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14. ABSTRACT: Background: Osteoarthritis (OA), the most common form of arthritis, is a leading cause of debilitating pain and disability, affecting > 32 million in the U.S. alone. The prevalence and incidence of OA have been continuously increasing due to increases in lifespan and obesity. The number of OA patients is expected to rise to over 78 million in the U.S. by 2040. In particular, OA has been a substantial burden among Veterans of the U.S. Armed Forces due to the high physical demands of their daily routines. More recently, serious concerns have been raised regarding OA-related chronic comorbid health conditions. For example, individuals with OA have a 2.5-times greater risk of having three or more other chronic diseases. Compared to civilians, veterans are far more vulnerable to a chronic comorbid health condition - more than a third of veterans suffer from at least two such chronic diseases (e.g., OA and depression). In particular, OA patients show a higher prevalence of the devasting symptoms of depression and anxiety than those without OA. Worse, the synergistic adverse effects of OA and depression have been recognized when they have comorbid conditions. Arthritic conditions and depression comorbidity magnify the persistent joint pain, which, in turn, worsens the depression. As OA is the most prevalent form of arthritis, depression is the most prevalent psychiatric disorder, ranking in the top five leading causes of disability worldwide. Every day, 17.6 U.S. veterans commit suicide, primarily due to the ramifications of untreated depression. These reports indicate an urgent unmet need to improve treatment strategies to manage OA symptoms and prevent depression, a comorbid chronic disease in OA patients.

15. SUBJECT TERMS

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The ultimate goal of our study is to evaluate oral treatments of our best pre-/probiotic combination (LA-synbiotic) for OA and associated depressive disease-modifying effects as preventive and/or therapeutic strategies. Successful completion of our study will set the stage for the discovery of mechanism-based new therapeutics involving the specific prebiotic/probiotic combination that is rapidly translatable to clinical settings.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Osteoarthritis Knee Joint Pain, Cartilage protection, *Lactobacillus acidophilus*(LA), Prebiotic, Intervention

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: To evaluate a pre-/probiotic combination, LA-synbiotic, for therapeutic efficacy in chronic comorbid disease, OA, and depressive disorder using a knee OA + depression animal model that aggravates OA joint pain.

Major Task: Evaluate rapid pain reduction, halted joint pathology and prevention of the development of depression by social isolation by LA-synbiotic over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.

Subtask 1: Generation of PTOA + comorbid depressive disorder animal model (by social isolation) in mice, Oral gavage (2X/week) of LA-synbiotic targeting different stages of OA progression over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.
Subtask 2: Weekly behavioral pain measurements with and without LA-synbiotic and documentation. Every 4 weeks, depression tests and documentation. We will discuss the results with our long-term collaborator, VA physician Dr. Gina Votta-Velis, for her clinical prospect for pain and depression-related behavior in our preclinical animal model.

Milestone(s) To Be Achieved:
We evaluate and validate the OA and comorbid depressive disease-modifying effect of LA-synbiotic for joint pain relief, cartilage protection, and inhibition of OA-associated development of depression by exploring the specific physiological effect.
Successful completion of our study will yield results (i) that provide essential information that will be rapidly translatable to clinical settings for LA-synbiotic; (ii) that change the OA treatment paradigm by addressing a critical issue addressing depression as a modifiable psychological factor for better treatment of OA and (iii) that are rapidly translatable to clinical settings not only for knee OA but

also potentially for a broad spectrum of musculoskeletal pain symptoms, including low back pain, and comorbid depressive disease. For example, our study will substantially impact the management of other diarthrodial joints, including the hip, shoulder, finger and ankle joints, and synarthrodial joints (for low back pain), which is also a major pain symptom Veterans suffer with a limited treatment option.

Specific Aim 2: To determine molecular mechanisms by which LA-synbiotics prevent/delay OA disease progression and prevent/reverse OA-associated depression.

Major Task: Detailed mechanisms of pro- and/or prebiotics that lead to improved joint health and depressive disease are not clear yet. We will determine the mechanisms by which LA-synbiotics mitigate joint pain and depression. (i) We will link depression markers in the brain (a-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments. (ii) We will measure key bacterial metabolites (SCFAs) altered by LA-synbiotic treatments.

Subtask 1: Tissue harvest from the animals in Aim 1 for *ex vivo*, *in vitro* histopathological analyses, quantitation of the results, correlate pain data, biochemical analyses from tissues (joints, gut, DRGs, spinal cord, blood, brain), tissue processing and decalcification, paraffin embedment. These harvested tissues will be quantitatively analyzed and documented in Specific Aim 2 for the mechanistic understanding of LA-synbiotic.

Subtask 2: We will link depression markers in the brain (α-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments.

Subtask 3: Stool samples collected from the animals in Aim 1 will be used to measure key bacterial metabolites (SCFAs), altered by LA-synbiotic treatments.

Milestone(s) To Be Achieved:
Our study will reveal how LA-synbiotic mechanisms mitigate joint pain, protect cartilage, and reverse depression. (i) Our results will connect the depression markers in the brain (a-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments. (ii) Our data will show altered key bacterial metabolites (SCFAs) by LA-synbiotic treatments and reveal specific SCFAs beneficial to OA pain, cartilage protection, and depression.

Specific Aim 3: To test if modulation of gut microbes by FMT can transform wild-type (*wt*) animals to *Flt1$^{fl/fl}$; RosaCreER^{+}*-like or *Flk1$^{fl/fl}$; RosaCreER^{+}*-like physiology (insensitive to OA pain or resistant to cartilage degeneration and depression).

