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AAO Foundation Final Report Form
(a/o 5/30/2021)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award, Biomedical Research Award

Name(s) of Principal Investigator(s): Sunil Kapila, BDS, MS, PhD.

Institution: UCLA School of Dentistry

Title of Project: The Role of Osteocyte-Mediated Bone Remodeling in Temporomandibular Joint Osteoarthritis

Period of AAOF Support (e.g. 07-01-2021 to 06-30-2022): 07/01/18 - 06/30/22

Amount of Funding: $30,000

Summary/Abstract

Temporomandibular joint disorders (TMJDs) are a highly prevalent spectrum of conditions occurring in about 6 to 12% of the adult US population totaling over 10 million people and costing billions of dollars in health care and lost productivity. TMJDs frequently present with pain, functional limitations and joint sounds associated with degenerative joint disease, an osteoarthritis (OA)-like condition that significantly affects the quality of life due to its impact on critical functions such as eating and speech. While the etiologies of the temporomandibular joint (TMJ) OA remain unknown, due to the propensity of these disorders in adolescent females- an age group that coincides with orthodontic treatment- orthodontic therapy has often been attributed as a causative or predisposing factor for TMJ OA. Thus, a deeper understanding of the pathogenesis and

mechanisms for TMJ degeneration would be highly valuable to clinicians and specifically to orthodontists.

Recent models of OA suggest a close interplay between bone and overlying cartilage composite through mechanical and / or biologic crosstalk in the progression of OA. More specifically the perturbation of osteocyte-mediated perilacunar remodeling (PLR) exacerbates OA in a surgical model of knee OA in mice. Whether this is true for TMJ which has a unique morphology, organization, development, cellularity and function to that of appendicular joints has not been investigated. The purpose of this study was to determine the contribution of osteocyte MMP13 which is involved in PLR in contributing to the progression of TMJ OA in an injury mouse model of TMJ osteoarthritis. We tested the hypothesis that defects in osteocyte-mediated bone remodeling via loss of MMP13 accelerates TMJ OA through the Specific Aims described below together with a summary of the goals achieved.

1. Establish and characterize a chemically induced TMJ OA model in mice. We will adapt a previously established monosodium iodoacetate (MIA)-induced mouse knee OA model to the TMJ by determining the monosodium iodoacetate (MIA) dose and temporal effects on the initiation and progression of mouse TMJ OA assessed histologically and by micro-computed tomographic (μCT) analyses. To ensure reproducible and predictable OA induction in the TMJ, we first performed pilot studies to refine the technique and confirm our ability to reproducibly administer the agent intra-articularly through administration of Fast Green dye into the TMJ. After several trials, we have established a reproducible technique (Fig. 1). For the second part of this Aim, we undertook MIA dose response experiments to determine what concentrations of MIA successfully and reliably induce osteoarthritis of the TMJ (Fig. 2).
2. Determine the contributions of osteocyte MMP13 and associated defects in PLR to the progression and severity of TMJ OA. TMJ OA was induced in osteocyte-specific MMP13 deficient (Cre+MMP13$^{OCY-/-}$) and wildtype (WT) Cre- mice using the optimal dose of MIA determined in Aim 1 and the severity of TMJ OA assayed histologically and by μCT. We have completed studies involving the administration of PBS and the selected MIA dose (0.1 mg) in WT and MMP13$^{OCY-/-}$ mice and assayed the tissues histologically and by μCT (Figs. 3 and 4).

Detailed results and inferences:

1. If the work has been published please attach a pdf of manuscript OR
2. Describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should be included. Please see summary of findings above and figures below.

Results

Aim 1A: We have confirmed the successful and repeatable intra-articular injection protocol using a 30-gauge 5/8'' needle placed parallel to zygomatic arch, directly inferior to the arch, as medial as possible and directly parallel to the mouse head (Figs. 1A and B). The needle is then inserted until it hubs against nose of mouse and lateral to mouse molars. When the needle hubs, the tip is nearly flush with cochlea; the needle is withdrawn about 0.5mm, when the fluid is deposited. Using this technique we have achieved consistently successful intra-articular injection through administration of Fast Green dye (Fig. 1C).

