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TITLE: **Endogenous and Exogenous Pericytes in the Pathobiology and Treatment of Osteoarthritis**

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Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Pdgfrb transgenic reporter animals represent a tool for the visualization of pericytes within joint associated tissues. Destabilization of the medial meniscus in the mouse induces osteoarthritic changes associated with dynamic changes in the vasculature and Pdgfrb reporter activity within the infrapatellar fat pad. These changes implicate endogenous pericytes as a key cellular player in post-traumatic osteoarthritis.

15. SUBJECT TERMS
Osteoarthritis, post-traumatic osteoarthritis, pericyte, perivascular cell

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	24	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Pericytes have mesenchymal progenitor cell properties and perform critical roles in tissue repair and immune regulation in diverse organ systems. The current project sought to explore the novel hypotheses that: *(1)* endogenous synovial pericytes may exert protective / immunomodulatory effects in osteoarthritis (OA), and *(2)* intra-articular administration of purified pericytes will improve OA disease progression. Aim 1 sought to develop and evaluate a post-traumatic osteoarthritis model within pericyte reporter mice. Next, Aim 2 evaluated the therapeutic potential of intra-articular pericyte delivery in mouse post-traumatic osteoarthritis.

2. KEYWORDS:

Arthritis, osteoarthritis, post-traumatic osteoarthritis, PTOA, inflammation, pericyte, perivascular stem cell, mesenchymal stem cell, MSC, immunomodulation, platelet derived growth factor receptor beta, PDGFRB.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

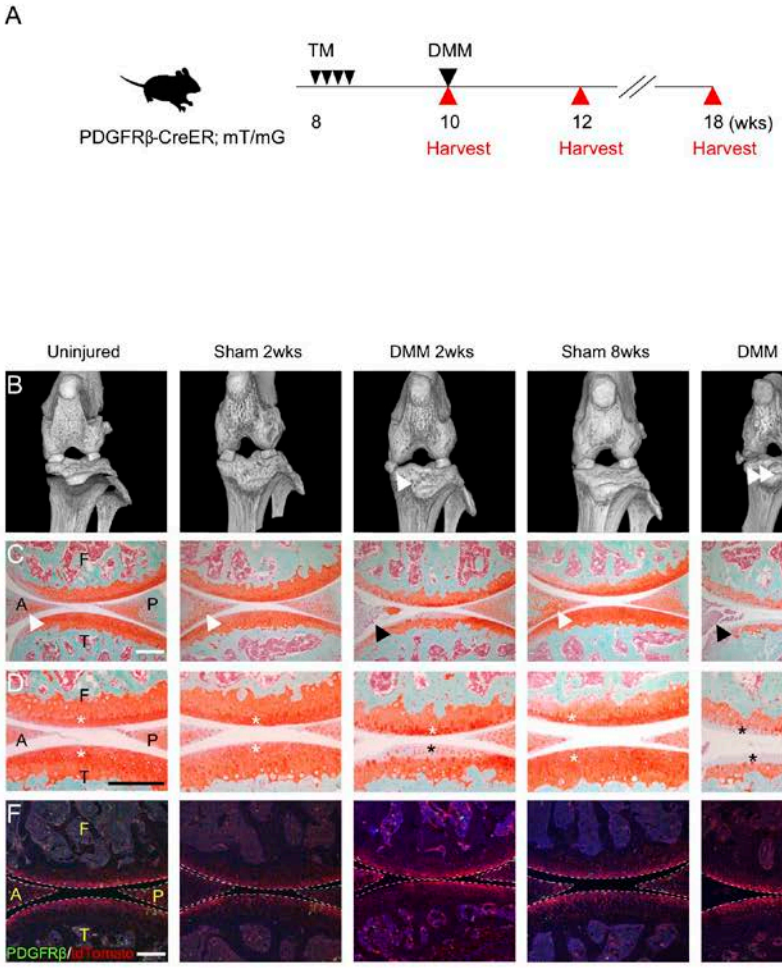
Major Task 1: Develop PTOA model in pericyte reporter animals
- 100% complete.

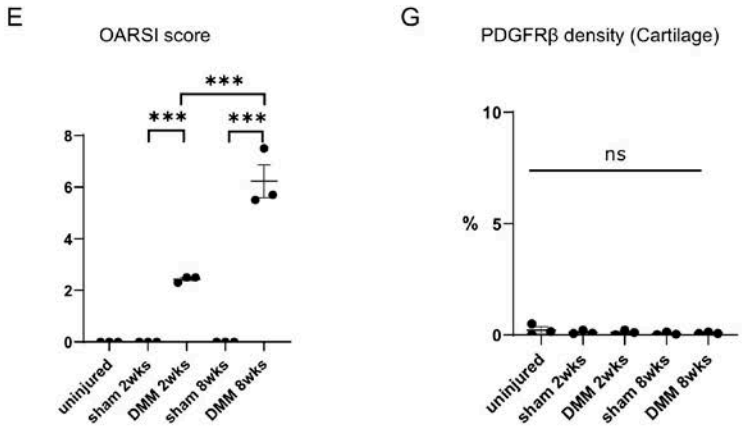
Major Task 2: Determine the therapeutic potential of intra-articular pericyte injection.
- 100% complete.

What was accomplished under these goals?