Major Task: One of the major targets of SCFAs is the VEGF signaling pathways. Global conditional deletion of *Flt1* in mice (*Flt1$^{fl/fl}$;RosaCreER*) showed no sign of OA pain, and deletion of *Flk1* in mice (*Flk1$^{fl/fl}$;RosaCreER*) showed no sign of depression in chronic OA condition. We will test if modulation of gut microbes by FMT can transform *wt* animals to *Flt1$^{fl/fl}$; RosaCreER^{+}*-like or *Flk1$^{fl/fl}$; RosaCreER^{+}*-like physiology.

Subtasks:

(i) Breeding of *Tg* mice to *Flt1$^{fl/fl}$; RosaCreER^{+}* and *Flk1$^{fl/fl}$; RosaCreER^{+}* and microbiome characterization.

(ii) Generation of OA + depression comorbidity in animals
(iii) Fecal transplant from donor to recipient
(iv) Longitudinal behavioral pain and depression tests
(v) SCFA analyses in fecal samples
(vi) Detection of depression markers in serum and brain

Milestone(s) To Be Achieved.
Together with other Aims (1~3), our study will take the field of OA research a giant step forward with high impact in the short term by increasing our understanding of biomarkers of pain and OA-associated depression which will lead to development of a novel strategy for treating OA and depression effectively and safely. In the longer term, we expect our results to provide a rationale for clinical trials to evaluate synbiotic-LA efficacy for treating OA patients who suffer comorbid OA-associated depressive disorder.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1 Major Task: Evaluate rapid pain reduction, halted joint pathology and prevention of the development of depression by social isolation by LA-synbiotic over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.

Subtask 1: Generation of PTOA + comorbid depressive disorder animal model (by social isolation) in mice, Oral gavage (2X/week) of LA-synbiotic targeting different stages of OA progression over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.

Subtask 2: Weekly behavioral pain measurements with and without LA-synbiotic and documentation.

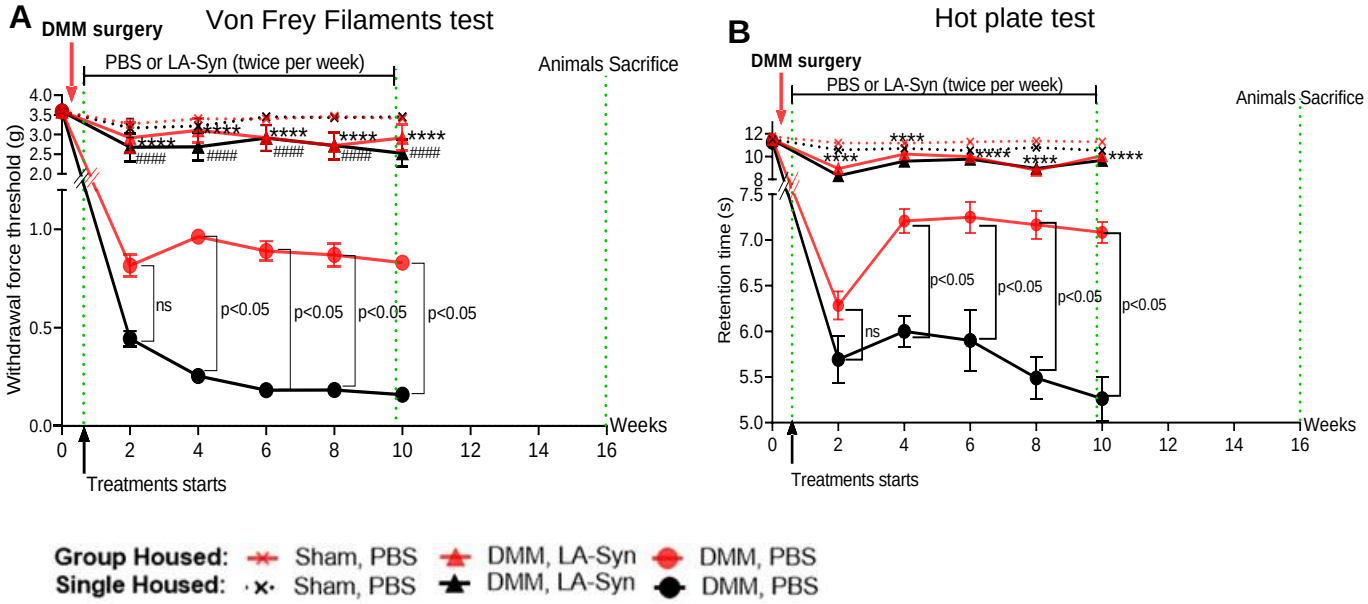
Figure 1 (i), (ii) and (iii)

Pharmacological impact of LA-Syn treatment on pain-related behavioral changes in a Mouse Model of Osteoarthritis (OA) and Depression co-morbidity. We assessed the development of mechanical sensitivity (using Von Frey filaments testing) and thermal sensitivity (using Hot plate testing) in the hind paw of mice subjected to different timepoint treatments with LA-Syn following destabilization of the medial meniscus (DMM) surgery.
These regimens included:

