair will be performed with 4.5-mm Corkscrew FT suture anchors (Arthrex, Naples, FL) loaded with #2 FiberWire sutures (Arthlex) based on the technique in our previous report[93]. Briefly, the medial-row anchors will be placed along the articular margin. Lateral-row anchors will be placed 10 mm apart from medial-row anchors. Sutures will be passed through the infraspinatus tendon and then tied the mattress configuration for the repair alone. For the augmentation repair, the suture will pass through both infraspinatus tendon and patch or sandwiched TFBC (**Fig 9**). Following repair, the humerus will be potted into a small block of polymethylmethacrylate, mounted and positioned at an incline of 135° to the long axis of the tendon to model the physiological pull of the infraspinatus tendon[85]. When the humerus is positioned, the free supraspinatus muscle will be held in a custom-made cryo-jaw at the muscle–tendon junction (**Fig. 5**). Using liquid carbon dioxide flowing through the cryo-jaw, the clamped muscle belly will be frozen to prevent failure at the tendon-grip interface and tissue slippage. The flow of the liquid carbon dioxide will be carefully controlled to prevent the tendon from freezing. Tensile testing will be carried with a 10-N preload applied, and the specimen will be loaded to failure at a rate of 1.0 mm/s[91]. The displacement in different regions will be measured by A Digital Image Correlation System (ARAMIS 4M, Trilion Quality Systems, Plymouth Meeting, PA) (**Fig 5**). Briefly, two CCD cameras equipped with 75mm lenses will be mounted on a tripod and positioned vertically in front of the loading fixture. The system will be calibrated to an 80x60x2cm capture volume (2358x1728 pixels). The relative position of the cameras with respect to each other will be calibrated using a high-precision 50x44mm calibration target. A pair of LED lamps will be used to illuminate the specimen and polarizing lens filters will be used to minimize glare and enhance the visibility of the surface pattern on the specimen. The mechanical testing protocol and Young's module calculation in different regions will be performed based on well-established protocols[94,102]. The failure mode, ultimate breaking strength and stiffness in repair will be also recorded and calculated.

C.1.e. Pitfalls and Alternatives

During this in vitro study, we do not anticipate any major difficulties in terms of the biomechanical testing and analysis because all methodologies are well established in our laboratory. However, the major concern is whether this in vitro investigation using a canine model would be applied to the human. Since the majority of human cadavers come from the older population; the rate of rotator cuff tear, injury, or degeneration is likely very high. Collecting a large number of normal cadaveric shoulders for the mechanical evaluations can be very time consuming. We have been using the canine model for tendon related research for over two decades and found the biomechanical and structural properties of canine tendon are very similar to humans. However, we will seek any possibility for obtaining young aged cadavers without shoulder problems for the material and structural analysis and compared to canine tissues. The double-row repair with or without patch augmentation will be performed with an open approach in our canine model, since there is no equipment that can be used for canine arthroscopic approach. This in vitro repair data can also serve as the time zero data to compare the results from Aim 2 in vivo study. Finally, although the double-row repair has been used in animal models[103,104], to our knowledge, double-row repair with suture anchors used in clinic has not been reported in animal model. We anticipate that the smallest available suture anchor (4.5 mm in diameter) will be appropriate to repair our TFBC (12 mm in width) with a double-row technique in canine model. However, if the anchor is too big to place four of those in a canine shoulder, we will custom-make small suture anchors for the use.

C.2. Specific Aim 2: To test engineered TFBC biological augmentation in canine vivo model

C.2.a. Rational and Hypothesis

Augmentation with biomaterials provides an effective way to reduce rotator cuff re-tear, especially for large or massive rotator cuff tear in current practice[21,34,99]. Tissue engineering approach offers an appealing technology

for augmentation material development[2,17]. Our preliminary studies have demonstrated that tendon could be disassembled with multilayer slices that maintain tendon mechanical properties and reassembled them with cell-based technology for tendon regeneration[95]. This proposal presents a novel and translational technology using an engineered native composite tissue to address this important issue in both clinical and research arena. We have characterized engineered TFBC mechanical and biological properties in our multiple preliminary studies that fully support our hypothesis that the TFBC engineered with BMSC sheet will mechanically enhance the repair strength and biologically augment healing, thus improving functional outcome following rotator repair. More encouragingly, our recent short-term in vivo study using our novel canine non-weight bearing shoulder model demonstrated that our engineered TFBC increased the healing ability compared to repair alone or TFBC alone augmentation. All repairs with TFBC+cell augmentation failed at the bone-bone interface, which indicated that TFBC-tendon healing was stronger than the TFBC-bone healing. This failure mode further reveals that bone-bone healing is faster and stronger than tendon-bone healing (control group), which has been also verified in other studies[105,106]. Therefore, a translational research with a long-term functional evaluation becomes critical for the engineered TFBC augmentation in rotator cuff repair before clinical application. The choice of a canine model are based upon: **1)** a common animal model for tendon research since 1941[107], **2)** our experience with the canine model including rotator cuff repair, flexor tendon repair and graft, postoperative care, and analytic methodologies, **3)** supportive preliminary studies in a canine model, **4)** size of canine rotator cuff makes surgical repair, especially with augmentation, more clinically relevant, and **5)** our novel free shoulder motion and non-weight bearing canine model not only mimics human shoulder function but also reduces the repair retear, which is a significant drawback for using the canine as a rotator cuff research animal model[75].

C.2.b. Supporting Preliminary Studies

C.2.b.1. Tendon Slices Seeded with BMSC (Unpublished data)

The TFBC improves tendon healing by converting a tendon end-to-end healing (small contact area) into a longitudinal surface-to-surface healing (like a sandwich). It is important to understand if the BMSC seeded between tendon slices can promote healing. Tendon slices 40x4x0.3mm were cut from decellularized canine Achilles tendon. Two tendon slices were stacked together with or without BMSC ($2x10^6$/slice) seeded between the slices. The slices were cultured for 0, 3, 7, and 14 days. After tissue culture, the slices were mounted on a custom-made microtester and the shear force was measured (**Fig 10**). The adhesion strength was calculated with the shear force divided by contact area. The shear force of the cell seeded slices was significantly increased after 7 days and also significantly higher than the slices without cells at 7 and 14 days (**Fig 11**). Fusion was observed between slices in the cell-seeded group (**Fig 12**). This study suggests that tendon healing is increased with BMSC transplant.

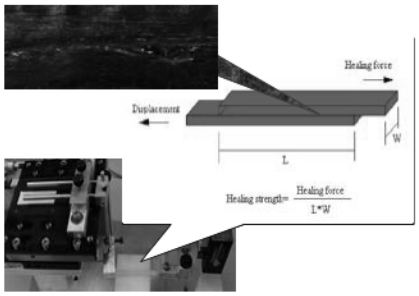

Fig 10. Stacked tendon slices were tested for shear strength to evaluate sliced tendon healing.