1) Major activities
    a. In reference to major task 1, we have used a Pdgfrb reporter animal in order to develop a pericyte reporter in a model of PTOA. We have examined Pdgfrb reporter activity within the stifle joint under uninjured, sham operated, or PTOA conditions (destabilization of the medial meniscus). As we will discuss, we have demonstrated that inducible Pdgfrb reporter mice represent a method to perform lineage tracing for pericytes / perivascular cells within the joint-associated subsynovial tissue. Further, we have observed dynamic changes in domains of Pdgfrb+ cellular descendants after DMM surgery. Next, we found that a subset of Pdgfrb+ cellular descendants convert to myofibroblasts after surgical destabilization of the joint. This is associated with a fibrotic gene signature among microdissected joint tissues. Finally, we have also determined that Pdgfrb reporter activity is not entirely specific to pericytes, and that reporter activity exists in other cell types within the joint, including most ligamentous cells and a small portion of synoviocytes.
    b. In reference to major task 2, we have isolated and applied Pdgfrb+ pericytes to the stifle joint of sham- or DMM-operated animals. Here, we again demonstrated the usefulness of Pdgfrb reporter animals in order to FACS isolate Pdgfrb+ perivascular cells. Next, we observed that a one-time, intra-articular dose of Pdgfrb+ cells led to a significant reduction in severity of osteoarthritis after joint destabilization.
2) Specific objectives
    a. Our first specific objective was to evaluate an inducible Pdgfrb reporter mouse as a pericyte reporter within the stifle joint. As we will describe, Pdgfrb reporter activity is highly localized to pericytes, but is also found in ligamentous cells and a portion of synoviocytes. Therefore, Pdgfrb reporter mice appear to be a strong pericyte reporter, but also highlights other morphologically distinct joint-associated cells.
    b. Our second objective was to map Pdgfrb reporter activity among sham-operated and DMM-operated conditions. As we will describe, DMM induced a dramatic change in Pdgfrb reporter activity. DMM induced microvascular proliferation within the subsynovium, with a concordant proliferation of Pdgfrb+ descendants, detachment of cells from vessel walls, and adoption of myofibroblastic markers at the gene and protein level.
    c. Our third objective was to isolate and apply Pdgfrb+ pericytes so as to mitigate DMM induced cartilage damage. We found that the isolation, purification, and culture propagation of Pdgfrb+ pericytes was feasible. We found that a one-time, intra-articular dose of Pdgfrb+ cell therapy led to a significant improvement in osteoarthritis severity as assessed using OARSI scoring systems.
3) Significant results
    a. First, we confirmed that Pdgfrb reporter animals undergoing degenerative changes after destabilization of the medial meniscus (DMM) surgery (Fig. 1). For these experiments, tamoxifen (TM) was administered to Pdgfrb-CreER ; mT/mG transgenic reporter mice at 8 weeks of age (Fig. 1A). Thereafter, animals were subjected to DMM or sham surgery at 10 weeks of age, and analyzed 2 and 8 weeks thereafter. Microcomputed tomography of the stifle joint confirmed destabilization of the medial meniscus among the DMM treatment groups at 2 and 8 weeks post-operative (Fig. 1B, white arrowheads). Sagittal histologic sections of the joint were next stained with Safranin O / Fast Green (Fig. 1C,D).

Figure 1

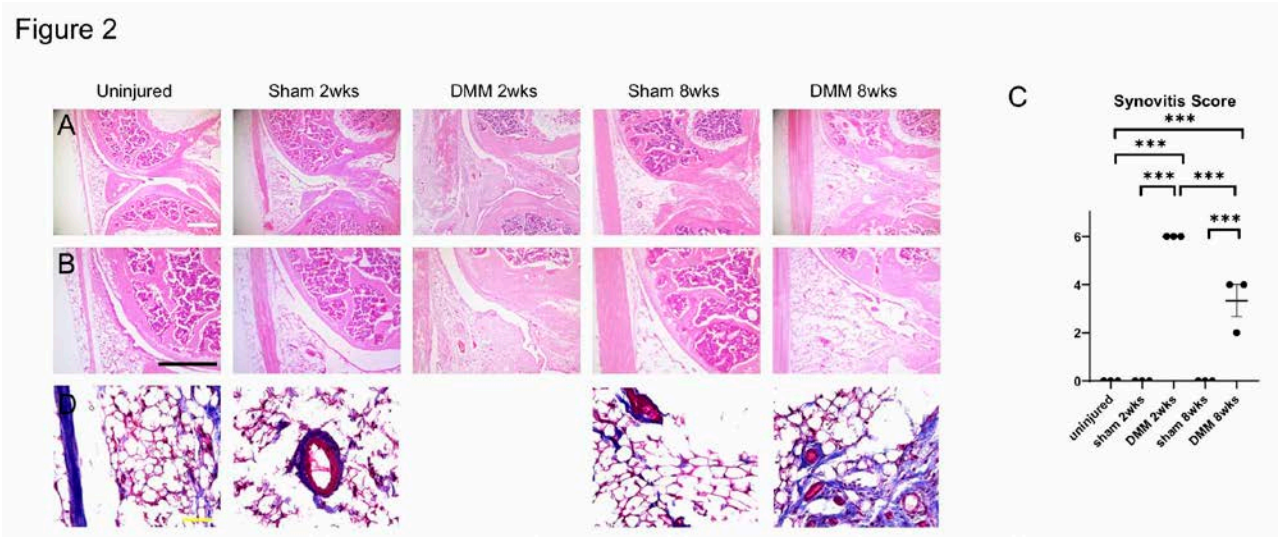

A
TM
DMM
PDGFRβ-CreER; mT/mG
8
10
Harvest
12
Harvest
18 (wks)
Harvest
Uninjured
Sham 2wks
DMM 2wks
Sham 8wks
DMM 8wks
B
C
D
F
F
A
P
T
PDGFRβ/tdTomato


E
OARSI score
***
***
***
8
6
4
2
0
uninjured
sham 2wks
DMM 2wks
sham 8wks
DMM 8wks
G
PDGFRβ density (Cartilage)
10
%
5
0
ns
uninjured
sham 2wks
DMM 2wks
sham 8wks
DMM 8wks

Results confirmed a significant and progressive loss of Safranin O staining among DMM but not sham operated animals, which was most notable within the proximal tibial articular cartilage (T), but also evident within the distal femoral cartilage (F). Pdgfrb reporter activity was examined across samples (Fig. 1E). Overall and as expected, minimal Pdgfrb reporter activity was observed among articular chondrocytes or bone-associated cells. Confirmation of degenerative changes was further obtained using the OARSI scoring system (Fig. 1F). Finally, lack of Pdgfrb reporter activity within articular chondrocytes was confirmed quantitatively (Fig. 1G).

b. Having confirmed osteoarthritic changes after DMM, we next examined in detail the histologic changes within the infrapatellar fat pad (IFP) after either sham or DMM surgery. For this purpose, sagittal sections of the knee joint were obtained at the level of the posterior cruciate ligament after either sham or DMM surgery (Fig. 2). Results using routine H&E stained sections showed dynamic changes in the appearance of the IFP (Fig. 2A,B). At two weeks after DMM, an inflammatory infiltrate was noted in the IFP, composed primarily of mononuclear inflammatory cells. At 8 weeks after DMM, increased fibrosis of the IFP was noted. Inflammatory changes within the IFP and overlying synovium were next quantified using a semi-quantitative scoring system (Fig. 2C). Synovitis score was highest at 2 weeks post DMM, and remained elevated above baseline at 8 weeks post DMM (Fig. 2C). Immunohistochemical confirmation of the cell composition of the inflammatory infiltrate is ongoing. In comparison and as expected, no significant synovitis was observed among uninjured or sham operated animals. Fibrotic change within the IFP was next confirmed by Masson's Trichrome staining (Fig. 2D). Here fibrotic change was most notable within the IFP at 8 weeks post DMM, while not observed under uninjured or sham operated conditions.