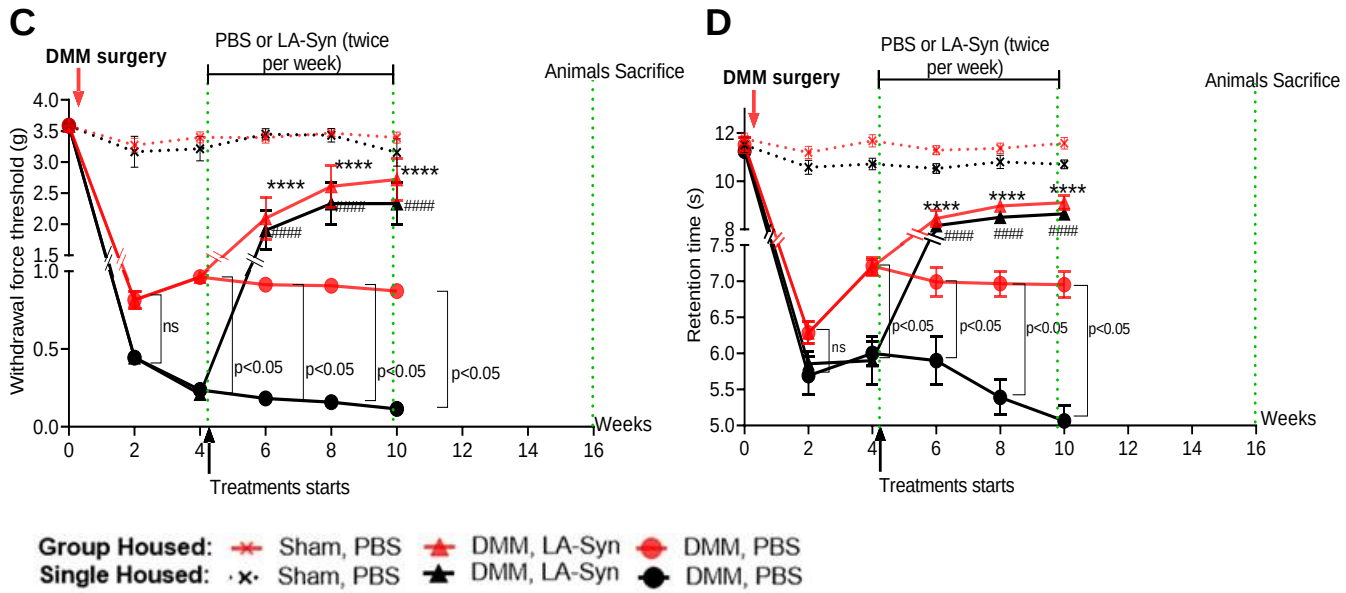
(i) LA-Syn administration starting from the inflammatory stage post-DMM surgery (A & B),
(ii) LA-Syn treatment starting from the early OA stage (C & D), and
(iii) LA-Syn treatment starting from the advanced OA stage up to 10 weeks post OA surgery (E & F). All values are expressed as mean ± SEM, n=12 mice of each experimental group. Statistical analyses were performed using two-way ANOVA followed by Tukey's multiple

comparison test. ****p<0.0001 *vs.* group-housed DMM, PBS and ####p<0.0001 *vs.* single-housed DMM, PBS.

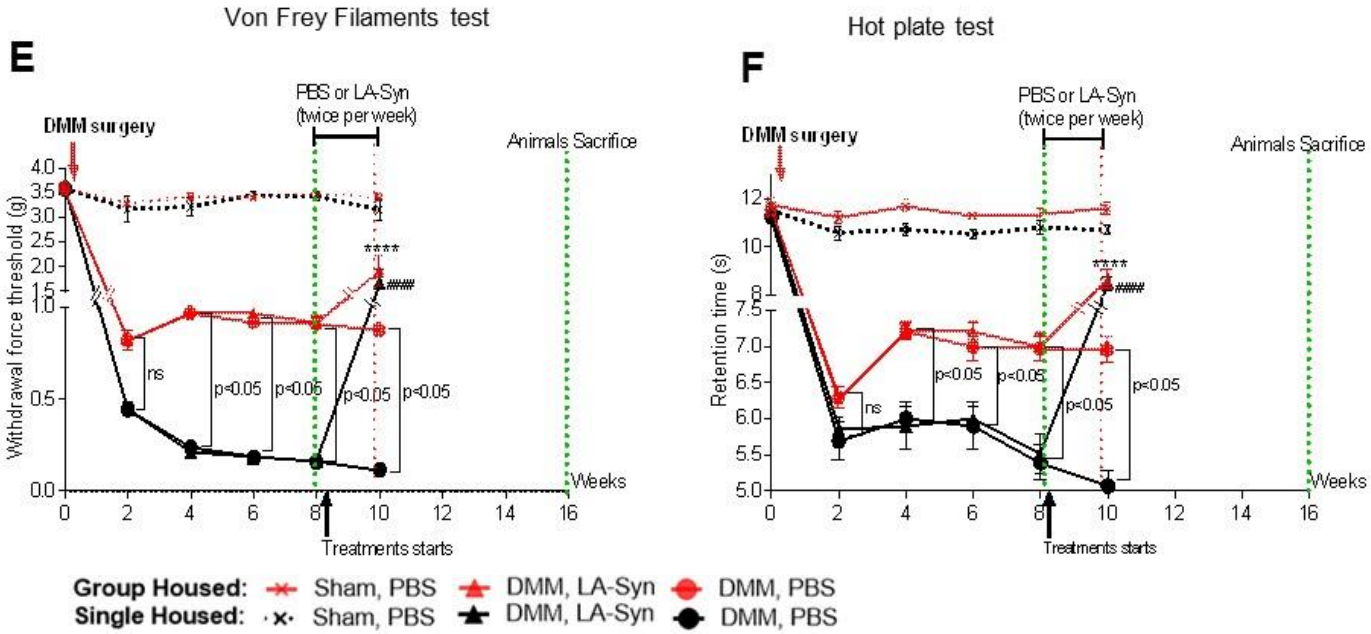
(i) LA-synbiotic treatment at the inflammatory pain stage using the preclinical PTOA mouse model.



(ii) LA-synbiotic treatment at the early-OA stage using the preclinical PTOA mouse model.



(iii) LA-synbiotic treatment at the advanced OA stage using the preclinical PTOA mouse model.