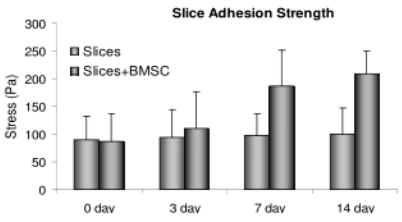

Fig 11. Shear strength of tendon slices

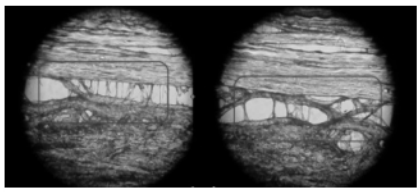

Fig 12. Tendon slices seeded with BMSC showed partial collagen fusion between slices.

C.2.b.2. FFBC revitalized with BMSC sheets for rotator cuff regeneration (unpublished data)

In this study, we investigated the biological and mechanical properties of an engineered TFBC patch with cyclic mechanical stimulation. Decellularized patellar TFBCs were seeded with MBSC sheets and subjected to mechanical stimulation which consisted of uniaxial 3.0 % strain at 0.2 Hz for 20 min of each hour, 12 h/day based on our well-developed protocols[95,101] for one week (**Fig 13**). The TFBC was then characterized by histology, immunohistochemistry, mechanical testing, and transcriptional regulation. Mechanically stimulated TFBC-BMSC constructs qualitatively displayed increased cell infiltration after 7 days of culture compared to static groups (**Fig 14A**). Young's modulus of the cell-seeded TFBC was significantly higher than the non-cell-seeded TFBC (**Fig 14B**). Tenogenic and chondrogenic genes including scleraxis, Sox-9, tenascin-C (TNC) were upregulated with mechanical stimulation (**Fig**

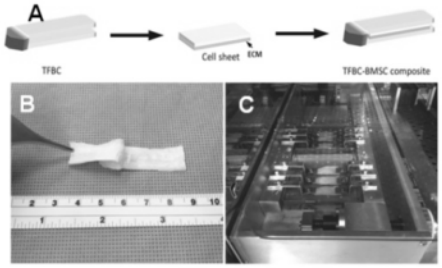

Fig 13. (**A**) Cell sheet with ECM sandwiched between tendon slices of the TFBC. (**B**) Patellar TFBC. (**C**) TFBC-BMSC composites placed in a custom-made bioreactor for dynamic culture.

14C). Given the increased cell infiltration and upregulated expression of tendon and cartilage genes, we believe that the TFBC-BMSC will provide a novel biological patch to improve outcomes of rotator cuff repair.

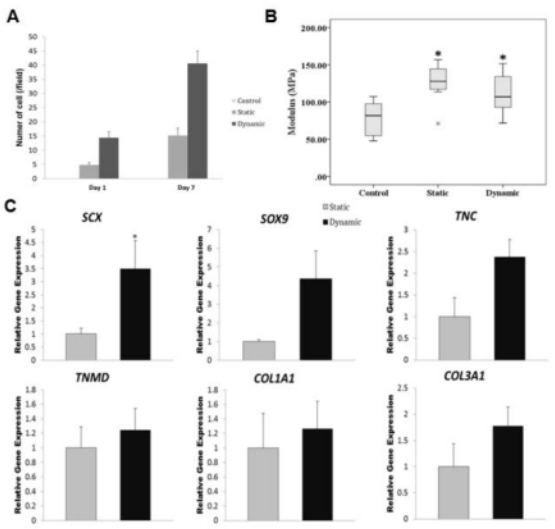

Fig 14. Cell number (**A**) and Young's modulus (**B**) increased under loading. **C:** Gene expression of scleraxis, SOX-9, TNC, tenomodulin, Col I and III

C.2.b.3. A Canine Non-Weight-Bearing Model with Radial Neurectomy for Rotator Cuff Repair[78]

The major concern of using a canine model to study rotator cuff repair is the high rate of repair retears. The purpose of this study was to test a non-weight-bearing canine shoulder model for rotator cuff repair research. First, in the in vitro study, 18 shoulders were randomized to 3 groups. Group-1) Full-width infraspinatus tendon transections repaired with modified Mason-Allen sutures using #3-0 polyglactin suture, Group-2) Group-1 repaired using #2 polyester braid suture, and Group-3) Partial-width transections leaving the superior a 2-mm infraspinatus tendon intact without repair. Second, in the in vivo study, the infraspinatus tendon from 6 dogs was partially transected as the same as the in vitro Group-3. A radial neurectomy was performed to prevent weight bearing. The operated limb was slung in a custom-made jacket for 4 weeks and then free activities allowed. Results showed that in the in vitro study, mean ultimate tensile load and stiffness in Group-2 were significantly higher than Group-1 and 3. There was no significant difference between partial transection group and #3-0 suture repair group **(Fig 15**). In the in vivo study, gross inspection and histology showed that the preserved superior 2-mm portion of the infraspinatus tendon remained intact with normal structure. If the strength of a 2-mm infraspinatus tendon bundle was similar between in vitro and in vivo, the rotator cuff repair even with #3-0 suture could bear the load without rupture during canine non-weight activities. From this study, we believe that the canine non-weight bearing model is an appropriate and useful model to investigate rotator cuff repair related research[78].

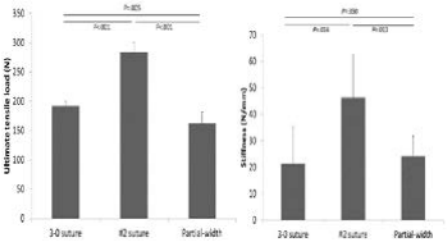

Fig 15. Ultimate tensile strength and stiffness in three groups

C.2.b.4. Rotator Cuff Repair Augmentation in a Rat Model with Multilayer Tendon Scaffold with Bone Marrow Stromal Cells[96]

The purpose of this study was to investigate a composite of multilayer tendon slices (COMTS) seeded with bone marrow stromal cells (BMSCs) for the rotator cuff augmentation using a rat model. Adult female Lewis rats underwent transection of the supraspinatus tendon and a 2-mm tendon resection at the distal end, followed by immediate repair to its bony insertion site under tension. Animals received 1 of 3 treatments at the repair site: (1) no augmentation, (2) COMTS augmentation alone, or (3) BMSC-seeded COMTS augmentation (**Fig 16**). BMSCs were labeled with fluorescent cell marker. Animals were euthanized 6 weeks after surgery for analyses. Histologic analysis showed robust fibrous tissue was observed in rats with BMSC-seeded COMTS augmentation. However, fibrous tissue was scarce within the gap in rats with no augmentation or COMTS-only augmentation (**Fig 17**). The labeled transplanted BMSCs were observed throughout the repair site (**Fig 18**). Biomechanical analysis showed that the repairs augmented with BMSC-seeded COMTS had significantly greater

