



Theses and Dissertations

2014-06-01

Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)

Elizabeth Murayama Chavez Matias
Brigham Young University - Provo

Follow this and additional works at: <https://scholarsarchive.byu.edu/etd>



Part of the [Cell and Developmental Biology Commons](#), and the [Physiology Commons](#)

BYU ScholarsArchive Citation

Chavez Matias, Elizabeth Murayama, "Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)" (2014). *Theses and Dissertations*. 5242.

<https://scholarsarchive.byu.edu/etd/5242>

This Thesis is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact ellen_amatangelo@byu.edu.

Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of
Mice with and Without Receptor for Advanced Glycation
End Products (RAGE)

Elizabeth Murayama Chávez Matías

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

David L. Kooyman, Chair
Paul R. Reynolds
Laura C. Bridgewater

Department of Physiology and Developmental Biology
Brigham Young University

June 2014

Copyright © 2014 Elizabeth Murayama Chávez Matías

All Rights Reserved

ABSTRACT

Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)

Elizabeth Murayama Chávez Matías
Department of Physiology and Developmental Biology, BYU
Master of Science

This thesis will be organized into three chapters discussing the mechanism underlying the onset and progression of osteoarthritis (OA) in the temporomandibular joint (TMJ). Understanding the mechanism of OA development in the TMJ helps in understanding how OA progresses and how to treat this disease. The goal of this investigation is to examine the process of cartilage degeneration and OA biomarker expression in the TMJ to understand their role in TMJ OA onset and development.

Chapter one covers mechanisms that are altered in TMJ OA during disease progression. Using animal models with different stressors such as mechanical disturbances, direct injury, and changes in the extracellular matrix composition revealed the role of the different mechanisms that are up-regulated and down regulated during cartilage destruction.

Chapter two will cover a paper I wrote that introduces a novel non-invasive technique applied to mice, which induces an early onset of OA in the TMJ. I developed this technique with the aim to provide a new mouse model where the onset and progression of OA more closely mimic the natural TMJ OA progression in humans. The histopathological analysis of the cartilage demonstrates that onset of OA starts at 2 weeks after treatment induction and is aggravated by week eight. This data demonstrated the effectiveness of our technique in inducing OA in the TMJ.

Chapter three will cover a second paper I wrote on the association of RAGE with the progression of OA in the TMJ of mice by using mice with and without RAGE expression. RAGE has been shown to contribute to the progression of OA by releasing several pro-inflammatory and catalytic cytokines. Additionally, RAGE has been shown to modulate the expression of specific OA biomarkers, including HtrA-1, Mmp-13, and Tgf- β 1 in knee cartilage. The objective of this study was to study the effect of knocking out RAGE on the expression of Mmp-13, HtrA-1, and Tgf- β 1 in the TMJ. After histopathological and quantitative analysis of biomarkers expression, the results demonstrated for the first time that absence of RAGE expression in the TMJ provides a protective effect against development of TMJ OA in mice.

Keywords: temporomandibular joint, murine, mice, osteoarthritis, RAGE, Mmp-13, HtrA-1, Tgf- β 1

ACKNOWLEDGEMENTS

I would like to thank Dr. David L. Kooyman my graduate chair, for giving me the opportunity to work in his lab and helping me with my research, and thesis. Thank you to Dr. Paul R. Reynolds and Dr. Laura C. Bridgewater for being on my graduate committee, and to all the undergraduate students who helped me with my research.

To my parents and siblings, thank you for your encouragement, patience, and love.

TABLE OF CONTENTS

| | |
|--|-----|
| TITLE PAGE | i |
| ABSTRACT | ii |
| ACKNOWLEDGEMENTS | iii |
| TABLE OF CONTENTS | iv |
| LIST OF FIGURES | vi |
| LIST OF TABLES | vii |
| CHAPTER 1: Introduction | 1 |
| References | 5 |
| CHAPTER 2: A Novel Mouse Model of Temporomandibular Joint Osteoarthritis | 8 |
| Abstract | 8 |
| Introduction | 9 |
| Methods and Materials | 11 |
| Facilities and Animals | 11 |
| Materials | 11 |
| Disruption of the Dental Occlusion | 12 |
| Tissue Harvesting and Processing | 14 |
| Histological Analysis | 14 |
| Statistical Analysis | 15 |
| Results | 15 |
| Discussion | 22 |
| Authors' Contributions | 24 |
| Funding | 24 |
| Competing Interests | 25 |
| Acknowledgments | 25 |
| References | 26 |
| CHAPTER 3: Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice With and Without Receptor for Advanced Glycation End Products (RAGE) | 30 |
| Abstract | 30 |
| Introduction | 31 |
| Methods and Materials | 34 |
| Facilities and Animals | 34 |
| Materials | 35 |
| Tissue Harvesting and Processing | 35 |

| | |
|-------------------------------------|----|
| Histological Analysis | 36 |
| Immunohistochemistry Analysis | 36 |
| Statistical Analysis..... | 37 |
| Results..... | 37 |
| Discussion..... | 38 |
| Authors' Contributions | 48 |
| Funding..... | 48 |
| Ethical Approval..... | 49 |
| Acknowledgments..... | 49 |
| References..... | 50 |
| CURRICULUM VITAE..... | 57 |

LIST OF FIGURES

| | |
|---|----|
| Figure 2.1: Tools Dental Platform | 13 |
| Figure 2.2: Wire Bonded to Teeth | 13 |
| Figure 2.3: Safranin O/Fast Green Staining | 17 |
| Figure 2.4: Modified Mankin Score | 18 |
| Figure 2.5: Cartilage Erosion..... | 19 |
| Figure 2.6: Chondrocyte Periphery Staining | 19 |
| Figure 2.7: Spatial Arrangement of Chondrocytes | 19 |
| Figure 2.8: Background Staining | 19 |
| Figure 2.9: Mice Weight | 20 |
| Figure 3.1: Wire Bonded to Teeth | 40 |
| Figure 3.2: Safranin O/Fast Green Staining..... | 41 |
| Figure 3.3: Modified Mankin Score WT-RAGE KO | 42 |
| Figure 3.4: IHC Staining - Mmp-13 | 43 |
| Figure 3.5: IHC Staining - HtrA-1 | 44 |
| Figure 3.6: IHC Staining - Tgf- β 1 | 45 |
| Figure 3.7: Osteoarthritis Biomarkers: Mmp-13, HtrA-1, and Tgf- β 1 | 46 |

LIST OF TABLES

| | |
|---|----|
| Table 2.1: Modified Mankin Score Table..... | 21 |
|---|----|

CHAPTER 1: Introduction

Osteoarthritis (OA) of the temporomandibular joint (TMJ) is the most common chronic degenerative disease (1, 2) that results from metabolic imbalances that lead to cartilage degeneration with thinning, fibrillation, fissuring, and loss of the condylar cartilage. The molecular mechanisms underlying this degenerative process indicate that early articular degeneration is characterized by chondrocyte proliferation, cluster formation, and over-production of extracellular matrix (ECM) elements, such as proteoglycans. Contrarily mid and late OA are characterized by progressive degradation of ECM elements, loss of proteoglycan content, and reduction in numbers of chondrocyte (hypoplasia) (3), due to apoptosis, terminal maturation or a combination of both. The molecular differences between OA stages result from the predominance of catabolic processes over anabolic ones as the disease progresses. The onset of these complex processes follows stress produced by mechanical disturbances, direct or indirect injury, and changes in the ECM composition.

Mechanical stress in patients, which is the result of missing teeth or parafunction, is the most frequent trigger of OA in the TMJ. Several studies using animal models such as mice, rats, rabbits, goats, and sheep demonstrated that mechanical stress leads to cartilage degradation (4-7). For example, after 8 and 12 weeks of experimental disruption of the alignment of the teeth on the dental arch of rats, which mimics the absence of the second molar, animals showed signs of cartilage degradation and increased chondrocyte death (4). Also, increasing or decreasing the loading of the TMJ by trimming the dental incisors (out of occlusion), producing a dental cross bite, changing diet, and by forcing the mouth opening resulted in loss of cartilage thickness, altered ECM composition, increased in chondrocyte proliferation, and subchondral bone growth

in the condylar cartilage (4-9). One study examined the effects of inducing a cross bite and changing the diet size for a period of 3 weeks in mice. Results showed that in the group with a large size diet and cross bites, there was an increased expression of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) mRNA (aggrecanase) in ECM, Receptor Activator of Nuclear Factor κ B (RANK) mRNA in subchondral bone; and a decreased expression of aggrecan mRNA, collagen type I alpha 2 (Col1a2) mRNA, and collagen type II all molecular results that are associated with increase percentage of cartilage degradation (5). Another study showed that after forcing mice mouth open for 1 hr/day for 5 days lead to chondrocyte proliferation and trabecular spacing, which was accompanied with an increase in Col1a2 mRNA, parathyroid hormone-related protein (Pthrp), and transcription factor SOX-9 (Sox9) expression (9).

While these studies revealed the involvement of some genes and proteins in the development of OA in TMJ after mechanical stress, studies made in animals after invasive treatment showed the involvement of more genes and proteins during OA progression (10-15). For example, some studies demonstrated that cartilage post-discectomy showed severe histological features that were coincident with the appearance and increase of discoidin domain receptors 2 (Ddr-2), matrix metalloproteinase-13 (Mmp-13), high temperature requirement protein (HtrA-1) expression, and degraded type II collagen in the cartilage (12, 16). Another study in contrast showed delayed condylar cartilage degeneration in a transgenic Ddr-2^{0/+} mice after surgery (11). All together, these studies demonstrate the key role of HtrA-1, Ddr-2 and Mmp-13 in condylar cartilage degradation.

The injection of chemicals into the joints of rats has also shown to promote cartilage degradation and expression of more proteins and pathways that are involved in TMJ OA

progression (17-21). One study showed that the injection of monosodium iodoacetate (MIA) into the TMJ of rats led to a large increase in the expression of ADAMTS-5 mRNA (aggrecanases), MMP3, MMP13, tumor necrosis factor alpha (TNF- α), Caspase 3/8, and proliferating cell nuclear antigen (PCNA), and to a decrease in the expression of mRNA of aggrecan, collagen I, collagen II, and tissue inhibitors of matrix metalloproteinases (TIMP 2). The increase in Caspase 3/8 (chondrocyte apoptosis) and patterns of gene expression were consistent with histological and radiological features characteristic of TMJ OA (22). Li et al. (2014) have shown that injecting collagenase into the rat joints produced mild damage to the TMJ cartilage, by significantly increasing the expression of ADAMTS-5 and ADAMTS-5mRNA and significantly decreasing the expression of TIMP-3 mRNA. Therefore, both the imbalance in protein and mRNA expression of aggrecanase and inhibitors of metalloproteinases have been shown to play an important role in the initial stage of condylar cartilage degradation (19). Although the injection of chemicals proved to induce TMJ OA in rats and rabbits, there is no report in the literature of injection- induced TMJ OA in mice.

Cartilage homeostasis is the result of the proper balance and interaction of the ECM elements. Several human chondrodysplasias are the result of mutations that alter the ECM composition, leading to cartilage degeneration and OA. Transgenic mouse models with mutations in collagen type II, IX and XI (Col2a1 (23, 24), Col9a1, Col11a1 (25)), biglycan and fibromodulin (Bgn^{-/-} Fmo^{-/-}) (26-28), and Ddr-1^{-/-}(29) genes develop TMJ OA via the activation of several signaling pathways that lead to the up-regulation of catalytic proteases. Thus, alteration of the ECM composition due to genetic mutations disrupts the homeostasis of the cartilage, which activates several mechanisms that promote the cartilage destruction. Jiao et al. (2014) showed that the over-expression of transforming growth factor beta 1 (Tgf β -1) in

subchondral bone also induces TMJ OA (30). Their findings support the theory that subchondral bone homeostasis plays an important role in OA development in the TMJ. Therefore, OA in TMJ is consequence of direct or indirect alterations of ECM elements.

The study of the TMJ OA in animal models has shown the complexity of this incurable disease that affects many people. Further research will help to elucidate mechanisms and molecules involved in disease progression. This knowledge may lead to the development of therapies for the prevention and cure of OA.