Result Description: Clinical studies reported that patients with OA diagnosed with comorbidities experienced more pain[1]. In this study, to mimic the human OA condition, we developed a novel model of OA-related depression by single-housing animals that had undergone DMM surgery. Our findings indicate that the combination of OA and social isolation substantially exacerbated the joint pain perception in the animals ($p<0.05$, comparing the group-housed DMM, PBS group to the single-housed DMM, PBS group) (Figure 1). Furthermore, we determined the therapeutic efficacy of LA-Syn in mitigating the co-morbidity model of OA and depression. We observed that LA-Syn treatment during the inflammatory pain phase resulted in an immediate reduction in pain level ($p<0.0001$) in both group-housed and single-housed animal models, with consistent pain alleviation observed for up to 10 weeks (see Figure 1A & B). When LA-Syn treatment was started at the early OA stage, it consistently reduced pain levels up to the 10th week following OA induction in both group-housed and single-housed animal models (see Figure 1C & D). In cases of advanced OA, treatment with LA-Syn in both models significantly diminished pain levels compared to the DMM and PBS groups. However, the extent of pain reduction was less pronounced than when treatment started during the inflammatory and early OA stages (see Figure 1E & F).

Reference:

1. van Dijk, G.M., Veenhof, C., Schellevis, F., Hulsmans, H., Bakker, J.P., Arwert, H., Dekker, J.H., Lankhorst, G.J. and Dekker, J., 2008. Comorbidity, limitations in activities and pain in patients with osteoarthritis of the hip or knee. BMC musculoskeletal disorders, 9(1), pp.1-10.

Subtask 3: IVIS *in vivo* imaging analyses for MMP activity, inflammation and angiogenesis, *in vitro* histopathological analyses

In progress

Milestone(s) Achieved:
We evaluated and validated the OA and comorbid depressive disease modifying-effect of LA-synbiotic for joint pain relief. In the next progress report, we should be able to provide further detailed information for the OA disease-modifying effects of LA-synbiotic showing cartilage protection, and inhibition of OA-associated development of depression by exploring the specific physiological effect (behavioral assessments for pain and depression, serum markers for depression). Our study will yield critical results (i) that provide essential information that will be rapidly translatable to clinical settings for LA-synbiotic; (ii) that change the OA treatment paradigm by addressing a critical issue addressing depression as a modifiable psychological factor for better treatment of OA and (iii) that are rapidly translatable to clinical settings not only for knee OA but also potentially for a broad spectrum of musculoskeletal pain symptoms, including low back pain, and comorbid depressive disease. For example, our study will substantially impact the management of other diarthrodial joints, including the hip, shoulder, finger and ankle joints, and synarthrodial joints (for low back pain), which is also major pain symptom Veterans suffer from a limited treatment option.

Aim 2 Major Tasks: Detailed mechanisms of pro- and/or prebiotics that lead to improved joint health and depressive disease are not clear yet. We will determine the mechanisms by which LA-synbiotic mitigate joint pain and depression. (i) We will link depression markers in the brain (α-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments. (ii) We will measure key bacterial metabolites (SCFAs) altered by LA-synbiotic treatments.

Subtask 1: Tissue harvest from the animals in Aim 1 for *ex vivo*, *in vitro* histopathological analyses, quantitation of the results, correlate pain data, biochemical analyses from tissues (joints, gut, DRGs, spinal cord, blood, brain), tissue processing and decalcification, paraffin embedment. These harvested tissues will be quantitatively analyzed and documented in Specific Aim 2 for the mechanistic understanding of LA-synbiotic.

In progress

Subtask 2: We will link depression markers in the brain (α-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments.

In progress

Subtask 3: Stool samples collected from the animals in Aim 1 will be used to measure key bacterial metabolites (SCFAs), altered by LA-synbiotic treatments.

In progress

Aim 3 Major Tasks: One of the major targets of SCFAs is the VEGF signaling pathways. Global conditional deletion of *Flt1* in mice (*Flt1*$^{fl/fl}$*;RosaCreER*) showed no sign of OA pain, and deletion of *Flk1* in mice (*Flk1*$^{fl/fl}$*;RosaCreER*) showed no sign of depression in chronic OA condition. We will test if modulation of gut microbes by FMT can transform *wt* animals to *Flt1*$^{fl/fl}$*Flk1*$^{fl/fl}$*; RosaCreER*$^{+}$-like physiology (resistant to OA pain, cartilage degeneration and depression).

We realized that double conditional knockout mice, *Flt1*$^{fl/fl}$*Flk1*$^{fl/fl}$*; RosaCreER* are not bred well – either die earlier or generate no pups. We slightly modified the protocol, and we generated global conditional deletion of *Flt1* in mice (*Flt1*$^{fl/fl}$*;RosaCreER*) that show no sign of OA pain and depression or deletion of *Flk1* in mice (*Flk1*$^{fl/fl}$*;RosaCreER*) that show cartilage protection after OA induction. We tested if modulation of gut microbes by FMT can transform *wt* animals to *Flt1*$^{fl/fl}$*; RosaCreER*$^{+}$-like physiology (resistant to OA pain and depression) or *Flk1*$^{fl/fl}$*; RosaCreER*$^{+}$-like physiology (resistant to OA cartilage degeneration). We observed interesting results which are summarized below.

(i) Modulation of gut microbiota by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces can transform *wt* animals to *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ - like physiology (insensitive to OA pain).