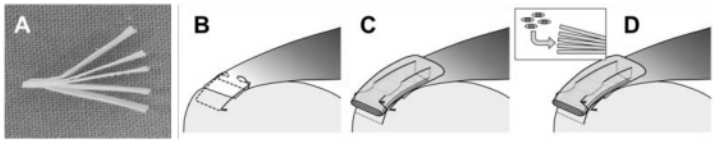

Fig 16. A: Composite of tendon slices; Three treatments: **B:** repair alone, **C:** repair+COMTS, and **D:** repair+COMTS+BMSCs

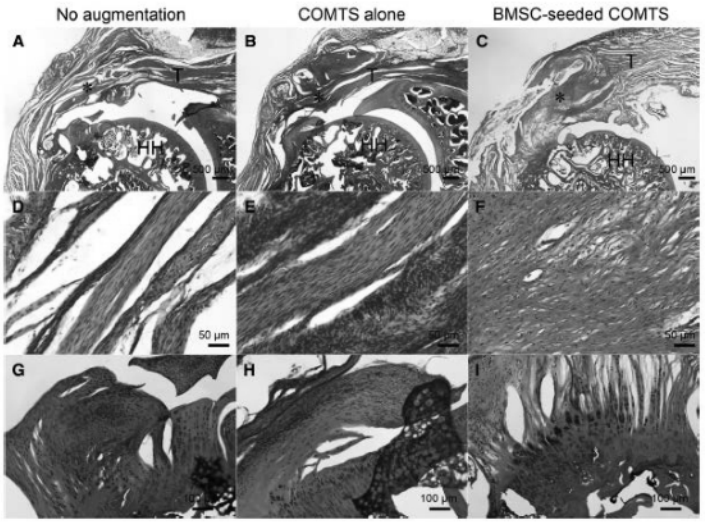

Fig 17. (**A–C**), Repaired supraspinatus tendon and the bony insertion site (×20). (**D–F**) Fibrous tissue in the gap (×200), as indicated by the * in panels **A–C**. HH, humeral head; T, tendon. (**G–I**) Tendon-to-bone insertion point (×100).

ultimate load to failure and stiffness compared with other treatments. However, baseline (time 0) data showed that COMTS-only augmentation did not increase mechanical strength of the repair site. In conclusion, although the COMTS scaffold did not increase the initial repair strength, the BMSC-seeded scaffold increased healing strength and stiffness 6 weeks after rotator cuff repair in a rat model[96].

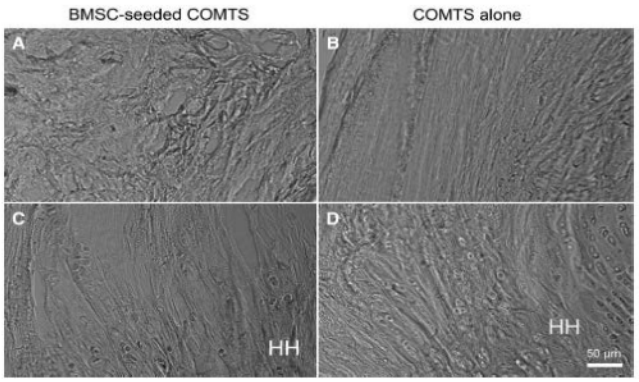

Fig 18. Confocal laser microscopy images show DiI cell labeling in the tendon (**A** & **B**) and insertion site (**C** & **D**)

C.2.b.5. Characterization of Canine Bone Marrow Derived Stem Cells and Use for Tendon Field

Mesenchymal stem cells (MSCs) have been successfully identified and isolated from a variety of tissues including bone marrow, skin, adipose tissue, tendon, lung, umbilical cord, skeletal muscle, etc., among which bone marrow-derived MSCs (BMSCs) is most commonly used for tissue regeneration and cell therapy because of their easy accessibility, expandability, and multipotency under certain biological or mechanical stimulations[108,109]. We have characterized the BMSC at different passages, surface antigen expression, proliferation/migration capacity, and multilineage differentiation potentials, and most importantly, identified the canine BMSC tenogenic potential under tenogenic biological stimulation[7,96,110,111], under tendon scaffold environment[61,90,100], or mechanical stimulation[95,101]. Although some of our studies showed muscle derived stem cells might be more tenogenic compared to BMSC, we did not find significantly improvement for tendon healing between these two cell lines[7,111]. Considering accessibility, we have extensively used BMSCs for tendon repair and regeneration[112-115] (**Fig 19**).

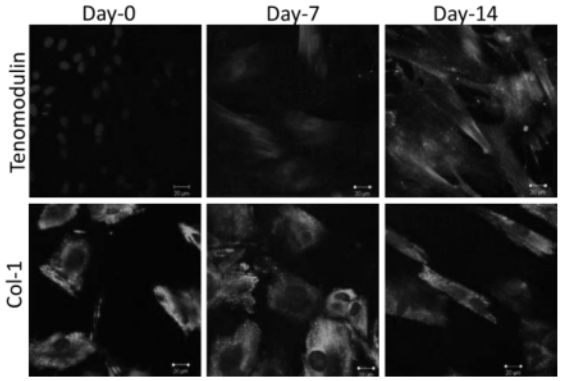

Fig 19. Canine BMSCs immunohistochemistry for tenogenesis (Tenomodulin and Col1)[7]

C.2.b.6. Engineered TFBC for Rotator Cuff Augmentation in a Canine In Vivo Model (unpublished data)

In this pilot study, we used our non-weight bearing canine shoulder model to study the effects of engineered TFBC augmentation on rotator cuff repair. The TFBC was prepared according to the protocol described in ***C.1.d.1*** and autologous? BMSCs were harvested three weeks prior to the surgery to make a cell sheet (CS) (***C.2.d.2***). Thirty-six dogs were used and infraspinatus tendon from each dog was sharply transected and repaired randomly with the following three groups. Group 1: repair alone, Group 2: repair with TFBC augmentation, and Group 3 repair with TFBC+SC. Mason-Allan repair technique was used (***C.1.b.1.***). **Fig 20** illustrated and described surgical procedures in details. Following rotator cuff repair, the radial nerve was exposed in the upper leg and sharply transected to paralyze forearm extensor muscles to prevent the operative limb from weight bearing. A custom-made comfort sling was applied for four weeks and then surgical shoulder was allowed free activities. Six weeks after surgery, the dogs were sacrificed and rotator cuff repairs were tested in repair strength, cell viability, and histology. The metal wire in both TFBC augmentation groups was removed before mechanical testing to eliminate its effects on repair strength. There was no repair rupture after six weeks of surgery in all groups which indicates this non-weight-bearing canine model is a reliable animal model. During the mechanical testing, all samples in the TFBC+CS group were ruptured at the bone-bone