Figure 2



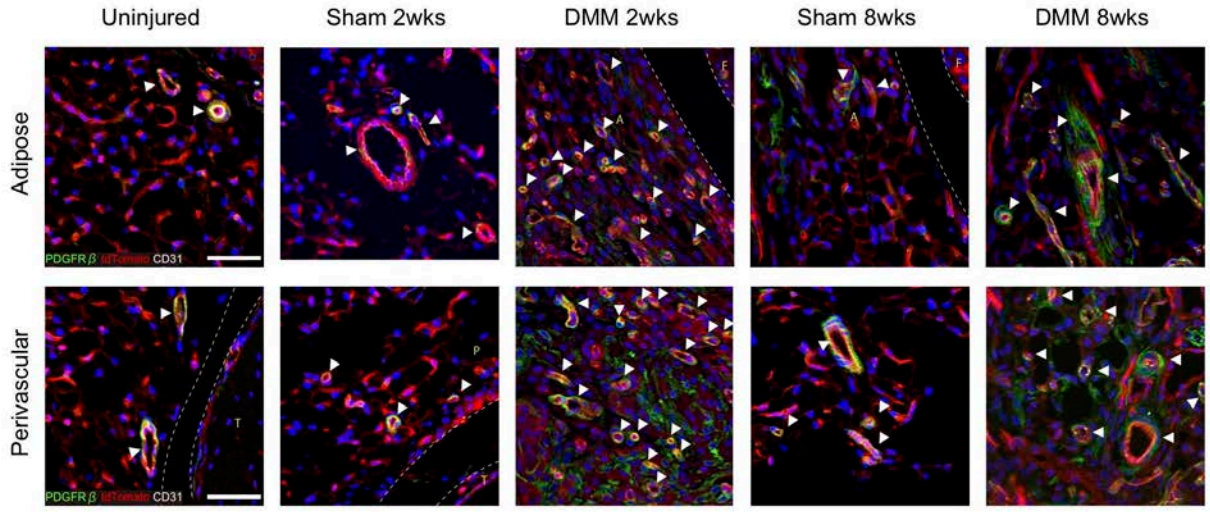
c. Having confirmed temporally patterned changes within the IFP elicited by DMM surgery, we next set out to examine pericyte reporter activity within the IFP using Pdgfrb reporter animals. For this purpose, the IFP was separated for analysis into adipose and perivascular areas (Fig. 3). Immunohistochemical staining for CD31 was performed to mark endothelium (appearing red), while Pdgfrb reporter activity appears green. Under uninjured conditions within the IFP, Pdgfrb reporter activity was found essentially exclusively within an abluminal 'pericytic' location. These findings were essentially confirmed among the IFP of sham-operated animals at both 2 and 8 weeks post-operative. In contrast, vascular changes and perivascular reporter activity showed dynamic changes after DMM surgery. At two weeks, numerous thin-caliber, capillary type vessels were observed within the IFP, either within adipose or perivascular locations (white arrowheads, middle column). These apparent increase in microvascular density was accompanied by a robust increase in the number of Pdgfrb reporter positive cells. Interestingly, DMM induced a significant expansion of Pdgfrb positive cellular descendants which was most notable at 2 weeks post-destabilization. Interestingly, cellular descents of Pdgfrb reporter positive cells were frequent both in a pericytic location, but also in cells completely unassociated with microvessels. Similar observations were observed at 8 weeks after DMM (far right column). Here, microvessels were still more apparent in comparison to uninjured or sham-operated conditions. Pericyte Pdgfrb reporter activity was likewise increased over baseline. Again, both vascular-associated with non-vascular mGFP reporter activity was observed. These findings led us to several conclusions:

1 – Pdgfrb reporter activity is specific to pericytes within the infrapatellar fat pad

2 – Minimal changes in vascular patterning or Pdgfrb reporter activity are seen within sham-operated conditions.

3 – DMM induces temporally dynamic changes in vascular patterning and Pdgfrb reporter activity, including a pronounced microvascular proliferation accompanied by expansion of Pdgfrb reporter activity.

Figure 3



d. These qualitative changes in vascular patterning as well as cellular descendants of Pdgfrb-expressing pericytes induced by DMM were next quantified by histomorphometric analysis. First, vascular histomorphometry was performed on serial sections of IFPs under each treatment condition (Fig. 4A-C). Vascular histomorphometry was performed specifically within adipose areas (left column), perivascular areas (middle column), or total area (right column). A significant increase in vascular numbers per high powered field was observed at both 2 and 8 weeks post DMM in comparison to either uninjured or sham-operated conditions (Fig. 4A). No statistically significant changes in mean vascular density were found (Fig. 4B). Total vascular area showed a significant increase at both 2 and 8 weeks post DMM in comparison to either uninjured or sham-operated conditions (Fig. 4C). Pdgfrb pericyte reporter activity was next quantitatively examined within the IFP under each treatment condition and timepoint (Fig. 4D-F). Consistent with our prior observation, the density of Pdgfrb reporter activity was most notably increased at 2 weeks post DMM (Fig. 4D). A non-significant trend toward increased Pdgfrb reporter activity was also seen at 8 weeks post-DMM (Fig. 4D). The density of Pdgfrb reporter activity that was not associated with the vasculature was next quantified (Fig. 4E). Consistent with our prior observations, uninjured animals essentially showed minimal non-vascular Pdgfrb reporter activity within the IFP. These findings of rare to absent non-vascular reporter activity were likewise observed under sham-operated conditions. In contrast, significant increased numbers of non-vascular Pdgfrb reporter activity was observed at 2 weeks post-DMM (Fig. 4E). This remained elevated above baseline at 8 weeks post-DMM, albeit without statistical significance (Fig. 4E). Finally, the frequency of Pdgfrb+ pericytic coverage of microvessels within the IFP was assessed (Fig. 4F). Under uninjured conditions, essential 100% coverage of microvessels by Pdgfrb+ pericytes was observed. These findings of near universal Pdgfrb+ pericyte coverage were conserved among sham-operated animals. In contrast, Pdgfrb+ pericytic coverage was reduced after DMM. This finding was most notable at 2 weeks after injury, but found at 8 weeks post-DMM as well.