References

1. Stegenga B, de Bont LG, Boering G. Osteoarthritis as the cause of craniomandibular pain and dysfunction: a unifying concept. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons*. 1989 Mar;47(3):249-56. PubMed PMID: 2646405. Epub 1989/03/01. eng.
2. Israel HA, Saed-Nejad F, Ratcliffe A. Early diagnosis of osteoarthritis of the temporomandibular joint: correlation between arthroscopic diagnosis and keratan sulfate levels in the synovial fluid. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons*. 1991 Jul;49(7):708-11; discussion 12. PubMed PMID: 2056369. Epub 1991/07/01. eng.
3. Fernandez-Criado C, Martos-Rodriguez A, Santos-Alvarez I, Garcia-Ruiz JP, Delgado-Baeza E. The fate of chondrocyte in osteoarthritic cartilage of transgenic mice expressing bovine GH. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2004 Jul;12(7):543-51. PubMed PMID: 15219569. Epub 2004/06/29. eng.
4. Jiao K, Wang MQ, Niu LN, Dai J, Yu SB, Liu XD, et al. Death and proliferation of chondrocytes in the degraded mandibular condylar cartilage of rats induced by experimentally created disordered occlusion. *Apoptosis : an international journal on programmed cell death*. 2009 Jan;14(1):22-30. PubMed PMID: 19052875. Epub 2008/12/05. eng.
5. Liu YD, Liao LF, Zhang HY, Lu L, Jiao K, Zhang M, et al. Reducing dietary loading decreases mouse temporomandibular joint degradation induced by anterior crossbite prosthesis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2013 Dec 5. PubMed PMID: 24316289. Epub 2013/12/10. Eng.
6. Fujisawa T, Kuboki T, Kasai T, Sonoyama W, Kojima S, Uehara J, et al. A repetitive, steady mouth opening induced an osteoarthritis-like lesion in the rabbit temporomandibular joint. *Journal of dental research*. 2003 Sep;82(9):731-5. PubMed PMID: 12939359. Epub 2003/08/27. eng.
7. Kawai N, Tanaka E, Langenbach GE, van Wessel T, Sano R, van Eijden TM, et al. Jaw-muscle activity changes after the induction of osteoarthritis in the temporomandibular joint by mechanical loading. *Journal of orofacial pain*. 2008 Spring;22(2):153-62. PubMed PMID: 18548845. Epub 2008/06/14. eng.
8. Luder HU, Leblond CP, von der Mark K. Cellular stages in cartilage formation as revealed by morphometry, radioautography and type II collagen immunostaining of the mandibular condyle from weanling rats. *The American journal of anatomy*. 1988 Jul;182(3):197-214. PubMed PMID: 3213819. Epub 1988/07/01. eng.
9. Sobue T, Yeh WC, Chhibber A, Utreja A, Diaz-Doran V, Adams D, et al. Murine TMJ loading causes increased proliferation and chondrocyte maturation. *Journal of dental research*. 2011 Apr;90(4):512-6. PubMed PMID: 21248355. Pubmed Central PMCID: PMC3065547. Epub 2011/01/21. eng.
10. Ying B, Chen K, Hu J, Man C, Feng G, Zhang B, et al. Effect of different doses of transforming growth factor-beta(1) on cartilage and subchondral bone in osteoarthritic temporomandibular joints. *The British journal of oral & maxillofacial surgery*. 2013 Apr;51(3):241-6. PubMed PMID: 22763343. Epub 2012/07/06. eng.
11. Salazar A, Polur I, Servais JM, Li Y, Xu L. Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular

- joints of osteoarthritic mouse models. *Journal of Oral Pathology & Medicine*. 2013:n/a-n/a.
12. Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2009 Jul;17(7):917-22. PubMed PMID: 19230720. Pubmed Central PMCID: 2941347.
 13. Ishimaru J, Handa Y, Kurita K, Goss AN. The effect of occlusal loss on normal and pathological temporomandibular joints: an animal study. *Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery*. 1994 Apr;22(2):95-102. PubMed PMID: 8021325. Epub 1994/04/01. eng.
 14. Axelsson S, Holmlund A, Hjerpe A. An experimental model of osteoarthrosis in the temporomandibular joint of the rabbit. *Acta odontologica Scandinavica*. 1992 Oct;50(5):273-80. PubMed PMID: 1441931. Epub 1992/10/01. eng.
 15. Lekkas C, Honee GL, van den Hooff A. Effects of experimental defects of the articular disc of the temporomandibular joint in rats. *J Oral Rehabil*. 1988 Mar;15(2):141-8. PubMed PMID: 3163731. Epub 1988/03/01. eng.
 16. Polur I, Lee PL, Servais JM, Xu L, Li Y. Role of HTRA1, a serine protease, in the progression of articular cartilage degeneration. *Histol Histopathol*. 2010 May;25(5):599-608. PubMed PMID: 20238298. Pubmed Central PMCID: 2894561. Epub 2010/03/20. eng.
 17. Grossin L, Cournil-Henrionnet C, Pinzano A, Gaborit N, Dumas D, Etienne S, et al. Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis. *Faseb J*. 2006 Jan;20(1):65-75. PubMed PMID: 16394269. Epub 2006/01/06. eng.
 18. Imada M, Tanimoto K, Ohno S, Sasaki A, Sugiyama H, Tanne K. Changes in urinary bone resorption markers (pyridinoline, deoxypyridinoline) resulting from experimentally-induced osteoarthritis in the temporomandibular joint of rats. *Cranio*. 2003 Jan;21(1):38-45. PubMed PMID: 12555930. Epub 2003/01/31. eng.
 19. Li W, Wu M, Jiang S, Ding W, Luo Q, Shi J. Expression of ADAMTs-5 and TIMP-3 in the condylar cartilage of rats induced by experimentally created osteoarthritis. *Archives of oral biology*. 2014 May;59(5):524-9. PubMed PMID: 24632095. Epub 2014/03/19. eng.
 20. Harvey VL, Dickenson AH. Behavioural and electrophysiological characterisation of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice. *Molecular pain*. 2009;5:18. PubMed PMID: 19379487. Pubmed Central PMCID: PMC2678995. Epub 2009/04/22. eng.
 21. Wang ZY, Xu LS, Gao J, Liu JZ, Zhang HJ, Liu Q, et al. An efficient chemoenzymatic method to prepare optically active O-methyl-L-serine. *Tetrahedron-Asymmetr*. 2012 Dec 31;23(24):1653-6. PubMed PMID: WOS:000312607200006. English.
 22. He W, Liu YJ, Wang ZG, Guo ZK, Wang MX, Wang N. Enhancement of meniscal repair in the avascular zone using connective tissue growth factor in a rabbit model. *Chinese medical journal*. 2011 Dec;124(23):3968-75. PubMed PMID: 22340326. Epub 2012/02/22. eng.
 23. Ricks ML, Farrell JT, Falk DJ, Holt DW, Rees M, Carr J, et al. Osteoarthritis in temporomandibular joint of Col2a1 mutant mice. *Archives of oral biology*. 2013

- Sep;58(9):1092-9. PubMed PMID: 23518238. Pubmed Central PMCID: PMC3716833. Epub 2013/03/23. eng.
24. Holt DW, Henderson ML, Stockdale CE, Farrell JT, Kooyman DL, Bridgewater LC, et al. Osteoarthritis-like changes in the heterozygous sedc mouse associated with the HtrA1-Ddr2-Mmp-13 degradative pathway: a new model of osteoarthritis. *Osteoarthr Cartilage*. 2012 May;20(5):430-9. PubMed PMID: WOS:000303297100013. English.
 25. Lam NP, Li Y, Waldman AB, Brussiau J, Lee PL, Olsen BR, et al. Age-dependent increase of discoidin domain receptor 2 and matrix metalloproteinase 13 expression in temporomandibular joint cartilage of type IX and type XI collagen-deficient mice. *Archives of oral biology*. 2007 Jun;52(6):579-84. PubMed PMID: 17125729. Pubmed Central PMCID: PMC3825249. Epub 2006/11/28. eng.
 26. Embree MC, Kilts TM, Ono M, Inkson CA, Syed-Picard F, Karsdal MA, et al. Biglycan and Fibromodulin Have Essential Roles in Regulating Chondrogenesis and Extracellular Matrix Turnover in Temporomandibular Joint Osteoarthritis. *Am J Pathol*. 2010 Feb;176(2):812-26. PubMed PMID: WOS:000274111400030. English.
 27. Chen J, Gupta T, Barasz JA, Kalajzic Z, Yeh WC, Drissi H, et al. Analysis of microarchitectural changes in a mouse temporomandibular joint osteoarthritis model. *Archives of oral biology*. 2009 Dec;54(12):1091-8. PubMed PMID: 19896116. Pubmed Central PMCID: 2787630.
 28. Wadhwa S, Embree MC, Kilts T, Young MF, Ameye LG. Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2005 Sep;13(9):817-27. PubMed PMID: 16006154.
 29. Schminke B, Muhammad H, Bode C, Sadowski B, Gerter R, Gersdorff N, et al. A discoidin domain receptor 1 knock-out mouse as a novel model for osteoarthritis of the temporomandibular joint. *Cellular and molecular life sciences : CMLS*. 2013 Aug 4. PubMed PMID: 23912900. Epub 2013/08/06. Eng.
 30. Jiao K, Zhang M, Niu L, Yu S, Zhen G, Xian L, et al. Overexpressed TGF-beta in Subchondral Bone Leads to Mandibular Condyle Degradation. *Journal of dental research*. 2014 Feb;93(2):140-7. PubMed PMID: 24309371. Epub 2013/12/07. eng.

CHAPTER 2: A Novel Mouse Model of Temporomandibular Joint Osteoarthritis

E.M. Chavez M.^a, D.K. Mecham^a, C.S. Black^a, J.W. Graf^a, S.K. Wilhelm^a, K.M. Andersen^a, J.A. Mitchell^a, J.R. Macdonald^a, E.S. Finlay^a, P.R. Reynolds^a, D.L. Kooyman^a

^a Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT 84602, USA

Abstract

Objective: Mice present advantages as models to study Osteoarthritis (OA) etiology and pathology. The present study introduces a novel mouse temporomandibular joint (TMJ) model that more closely mimics natural OA progression of the TMJ in humans.

Design: A wire was bound to the right maxillary molars of mice in order to induce a malocclusion, which leads to development of temporomandibular joint dysfunction (TMD) and associated OA. Tissues were histologically analyzed for osteoarthritic characteristics, scored using a standard Modified Mankin system, and compared with an age matched control group.

Results: Experimental mice demonstrated more surface abrasions, hypercellularity and chondrocyte clustering, and decreased proteoglycan staining when compared to control mice. In comparison to controls, summed modified Mankin scores in experimental animals were statistically higher at all time points examined. Confirmatory histological evaluations revealed that experimental mice displayed early onset of OA.

Conclusions: Bonding a wire to induce a malocclusion in mice is an effective inexpensive, novel method to produce an OA model that can be used in future studies to elucidate disease pathogenesis and treatment.

Keywords: temporomandibular joint, murine, osteoarthritis

Introduction

Osteoarthritis (OA) is one of the most common chronic diseases characterized by joint pain, effusion, loss of mobility, and deformity that progresses to functional joint failure. To date there is no treatment to slow or stop its progression. Consequently, OA has become the most common cause of long term disability or physical impairment. It is now considered a major life-altering disorder, and its prevalence in the general population is statistically comparable to major end-stage kidney disease and heart failure. Epidemiological studies shows that over 20 million Americans are affected by OA and that over 500,000 joint replacements are performed annually in United States (1). It has also been reported that more than 80% of people older than 65 years are symptomatic for OA (2-4) and that its incidence is progressively increasing in the middle-aged population (5).

Temporomandibular joint disorder (TMD) is a term encompassing a number of pathological conditions that are primarily caused by the disruption of the dental occlusion. This disorder affects the masticatory musculature, temporomandibular joint, and/or associated structures (6). According to the NIH, approximately 10 million Americans suffer from TMD (7). Symptoms of TMD include severe pain in the soft and/or hard tissues, asymmetrical and/or limited movement of the jaw, joint sounds (clicking), muscular stiffness, and displacement or perforation of the condylar disc. Though symptoms are different for each patient, TMD and the associated malocclusion ultimately result in the development of OA in the TMJ (TMJ OA).