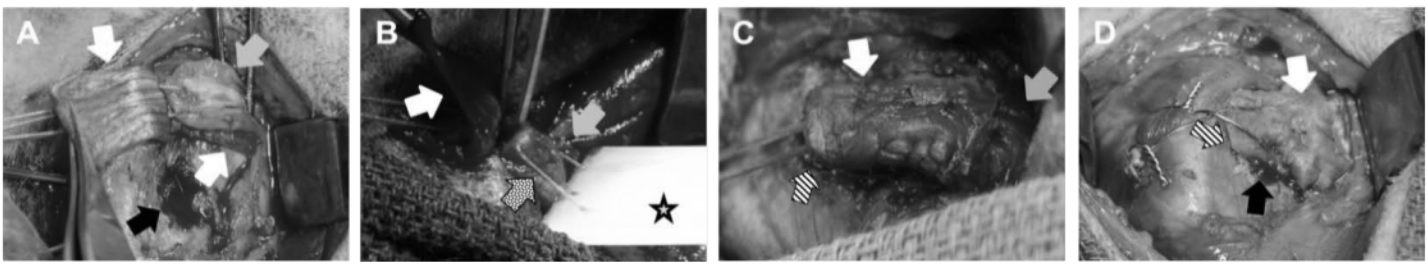

Fig 20. TFBC repair method: Infraspinatus tendon (grew arrow was fed between two TFBC slices (white arrows). Cortical bone from the foot print of infraspinatus was removed (black arrow) to create a good bone to bone healing interface (**A**). Cell sheet (dot arrow) was placed between tendon slices with a cell scraper (star) (**B**). Two tendon slices were closed to wrap the infraspinatus tendon with sutures to form a tendon-fibrocartilage-bone composite (**C**). Then, the TFBC-infraspinatus composite was repaired with Mason-Allan technique and an extra metal wire was used to fix the bony part of the TFBC (**D**).

conjunction, and all repairs in the other groups were failed at the tendon to bone (repair alone) or tendon to tendon repairs (TFBC alone). The ultimate strength and stiffness of the TFBC+CS group was significantly higher than the other groups, although all repair groups was far below intact tendon strength (**Fig 21**). The BMSCs tracked with DiI prior to surgery were observed under confocal microscope after six weeks surgery (**Fig 22**). Histological images demonstrated that tendon-to-tendon healing in the TFBC+CS group was solid fused, but in TFBC alone group showed the gap between tendon layers (**Fig 23**). At the tendon-bon interface, the repaired tendon was incorporated with bone in all three groups, but with different appearance. The normal infraspinatus tendon enthesis presents a well-alignment fibrocartilage zone between tendon and bone (**Fig 24A,B,&C**). In the repair group, there was no identifiable fibrocartilage zone at the tendon bone interface (**Fig 24E**). Sharpey's fibers were anchored directly into bone (**Fig 24D**) and visible gap was observed at the tendon bone interface (**Fig 24F**). Both TFBC groups displayed the fibrocartilage zone at the tendon-bone interface (**Fig 24H,I,K,&L**). The tendon in the TFBC alone group showed a hypocellular feature (**Fig 24G**), but the layers of cells presented in the TFBC+CS group (**Fig 24J**). This pilot study fully supports that our non-weight bearing canine model can be successfully used for rotator cuff research. It also revealed that the tendon to bone healing following six weeks of rotator cuff repair (control group) did not lead to the repair strength increased compared to the time-0 repair strength. Although TFBC without cells seemed increasing the healing strength (not significant), the conjunction at the TFBC and infraspinatus tendon was the weak point compared to the TFBC to bone healing. However, in the TFBC+CS group, TFBC to tendon healing was improved significantly evidenced by all repairs failed at the bone-bone conjunction. From this study, we concluded that our engineered TFBC could be a novel and advanced material to augment rotator cuff biological healing. Functional outcomes for a long-term study need to be investigated.

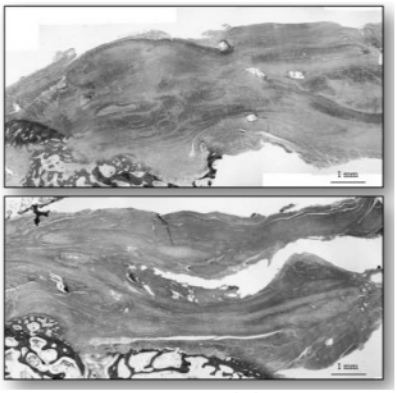

Fig 23. Masson's Trichrome staining showed FBC+CS with solid tendon fusion (A), but TFBC alone with gap between tendon layers (B).

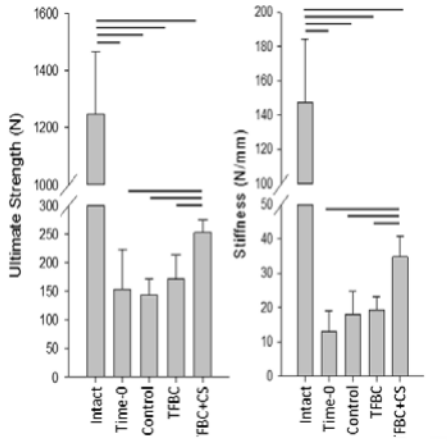

Fig 21. Repair maximal failure strength and stiffness

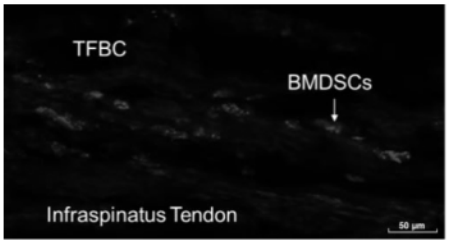

Fig 22. Dil labeled BMSCs were observed under confocal microscopy (blue: DAPI with cell nuclear stinging)

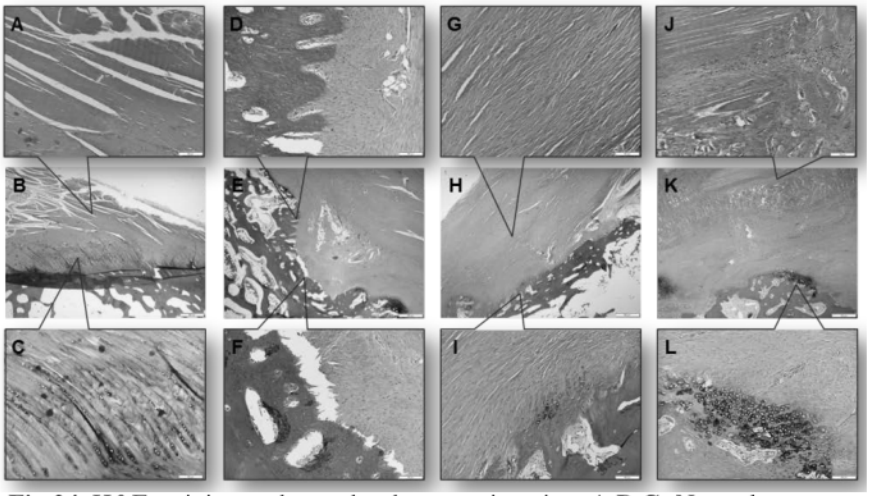

Fig 24. H&E staining at the tendon-bone conjunction. **A.B.C:** Normal infraspinatus tendon enthesis, **D.E.F:** Repair alone showed Sharpey fibers and gap, **G.H.I**: Repair with TFBC alone displayed acellular tendon, **J.K.L:** Repair with TFBC+CS revealed clear fibrocartilage zone and cellular TFBC. Middle row: 20x magnification; top and bottom rows: 100x magnification