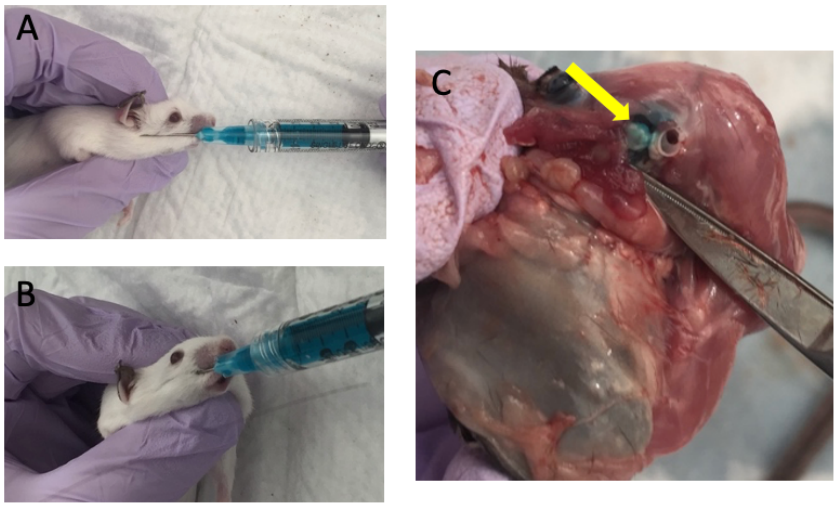

Figure 1. Characterizing and verifying an optimal method for intra-articular TMJ injection in the mouse. Lateral (A) and anterior (B) views showing direction of needle insertion. (C) Dissected mouse head showing Fastgreen staining of condylar head and TMJ fossa (arrow).

Aim 1B: Next we performed dose response experiments to determine the optimal dose of MIA that results in reproducible OA of the TMJ. Using doses calculated from previous experiments in the mouse knee and rat TMJ, we administered either 0.05 mg or 0.1 mg MIA in 10 μl of PBS or PBS alone to four WT mice in each of the groups. After 28 days following the intra-articular administration of MIA or PBS, the tissues were retrieved for histology and μCT. Specimens from these experiments have undergone histologic processing and quantitative analyses using the modified Mankin Score. The findings demonstrate that 0.1 mg MIA results in a reproducible and substantial TMJ changes mimicking osteoarthritis (Fig. 2).

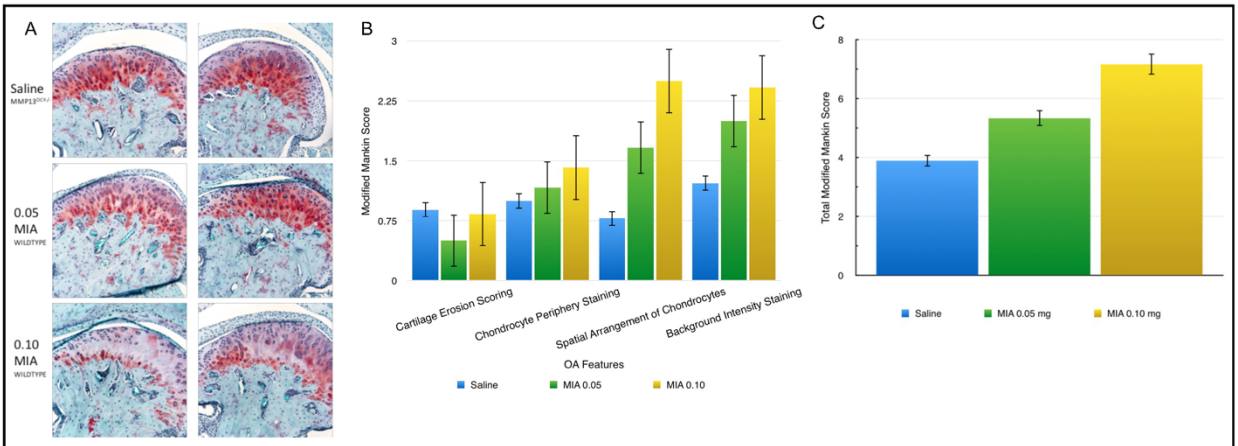

Figure 2: MIA dose effects on severity of TMJ arthritis. (A) Representative photomicrographs of coronal sections of TMJs from control PBS MMP13$^{OCY-/-}$ (upper panel), 0.05 mg MIA WT (middle panel) and 0.10 mg MIA WT mice (lower panel) at 28 days post injection (20x magnification). Mean (± SD) arthritis severity quantified by modified Mankin scores from PBS control MMP13$^{OCY-/-}$ vs 0.05 mg MIA WT and 0.10 mg MIA WT mice by individual criteria (B) and total modified Mankin scores (C).