OA is a metabolically active process, where the homeostasis between synthesis and degradation of several extra cellular matrix (ECM) cartilage components is irreversibly disrupted, leading to a loss of cartilage integrity. Despite extensive research, the pathogenesis of the disease is still poorly understood, making effective universal treatment non-existent. These

unknowns necessitate the use of various animal models that induce TMJ OA in order to better understand the biomolecular progression of the disease. Methods currently used to induce TMJ OA include spontaneous, surgical, drug-induced, and mechanical models. Spontaneous models involve the use of genetically altered mice. Researchers have reported TMJ OA in mice with mutations of the *Col2a1* (8, 9), Tgf- β 1(10), DDR-1(11), Fgfr^{3P244R}(13), *Col9a1* and *Coll1a1* (14), biglycan and fibromodulin genes (15-17). Drug induced models often involve the injection of solutions like monosodium iodoacetate (MIA) or collagenases (18) into the joint (19, 20). Invasive surgical models include the removal of part or all of the articular disc (12, 21, 22), and perforation of the disc (23).

While all of these models have the general advantage in that they lead to TMJ OA, there are also disadvantages. For instance, a spontaneous model with a genetic mutation may result in blockage of the endoplasmic reticulum or over-activation of pathways that while leading to OA, do not represent common pathways of induction in humans. In addition, inactivating genes in knockout models may interfere with normal early development of healthy cartilage. For surgically invasive models, contamination of the joint is possible, and use of analgesic drugs after surgeries might interfere with the natural progression of OA. Additionally, current surgical models are overly invasive such that although the animal develops early TMJ OA, they do not mimic the natural onset and progression of the disease in humans. Our goal was to create a mouse model that results in development of TMJ OA in a more natural manner to better mimic the progression of the disease in humans. Consequently, our model can be used in future research as an inexpensive, quick option for animal studies that focus on discovering biomolecular components associated with OA, leading to viable treatment options.

Researchers have previously used several techniques to disrupt the dental occlusion in mice, rats, and rabbits (24-27) to study temporomandibular disease (TMD) (26, 28). Inducing such disruptions in mice can prove difficult, due to their small anatomy and the absence of appropriately sized dental tools. However, we developed tools and a quick (7 min), efficient method to alter the dental occlusion in mice. This novel technique is reversible and consists of bonding a wire to the upper maxillary molars of mice at eight weeks of age. Mice were not given an analgesic and their diets were unaltered, allowing for the malocclusion to naturally induce OA. After histological joint analysis and comparison with a control group of age matched mice, we observed the development of TMJ OA as early as two weeks post- misalignment. This novel OA model allows for the study of the expression of OA at the gross and cellular levels during its onset and development in a manner similar to the natural development of TMJ OA in humans. This new technique could aid greatly in elucidating disease pathogenesis and treatment.

Methods and Materials

Facilities and Animals

The experimental procedure was performed on 8-week-old mice, when mice are considered fully mature. Mice were maintained in the animal care facility of Brigham Young University. All experimental procedures were performed following protocol #12-0801, approved by the Brigham Young University IACUC.

Materials

In each of the mice, we disrupted the dental occlusion by bonding a wire to the maxillary molar teeth, similar to the procedure used by Walker et al. (28). We modified the technique by bonding the wire to the molars indefinitely. In addition, we invented a dental platform (Figure 2.1) that allowed the mouse to be restrained during the procedure and tools that helped to provide

a clear operatory field. One tool, referred to as a dental mandibular separator (Figure 2.2), helped by keeping the mouth open during the entire procedure. Another tool, a cheek retractor (Figure 2.2), helped to separate the cheek from the teeth to allow for proper placement of the wire on the molars. Stainless steel wire (0.012" diameter) was cut into 5 mm sections and folded in half.

Disruption of the Dental Occlusion

Mice were anesthetized with intra-peritoneal anesthetic, that contained 1 ml of Ketamine (100 mg/ml), 0.1 ml of Xylazine (100 mg/ml), and 8.9 ml of sterile water. The dose injected was 0.01 ml per 1 gram of mouse body weight. Ophthalmic ointment was applied prior to and post-wire placement to the mouse eyes. Once on the dental platform the mouth was opened with the mandibular separator and cheek was retracted with the cheek retractor. We put drops of 37.5% phosphoric acid gel (Kerr Gel Etchant, Orange, CA, USA) on the maxillary molars and after 20 seconds, we rinsed them and aspirated simultaneously. We dried the teeth by blowing air, after which we added composite adhesive (OptiBond Solo Plus Kerr, Orange, CA, USA), prior to placing a stainless steel wire. The composite was light cured with a micro dental curing light (quartz-tungsten-halogen curing light with radiance of 500Mw/cm²) for 20 seconds. Finally we applied flowable composite (N'Durance Dimer Flow, Septodont, Louisville, CO, USA) and light cured it for 30 seconds (Figure 2.2). Mice were placed in a cage that had a heater pad until they recovered from the anesthetic. After that, they were returned to their proper cages. They did not receive any analgesic prior to and after the procedure. Mice were weighed before the procedure and every week for the duration of the study. In addition, permanence of the wire was checked every week. A pain scoring rubric was used to assess level of discomfort with the wire bonded to the tooth.

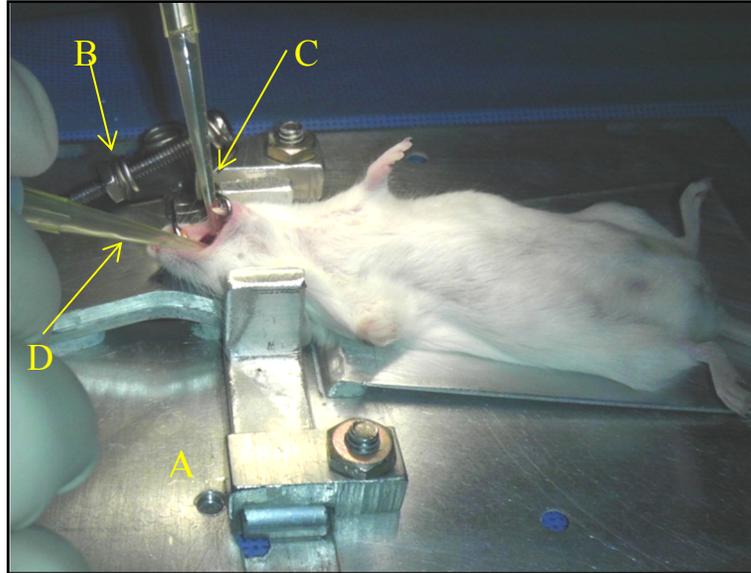


Figure 2.1: Tools Dental Platform.
The dental platform (A) restricts movement and the mandibular separator (B) holds the mouse's mouth open during the entire procedure. Pipet tips connected to the portable dental unit (C, D)

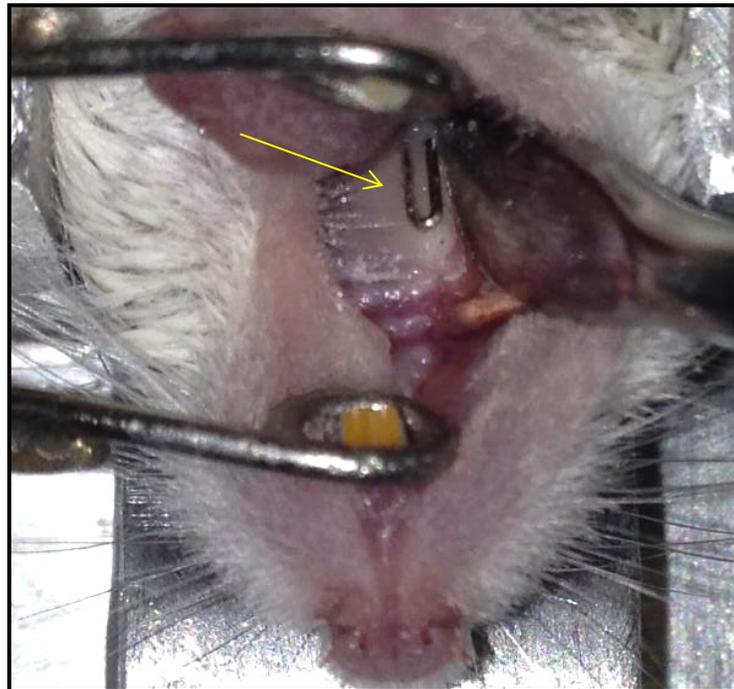


Figure 2.2: Wire Bonded to Teeth.
The wire was bonded to the right upper maxillary molars (A). The murine mandibular separator (B) and cheek retractor (C) were used.

Tissue Harvesting and Processing

Mice 2, 4, 6, and 8 weeks post placement of wire were euthanized using CO₂ and heads were excised and fixed in 4% paraformaldehyde overnight. Each sample was then washed with water every 30 minute over a period of 6 hours. Tissues were decalcified in a decal solution (1:1 ratio of 45% formic acid and 20% sodium citrate), that was changed every 2-3 days for a period of 2-3 weeks. Decalcification of each sample was confirmed through performance of an ammonium oxalate calcium precipitation reaction. They were then dehydrated and embedded in paraffin wax using an automated tissue processor (ThermoFisher Scientific, Waltham, MA, USA). Paraffin blocks were prepared, and TMJs were embedded in paraffin wax with the joint flush with the cutting surface to achieve a frontal cut. TMJ blocks were sectioned at 6 µm through the entire joint from the anterior surface to the posterior part using a Micro HM 325 microtome (Thermo Scientific, Kalamazoo, MI, USA). Four sections were placed per glass microscope slide, yielding approximately 15-20 slides, or approximately 60-80 sections per joint, depending on the animal.

Histological Analysis

Slides from control and experimental mice at each time point were stained with Safranin-O/Fast Green to evaluate the histopathological state of the TMJ. Using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA, USA), photographs of each joint were taken at 10X, 20X, and 40X. The joint cartilage sections were analyzed using a modified Mankin score system (9, 29-32) to quantify the cartilage's pathological state. The modified Mankin scoring system is based on a subset of scores, including cartilage erosion scoring (0-6), chondrocyte periphery staining (0-2), spatial arrangement of chondrocytes (0-3), and background staining intensity (0-3) (33-35). Scores were calculated by

summing the four sub-criteria scores, where zero represented unaltered articular cartilage and 14 represented severe OA.

Statistical Analysis

Statistical significance of the combined Mankin scores at each time point for experimental and control groups was performed using a two-way analysis of variance test (ANOVA) conducted by the department of statistic at BYU.

Results

Modified Mankin scoring used for the semiquantitative assessment of histological OA showed an overall higher score in experimental mice than of control mice at all times points (Figure 2.3 and 2.4). The experimental mice summed scores increased over time, reaching a peak at 8 weeks (W) post disruption of normal occlusion. There was a statistically significant difference between Mankin scores of the two groups of mice at 2 ($p=0.032$), 4 ($p<.0001$), 6 ($p<.0001$), and 8W ($p<.0001$).

The cartilage erosion scores of experimental group compared with that of the control group were higher at all times points (Figure 2.5). However, there was a statistically significant increase at 4W ($p=0.026$) in experimental when compared with control. Interestingly, scores in experimental and control group showed an increase at 4W, decrease at 6W and increase again at 8W. There was also a statistically significant difference ($p=0.017$) when cartilage erosion in 8W experimental mice were compared to 2W experimental mice.

The chondrocyte periphery staining of the experimental group showed an increase at 2W, its highest value at 4W, and a dramatic drop during the following weeks (Figure 2.6). These scores, with the exception of 6W scores, were always higher in mice after placement of wire when compared to control mice. There was a statistically significant increase in chondrocyte

periphery staining at 4W ($p=0.0007$) in the experimental group compared with that of the control group.

Spatial arrangement: Scores for spatial arrangement of chondrocytes in the experimental group increased over time and were always higher than that of the control group (Figure 2.7). These values were significantly higher at 4 ($p=0.0005$), 6 ($p<.0001$), and 8 ($p<.0001$) weeks in mice after placement of wire when compared with the control group. The overall scores for the control groups were lower at all time points.