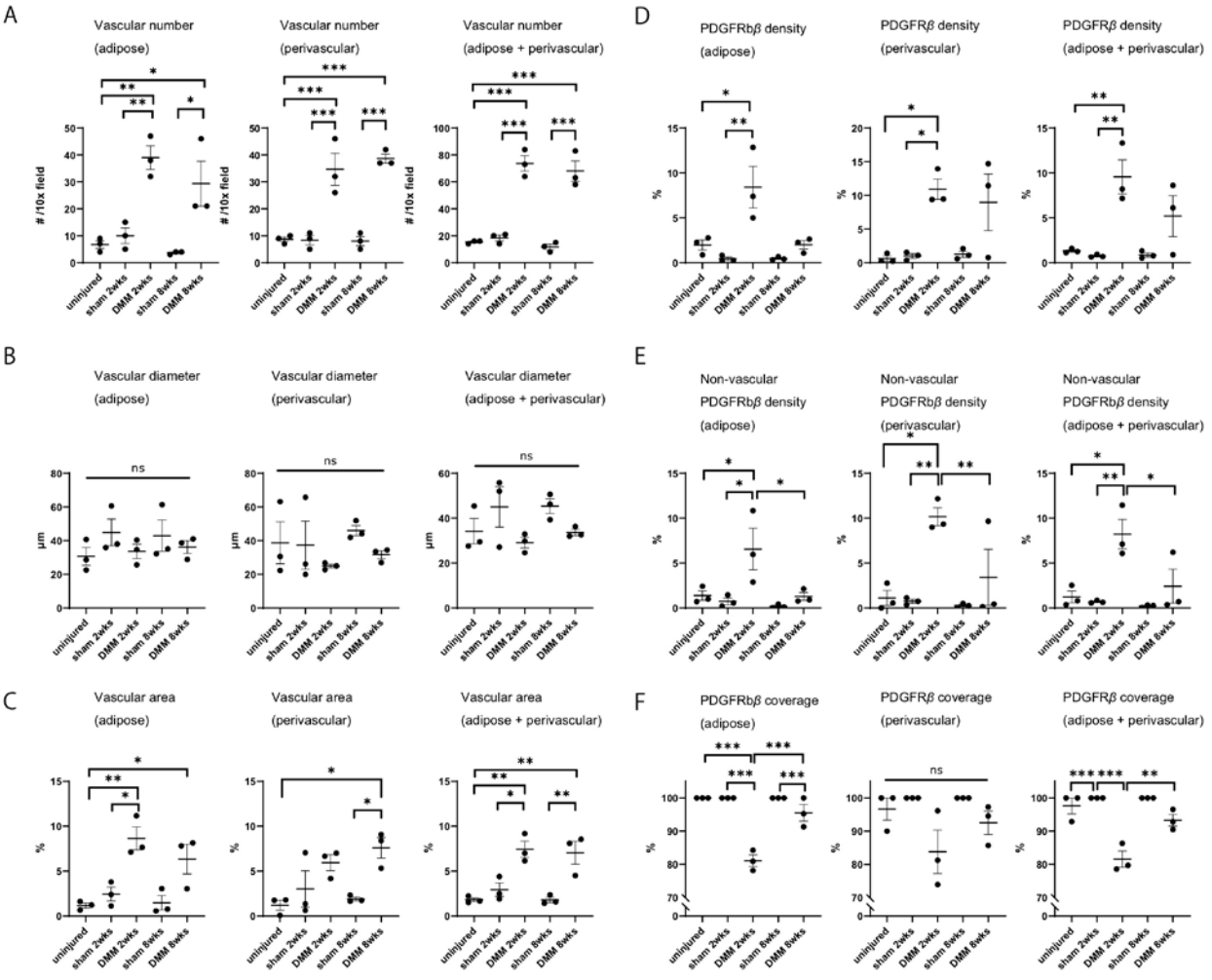
These findings led us to several conclusions:

1 – Pdgfrb-expressing pericyte coverage is a near universal feature of microvessels within the IFP at baseline.

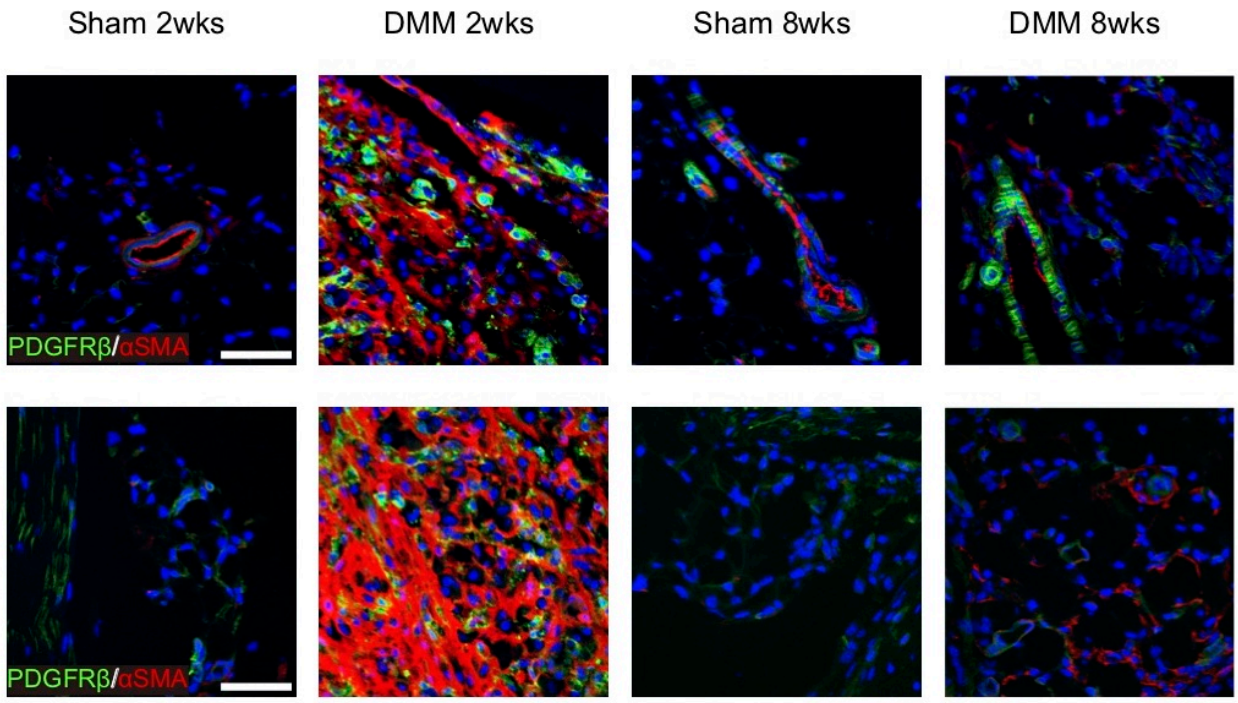
2 – DMM induces acute changes in the IFP at two weeks post-injury, including increases in microvascular density and area, accompanied by increased detachment of Pdgfrb-expressing cellular descendants from vessel walls, and reduced coverage of vessels by Pdgfrb-expressing pericytes.