Aim 2: Histologic analyses of WT and MMP13$^{OCY-/-}$ mice unexpectedly revealed that while MIA administration results in significant increase ($p<0.05$; N= 5 per group) in aggregate Mankin score in WT mice at 28 days post injury, the absence of osteocyte MMP13 mitigated this response (Fig. 3A and B). In further characterizing the criteria that contributed to MIA-mediated arthritic changes in the WT group, we found significantly enhanced chondrocyte periphery staining, altered spatial arrangement of chondrocytes with increased in diffuse hypocellularity and more vague demarcation of cartilage in the MIA WT group compared to the sham WT and both sham and MIA MMP13$^{OCY\ -/-}$ samples (Fig 3C). In contrast, sections from MMP13$^{OCY\ -/-}$ mice show similar degrees of Safranin-O intensity staining, spatial arrangement of chondrocytes, and little to no cartilage erosion between the PBS sham and MIA-injected groups. Finally, within the WT group, the MIA-administered mice showed significantly greater ($p<0.05$) staining than the PBS sham mice. The statistical analysis using ANOVA with at least a n=5 per group phenotypic findings from the histology sections and demonstrates a significant difference between sham and MIA treated WT mice, but not between the two treatments in the MMP13$^{Ocy\ -/-}$ mice. Thus, the subcategories of Modified Mankin scoring revealed that the chondrocyte periphery staining, spatial arrangement of chondrocytes, background staining intensity, and demarcation between the cartilage and bone were the primary factors which drove the differences in the overall Mankin Scoring numbers.

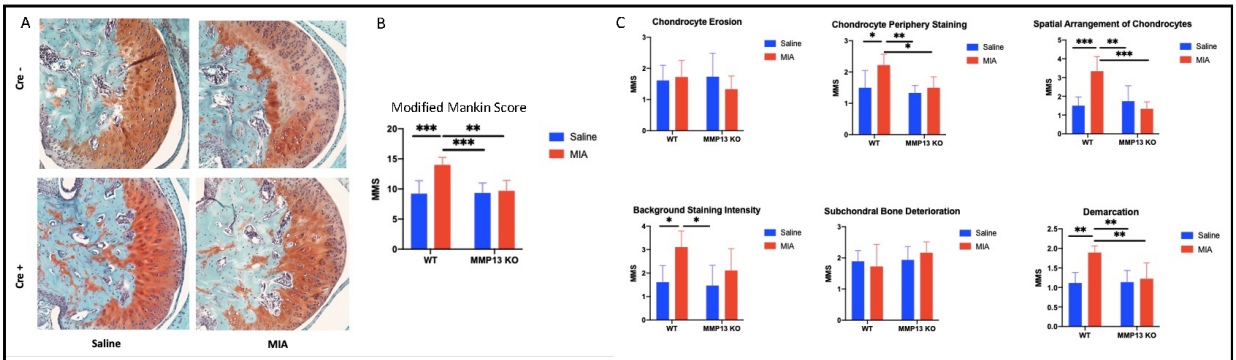

Figure 3: Absence of osteocyte MMP13 mitigates MIA-induced TMJ arthritis. (A) Representative photomicrographs from Cre- WT and Cre+ MMP13$^{OCY-/-}$ mice intraarticularly administered normal saline or MIA. (B) Mean (± SD) of total Modified Mankin score (MMS) demonstrating statistically significant increases in the arthritis for Cre- WT MIA (0.1 mg)-treated mice relative all other groups of animals. (C) Mean (+/- SD) of individual criteria of modified Mankin demonstrates statistically significant increases in chondrocyte periphery staining, spatial arrangement of chondrocytes, background staining intensity, and demarcation between cartilage and bone for WT MIA treated mice relative to one or more other groups of animals (*$p<0.05$; **$p<0.01$; ***$p<0.001$ by ANOVA and Tukey post hoc test).

The normal control, sham-treated, and MIA treated groups were analyzed for changes in bone quality and quantity using μCT. There were no significant differences for any of the measured parameters namely subchondral bone volume fraction, bone mineral density, tissue mineral density, trabecular number, trabecular thickness, and trabecular spacing between WT and MMP13$^{OCY\ -/-}$ sham and MIA mice and between these two genotypes (Fig. 4). However, MMP13$^{OCY\ -/-}$ samples have qualitatively denser bone and had fewer trabecular spaces below the subchondral bone and MIA appears to increase spaces within the condylar bone in WT MIA mice than in sham WT mice confirming the histologic observations. These images provide greater detail about the condylar bone, which was not captured in the quantitative μCT analysis conducted for this study. Additional analyses are ongoing to quantitate bone phenotype in a deeper portion of the condyle.