Background staining scores in mice after the wire was bonded were similar in experimental mice at 2 and 4W. However, they increased in the following weeks, reaching a peak value at 8W. Control group scores reached their peaks at week 4, decreased at week 6, and increased again at week 8. Experimental scores, with the exception of scores at 4W, were higher when compared with that of control scores. However, these differences were not statistically significant. There was a statistically significant difference between scores at 2W in the control and experimental groups when compared with 8 week scores of the experimental group ($p=0.0017$, $p=0.0022$) (Figure 2.8).

Mice in our study showed a decrease in total body weight by the first week after misalignment of the TMJ. However, they recovered and increased in weight to normal levels (36) during the following weeks (Figure 2.9). This showed that mice are capable of adapting to the induced malocclusion. Even though mice were developing OA in the TMJ they did not appear to suffer any pain or change dietary habits.

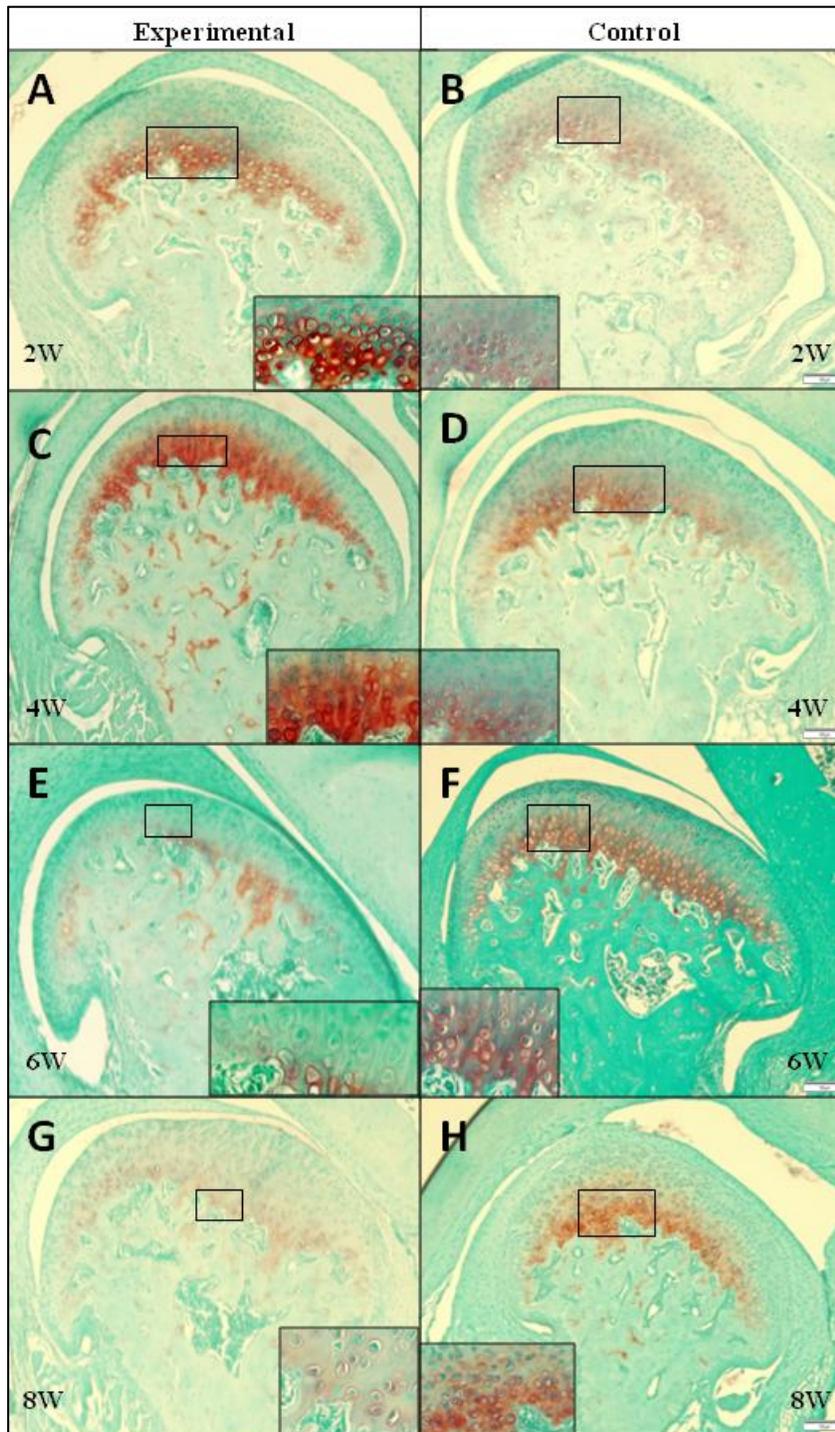


Figure 2.3: Safranin O/ Fast Green Staining.

Proteoglycan expression observed by SO staining in experimental and control mice. An increase in proteoglycan expression was observed in experimental mice at 4W (week) (C) as compared with control mice (D). However, proteoglycan expression decreased at 6W (E) and 8W (G) in experimental mice, as compared with control mice at 6W (F) and 8W (H). At 4W hypercellularity is accompanied with the appearance of clusters in experimental mice (C), features that are missing in control mice of the same age group (D). However, hypocellularity was observed in 8W experimental mice (G) when compared with 8W control mice (H).

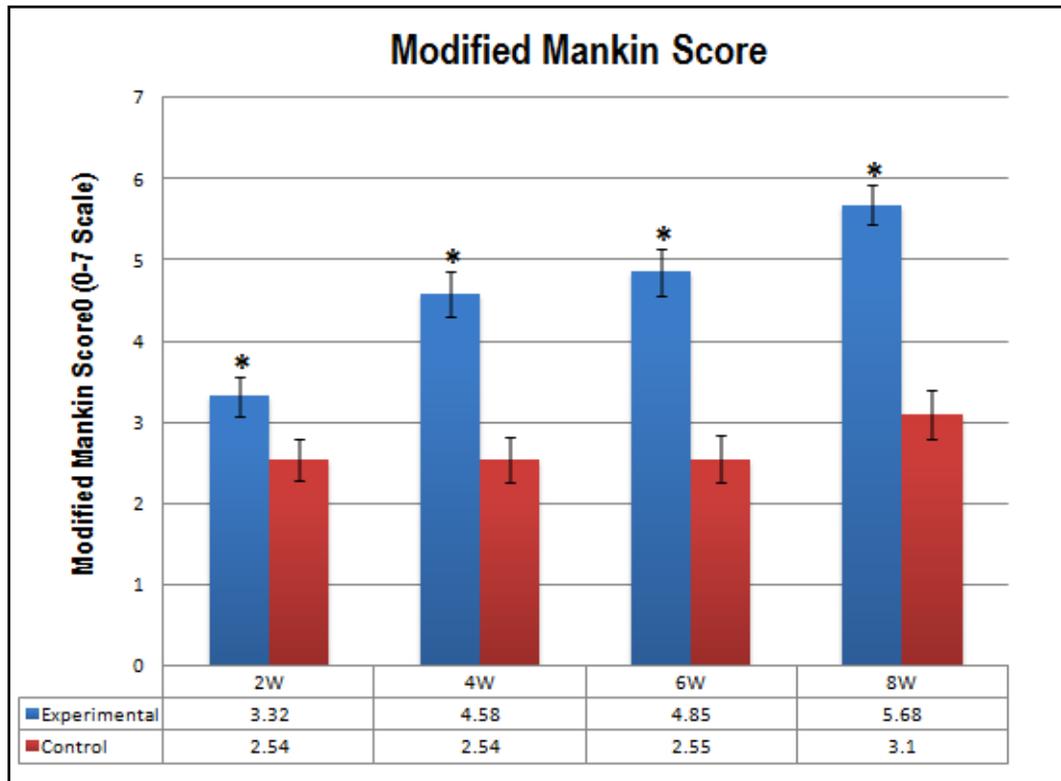


Figure 2.4: Modified Mankin Score.

Modified Mankin score shows an overall higher score in experimental group when compared with that of control mice at all time points. These differences are statistically significant at 2W ($p=0.032$), 4W ($p<0.0001$), 6W ($p<0.0001$), and 8W ($p<0.0001$).

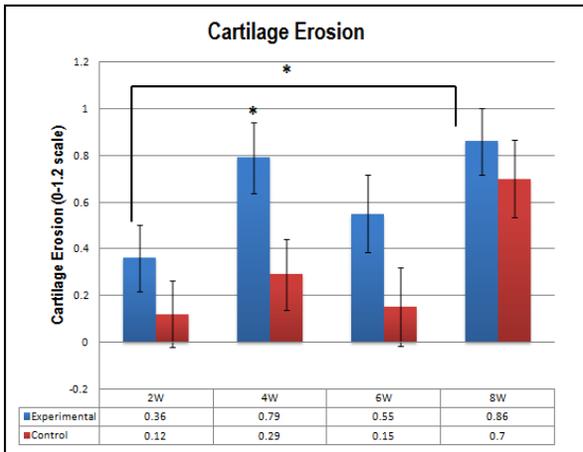


Figure 2.5: Cartilage Erosion. Cartilage erosion scores demonstrated a significant increase in the experimental group when compared with that of the control group at four weeks ($p=0.026$), and between 2W and 8W experimental mice ($p=0.017$). Increased erosion scores in experimental mice are indicative of cartilage fibrillations, OA.

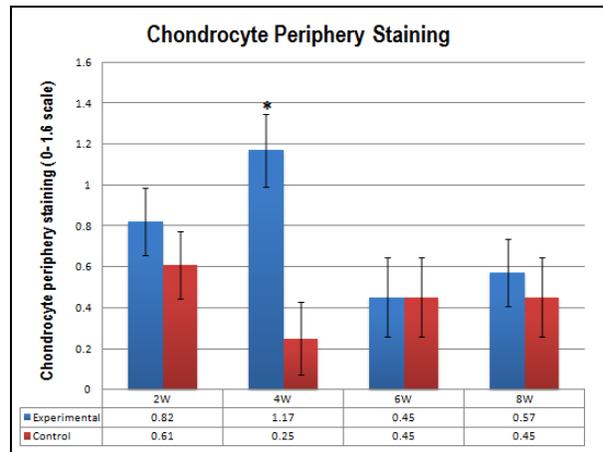


Figure 2.6: Chondrocyte Periphery Staining. There is a significant difference in the chondrocyte periphery staining scores of 4W experimental group ($p=0.0007$) when compared with control. This indicates that stressed chondrocytes of experimental mice by altered occlusion increased proteoglycan expression.

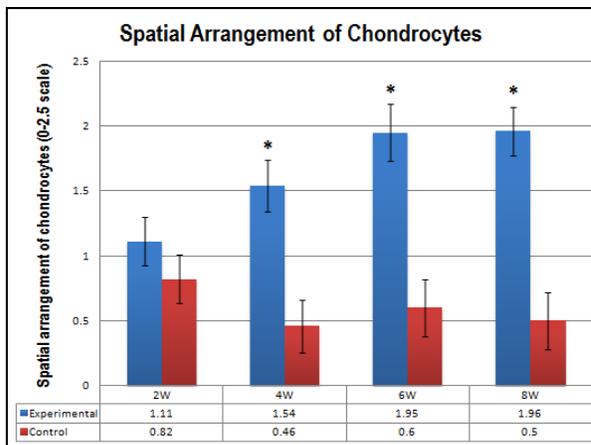


Figure 2.7: Spatial Arrangement of Chondrocytes. Spatial arrangement scores are statistically different between 4 ($p=0.0005$), 6 ($p<0.0001$) and 8 ($p<0.0001$) groups of mice. This difference is caused by increased hyperplasia and clustering of chondrocytes in experimental mice. These changes worsen over time and are characteristic of osteoarthritic cartilage.

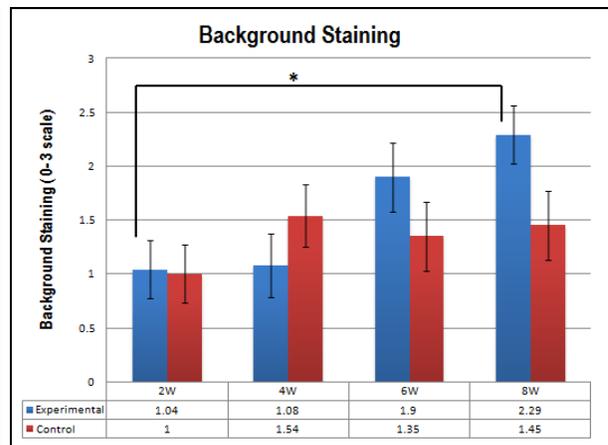


Figure 2.8: Background Staining. There is a significant increase in the background staining scores of 2W and 8W experimental groups of mice ($p=0.0017$). This increase is caused by the development of OA and associated decrease in proteoglycan expression and chondrocyte quantity.