3 – Finally, timepoint corresponding to later cartilage catabolism demonstrate further vascular changes, including a sustained increase in vascular numbers and vascular area, but also an apparent ‘maturation’ of blood vessels, with reduced non-vascular Pdgfrb-expressing cells, and a return to Pdgfrb-expressing pericyte coverage of IFP microvessels.

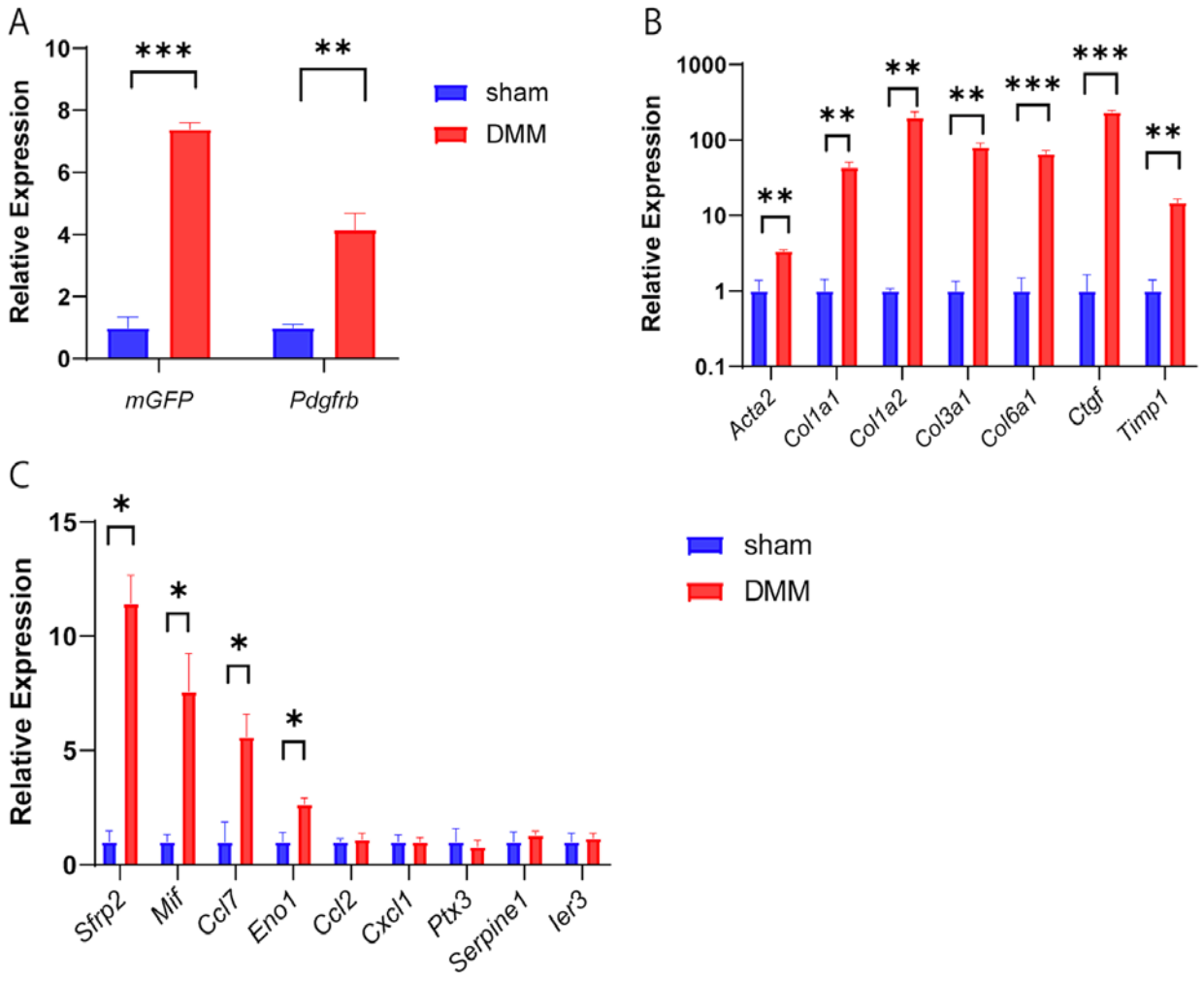
Figure 4


A
Vascular number (adipose)
Vascular number (perivascular)
Vascular number (adipose + perivascular)
/10x field
B
Vascular diameter (adipose)
Vascular diameter (perivascular)
Vascular diameter (adipose + perivascular)
ns
μm
C
Vascular area (adipose)
Vascular area (perivascular)
Vascular area (adipose + perivascular)
%
D
PDGFRbβ density (adipose)
PDGFRβ density (perivascular)
PDGFRβ density (adipose + perivascular)
E
Non-vascular PDGFRbβ density (adipose)
Non-vascular PDGFRbβ density (perivascular)
Non-vascular PDGFRbβ density (adipose + perivascular)
F
PDGFRbβ coverage (adipose)
PDGFRβ coverage (perivascular)
PDGFRβ coverage (adipose + perivascular)
uninjured
sham 2wks
DMM 2wks
sham 8wks
DMM 8wks