C.2.c. Rigorous Study Design

In our preliminary study (***C.2.b.6.***), rotator cuff repair alone after six weeks remained intact indicating our non-weight bearing shoulder model is reliable. However, its failure strength was equivalent to the time zero repair strength denoting tendon to bone healing is insufficient at six weeks after repair compared to tendon to tendon healing in which the healing strength increased about 60% compared to time zero in flexor tendon healing in the canine model[77,116]. With engineered TFBC augmentation, surprisingly, the repair strength increased over 60% compared to the time zero and repair alone. This promising preliminary data encouraged us to pursue TFBC investigation in a long-term follow up with a double-row technique, the common surgical practice to date. Based on our preliminary data[33], rotator cuff repair following 6 months in a canine model showed functional tissue regeneration. Therefore, we will choose 6 months as the postoperative survival time point for evaluating the functional outcomes. A total of 90 mongrel dogs (1-2 year old, 20-30 kg weight, both

sexes) will be randomly divided into 5 groups with 18 dogs in each group including **1)** rotator repair alone; **2)** repair with GrafJacket augmentation; **3)** repair with TFBC augmentation; **4)** repair with TFBC+CS augmentation, and **5)** Group 4 with mechanical stimulation before surgery. Although our preliminary data (**C.2.b.2.**) showed that the mechanical stimulation of engineered resulted in better tenogenesis of the TFBC, the complexity of the stimulation experiment could be a huge hurdle for clinical translation. We also believe that non-restricted shoulder activities, indeed, provide mechanical stimulation in vivo due to the action of the muscles of the rotator cuff. However, to better understand if pre-implantation stimulation of BMSCs would result in better outcomes, we incorporated group 5 to the study design. All dogs will undergo bone marrow harvesting three weeks prior to the surgery to eliminate possible confounding effects by bone marrow harvesting. Then the dogs will have unilateral surgery randomized either left or right shoulders. The dogs will be sacrificed at 6 months after surgery and the surgical shoulder will be evaluated in mechanical properties (n=10) and biological conditions (n=8) detailed below.

C.2.d. Methods

C.2.d.1. TFBC Preparation and Decellularization

TFBCs will be prepared as described in ***C.1.d.1.*** We have been collecting the patellar tibial composite tissues from the dogs that have been used for other studies, which have been approved by our Institutional Animal Care and Use Committee (IACUC).

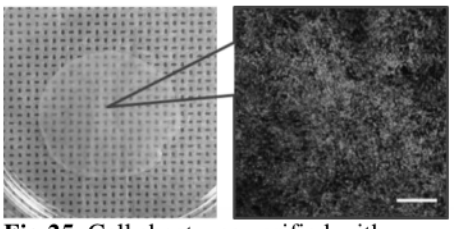

Fig 25. Cell sheet was verified with Calcein AM showing live cells in green.

C.2.d.2. BMSC Sheet Construction and Characterization

10 mL of bone marrow will be aspirated from the tibia and BMSCs will be harvested based on our standard protocol[115]. BMSCs from passage 3 will be used to prepare cell sheets. BMSCs will be seeded in 60 mm dishes at $1x10^6$ cells/dish and cultured in standard medium. 50 μg/ml L-ascorbic acid (vitamin C; Vc, Sigma, USA) will be added to the standard medium to produce cell sheet formation[117], which also promotes tenogenesis[7]. After 1 week of culture, a cell sheet will be formed with roughly $2x10^6$ cells (doubled with initial cell population: $1x10^6$ cells) in each sheet, which will be released from the culture plate by lifting with a cell scraper for the use (**Fig 25 & 20**). Group 5 will be mechanically stimulated for one week before surgery as described in ***C.2.b.2.*** To characterize the BMSCs at the time of BMSC sheet harvest, we will characterize a portion of the cells. The characterization will focus on BMSC stemness (CD44, CD45, CD90, CD105), lineage analysis and function (proliferation, migration, production of Col1 and Col3)[7,95,118]. BMSC characterization will provide us valuable information to better understand the biological variables that may influence differences in healing between each individual dog by comparing the characters of the BMSCs with final outcome analyses.

C.2.d.3. Surgical Procedure and Postoperative Management

Dogs will be anesthetized with intravenous ketamine (10 mg/kg) and diazepam (0.5 mg/kg) solution followed by vaporized isoflurane in oxygen. A 5-cm incision will be made through the skin over the selected shoulder and dissection will proceed to the deltoid muscle, which will be split in line with its fibers and retracted to expose the infraspinatus tendon. The infraspinatus tendon will be sharply transected at its insertion, along with the capsule beneath, to expose the glenohumeral joint to simulate an intrasynovial tendon injury condition in the clinical scenario. A 10-mm tendon at the distal infraspinatus tendon will be transected to create a tensional repair for all dogs. Double-row repair will be performed with 4.5 mm Corkscrew FT suture anchors (Arthrex, Naples, FL) loaded with #2 FiberWire sutures (Arthlex) described in ***C.1.d.2.*** For the GrafJacket patch augmentation, sutures will pass through the lacerated infraspinatus tendon and the patch augmentation to the greater tuberosity. For the three TFBC augmentation groups including TFBC alone, TFBC+cell, and TFBC+cell+stimulation will follow the same procedures described in ***C.2.b.6.*** Briefly, the transected infraspinatus tendon will be fed between two TFBC slices with or without cell sheets. The TFBC and infraspinatus tendon will be sutured together to make an infraspinatus tendon-fibrocartilage-bone composite (**Fig 20A. B. & C**). The TFBC will then be repaired back to its foot print on greater tuberosity with the double-row technique that was described in ***C.1.d.2*** and illustrated in **Fig 9**. Following rotator cuff repair, two 1-mm metal markers will be buried in greater tuberosity and distal infraspinatus tendon respectively, which will be used to monitor the repair status under a fluoroscopy. The distance of these two markers will be measured with the shoulder in a neutral position immediately after surgery serving as a baseline to estimate the gap or repair rupture. Following rotator cuff repair, a radial neurectomy will be performed. By denervating the elbow and wrist extensors, the dogs will be unable to bear weight on the operated limb but allow free shoulder motion, which is similar to the human shoulder condition and function.