A partial medial meniscectomy (PMM) was done to induce OA in *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ and *wt* (*Flt1*$^{fl/fl}$) mice. Fresh feces from *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice (donor, no OA) were collected daily from week 7. Fresh stool (10 mg) was diluted in 100 mL sterile PBS solution. 100 µL of slurry solution was daily gavaged to *wt* mice in which unilateral knee OA was induced (FMT Recipient) from week 7. The groups not receiving FMT were gavaged with PBS 100 µL (control). The behavioral pain testings (von Frey and hot plate) showed conditional deletion of *Flt1* (*Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$) dramatically reduced joint pain in the PMM model, and this analgesic effect sustained throughout the 12 weeks post-PMM (the end of the experimental time period). We observed a similar joint pain reduction effect by modulation of gut microbiota in *wt* mice with knee OA (induced by PMM) by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces (donor, no OA). Those recipients *wt* mice significantly alleviated PMM-induced OA joint pian after FMT. Our results demonstrate the critical role of gut microbiome in pain sensation.

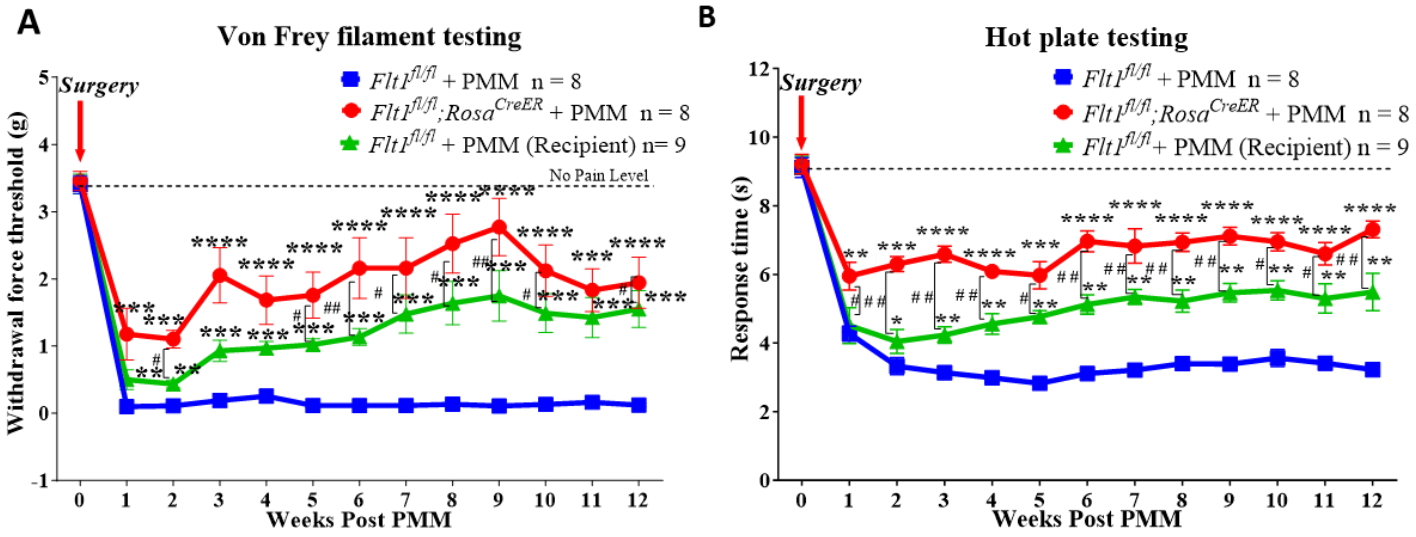


Figure 2. The development of mechanical allodynia and thermal pain was measured weekly by von Frey filaments (**A**) and hot plate testing (**B**) after PMM in the ipsilateral hind paw (n= 8 or 9). Statistical analysis was performed using one-way ANOVA with Tukey-Kramer test. Data are presented as means ± SEM (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared between *wt*

with FMT treatment (Recipient) or *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ and *wt* group mice that underwent PMM, #p<0.05, ##p<0.01 compared between *wt* with FMT treatment (Recipient) and *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ group mice that underwent PMM).

(ii) Alteration of gut microbiota by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces transforms *wt* animals to *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ - like behavior (attenuated chronic joint pain-associated depression-like behavior).

We observed that *wt* mice with OA develop chronic pain-associated depression. Conditional deletion of *Flt1* (*Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$) significantly reduced chronic joint pain-associated depressive-like behaviors compared to matched *wt* mice when measured at the advanced OA stage (12-weeks post PMM). Similarly, modulation of gut microbiota by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces also significantly alleviated chronic pain-associated depression in the FMT recipient *wt* mice represented by sucrose preference test, the most common and frequently used depression assessments (Figure 3A, #p<0.05).

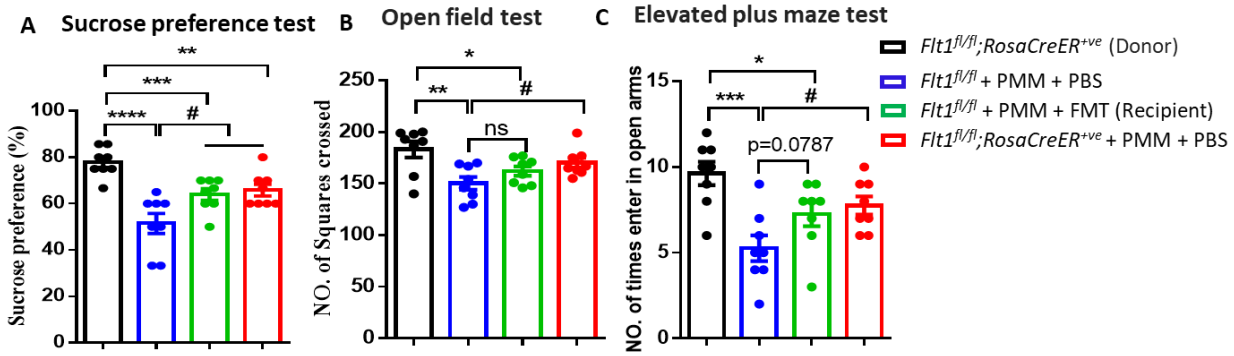


Figure 3. Sucrose Preference test (A), Open Field test (B) and Elevated Plus Maze test (C) were examined in mice (n=8) at week 12 post PMM. Data are expressed as mean ± S.E.M. Statistical analysis was conducted using the unpaired t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared between Sham and PMM groups. #p<0.05 compared between *wt* with FMT treatment (Recipient) or *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ and *wt* group mice that underwent PMM.