e. Our findings thus far suggested that DMM induces Pdgfrb+ pericyte vascular detachment and expansion within the IFP, which was followed by fibrotic change of the IFP. These findings suggested the potential transdifferentiation of Pdgfrb-expressing pericytes to myofibroblasts within the IFP. To begin to investigate this possibility, immunohistochemistry for Smooth Muscle Actin (SMA) was performed on the IFP of sham-operated or DMM-operated animals (Fig. 5). Results among sham-operated animals showed that SMA immunostaining highlighted only a thin-rim of tissue within a pericytic / perivascular location (observed at both 2 and 8 weeks after sham-surgery). In marked contrast, a high density of SMA immunoreactivity was observed at 2 weeks post-DMM, again corresponding to non-vascular mGFP/Pdgfrb reporter activity. At 8 weeks post DMM, some residual SMA immunohistochemical staining was observed within non-vascular components of the IFP. Thus, transient and robust SMA expression is observed within pericyte descendants within the IFP. These findings are suggestive of pericyte-to-myofibroblast transdifferentiation within the IFP elicited by destabilization surgery. Additional myofibroblastic markers showed the same trend after immunostaining, including TGFB1 and Fibronectin (Sono et al., JOR 2020).

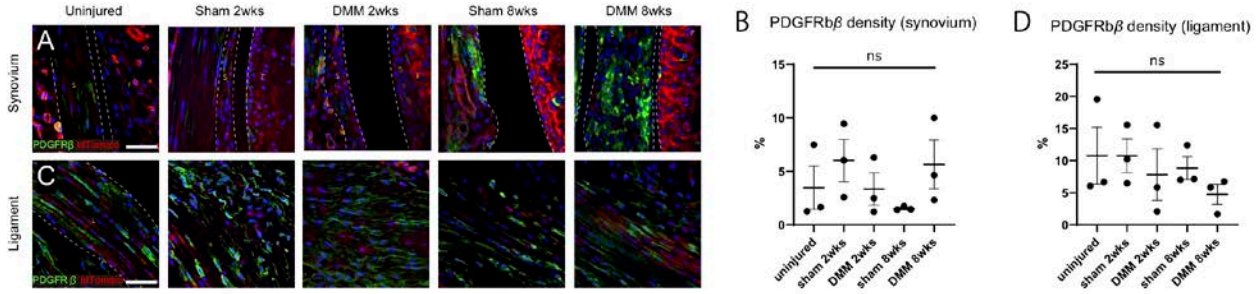


f. Our findings thus far highly suggested that Pdgfrb+ pericytes undergo perivascular-to-myofibroblastic differentiation after joint destabilization. In order to confirm this, IFPs from sham-operated or DMM-operated mice were microdissected at 2 weeks post-injury and gene expression was analyzed by qPCR (see below). An increase in reporter activity was confirmed via increase in mGFP transcript, along with increased transcripts for Pdgfrb (below, part A). Additional genes markers of fibrosis were examined among microdissected IFPs (below, part B). Briefly, a 3.37-fold change in *Acta2* was observed (encoding *αSMA*), as well as a 43.4 - 198-fold change in genes encoding collagens associated with fibrotic repair (*Col1a1*, *Col1a2*, *Col3a1*, *Col6a1*). In addition, a 235-fold change in connective tissue growth factor (*Ctgf*) expression and a 14.9-fold change in tissue inhibitor of metalloproteinase 1 (*Timp1*) was observed. We next examined markers upregulated in skeletal muscle fibrosis (below, part C). This included chemokines and inflammatory regulatory factors such as *Ccl2* (C-c motif chemokine ligand 2), *Ccl7*, *Cxcl1 (*C-x-c motif chemokine ligand 1), *Ptx3* (pentraxin3), and *Mif* (macrophage migration inhibitory factor), an inhibitor of metalloproteinase such as *Timp1*, fibrinolysis related genes including *Serpine1* (serpin family E member 1) and enolase 1 (*Eno1*), the Wnt signaling inhibitor *Sfrp2 (*secreted frizzled related protein 2), and apoptosis related genes *Ier3* (immediate early response 3). Five of ten genes showed upregulation in microdissected IFPs after DMM. This included significant increases in *Ccl7*, *Mif*, *Timp1*, *Eno1*, and *Sfrp2* (2.64 -14.9-fold changes).

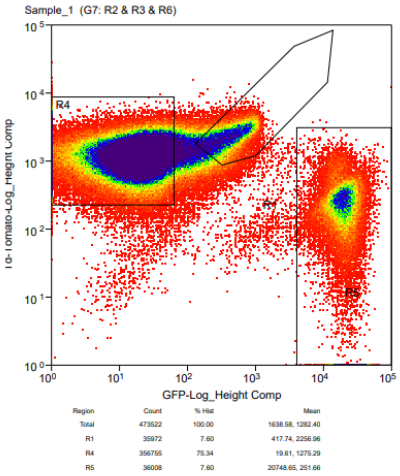


g. The specificity of Pdgfrb reporter for putative ‘pericytes’ within joint-associated tissues was next assessed. Previously we found that Pdgfrb reporter activity was indeed a highly specific marker of ‘pericytes’ within the IFP. Our findings did however show that Pdgfrb reporter activity was present in other cell types within joint-associated tissues. First, a small population of synoviocytes demonstrated Pdgfrb reporter activity (Fig. 6A,B). Interesting, a slight and non-significant increase in numbers of Pdgfrb+ synoviocytes was observed after DMM surgery, especially at the 8 week timepoint. Second, a significant portion of intra-articular ligamentous cells were found to have Pdgfrb reporter activity (Fig. 6C,D). No significant changes in Pdgfrb frequency within ligamentous cells was observed across treatment groups or timepoints. In summary, transgenic Pdgfrb reporter animals represent an excellent means to track pericytic cells within the IFP. As with many reporter animals, however, cell types within our tissue compartments also demonstrate reporter activity, and care must be taken to appropriately interpret histologic findings.