After surgery, the animals will be monitored intensively for 24 hours in the recovery room postoperatively and then housed in metabolic cages for 14 days to limit activity. The operated paw will be kept elevated by our custom-made chest-arm sling. The sling will be removed daily and each dog will be allowed free activities in the canine hosting room for about 30 minutes under close observation. This daily excise effectively prevents joint contractures based on our experiences in flexor tendon studies using the canine model. Four weeks after surgery, the sling will be removed to allow free arm motion while remaining non-weight bearing due to denervation until sacrifice at 6 months postoperatively. A fluoroscopy will be used every 4 weeks to monitor the repair status according to the distance between two metal markers described above. The dogs will be sacrificed and both shoulders (surgical and non-surgical severed as the normal control) will be harvested for testing described below.

C.2.d.4. Mechanical Evaluation of Rotator Cuff Repair

Ten dogs in each group will be used for the mechanical evaluations. The surgical shoulders will be carefully dissected to preserve the humeral upper portion and repair the infraspinatus tendon with its muscle without destructing any tissues around repair site. The infraspinatus tendon and repair will be measured in their thickness and width for the calculation of cross sectional area, and then the specimen will undergo a mechanical failure test using the same methods as described in ***C.1.d.2.***

C.2.d.5. Histological, Immunohistochemistry, and Structural Analysis

The repair site with infraspinatus tendon and its bony insertion will be carefully dissected from eight dogs in each group including non-surgical normal shoulder. The tissue samples will be sharply divided in half along the long axis of the muscle-tendon-bone. One half will be used for histology analysis and the other half will be used for Western Blot evaluation described below. The histology, immunohistochemistry, and structure will be assessed with well-established from literatures and our previous studies[45,83,96]. The specimens will be decalcified with 15% EDTA and embedded in optimum cutting temperature compound. Coronal sections (10-µm thick) will be cut with a cryostat (Leica CM1850). The slides will be stained with hematoxylin and eosin for morphological analysis, Safranin O staining for fibrocartilage, Sirius red staining for collagen fiber orientation, and Alcian blue for ground substance analysis. All images will be quantified with Bonar score, which has been well established[119]. Briefly, grade 0-3 scoring system will be used to quantify cell morphology, cellularity, ground substance, collagen alignment, and vascularity[119]. Fibrocartilage zone will be quantified with the area identified by Safranin O staining using ImageJ software. Extracellular matrix including type I, II, and III collagens (major collagens in musculoskeletal tissues), decorin (major proteoglycan in connective tissues), and TGF-β (major cytokine in tissues) will be identified with immunohistochemistry based our established protocols[120-123], and staining intensity will be quantified with ImageJ software. The PI (CZ) and co-I (AG) will quantitatively evaluate the histological scores in a blinded manner from both observe/samples and observers/observer to reduce subjective bias and inter- and inter observer variables.

C.2.d.6. Extracellular Matrix Analysis by Western Blotting

The other half of rotator cuff repair samples will be harvested, dissected, and stored at -80° C for Western Blotting analysis including tenogenic markers (tenomodulin, scleraxis, tenascin-C, Col-I & III, chondrogenic markers (SOX-9, Col-II & X), and proliferative-remodeling cytokines (TGF-β, MMP2, MMP3 and MMP13) based on our well-established protocols[7,61,95,124].

C.2.d.7. Transplanted Cell Tracking

Although we have observed transplanted cells after six weeks of surgery, the cell fate in a long-term follow up is not clear. Therefore, two samples from the histology samples in the TFBC+CS group will be tracked with cell markers based on our established methods[101]. Briefly, BMSCs will be labeled with 1, 1'-dioctadecyl-3, 3, 3', 3'-tetramethylindocarbocyanine (DiI) (Molecular Probes, Eugene, OR) following the manufacturer's instructions before forming a cell sheet. After sacrificing the dogs, the samples will be decalcified and embedded in OCT compound and sectioned longitudinally into 10 µm serial slices. The sections will be fixed in 4% paraformaldehyde and incubated in 10% sheep serum in 2% Triton-PBS, and nuclei will be stained with 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St. Louis, MO). A laser scanning confocal microscope (LSM310, Zeiss) will be used to observe fluorescence label of all sections.

C.2.e. Potential Problems and Alternative Strategies

First, the TFBC composite tissue is allogenic which may induce an immune response. However, we do not anticipate the long-term immune rejection, since patellar tendon bone composite has been clinically used for ACL reconstruction[125]. First, our preliminary data (***C.2.b.6.***) also showed no immune response after six weeks of TFBC transplantation. Second, the radial nerve denervation effectively prevented operated forelegs from

weight bearing for six weeks, but it is unknown if it could be effective for six months. However, the radial nerve is unlikely regenerated within 6 months without repair. If weight-bearing capabilities of the operative leg restores within six months postoperatively, the transection of the triceps tendon will be performed to prevent elbow extension. Finally, for the purpose of the clinical application in the future, the width of the patellar tendon (around 30 mm in human)[126] may not be wide enough to repair a massive tear with a wide defect. Two potential strategies may be applied for the TFBC augmentation if it can be translated into clinical practice in the future: **1)** using the Margin Convergence repair technique to reduce strain and narrow down the tear size, which has been used in the clinic[88,127], and **2)** using two parallel TFBCs to cover the massive tear.

C.3. Sample Size Justification and Statistics

The key quantitative evaluations will be grafting material mechanical properties, augmentation strength, and biological analysis including histology and Western Blot analysis. The sample size justification is calculated based on available data for these parameters obtained from our previous studies. For the mechanical evaluation, our pilot studies have shown that the maximum strength of rotator cuff repair with and without TFBC was 272.8 ± 49 N and 365.5 ± 36.5 N, respectively[85]. Healing strength in vivo data at 24 weeks after autologous grafting was about 925.5 N ± 425 N for the allograft and 525 N ± 230 N for GraftJacket graft[33]. A sample size of 10 in the mechanical evaluation section will be sufficient to detect a significant difference with an 80% power at a significance level of $p<0.05$. For the Western Blot, cell viability, and histological analyses, (based on preliminary studies[61,124,128,129]), we need a sample size of 8 tendons per group to achieve 80% power at a significance level of 0.05 to detect a 50% difference. Therefore, a sample size of 18 shoulders will be allocated in 5 groups. Thus, a total of 90 dogs will be needed to satisfy the statistical analysis. However, based on our previous canine tendon research, 10% failure rate needs to be added due to some unexpected issues detailed in **Vertebrate Animal** section. Therefore, 9 dogs will be supplemented for failure resulting in a total of 99 dogs needed for this study. Results of quantitative studies will be expressed as mean with standard deviation. The data from the measures of interest (maximum strength, stiffness, Bonar score, and protein expression) will be analyzed using one-factor repeated measures ANOVA. In all cases, a level of $p < 0.05$ will be considered to be statistically significant. All statistical analyses will be carried out in consultation with statisticians at Mayo Clinic and will be performed using SAS-9.4 software (SAS institute Inc., Cary, NC).