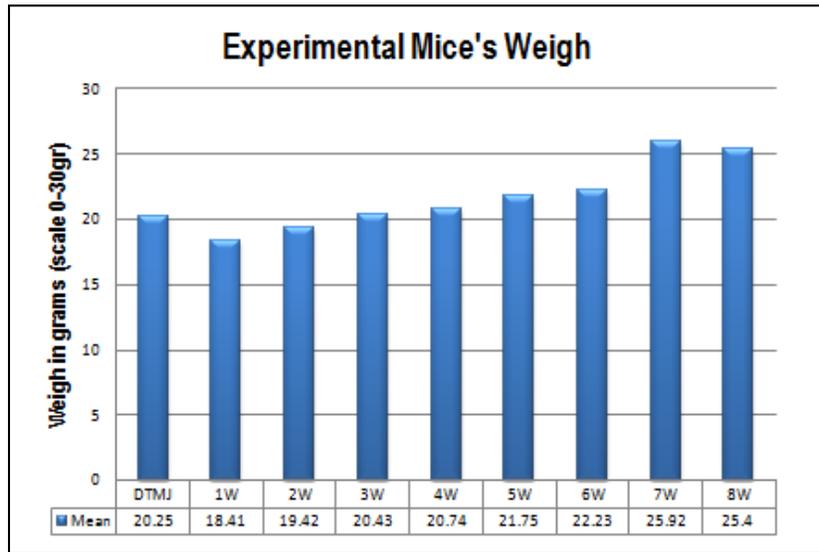


Figure 2.9: Mice Weight.
 Experimental mice showed a decrease in weight after alteration of the dental occlusion and then a gradual increase in the following weeks.

Table 2.1: Modified Mankin Score Table.

| | |
|--|---|
| Cartilage Erosion Scoring | |
| Smooth non-eroded Cartilage | 0 |
| Rough non-eroded Cartilage | 1 |
| Superficial fibrillation | 2 |
| Separation of uncalcified from calcified cartilage | 3 |
| Erosion of uncalcified cartilage only | 4 |
| Erosion extending into calcified cartilage | 5 |
| Erosion down to subchondral bone | 6 |
| Chondrocyte periphery staining | |
| Normal | 0 |
| Slightly enhanced | 1 |
| Intensely enhanced | 2 |
| Spatial arrangement of chondrocytes | |
| Normal | 0 |
| Diffuse hypercellularity | 1 |
| Clustering | 2 |
| Hypocellularity | 3 |
| Background staining intensity | |
| Normal | 0 |
| Slight reduction | 1 |
| Moderate reduction | 2 |
| Severe reduction | 3 |
| No dye noted | 4 |

Discussion

In this study, we analyzed the tissue to determine whether our model induced the development of TMJ OA. The histology of the condylar cartilage consists of the articular proliferative, chondroblastic, and hypertrophic layers. Disruptions of cartilage homeostasis are reflected in changes in the cartilage surface, cellular, and extracellular arrangement.

In our study the superficial cartilage in the control group after 2W was smooth compared with that of the experimental group at 8W, where the surface was rough and superficial fibrillations were present (Figure 2.3G). The cartilage erosion score showed an interesting pattern of behavior such that erosion increased at 4W, decreased at 6W, and increased again at 8W in the experimental and control groups (Figure 2.5). We hypothesize that cartilage repair mechanisms were elevated after initial damage to the cartilage surface at 4W. This repair process is reflected in the decrease of the surface erosion score at 6 weeks. However, the homeostatic imbalance caused by the malocclusion prevents long term repair, resulting in increased surface erosion that is typical of OA at 8W in experimental mice. Knee cartilage is articular hyaline cartilage consisting solely of Type II collagen whereas the cartilage of the TMJ is condylar fibrocartilage, composed of Type I and Type II collagen (37, 38). It has been suggested that condylar cartilage is unique in that it has the ability to repair itself, unlike cartilage of the knee (39). This may explain the sudden drop in severity of surface erosion in the group of 6 week experimental mice, but this phenomenon needs to be explored further in future studies.

Even though the cartilage of experimental mice showed surface fibrillation (Figure 2.3G), we did not see any erosion or separation of uncalcified cartilage and erosion extending to the calcified region or subchondral bone. Our study only monitored mice until 8W post malocclusion, at which point experimental mice demonstrated surface abrasions, markedly

decreased proteoglycan levels, and hypocellularity with clustering of chondrocytes (Figure 2.3E, and G). These factors are typical of osteoarthritic tissues and imply that given more time, more dramatic destruction of cartilage surface would occur if monitored to a further time point (40).

Mature chondrocytes reside in the chondroblastic layer (41, 42) and they actively produce collagen type I, II, proteoglycans, and various other ECM components (38, 43). In our study, Safranin –O staining showed a strong staining for proteoglycans in the chondroblastic and hypertrophic layer at 2W (Figure 2.3A and C), which reached its maximum value at 4W in the experimental group of mice (Figure 2.3C and Figure 2.6). This increase in proteoglycan concentration shows hyperactivity in stressed chondrocytes after inducing the malocclusion in our model and is compatible with early onset of OA (9, 44, 45). In the following weeks, the proteoglycan expression decreased dramatically in the experimental group (Figure 2. 3E and G, and Figure 2.6) showing a failed attempt of self-repair and chondrocyte death. The decrease of proteoglycans means that catabolic processes are taking place, causing the cartilage to be unable to repair itself. These features are characteristic of osteoarthritic cartilage shown in our mouse model.

Experimental mice scores in comparison with that of the control mice showed a statistically significant increase for spatial arrangements of chondrocytes at 4, 6, and 8W (Figure 2.7). In response to stress, chondrocytes proliferate and increase in number as an attempt to self-repair and to fix the homeostatic imbalance associated with OA (46). This may explain why tissues at 2W in the experimental group show chondrocyte hyperplasia in comparison to 2W control mouse tissues (Figure 2.3A and B). At 4 weeks, there is a considerable increase in clusters in the proliferative and hypertrophic layers of the experimental group when compared with that of the control group (Figure 2.3C and D). However, at 6 and 8W there is a dramatic

decrease of chondrocytes in the hypertrophic, chondroblastic, and proliferative layers of the experimental mice (Figure 2.3E and G). These cellular changes are compatible with the progression of osteoarthritis that leads to cartilage atrophy (9, 17).

The background staining decreases at 8W in experimental group due to the lack of chondrocytes (Figure 2.3G) and likely increase in catabolic factors, demonstrating dramatically reduced proteoglycan expression in the cartilage. These changes are compatible with the development of TMJ OA in other mice models (8, 17). There is a significant statistical difference in the background staining score between 2 and 8W experimental mice (Figure 2.8). This shows that mouse cartilage, after 8W of malocclusion, develops OA-like changes.

Our hypothesis that the disruption of the occlusion would lead to OA in the TMJ was successfully demonstrated, as shown by histological and statistical analysis. This study introduces a novel TMJ OA mouse model that involves an inexpensive, quick, and replicable procedure. The mechanical nature of the malocclusion resembles the natural development of OA in humans, making this an ideal model in future studies that aim to elucidate the pathogenesis of TMJ OA and discover a treatment.

Authors' Contributions

DLK, EMCM designed the experiment. EMCM designed the mouse model, tools and perform all experimental procedures. PRR provided the mice. DLK, EMCM, PRR and DKM contributed to writing the manuscript. All the authors assisted in carrying out experimental procedures and interpreting the results.

Funding

This work was supported by a Mentoring Environments Grant from the Brigham Young University Office of Research and Creative Activities.

Competing Interests

No author had competing or conflicting interests during the duration of this study.

Acknowledgments

The authors thank Dr. Dennis Eggett (Department of Statistics, Brigham Young University) for conducting statistical analyses, Dr. Craig Hollis for kindly donating a curing light.

References

1. Abramson S, Krasnokutsky S. Biomarkers in osteoarthritis. *Bulletin of the NYU hospital for joint diseases*. 2006;64(1-2):77-81. PubMed PMID: 17121495. Epub 2006/11/24. eng.
2. Oka K, Oka S, Hosokawa R, Bringas P, Jr., Brockhoff HC, 2nd, Nonaka K, et al. TGF-beta mediated Dlx5 signaling plays a crucial role in osteo-chondroprogenitor cell lineage determination during mandible development. *Developmental biology*. 2008 Sep 15;321(2):303-9. PubMed PMID: 18684439. Pubmed Central PMCID: PMC3378386. Epub 2008/08/08. eng.
3. Kaushik AP, Martin JA, Zhang Q, Sheffield VC, Morcuende JA. Cartilage abnormalities associated with defects of chondrocytic primary cilia in Bardet-Biedl syndrome mutant mice. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2009 Aug;27(8):1093-9. PubMed PMID: 19195025. Epub 2009/02/06. eng.
4. Chien J, Ota T, Aletti G, Shridhar R, Boccellino M, Quagliuolo L, et al. Serine protease HtrA1 associates with microtubules and inhibits cell migration. *Mol Cell Biol*. 2009 Aug;29(15):4177-87. PubMed PMID: 19470753. Pubmed Central PMCID: 2715801. Epub 2009/05/28. eng.
5. Mobasheri A. Osteoarthritis year 2012 in review: biomarkers. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2012 Dec;20(12):1451-64. PubMed PMID: 22842200. Epub 2012/07/31. eng.
6. Slavkin. A lifetime of motion: Temporomandibular joints. *J Am Dent Assoc*. 1996 Jul;127(7):1093-8. PubMed PMID: WOS:A1996UX17500028. English.
7. National Institute of Dental and Craniofacial Research. *TMJ Disorders Maryland 2013* [updated January 06, 2014; cited 2014 February 22, 2014]. NIH Publication 13-3487:[Available from: <http://nidcr.nih.gov/OralHealth/Topics/TMJ/TMJDisorders.htm>.
8. Ricks ML, Farrell JT, Falk DJ, Holt DW, Rees M, Carr J, et al. Osteoarthritis in temporomandibular joint of Col2a1 mutant mice. *Archives of oral biology*. 2013 Sep;58(9):1092-9. PubMed PMID: 23518238. Pubmed Central PMCID: PMC3716833. Epub 2013/03/23. eng.
9. Holt DW, Henderson ML, Stockdale CE, Farrell JT, Kooyman DL, Bridgewater LC, et al. Osteoarthritis-like changes in the heterozygous sedc mouse associated with the HtrA1-Ddr2-Mmp-13 degradative pathway: a new model of osteoarthritis. *Osteoarthr Cartilage*. 2012 May;20(5):430-9. PubMed PMID: 22155431. Epub 2011/12/14. eng.
10. Jiao K, Zhang M, Niu L, Yu S, Zhen G, Xian L, et al. Overexpressed TGF-beta in Subchondral Bone Leads to Mandibular Condyle Degradation. *Journal of dental research*. 2014 Feb;93(2):140-7. PubMed PMID: 24309371. Epub 2013/12/07. eng.
11. Schminke B, Muhammad H, Bode C, Sadowski B, Gerter R, Gersdorff N, et al. A discoidin domain receptor 1 knock-out mouse as a novel model for osteoarthritis of the temporomandibular joint. *Cellular and molecular life sciences : CMLS*. 2013 Aug 4. PubMed PMID: 23912900. Epub 2013/08/06. Eng.
12. Salazar A, Polur I, Servais JM, Li Y, Xu L. Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular joints of osteoarthritic mouse models. *Journal of Oral Pathology & Medicine*. 2013:n/a-n/a.