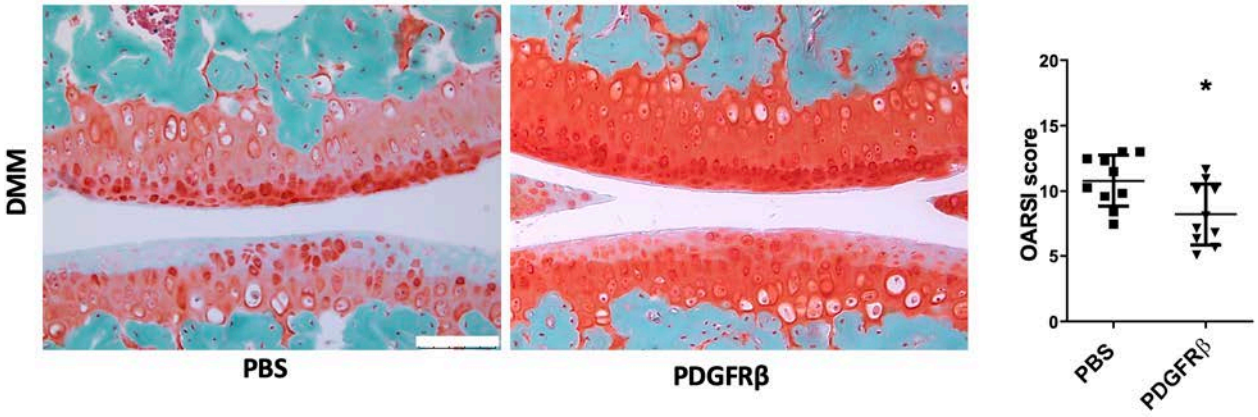
Figure 6



h. Finally, we examined the role of exogenous perivascular cells in treating DMM-induced arthritis. To address the therapeutic potential of PDGFRβ-expressing perivascular cells, in our Aim 2 supported studies we performed intra-articular injection of PDGFRβ^{+} perivascular cell subsets. Briefly, we FACS isolated PDGFRβ^{+} cells from adipose tissue (see below), and observed that the frequency of mGFP+ cells ranged from 7-15% of total adipose stromal cells.



i. Next, mGFP+ cells from the inguinal fat pads of PDGFRβ $^{mT/mG}$ reporter animals were injected as a one-time intra-articular dose (0.5 M total cells), and compared to PBS control injection. All knee joints were analyzed at 8 weeks after DMM (see below). Typical degenerative changes to the femoral and tibial articular cartilage were observed among PBS control-treated animals after DMM. PDGFRβ^{+} cell injection led to a slight improvement in arthritic change, with less prominent loss of safranin O staining among superficial articular cartilage. OARSI scoring confirmed these observations, with PDGFRβ^{+} cell therapy demonstrating an improvement in comparison to PBS control. Thus, PDGFRβ^{+} perivascular cell therapy as a one-time intra-articular dose demonstrates some modest improvements in osteoarthritis development after joint destabilization.



4) Other achievements
No additional.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

N/A.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

Our findings that dynamic vascular and perivascular changes occur in joint-associated tissue during the progression of osteoarthritis. Interestingly, these changes are somewhat transient and occur at early stages that precede the more obvious changes in cartilage degradation, and are associated with a newly described observation of perivascular-to-myofibroblast transdifferentiation. In addition, we have found that purified perivascular cells demonstrate some disease modifying efficacy when injected into the joint capsule after joint destabilization.

What was the impact on other disciplines?

Our findings strongly suggest that tissue injury induces a pericyte-to-myofibroblast transdifferentiation, and that transgenic Pdgfrb reporter animals represent an excellent tool to examine this phenomenon. This has significant potential impact in other disease of fibrosis, including wound healing or fibrosis of internal organs such as the liver, lung or kidney.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Journal publications.

1. Sono T, Hsu CY, Negri S, Miller S, Wang Y, Xu J, Meyers CA, Peault B, **James AW**. Platelet derived growth factor receptor-beta (Pdgfrb) lineage tracing highlights perivascular to myofibroblast transdifferentiation during post-traumatic osteoarthritis. J Orthop Res, Mar 2020, Epub ahead of print. 2. Sono T, Hsu CY, Negri S, Wang Y, Xu J, Marini S, Miller S, Levi B, Peault B, **James AW**. Perivascular fibroadipoprogenitor lineage tracing during post-traumatic osteoarthritis. American J Pathol, Mar 2020, In Resubmission. 3. Wang Y, Xu J, Meyers CA, Gao Y, Tian Y, Broderick K, Peault B, **James AW**. PDGFRa marks distinct perivascular populations with different osteogenic potential within adipose tissue. Stem Cells, Feb 2020; 38(2): 276-290.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

1. Sono T, Hsu CY, Negri S, Miller S, Wang Y, Xu J, Meyers CA, Peault B, **James AW**. Platelet derived growth factor receptor-beta (Pdgfrb) lineage tracing highlights pericyte to myofibroblast transdifferentiation during post-traumatic osteoarthritis. ORS 2020, Oral presentation, Phoenix, AZ. 2. Wang Y, Xu J, Meyers CA, Miller S, Tian Y, Gao Y, Ding C, Broderick K, Peault B, **James AW**. Pdgfra marks distinct perivascular osteoprogenitor populations within adipose tissue. MHSRS 2019, Aug 2019. Poster.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Aaron W. James*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-2002-622X
Nearest person month worked:	*1*
Contribution to Project:	*Dr. James is responsible for the overall conduct of the project.*
Funding Support:	*NIH, DoD, American Cancer Society, MTF Biologics, Maryland Stem Cell Research Fund.*

Name:	*Stefano Negri*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-2218-6654
Nearest person month worked:	*6*
Contribution to Project:	*Stefano is responsible for all technical aspects of the project, including performing surgical models, and postmortem analyses.*
Funding Support:	*NIH, DoD*

Name:	*Seungyong Lee*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Seungyong is responsible for aspects of the project, including performing histology and radiology.*
Funding Support:	*NIH, DoD*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Endogenous and Exogenous Pericytes in the Pathobiology and Treatment of Osteoarthritis