(iii) Alteration of gut microbiota by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces reduces VEGFA and corticosterone, well-known depression biomarkers in the serum in the *wt* animals similar to those in the conditional deletion of *Flt1* in mice (*Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$).

At the end of experimental period (12-weeks post PMM, advanced OA stage), blood from the animals were collected before euthanization. These animal groups include:

- Gp1: *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice (donor, intact)
- Gp2: *Flt1*$^{fl/fl}$ + PMM with PBS gavage as a control group (no FMT)
- Gp3: *Flt1*$^{fl/fl}$ + PMM + FMT (recipient)

- Gp4: *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ + PMM with PBS gavage (no FMT)

Our data show that conditional deletion of *Flt1* (*Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$) significantly reduces the expression of depression markers in plasma. Similarly, modulation of gut microbiota in *wt* mice (OA was induced) by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice feces also significantly reduces the expression of depression markers in plasma (Figure 4A and B).

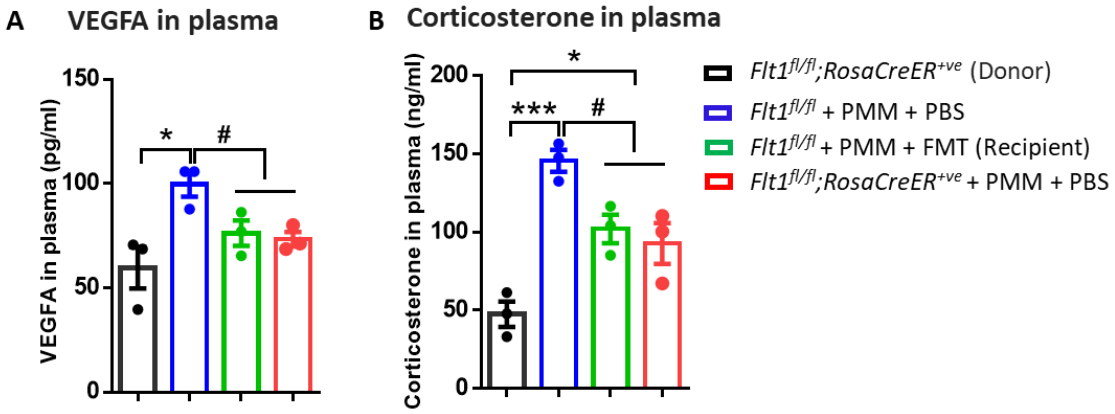


Figure 4. VEGFA (A) and corticosterone (B) level in plasma were examined in mice (n=3) using ELISA kits at week 12 post PMM. Data are expressed as mean ± S.E.M. Statistical analysis was conducted using the unpaired t-test. *p<0.05, ***p<0.001 compared between Sham and PMM groups. #p<0.05 compared between *wt* with FMT treatment (Recipient) or *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ and *wt* group mice that underwent PMM.

(iv) Alterations of gut microbiota by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ donor mice feces did not inhibit cartilage from injury-induced degeneration similar to those seen in conditional deletion of *Flt1* (*Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$) in mice.

We determined specific roles for Flt1/VEGFR1 for pain transmission and Flk1/VEGFR2 for cartilage degeneration (Ma *et al*., 2023a; Ma *et al*., 2023b). Inhibition of Flt1 signaling pathway showed resistance to OA-induced joint pain but without blocking cartilage degeneration (Qiu et al., 2020; Das et al., 2018; Ma *et al*., 2023a; Ma *et al*., 2023b). Inhibition of Flk1 signaling pathway attenuated cartilage degeneration which eventually reduced joint pain due to cartilage protection. We examined the effect of the alterations of gut microbiota of *wt* mice with OA (recipient) by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ mice donor feces on cartilage degeneration via histopathological analyses of knee joints at 12-weeks post PMM. Pathological grading was quantified using the Osteoarthritis Research Society International (OARSI) scoring system. The safranin-O fast green staining displayed that conditional deletion of *Flt1* in mice with PMM (▭) developed similar cartilage damage as *wt* mice (▭) without protection. Alterations of gut microbiota of *wt* mice (recipient, ▭) by FMT using *Flt1*$^{fl/fl}$*; RosaCreER*$^{+ve}$ donor mice feces did not protect cartilage from injury-induced degeneration (Figure 5). Our data consistently suggest that the specific role of Flt1/VEGFR1 in joint pain transmission, and inhibition of the Flt1 signaling pathways do not attenuate pathological progression as we previously reported (Qiu et al., 2020; Das et al., 2018; Ma *et al*., 2023a; Ma *et al*., 2023b).