13. Yasuda T, Nah HD, Laurita J, Kinumatsu T, Shibukawa Y, Shibutani T, et al. Muenke syndrome mutation, FgfR3P(2)(4)(4)R, causes TMJ defects. *Journal of dental research*. 2012 Jul;91(7):683-9. PubMed PMID: 22622662. Pubmed Central PMCID: 3383850.
14. Lam NP, Li Y, Waldman AB, Brussiau J, Lee PL, Olsen BR, et al. Age-dependent increase of discoidin domain receptor 2 and matrix metalloproteinase 13 expression in temporomandibular joint cartilage of type IX and type XI collagen-deficient mice. *Archives of oral biology*. 2007 Jun;52(6):579-84. PubMed PMID: 17125729. Pubmed Central PMCID: PMC3825249. Epub 2006/11/28. eng.
15. Embree MC, Kilts TM, Ono M, Inkson CA, Syed-Picard F, Karsdal MA, et al. Biglycan and Fibromodulin Have Essential Roles in Regulating Chondrogenesis and Extracellular Matrix Turnover in Temporomandibular Joint Osteoarthritis. *Am J Pathol*. 2010 Feb;176(2):812-26. PubMed PMID: WOS:000274111400030. English.
16. Chen J, Gupta T, Barasz JA, Kalajzic Z, Yeh WC, Drissi H, et al. Analysis of microarchitectural changes in a mouse temporomandibular joint osteoarthritis model. *Archives of oral biology*. 2009 Dec;54(12):1091-8. PubMed PMID: 19896116. Pubmed Central PMCID: 2787630.
17. Wadhwa S, Embree MC, Kilts T, Young MF, Ameye LG. Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2005 Sep;13(9):817-27. PubMed PMID: 16006154.
18. Imada M, Tanimoto K, Ohno S, Sasaki A, Sugiyama H, Tanne K. Changes in urinary bone resorption markers (pyridinoline, deoxypyridinoline) resulting from experimentally-induced osteoarthritis in the temporomandibular joint of rats. *Cranio*. 2003 Jan;21(1):38-45. PubMed PMID: 12555930. Epub 2003/01/31. eng.
19. Harvey VL, Dickenson AH. Behavioural and electrophysiological characterisation of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice. *Molecular pain*. 2009;5:18. PubMed PMID: 19379487. Pubmed Central PMCID: PMC2678995. Epub 2009/04/22. eng.
20. Wang XD, Kou XX, He DQ, Zeng MM, Meng Z, Bi RY, et al. Progression of cartilage degradation, bone resorption and pain in rat temporomandibular joint osteoarthritis induced by injection of iodoacetate. *Plos One*. 2012;7(9):e45036. PubMed PMID: 22984604. Pubmed Central PMCID: PMC3439407. Epub 2012/09/18. eng.
21. Ying B, Chen K, Hu J, Man C, Feng G, Zhang B, et al. Effect of different doses of transforming growth factor-beta(1) on cartilage and subchondral bone in osteoarthritic temporomandibular joints. *The British journal of oral & maxillofacial surgery*. 2013 Apr;51(3):241-6. PubMed PMID: 22763343. Epub 2012/07/06. eng.
22. Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2009 Jul;17(7):917-22. PubMed PMID: 19230720. Pubmed Central PMCID: 2941347.
23. Ying B, Chen K, Hu J, Man C, Feng G, Zhang B, et al. Effect of different doses of transforming growth factor-beta(1) on cartilage and subchondral bone in osteoarthritic temporomandibular joints. *The British journal of oral & maxillofacial surgery*. 2012 Jul 2. PubMed PMID: 22763343.
24. Sobue T, Yeh WC, Chhibber A, Utreja A, Diaz-Doran V, Adams D, et al. Murine TMJ loading causes increased proliferation and chondrocyte maturation. *Journal of dental*

- research. 2011 Apr;90(4):512-6. PubMed PMID: 21248355. Pubmed Central PMCID: PMC3065547. Epub 2011/01/21. eng.
25. Liu YD, Liao LF, Zhang HY, Lu L, Jiao K, Zhang M, et al. Reducing dietary loading decreases mouse temporomandibular joint degradation induced by anterior crossbite prosthesis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2013 Dec 5. PubMed PMID: 24316289. Epub 2013/12/10. Eng.
 26. Jiao K, Wang MQ, Niu LN, Dai J, Yu SB, Liu XD, et al. Death and proliferation of chondrocytes in the degraded mandibular condylar cartilage of rats induced by experimentally created disordered occlusion. *Apoptosis : an international journal on programmed cell death*. 2009 Jan;14(1):22-30. PubMed PMID: 19052875. Epub 2008/12/05. eng.
 27. Chen J, Sorensen KP, Gupta T, Kilts T, Young M, Wadhwa S. Altered functional loading causes differential effects in the subchondral bone and condylar cartilage in the temporomandibular joint from young mice. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2009 Mar;17(3):354-61. PubMed PMID: 18789726. Pubmed Central PMCID: PMC2646810. Epub 2008/09/16. eng.
 28. Walker CG, Ito Y, Dangaria S, Luan X, Diekwisch TG. RANKL, osteopontin, and osteoclast homeostasis in a hyperocclusion mouse model. *European journal of oral sciences*. 2008 Aug;116(4):312-8. PubMed PMID: 18705798. Pubmed Central PMCID: PMC2597431. Epub 2008/08/19. eng.
 29. Bertram S, Rudisch A, Innerhofer K, Pumpel E, Grubwieser G, Emshoff R. Diagnosing TMJ internal derangement and osteoarthritis with magnetic resonance imaging. *J Am Dent Assoc*. 2001 Jun;132(6):753-61. PubMed PMID: 11433854. Epub 2001/07/04. eng.
 30. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. *Instructional course lectures*. 1998;47:477-86. PubMed PMID: 9571449. Epub 1998/05/08. eng.
 31. Sunk IG, Bobacz K, Hofstaetter JG, Amoyo L, Soleiman A, Smolen J, et al. Increased expression of discoidin domain receptor 2 is linked to the degree of cartilage damage in human knee joints: a potential role in osteoarthritis pathogenesis. *Arthritis and rheumatism*. 2007 Nov;56(11):3685-92. PubMed PMID: 17968949. Epub 2007/10/31. eng.
 32. Lippiello L, Hall D, Mankin HJ. Collagen synthesis in normal and osteoarthritic human cartilage. *The Journal of clinical investigation*. 1977 Apr;59(4):593-600. PubMed PMID: 845251. Pubmed Central PMCID: PMC372262. Epub 1977/04/01. eng.
 33. Bomsta BD, Bridgewater LC, Seegmiller RE. Premature osteoarthritis in the Disproportionate micromelia (Dmm) mouse. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2006 May;14(5):477-85. PubMed PMID: 16431140. Epub 2006/01/25. eng.
 34. Xu L, Flahiff CM, Waldman BA, Wu D, Olsen BR, Setton LA, et al. Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho). *Arthritis and rheumatism*. 2003 Sep;48(9):2509-18. PubMed PMID: 13130470. Epub 2003/09/18. eng.
 35. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *The Journal of bone and joint surgery American volume*. 1971 Apr;53(3):523-37. PubMed PMID: 5580011. Epub 1971/04/01. eng.

36. The Jackson Laboratory. Body weight information Maine: The Jackson Laboratory; 2014 [cited 2014 February 22, 2014]. Available from: <http://jaxmice.jax.org/support/weight/000686.html>.
37. Wadhwa S, Kapila S. TMJ disorders: Future innovations in diagnostics and therapeutics. *J Dent Educ.* 2008 Aug;72(8):930-47. PubMed PMID: WOS:000259183100008. English.
38. Shibukawa Y, Young B, Wu C, Yamada S, Long F, Pacifici M, et al. Temporomandibular joint formation and condyle growth require Indian hedgehog signaling. *Developmental dynamics : an official publication of the American Association of Anatomists.* 2007 Feb;236(2):426-34. PubMed PMID: 17191253.
39. Robinson PD. Articular cartilage of the temporomandibular joint: can it regenerate? *Annals of the Royal College of Surgeons of England.* 1993 Jul;75(4):231-6. PubMed PMID: 8379622. Pubmed Central PMCID: PMC2497923. Epub 1993/07/01. eng.
40. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2010 Oct;18 Suppl 3:S17-23. PubMed PMID: 20864019. Epub 2010/10/01. eng.
41. Rabie AB, Tsai MJ, Hagg U, Du X, Chou BW. The correlation of replicating cells and osteogenesis in the condyle during stepwise advancement. *The Angle orthodontist.* 2003 Aug;73(4):457-65. PubMed PMID: 12940568.
42. Rabie AB, Hagg U. Factors regulating mandibular condylar growth. *American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics.* 2002 Oct;122(4):401-9. PubMed PMID: 12411886. Epub 2002/11/02. eng.
43. Pirttiniemi P, Kantomaa T, Salo L, Tuominen M. Effect of reduced articular function on deposition of type I and type II collagens in the mandibular condylar cartilage of the rat. *Archives of oral biology.* 1996 Jan;41(1):127-31. PubMed PMID: 8833602. Epub 1996/01/01. eng.
44. Adams ME, Matyas JR, Huang D, Dourado GS. Expression of proteoglycans and collagen in the hypertrophic phase of experimental osteoarthritis. *The Journal of rheumatology Supplement.* 1995 Feb;43:94-7. PubMed PMID: 7752150. Epub 1995/02/01. eng.
45. Venn G, Billingham ME, Hardingham TE. Increased proteoglycan synthesis in cartilage in experimental canine osteoarthritis does not reflect a permanent change in chondrocyte phenotype. *Arthritis and rheumatism.* 1995 Apr;38(4):525-32. PubMed PMID: 7718006. Epub 1995/04/01. eng.
46. Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Therapeutic advances in musculoskeletal disease.* 2012 Aug;4(4):269-85. PubMed PMID: 22859926. Pubmed Central PMCID: PMC3403254. Epub 2012/08/04. eng.

CHAPTER 3: Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)

E.M. Chavez M., D.K. Mecham, E.S. Finlay, J.W. Graf, J.A. Mitchell, R. Wood, S.K. Wilhelm, C.S. Black, K.M. Andersen, J.R. Macdonald, R. Wood, P.R. Reynolds, D.L. Kooyman

Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT 84602, USA

Abstract

Objective: Osteoarthritis (OA) in the temporomandibular joint (TMJ) is a chronic degenerative disease that may be the result of sustained chronic inflammation. Recent experiments have shown that the receptor for advanced glycation end products (RAGE) is involved in OA progression. The objective of this research was to study the expression of Mmp-13, HtrA-1, and Tgf- β 1, OA biomarkers in TMJ of mice with and without RAGE expression after induced TMJ misalignment.

Design: We induced a non invasive TMJ misalignment on 8-week-old RAGE knockout (KO) and wild type (WT) mice. We assayed for Mmp-13, HtrA-1, and Tgf- β 1 biomarkers in condylar cartilage at 2 and 8 weeks post-misalignment by immunohistochemistry of the right TMJs.

Results: Immunohistochemistry and histopathological tissue analysis in RAGE KO mice after misalignment showed lower Mmp-13 and HtrA-1 expression, as well as lower summed modified Mankin scores in condylar joints when compared to those of WT.

Conclusions: We conclude that knocking out RAGE protects the TMJ from the typical development of OA by decreasing Mmp-13 and HtrA-1 expression.

Keywords: temporomandibular joint, murine, mice, osteoarthritis, RAGE, Mmp-13, HtrA-1, Tgf- β 1.

Introduction

Osteoarthritis (OA) is a chronic degenerative disease that affects the joints, including the temporomandibular joint (TMJ). Even though OA is associated with several inherited chondrodysplasia syndromes, in most adults it seems to be associated with risk factors such as aging, obesity, repetitive stress, joint misalignment, acute injury, and genetic predisposition. OA results in a painful and progressive degeneration of the articular surface with thinning, fissuring, and ultimate loss of the protective articular surface of the joint.