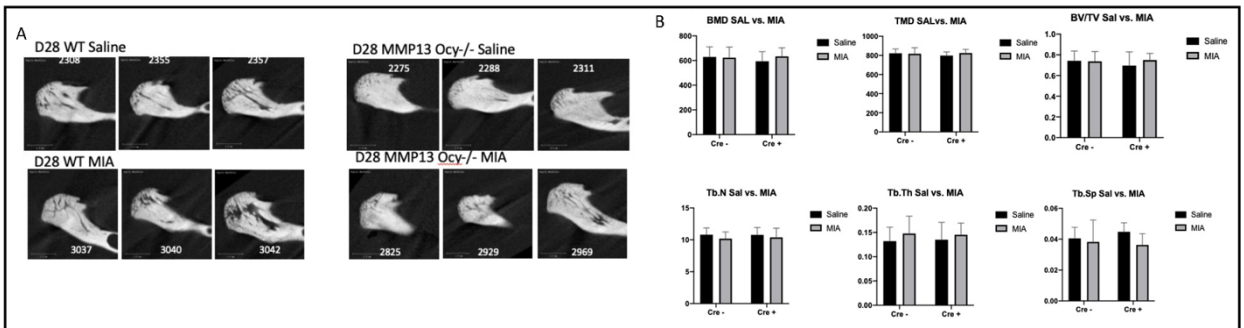

Figure 4: Absence of osteocyte MMP13 has minimal effect on subchondral bone quality and quantity in MIA-mediated TMJ arthritis. (A) Sagittal μCT sections of representative samples from Cre- WT and Cre+ MMP13$^{OCY-/-}$ mice intraarticularly administered normal saline or MIA showing increased subchondral intertrabecular space in condyles from WT mice administered MIA. (B) However, bone measures of bone quality and quantity (mean ±SD) show no statistically significant differences by ANOVA in these variables between any of the groups.

Inferences and Conclusions

Intra-articular administration of MIA resulted in histologic changes characteristic of OA in WT mice, it did not contribute to degenerative changes in cartilage or subchondral bone in MMP13$^{OCY-/-}$ mice. We also show that the lack of osteocytic MMP13 has a protective effect against MIA-induced TMJ OA. These findings contrast with those of the knee, where loss of osteocytic MMP13 increases the severity of OA in an injury model. Both the anatomic and biological differences between the knee and TMJ may explain the observed differences between the findings in the TMJ and knee joints. For example, the lack of a subchondral bone plate in the TMJ as opposed to presence of thick subchondral bone plate in the femur would facilitate diffusion of MMP13 in WT mice TMJs vs the knee joint resulting in damage to TMJ fibrocartilage from osteocyte MMP13 that would not be seen in the absence of osteocyte MMP13 suggesting the relative importance of bone MMP13 in cartilage-bone crosstalk TMJ degeneration. In contrast, it is likely that cartilage damage in the WT femoral condyle and its potentiation in the absence of osteocyte MMP13 reflects a different mechanism for this phenomenon in the knee joint that may rely more on the cartilage damage arising directly from factors released by the cartilage itself rather than the effects of osteocyte MMP13. These postulates require further study to be verified.

Besides providing fundamental information on osteochondral interactions and the role of each of these tissues to the initiation and / or progression of TMJ OA, this study offers a better understanding the pathogenesis of this disorder and provides insights into potential therapeutic targets to prevent or alleviate degenerative diseases of the TMJ. For example, approaches targeting the inhibition of octeocyte MMP13 may have therapeutic benefits for TMJ OA. Furthermore, determination of an effective MIA dose that generates TMJ OA has enabled us to establish a mice model for TMJ OA that could be valuable for future studies to identify disease mechanisms.

<u>Respond to the following questions</u>:

1. Were the original, specific aims of the proposal realized? *Yes*

2. Were the results published?
   - *a.* If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers. *Two MS theses by Dr. Marianne Demerdji and Dr. Alena Larios resulted from these studies. We are currently preparing a manuscript for publication.*
   - b. Was AAOF support acknowledged? *Yes*
   - *c.* If not, are there plans to publish? If not, why not? *Given the extensive nature of*

these studies and delays due to the COVID pandemic, our work progressed slower than anticipated. We are currently in the process of compiling a manuscript for publication.

3. Have the results of this proposal been presented?
    a. If so, list titles, author or co-authors of these presentation/s, year and locations. "*Differential responses of TMJ and Knee Osteochondral Tissues to Estrogen" Kapila, S. Presented at 2021 IADR/AADR/CADR Virtual Meeting during a symposium titled "Therapies for TMJ by Understanding Unique Characteristics Versus Appendicular Joints".*

    b. Was AAOF support acknowledged? *Yes*

    c. If not, are there plans to do so? If not, why not? *NA*

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career? *I plan to continue seeking funding for our projects in the future.*

Accounting for Project; (i.e.), any leftover funds, etc. *None (NA)*