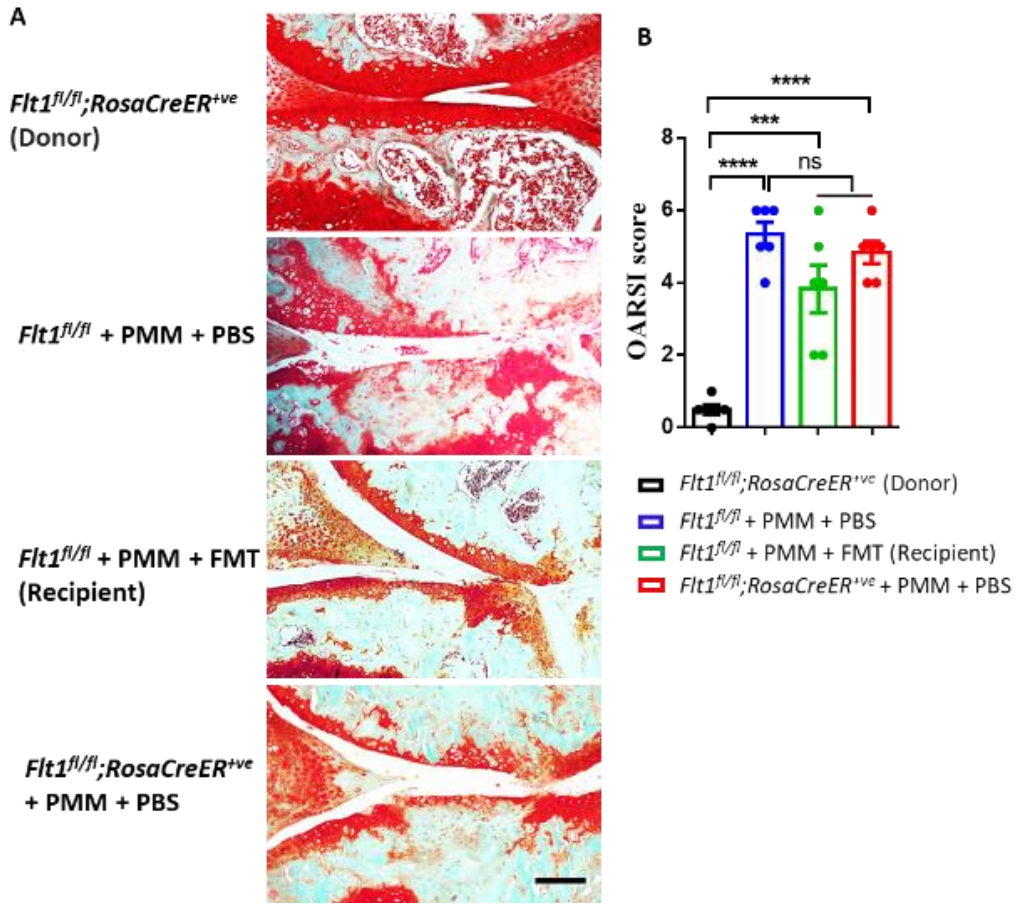


Figure 5. Modulation of gut microbiota fails to prevent cartilage from injury-induced degeneration by FMT using *Flt1$^{fl/fl}$; RosaCreER^{+ve}* mice feces. Representative images of safranin-O fast green staining of the knee joints (**A**). Graphs of average OARSI scores in the knee joints (**B**). Data are expressed as mean ± S.E.M. Statistical analysis was conducted using the unpaired t-test(n=5). **p<0.01, ***p<0.001, ****p<0.0001 compared between Sham and PMM groups, Scale bars: 200 μm.)

References:

Kaige Ma, Gurjit Singh, Jun Wang, InSug O-Sullivan, Gina Votta-Velis, Benjamin Bruce, Arivarasu Natarajan Anbazhagan, Andre J van Wijnen*, Hee-Jeong Im*. Targeting vascular endothelial growth factor receptors as a therapeutic strategy for osteoarthritis and associated pain. I J Biol Sci (IF: 12) 2023a, 19(2):675-690. ***Selected and featured as the Journal Cover (Editor-in-Chief's Choice) for scientific excellency.***

Kaige Ma, Tiep Pham, Jun Wang, InSug O-Sullivan, Amy DiCamillo, Shiyu Du, Fackson Mwale, Zeba Farooqui, Andre J. van Wijnen, Gina Votta-Velis, Benjamin Bruce, B. Duncan X. Lascelles, Ying Liu, Hee-Jeong Im. Nanoparticle-based Inhibition of Vascular Endothelial Growth Factor Receptors Resolves Osteoarthritis-Associated Pain and Cartilage Damage. Science Advances (IF: 14). 2023b (in revision).

Vaskar Das, Ranjan Kc, Andre J. van Wijnen, InSug O-Sullivan, Daniel Applegate, Richard L Ripper, Bronislaw Pytowski, Gina Votta-Velis, Jeffrey S. Kroin, Thomas J. Park, Hee-Jeong Im*. Vascular Endothelial Growth Factors (VEGF) Antibodies (MF-1 vs. DC101) Reveals a Novel Analgesic for Osteoarthritis-Induced Pain in Mammalian. Gene Reports 2018 11,94-100

Sujun Qiu, Changgui Shi, Arivarasu Natarajan Anbazhagan, Vaskar Das, Vipin Arora, Ranjan Kc, Xin Li, InSug O-Sullivan, Andre van Wijnen, Sudhakar Chintharlapalli, Gina Gott-Velis, Ripper Richard, Fackson Mwale, Masabumi Shibuya, Shaoxiong Min, Hee-Jeong Im*. Absence of VEGFR-1/Flt-1 signaling pathway in mice results in insensitivity to discogenic low back pain in an established disc injury mouse model. J Cell Phy 2020, 235(6):5305-5317.