OA is increasingly viewed as a metabolically, active dynamic process, where destruction and repair occur simultaneously, after being triggered by a variety of biochemical and mechanical insults (1, 2). This dynamic process is carried out by the expression of pro-inflammatory cytokines during OA onset, and its progression explains why OA is currently viewed as an inflammatory disease (3). Unfortunately, the exact inflammatory mechanism that is activated or up-regulated and the exact cytokine/s responsible for OA onset and development are not well understood. Loeser et al. showed that chondrocytes express the receptor for advanced glycation end-products (RAGE), which is up-regulated in OA in humans (4). RAGE, a member of the superfamily of pattern recognition receptors (PRR), is a transmembrane receptor that is composed of three immunoglobulins like domains. V and C domains provide a large cationic surface area that mediates the binding of most RAGE ligands. An additional transmembrane domain that is attached to a highly charged 43 amino acid intra-cytoplasmic domain mediates intracellular signal transduction [17]. RAGE binds to several ligands, such as advanced glycation end-products (AGEs)(5), high-mobility group box-1(HMGB1/amphoterin) (6, 7), several pro-inflammatory cytokine-like mediators of the S100/calgranulin family (8), β amyloid peptides, Mac-1 (9, 10), and specific DNA and RNA structures (7). After binding its ligands, RAGE

mediates gene expression and pro-inflammatory response through the activation of a variety of signal transcriptional pathways, including PI3K/Akt (11), RhoGTPases (12), Jak/STAT (13), Src family kinases (14), MAPKs/Erk1/2, JNK, p38 and IKK/NF- κ B (15). The downstream gene product results of its signal transcription include NF- κ B, Cox-2, pro-inflammatory cytokines TNF- α (16, 17), IL-1 β (8), IL-6(18), IL-8(19), MCP-1(19), INF- α (20), and matrix metalloproteinases, such as Mmp-13 (21). The release of these products is sustained by a positive feedback loop, a feature characteristic of RAGE signaling (22, 23). This is the reason why RAGE expression is up-regulated in chronic inflammatory states (24, 25) such as OA (4), diabetes (26), atherosclerosis, sepsis, rheumatoid arthritis, Alzheimer's Disease (7), lung fibrosis (27), acute respiratory distress syndrome (ARDS), polycystic kidney disease (28), and chronic obstructive pulmonary disease (COPD). In OA, the cartilage deterioration is due to sustained degradation of type II collagen that has been shown to be carried out by expression of Mmp-13 (collagenase 3), a downstream RAGE product that is increased as a result of RAGE activation (29).

We and others have recently demonstrated that knees of osteoarthritic mice show high levels of Mmp-13 and high temperature requirement A serine protease (HtrA-1), indicating their key role in the initiation and progression of OA (30-33). HtrA-1 is a serine protease that is involved in various aspects of protein quality and cell fate. During development, its expression is significantly increased; however, in adult articular cartilage, its expression is not significantly detectable (34). Pathological levels of HtrA-1 have been implicated with the development of several diseases, such as rheumatoid arthritis, OA, cancer, macular degeneration, muscular dystrophy, and aging (35-40). HtrA-1 has three mechanisms of action. First, it hydrolyzes multiple substrates present in the pericellular (PCM) (36) and extracellular matrix (ECM),

including aggrecan, biglycan, fibromodulin, decorin, cartilage oligomeric matrix protein (COMP), (41) and fibronectin (42, 43). Second, it induces expression of metalloproteases (MMPs) (34). Third, it inhibits growth factor family activity (34). Because of its mechanism of action, HtrA-1 expression has been implicated in the onset and progression of OA; patients with OA have been shown to express high levels of HtrA-1 in their synovial fluid and cartilage (35, 44). HtrA-1 has also been shown to be associated with centrosomal modulation of microtubule stability (45). This suggests that its aberrant expression plays a role in cytoskeletal disruption associated with cell death, or at least in pathological processes like OA. Upon destruction of the PCM by HtrA-1, the triple helical type 2 collagen makes contact with exposed discoidin domain receptor 2 (Ddr2) located in the chondrocyte cell membrane, thereby causing further up-regulation of this receptor (36) and subsequent expression of Mmp-13 (46). Thus, in OA, the up-regulation of Mmp-13 as a result of RAGE and HtrA-1 signaling accelerates cartilage degeneration. We and others have show that OA is additionally linked to low levels of an anabolic cytokine, Tgf- β 1(30), which is inhibited by high levels of HtrA-1.

Transforming growth factor (Tgf- β 1) is a multifunctional growth factor that modulates chondrocyte cell differentiation, proliferation, and synthesis of proteoglycans (47-50) and type II collagen (51, 52). Chondrocytes secrete inactive Tgf- β 1 (53), which once activated, triggers a series of reactions that result (54) in sustained cartilage integrity. Normal cartilage expresses moderate levels of Tgf- β 1, as opposed to osteoarthritic cartilage, where Tgf- β 1 expression is almost absent (55). Additionally, high levels of Tgf- β 1 have been show to promote osteophyte formation and synovial fibrotic features of OA (56). The overall effects of Tgf- β 1 counteract the catabolic effects of IL-1 (55), TNF- α , and Mmp-13 by stimulating synthesis of ECM (57), down

regulating the expression of cytokine (IL-1) receptors (51) and Mmp-13 (58, 59), and inhibiting chondrocyte terminal differentiation into osteophytes (60).

We have shown that Tgf- β 1 expression disappears as HtrA-1 expression appears following surgical destabilization of the knee and that these events are associated with an increase in Mankin scores of knee joints (30). Furthermore, we have observed that the expression of HtrA-1 and other catabolic factors are attenuated in a RAGE knockout (KO) mouse following surgical destabilization. These results support the theory that inflammation may provide the trigger for RAGE, HtrA-1 up-regulation, and Tgf- β 1 down regulation, which results in the up-regulation of Mmp-13 and onset of OA. Our lab and others have shown that Tgf- β 1, HtrA-1, and Mmp-13 play a key role in the development of TMJ OA in transgenic and invasive OA mouse models (44, 61).

In our present study, we investigate the interaction of RAGE, HtrA-1, Tgf- β 1, and Mmp-13 activity signaling in TMJ OA. Because these cytokines have been shown to play key roles in the onset and development of OA, we use mice with and without RAGE expression in order to elucidate the receptor's role in this process. Contrary to other experiments, we induce TMJ OA by producing a non-invasive misalignment of the TMJ (manuscript in review). To our knowledge, this is the first study to elucidate the role of RAGE expression in TMJ OA development after induction by a non-invasive trigger. We therefore hypothesize that the absence of RAGE expression will attenuate or prevent the TMJ from developing OA.

Methods and Materials

Facilities and Animals

Balb/C wild type mice (n=56) and RAGE knockout mice (n=56) were kindly provided by Dr. Paul Reynolds at Brigham Young University. The experimental procedure was performed

on 8-week-old mice, when mice are considered fully mature. Mice were maintained in the animal care facility of Brigham Young University. All experimental procedures were performed following protocol #12-0801, approved by the Brigham Young University IACUC.

Materials

In each of the mice, we produced a TMJ misalignment by disrupting the dental occlusion through bonding a wire to the right maxillary molar teeth, similar to the procedure used by Walker et al. (62). We modified the technique by bonding the wire to the molars indefinitely (manuscript in revision).

Tissue Harvesting and Processing

Mice 2, 4, 6, and 8 weeks post placement of wire were euthanized using CO₂ and heads were excised and fixed in 4% paraformaldehyde overnight. Each sample was then washed with water every 30 minute over a period of 6 hours. Tissues were decalcified in a solution (1:1 ratio of 45% formic acid and 20% sodium citrate) that was changed every 2-3 days for a period of 2-3 weeks. Decalcification of each sample was confirmed through performance of an ammonium oxalate calcium precipitation reaction. Tissues were then dehydrated and embedded in paraffin wax using an automated tissue processor (ThermoFisher Scientific, MA). Paraffin blocks were prepared, and TMJs were embedded in paraffin wax with the joint flush with the cutting surface to achieve a frontal cut. TMJ blocks were sectioned at 6 μm through the entire joint from the anterior surface to the posterior part using a Microm HM 325 microtome (Thermo Scientific, Kalamazoo, MI). Four sections were placed per glass microscope slide, yielding approximately 15-20 slides, or approximately 60-80 sections per joint, depending on the animal.

Histological Analysis

Slides from control and experimental mice at each time point were stained with Safranin-O/Fast Green to evaluate the histopathological state of the TMJ. Using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA, USA), photographs of each joint were taken at 10X, 20X, and 40X. The joint cartilage sections were analyzed using a modified Mankin score system (2, 63-66) to quantify the cartilage's pathological state. The modified Mankin scoring system is based on a subset of scores, including cartilage erosion scoring (0-6), chondrocyte periphery staining (0-2), spatial arrangement of chondrocytes (0-3), and background staining intensity (0-3) (67-69). Scores were calculated by summing the four sub-criteria scores, where zero represented unaltered articular cartilage and 14 represented severe OA.

Immunohistochemistry Analysis

Immunohistochemistry (HIC) was performed on sections of joints from WT and RAGE-KO mice at 2, 4, 6, and 8 weeks post-TMJ misalignment. Separate slides were stained with antibodies against HtrA-1, Tgf- β 1, and Mmp-13. Antibodies against HtrA-1 (ab38611) and Tgf- β 1 (ab92486) were purchased from Abcam (Cambridge, MA), and those against Mmp-13 (SC-8989) from Santa Cruz Biotechnology (Santa Cruz, Ca). Slides were deparafinized and then blocked for 1 hour. All antibodies were diluted, applied to specimens, and incubated overnight at 4°C. On the second day, samples were rinsed with PBS and then incubated with an avidin/biotin ABC mix (Vectastain elite ABC Kit, Vector Laboratories, Inc. Burlingame, CA). Slides were rinsed a second time with PBS and incubated with biotinylated secondary antibody. After a third PBS rinse, a color reaction was initiated to achieve a red/brown stain using a peroxidase

substrate VECTOR NovaRED substrate kit, (Vector Laboratories, Burlingame, CA). A cover slip was placed prior to photographing. Negative controls were prepared by staining without the addition of primary antibody. Positively stained cells were counted in an area of the lateral side of the joint, the side to which the wire was bonded. Following quantitative analysis, percentages were compared to wild type controls. Photographs of each joint were taken at 40X magnifications using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA).

Statistical Analysis

Statistical significance of the combined Mankin scores at each time point for experimental and control groups was performed using a two-way analysis of variance test (ANOVA). Staining was analyzed quantitatively by calculating the percentage of cells staining positive for the respective biomarkers in a defined area of the lateral side of the condylar cartilage. All quantitative analysis were performed using ImageJ (National Institutes of Health, Bethesda, MD). The quantitative results were subsequently analyzed statistically using ANOVA to detect differences in the mean percentages of positive staining for OA biomarkers between the RAGE KO and WT samples.

Results

Histopathological results at 8 weeks post-TMJ misalignment show that RAGE KO mice have statistically low modified Mankin scores when compared with WT mice of the same age ($p < .0036$) (Figure 3.3). We observed increased Safranin O staining for proteoglycans of the condylar cartilage 2 weeks after TMJ misalignment, which was greatly reduced in WT cartilage and slightly reduced in RAGE KO cartilage at 8 weeks after TMJ misalignment (Figure 3.1). In addition, controls group show an overall low Mankin score in comparison to experimental

groups, with the exception of RAGE KO at 8 weeks (16 weeks old), unexpectedly showed an elevated Mankin score that is not typical of OA OA (Figure 3.3).

Immunohistochemical analysis of Mmp-13 shows low percentages of positive cells (42.9%) at eight weeks post TMJ-misalignment in RAGE KO mice when compared with WT (70%) (Figure 3.7). However, results did not show a statistically significant difference ($p=.363$). Wild type experimental mouse Mmp-13 expression at 8 weeks shows a statistically higher value (70%) when compared with age matched WT control group (26%) ($p<.0091$). All control groups showed lower Mmp-13 expression than age matched experimental groups.

Percentages of positive HtrA-1 staining in WT mice at 2 and 8 weeks after TMJ misalignment are higher (59-62%) when compared with RAGE KO at 2 and 8 weeks post-TMJ misalignment (42-41%) (Figure 3.7). Interestingly, we found that control groups have elevated HtrA-1 expression at 2 week (10 weeks old) (62-67%), which subsequently decrease at 8 week (16 weeks old) (42-54%) (Figure 3.7). Tgf- β 1 positive chondrocyte staining shows comparable percentages in all mouse groups at 2 weeks after induced OA, which were slightly reduced by week 8, with the exception of RAGE KO controls, where values increased from 49% to 61% at week eight (Figure 3.7).