C.4. Scientific Rigor and Reproducibility

The scientific premise of this project is built upon our strong supporting data that use a novel engineering approach to address an important and challenging clinical and research issue, rotator cuff repair and regeneration. Scientific rigor is also supported by our sufficient preliminary studies that have shown the experimental feasibility and reproducibility. We will well control the experimental procedures and methods to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results through strict cell culture and passage control, experimental randomization, blinding evaluations, power analysis, well-described methods, and identified devices or reagents. We have also developed SOPs (standard operating procedures) with video clips for many procedures including canine bone marrow aspiration, BMSCs harvesting, TFBC fabrication, surgical procedures, and most outcome measures to reduce experimental errors and increase data processing accuracy, reproducibility, and quality control among different users. We have been using the canine model for musculoskeletal research over the past two decades and developed many IACUC protocols for animal husbandry, surgery, post-operative care with the support from Mayo Comparative Medicine. Biological variables will be controlled through a strict animal inclusion and exclusion criteria including age, sex, weight, health conditions (see Vertebrate Animal Section). Findings will be reported in per reviewed scientific literature.

C.5. Timetable

This project will be completed within five years. Two specific aims (Aim1: mechanical augmentation and Aim2: biological augmentation and enthesis regeneration) will start at the same time since they are independent. Specific Aim-1 is straightforward, which can be done within 1 year based on our experience in orthopedic biomechanics and our previous relative studies[85,88,93]. In Aim 2, we will request a total of 99 dogs, which will survive for average 27.2 weeks (3 weeks for BMSC, one week for mechanical stimulation in only one group (n=18), and 24 weeks followup). Thus, total dog-weeks will be 2693 (99 dogs x 27.2 weeks). Based on our experience from previous canine studies, we will be able to manage 12 dogs at any one time postoperatively. Thus, a minimum of 225 weeks (2693/12 cages) (about 4.3 years) will be needed to finish surgeries and postoperative care. Factoring in holidays and the time for TFBC fabrication, post functional and biological assessments, data analysis, and manuscript preparation, we need approximately 5 years to complete Aim 2.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

VERTEBATE ANIMAL

Department of Comparative Medicine at Mayo Clinic and our research team have successfully developed a series of protocols including animal ordering, transport, husbandry, anesthesia, surgical procedures, postoperative care, and euthanasia according to the IACUC policies and regulations; they are also conducted in accordance with the ILAR guide for the care and use of laboratory animals and the Mayo Animal Welfare Assurance. The short term protocol which validated the engineered TFBC for rotator cuff repair and regeneration using a canine non-weight bearing model is currently approved by Mayo Institutional Animal Care and Use Committee (IACUC), the modification to extend the survival time from six weeks to six months has been submitted and pending approval. We will provide the confirmation of approval after we hear from Mayo Clinic's IACUC committee.

1. Procedures

Adult (1-2 year old) adult mixed breed dogs of both sexes Redacted by agreement weighing 20-30 kg will be used for this study. Survival procedures are only performed in Aim 2 of the proposal. The choice of sample size, particularly for an animal model, represents a compromise on minimizing the number of animals that are needed to detect differences that are clinically important. As the major quantitative evaluations will be rotator cuff repair strength, which represents the healing and functional properties, along with histology, the sample size justification is calculated based on available data parameters obtained from our previous studies[33,61,124,128,129]. Based on our statistical analysis, a total of 90 dogs will be needed to satisfy the power-calculation; accounting for an estimated, 10% failure rate due to anesthesia accident, deep wound infection, repair failures due to fully weight-bearing (confirmed by x-ray with two metal markers elongate more than 5 cm), unsolved severe pain or distress, or any health issues where body weight lost is more than 20% in accordance with the IACUC policy. Based on our experience working with canine model for tendon research in the past two decades, the total failure rate is less than 10%. During the in vitro study in Aim 1, we will obtain cadaver canine shoulders and knees for TFBC biomechanical testing from dogs that were sacrificed from other IACUC approved protocols. We have started collecting samples so if funded we will have enough specimens to start Aim 1.

Anesthesia

Dogs will be anesthetized with the following protocol: premedication with Atropine 0.05 mg/kg, Acepromazine 0.05 mg/kg and Morphine 0.5 mg/kg IM (intramuscular), and then inducted with Ketamine 10 mg/kg and Diazepam 0.6 mg/kg IV (intravenous). Intubation of trachea with an endotracheal tube will be performed. Anesthesia will be maintained using an animal anesthesia machine with a circle system administering isoflurane 1-2% in 100% oxygen (minimum of 20ml/kg/min). In addition, positive ventilation will be applied to keep end tidal CO2 between 35-45mmHg. Monitoring will be achieved with ECG, capnography, thermometer, direct blood pressure.

Bone Marrow Harvesting Procedure

Following general anesthesia, both hind legs will be shaved at the knee and scrubbed with povidone-iodine and sterilely draped. 8-10 ml of bone marrow from both proximal heads of the tibia will be aspirated using a bone aspiration needle that is 16ga x 2.668 in. Bone marrow will be immediately processed for bone marrow derived stem cell culture and cell-sheet making according the protocol described in ***C.2.d.2.***

Surgical Procedure

Under general anesthesia, one shoulder will be randomly selected for surgery in each dog. The surgery leg will be shaved and scrubbed with povidone-iodine, sterilely draped and surgery will begin on the selected shoulder. An incision will be made on the shoulder posterior-lateral to the acromion. The deltoid muscle will be divided along its muscle fiber to expose the infraspinatus tendon at its insertion site. The infraspinatus tendon will be transected at its insertion, and a 5 mm portion of the underlying joint capsule will be excised to model an intra-articular injury. 10 mm distal infraspinatus tendon will be resected to induce a tensional repair condition to mimic a large rotator cuff repair in clinical scenario. The lacerated tendon will be repaired with double-row technique with or without GraftJacket or TFBC augmentation according to the repair procedure described in detail in section ***C.2.d.3.*** After rotator cuff repair, two metal markers will be inserted in to the greater tuberosity and distal infraspinatus tendon respectively to monitor the repair status with a fluoroscopy immediately after surgery and then every 4 weeks postoperatively. The wound will be closed in layers with interrupted 4-0 Vicryl (Ethicon) sutures, followed by a running subcuticular skin closure with 4-0 Monocryl (Ethicon) sutures. Following rotator cuff repair and within the same incision there will be a high radial neurectomy. The triceps muscle will be longitudinally separated within the intramuscular fibers to expose the radial nerve. The radial

nerve will be sharply transected above the triceps brachii causing the denervation of the extensor muscles in the leg thus preventing elbow and wrist extension and thereby preventing weight bearing postoperatively. The incision will then be closed in order of muscle, subcutaneous and cutaneous with 2-0 Vicryl using the interrupted suture pattern.