Percentage of Completion for the Major Tasks/Sub-tasks:

Project Description, Objectives:		**Percentage of Completion**	
Specific Aim 1: To evaluate a pre-/probiotic combination, LA-synbiotic, for therapeutic efficacy in chronic comorbid disease, OA, and depressive disorder using a knee OA + depression animal model that aggravates OA joint pain. Major Task: Evaluate rapid pain reduction, halted joint pathology and prevention of the development of depression by social isolation by LA-synbiotic over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.	**Timeline 25 (Months)**		
Subtask 1: Generation of PTOA (by PMM) + comorbid depressive disorder animal model (by social isolation) in mice, Oral gavage (2X/week) of LA-synbiotic targeting different stages of OA progression over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.	25 Months	55% (ongoing)	
Subtask 2: Weekly behavioral pain measurements with and without LA-synbiotic and documentation. Depression tests and documentation; We will discuss the results with our long-term collaborator, VA physician Dr. Gina Votta-Velis for her clinical prospect for pain and depression-related behavior in our preclinical animal model.	25 Months	50% (ongoing)	
Subtask 3: IVIS *in vivo* imaging analyses for MMP activity, inflammation and angiogenesis, *in vitro* histopathological analyses	25 Months	Not initiated	
Specific Aim 2: To determine molecular mechanisms by which LA-synbiotics prevent/delay OA disease progression and prevent/reverse OA-associated depression. Major Task: Detailed mechanisms of pro- and/or prebiotics that lead to improved joint health and depressive disease are not clear yet. We will determine the mechanisms by which LA-synbiotics mitigate joint pain and depression. (i) We will link depression markers in the brain (α-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes	**Timeline 36 (Months)**	Not Initiated (Samples are collected)	

by comparing with and without LA-synbiotic treatments. (ii) We will measure key bacterial metabolites (SCFAs), altered by LA-synbiotic treatments.			
Subtask 1: Tissue harvest from the animals in Aim 1 for *ex vivo*, *in vitro* histopathological analyses, quantitation of the results, correlate pain data, biochemical analyses from tissues (joints, gut, DRGs, spinal cord, blood, brain), tissue processing and decalcification, paraffin embedment. These harvested issues will be quantitatively analyzed and documented in Specific Aim 2 for the mechanistic understanding of LA-synbiotic.	24 Months	Not Initiated (Samples are collected)	
Subtask 2: We will link depression markers in the brain (α-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments.	12 Months	Not Initiated (Samples are collected)	
Subtask 3: Stool samples collected from the animals in Aim 1 will be used to measure key bacterial metabolites (SCFAs), altered by LA-synbiotic treatments.	36 Months	Not Initiated (Samples are collected)	
Specific Aim 3: To test if modulation of gut microbes by FMT can transform wild-type (*wt*) animals to *Flt1$^{fl/fl}$Flk1$^{fl/fl}$; RosaCreER^{+}*-like physiology (insensitive to OA pain, resistant to cartilage degeneration and depression). Major Task: One of the major targets of SCFAs is the VEGF signaling pathways. Global conditional deletion of *Flt1* in mice (*Flt1$^{fl/fl}$;RosaCreER*) showed no sign of OA pain, and deletion of *Flk1* in mice (*Flk1$^{fl/fl}$;RosaCreER*) showed no sign of depression in chronic OA condition. We will test if modulation of gut microbes by FMT can transform *wt* animals to *Flt1$^{fl/fl}$Flk1$^{fl/fl}$; RosaCreER^{+}*-like physiology (resistant to OA pain, cartilage degeneration and depression).	**Timeline 48 (Months)**	25% (ongoing)	
Subtasks: (i) Breeding of Tg mice *Flt1$^{fl/fl}$; RosaCreER* and *Flk1$^{fl/fl}$; RosaCreER*, and microbiome characterization. (ii) Generation of OA + depression comorbidity in animals (iii) Fecal transplant from donor to recipient (iv) Longitudinal behavioral pain and depression tests (v) SCFA analyses in fecal samples (vi) Detection of depression markers in serum and brain	48 Months	25% (ongoing)	

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training"

activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

"Nothing to Report."

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

"Nothing to Report."

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Aim 1 Major Task: Evaluate rapid pain reduction, halted joint pathology and prevention of the development of depression by social isolation by LA-synbiotic over the course of disease progression (inflammatory-, early- and advanced OA) in our preclinical PTOA + depression animal model.

Aim 1, Subtask 2: We will generate depression-like behavioral measurements with and without LA-synbiotic and documentation.
Aim 1, Subtask 3: We will complete IVIS *in vivo* imaging analyses for MMP activity, inflammation and angiogenesis.

Aim 2 Major Task: Detailed mechanisms of pro- and/or prebiotics that lead to improved joint health and depressive disease are not clear yet. We will determine the mechanisms by which LA-synbiotic mitigate joint pain and depression. (i) We will link depression markers in the brain (a-Tubulin acetylation) and serum (VEGF and corticosterone) to physiological changes by comparing with and without LA-synbiotic treatments. (ii) We will measure key bacterial metabolites (SCFAs) altered by LA-synbiotic treatments.

Aim 2, Subtask 1: We will harvest tissues from the animals in Aim 1 for *ex vivo*, *in vitro* histopathological analyses, quantitation of the results, correlate pain data, biochemical analyses from tissues (joints, gut, DRGs, spinal cord, blood, brain), tissue processing and decalcification, paraffin embedment. These harvested tissues will be quantitatively analyzed and documented in Specific Aim 2 for the mechanistic understanding of LA-synbiotic.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

"Nothing to Report."

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

"Nothing to Report."

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

"Nothing to Report."

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

"Nothing to Report."

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

"Nothing to Report."

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We do not have any obstacles, and we expect to complete the project on time.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

"Nothing to Report."

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  "Nothing to Report."

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  "Nothing to Report."

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

"Nothing to Report."

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

"Nothing to Report."

- **[illegible]**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

"Nothing to Report."

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

"Nothing to Report."

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). ***If information is unchanged from a previous submission, provide the name only and indicate "no change****".*

"No Change"
(1) ***PI:*** *Hee-Jeong Im Sampen*
(2) ***Co-****Is: Mark M. Rasenick, Arivarasu N Anbazhagan*
(3) ***Postdoctoral scientist:*** *Gurjit Singh*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

"Nothing to Report."

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial

or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

"Nothing to Report."

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*