PR170080

W81XWH-18-0121

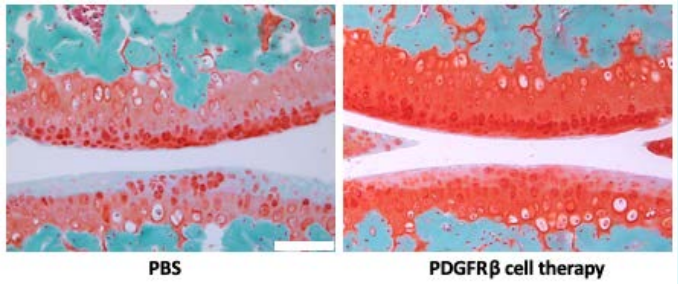
PI: Aaron W. James, MD, PhD **Org:** Johns Hopkins University **Award Amount:** $322,680

Study/Product Aim(s)

- Develop PTOA model in pericyte reporter animals (Aim 1)
- Determine the therapeutic potential of intra-articular pericyte delivery in mouse PTOA (Aim 2)

Approach

The present award seeks to explore the entirely novel hypotheses endogenous synovial pericytes may exert regulatory effects in the development osteoarthritis, and that intra-articular delivery of purified pericytes will improve OA disease progression. The current work will systematically evaluate the location, function, and therapeutic potential of endogenous and exogenous pericytes using a Pdgfrb transgenic reporter animal.



The culminated work validates Pdgfrb cell therapy to prevent the development of osteoarthritis in mice. Safranin O stained sections demonstrate improvement in OA after cell injection.

Accomplishment: In our first year, we have validated a pericyte reporter in osteoarthritis. In our second year, we observed that purified pericytes have benefit in the reduction of post-traumatic OA severity after intra—articular injection.

Timeline and Cost

Activities CY	18	19
Develop PTOA model in pericyte reporter animals		
Determine the therapeutic potential of intra-articular pericyte delivery in mouse PTOA		
Estimated Budget ($K)	**$252**	**$71**

Updated: 04/15/2020

Goals/Milestones

CY18 Goal – Develop PTOA model in pericyte reporter animal

- ☑ Perform pilot PTOA studies in pericyte reporter animals
- ☑ Perform histologic analysis
- ☑ Perform flow cytometry, PCR and immunohistochemical analyses

CY19 Goals – Evaluate the therapeutic potential of intra-articular pericyte delivery in mouse post-traumatic osteoarthritis

- ☑ Isolate adipose mouse pericytes
- ☑ Perform pilot PTOA studies with or without pericyte intra-articular injection
- ☑ Perform histologic analysis

Comments/Challenges/Issues/Concerns

- None.

Budget Expenditure to Date

Projected Expenditure: $322,680

Actual Expenditure: $321,361

PR170080: Endogenous and Exogenous Pericytes in the Pathobiology and Treatment of Osteoarthritis

PI: Aaron W. James, Johns Hopkins University, MD **Budget:** $322,680

Topic Area: Arthritis **Mechanism:** W81XWH-18-0121

Research Area(s): 0400 **Award Status:** 01 July 2018 – 30 Dec 2019

Study Goals:

The present Discovery award seeks to explore the entirely novel hypotheses that: *(1)* endogenous synovial pericytes may exert protective / immunomodulatory effects in osteoarthritis (OA), and *(2)* intra-articular administration of purified pericytes will improve OA disease progression. The current proposal will systematically evaluate the location and immunomodulatory function of pericytes within the OA afflicted synovium. Next, we will perform a pilot study examining the therapeutic potential of purified pericytes in OA.

Specific Aims:

Aim 1: Develop and evaluate a post-traumatic osteoarthritis model within pericyte reporter mice.
Aim 2: Evaluate the therapeutic potential of intra-articular pericyte delivery in mouse post-traumatic osteoarthritis.

Key Accomplishments and Outcomes:

Publications: 1. Sono T, Hsu CY, Negri S, Miller S, Wang Y, Xu Y, Meyers CA, Peault B, James AW. Platelet derived growth factor receptor-beta (Pdgfrb) lineage tracing highlights perivascular to myofibroblast transdifferentiation during post-traumatic osteoarthritis. J Orthop Res, Mar 2020, Epub ahead of print.
2. Sono T, Hsu CY, Negri S, Wang Y, Xu J, Marini S, Miller S, Levi B,. Peault B, James AW. Perivascilar fibroadipoprogenitor lineage tracing during post-traumatic osteoarthritis. Am J Pathol, Mar 2020, In Resubmission.
Presentations: 1. Sono T, Hsu CY, Negri S, Miller S, Wang Y, Xu J, Meyers CA, Peault B, James AW. Platelet derived growth factor receptor-beta (Pdgfrb) lineage tracing highlights pericyte to myofibroblast transdifferentiation during post-traumatic osteoarthritis. ORS 2020, Oral presentation, Phoenix, AZ.
Patents: none to date
Funding Obtained: none to date

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

Our finding suggest that:
1) Pdgfrb reporter mice are a valid means to perform lineage tracing of pericytes during the disease course of osteoarthritis (OA).
2) Pdgfrb+ pericytes change dramatically during the course of OA development in terms of location, frequency, and expression profile, and this may impact the disease.
3) Pdgfrb+ cell therapies when injected locally lessen the severity of osteoarthritis in a mouse model.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.

Yes. We have submitted a pre-application for an expansion award for this project.
(a) Department of Defense, PRMRP
(b) Budget requested: $500,000 direct costs
(c) Title: Refining perivascular adipose cell therapies for prevention or reversal of osteoarthritis

4. What will be **the next step(s)** for this project?

Here we observed that a one-time dose of a cell therapy improved the severity of osteoarthritis. The next steps would be to optimize this therapy, in terms of cell numbers, frequency of administration and timing relative to induction of arthritis.

5. How would you classify your **lead candidate product**? a
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Cell/Gene Therapy
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

Osteoarthritis is common among the Warfighter, Veteran, Beneficiary, and the General Population. Intra-articular injection of the tested cellular therapy led to an improvement in the severity of osteoarthritis in a mouse model. Use of intra-articular stem/stromal cell therapies for arthritis has potential high impact to improve the course of disease.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

The past Discovery award provided funds for a proof-of-principle study. We must now optimize this cellular therapy, examining factors such as cell dose, frequency of administration, comparison to other cell fractions, and timing of cell therapy in relation to disease onset.