Postoperative Care

After surgery, the animals will be monitored intensively for 24 hours in the recovery room and then housed in metabolic cages for 14 days to limit activity. The operated paw will be kept elevated by our custom-made soft chest-arm sling. The combination of denervation and the canine sling makes postoperative cares much easier to be performed, as compared to using a spica. These combined efforts also reduce the chance of repair rupture due to the surgical arm remaining non-weight-bearing; we have successfully used these techniques in our previous and current studies of flexor tendon repair and grafting[45,77]. Postoperative care, including antibiotic (cephalexin 20 mg/kg) and analgesic use (buprenorphine 0.02 mg/ kg), will be coordinated through the Mayo Clinic's Department of Comparative Medicine. The sling will be removed daily and dogs will be allowed free activities in the canine hosting room for about 30 minutes under close observation. The operative foreleg cannot bear weight during activities due to radial nerve denervation, but this daily excise effectively prevents joint contractures based on our experiences in flexor tendon repair and graft in the canine model. Four weeks after surgery, the sling will be removed to allow free arm motion while remaining non-weight bearing due to denervation until sacrifice at 6 months postoperatively. A fluoroscopy will be used every 4 weeks to monitor the repair status according to the distance between two metal markers described above. The dogs will be sacrificed with an intravenous overdose of pentobarbital. Both Shoulders will be harvested for evaluations, the nonsurgical shoulder to serve as the control.

Dogs will be examined daily for the first two weeks, and daily surgical and postoperative records will be maintained. Any problems will be reported promptly to the attending veterinarian. Any complications such as cage sores, wound infections, or wound dehiscence will be reviewed with the veterinarian and managed in a way designed to minimize discomfort or suffering of the experimental animals. If a problem cannot be resolved promptly, the animal will be removed from the study for appropriate veterinary care.

2. Justification

The proposed project is to develop a novel and functional engineered tendon composite to improve rotator cuff surgical repair. This preliminary study cannot be conducted in humans. As the project is translational research, a large animal model is appreciated and appropriate. The choice of using a canine model in this project are based upon: 1) the canine has been a common animal model for tendon related research since 1941[107], 2) our experience with the canine model including rotator cuff repair, flexor tendon repair and graft, postoperative care, and analytic methodologies, 3) supportive preliminary studies in a canine model, 4) the size of the rotator cuff in canine is clinically relevant, 5) our non-weight bearing canine shoulder model reduces the repair re-tear which is a fatal drawback for using canine as a rotator cuff research animal model[75].

3. Minimization of Pain and Distress

The animals will be housed at Redacted by agreement for one week before surgery for acclimation. Pre-operative analgesics Carprofen 4 mg/kg SC will be administered on the day of surgery. Postoperative analgesia Buprenorphine SR (slow release) 0.12 mg/kg SC (once right after surgery) and Carprofen 4 mg/kg PO daily for one week will be administrated. Dogs will be given an antibiotic of Cefazolin 40 mgs/kg IV once pre-op and Simplicef 10 mg/kg PO daily for five days postoperatively. Dogs will be weighed weekly and monitored for signs of discomfort, including lethargy and lack of appetite daily. Analgesics will be administered to any animal showing these signs. Operative incisions will be checked regularly for redness, swelling, and oozing. Animals whose pain or distress cannot be alleviated will be euthanized in a humane fashion by veterinary staff.

4. Method of Euthanasia

Following six months surgery, the dogs will be euthanized using a method consistent with the recommendations of the AVMA Guidelines on Euthanasia. Specifically, this will be achieved with intravenous injection of an overdose of Pentobarbital.

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AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

1. Animal Model and Cell Culture

The canine as a research model has been used for over a century as it is one of the closest mammal species to human. We have used this model for over two decades. We will purchase the dogs that are bred for medical research purpose from Redacted by agreement The mixed breed (mongrel) dogs will be 1-2 year old (skeletally mature), 20-30 kg body-weight, and both sexes. Mayo Comparative Medicine has strict policies, regulation and standard processes for purchasing, hosting, and caring for dogs that are approved by IACUC (Institutional Animal Care and Use Committee). Our proposed animal protocol has been approved by IACUC. Our animal experiment will follow the "Guide for the Care and Use of Laboratory Animals" provided by the Institute for Laboratory Animal Research, National Research Council (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf). All surgical procedures including bone marrow harvesting will be performed fully under the sterile conditions. Postoperative care, including antibiotic and analgesic treatment, will be coordinated through the Mayo Clinic's Department of Comparative Medicine. Details for animal are described in **Vertebrate Animal** Section. We have been using culture bone marrow derived stem cell from dogs for musculoskeletal research for many years and developing a series of protocols to standardize the procedures including standard cell culture media, bone marrow harvesting, cell culture, cell-sheet making, and cell transplantation. We have characterized BMSCs for regenerative potential in multiple studies in both in vitro and in vivo models.

2. Bioreagents and Chemicals

The bioreagents and chemicals in this proposal have been used in our previous publications or described in our preliminary unpublished data.

- Lyophilizer for tissue processing (Millrock Technology Inc, Kingston, NY)
- Fetal bovine serum, and 1% antibiotics and trypsin-EDTA (antibiotic–antimycotic; gibco®).
- L-ascorbic acid (vitamin C; Vc, Sigma, USA)
- Nuclease solution (RNase and DNase) (Roche Diagnostic, Indianapolis, IN)
- Surface marker antibodies for Western Blot (TheromFisher Scientific, Waltham, MA)
- OCT compound (Tissue-Tek®, Sakura Finetek USA, Torrance, CA)
- Live/Dead Viability/Cytotoxicity Kit (Molecular Probes, Carlsbad, CA)
- Decalcifying Solution B (Protocol, Fisher HealthCareTM)
- 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St. Louis, MO)
- Picrosirius Red solution (Polysciences, Inc.)
- Immunohistochemistry to identify and quantify the extracellular matrix including type I, II, and III collagens, decorin, and TGF-β will be purchased from TheromFisher Scientific, Waltham, MA

3. Biomechanical Evaluation Methods and Tools

Mayo Biomechanics Lab is one of the top labs across the world to carry out musculoskeletal biomechanics related research. It has developed many biomechanics methods and tools to evaluate orthopedic biomechanics for the past over four decades. In this proposal, the major biomechanical evaluation is to measure the mechanical properties of the rotator cuff repair and augmentation, which has been well established in our lab including a servohydraulic test machine (858 MiniBionix II; MTS Systems Corp, Eden Prairie, MN) and a Digital Image Correlation System (DICS) (ARAMIS 4M, Trilion Quality Systems, Plymouth Meeting, PA). We have also developed several software program using MATLAB (MathWorks©) to eliminate manual analytic errors.