Discussion

Early onset of osteoarthritis is characterized by over-production of proteoglycans, hypercellularity, and chondrocyte clustering. Contrary to other invasive TMJ OA mouse models where these features appear at 4 weeks post-OA induction, we found that the same OA features appeared at 2 weeks after a non-invasive OA induction in both RAGE KO and WT mice (Figure 3.2)(31, 70). In subsequent weeks, we observed gradual loss of proteoglycan expression, a reduction in chondrocyte number, and the appearance of superficial fibrillations. At 8 weeks,

RAGE KO mice expressed more proteoglycans and clustering than WT mice, which had significantly reduced proteoglycan expression and chondrocyte number (Figure 3.2). In order to evaluate if there was a statistical difference between the morphological conditions of the condylar cartilage in both mice, we analyzed them using a modified Mankin score system (30, 31, 67). The results revealed that there is a statistical difference between RAGE KO and WT scores at 8 weeks post experimental OA induction (Figure 3.3). This finding demonstrates and agrees with previous work in knee OA that postulates that absence of RAGE does provide a protective effect against OA progression (30). We found an unexpectedly high Mankin score in RAGE KO control mice at 16 weeks. We attribute this to reduced background staining due to low proteoglycan expression, which is accompanied by chondrocyte hypocellularity. This finding could be explained by the absence of RAGE expression during development, which appears to result in reduced chondrocyte number. A reduction in the number of chondrocytes appears to subsequently alter proteoglycan expression in a process that is reversed by the stress induced by TMJ misalignment.

In order to elucidate the underlying molecular events through the expression of catabolic and anabolic cytokines in the onset and development of OA in TMJ and link them with the expression of RAGE, we examined the expression patterns of Mmp-13, HtrA-1, and Tgf- β 1. Mmp-13 is a downstream product of RAGE activation and sustained up-regulation (4, 29). Our results confirm our hypothesis that knocking out RAGE decreases Mmp-13 expression from 70% to 40% (Figure 3.4 and 3.7). We are the first to effectively demonstrate that after a non-invasive TMJ OA induction, RAGE KO mice, when compared with WT, showed low Mmp-13 expression in TMJ. This result coincides with observations made by Larkin et al, who studied similar molecular processes in articular cartilage of knee joints (30). These findings show the role of

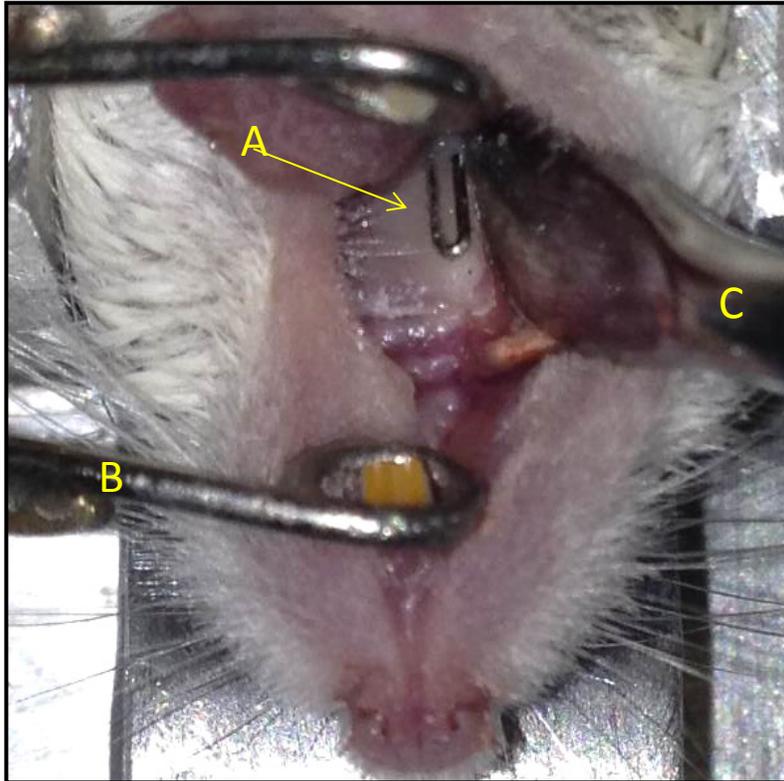


Figure 3.1: Wire Bonded to Teeth.
Misalignment of the temporomandibular joint was produced by bonding a stainless steel wire to the right upper maxillary molars (A). The murine mandibular separator (B) and cheek retractor (C) were used (manuscript in revision).

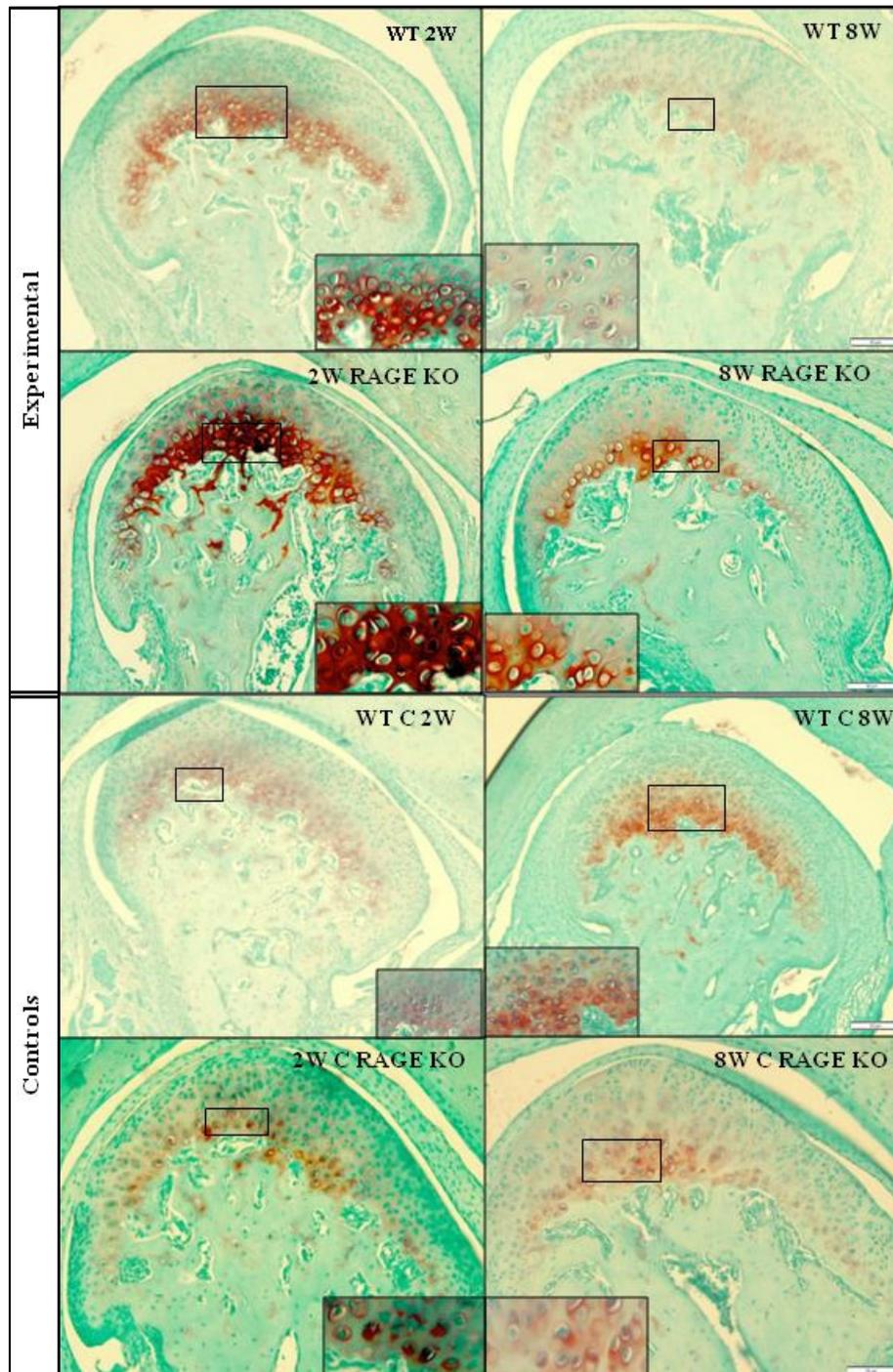


Figure 3.2: Safranin-O/Fast Green Staining. Safranin-O/Fast Green staining of proteoglycan expression of experimental and control WT and RAGE KO mice. 2 week samples after TMJ misalignment demonstrated intense proteoglycan staining clustering formation, and hypercellularity features typical of early OA. 8 week WT experimental mice showed less proteoglycan staining, and hypocellularity than RAGE KO experimental mice. Control group show steady proteoglycans expression.

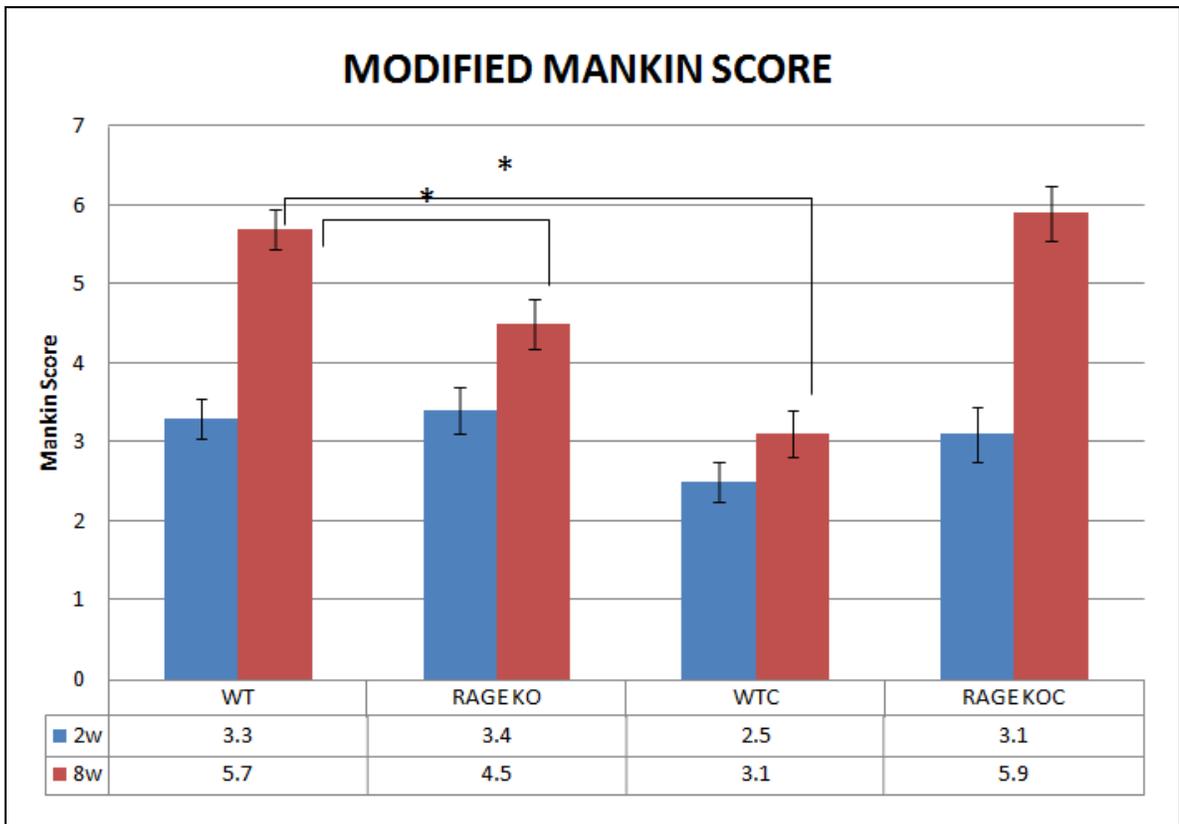


Figure 3.3: Modified Mankin Score WT-RAGE KO.

Modified Mankin score totals of all groups. There is a statistically significant difference between Mankin scores of the experimental WT with control WT mice at 8 weeks ($p < .0001$), demonstrating the effectiveness of the misalignment model in inducing early OA in the TMJ. 8 week experimental RAGE KO mice had statistically lower scores than 8 week WT ($p < .0036$), demonstrating a slight protective effect by knocking out RAGE receptors. 8 week RAGE KO control mice scored surprisingly high, we attributed this to a lack of receptor expression from birth, resulting in fewer chondrocytes and subsequent reduced proteoglycan expression.

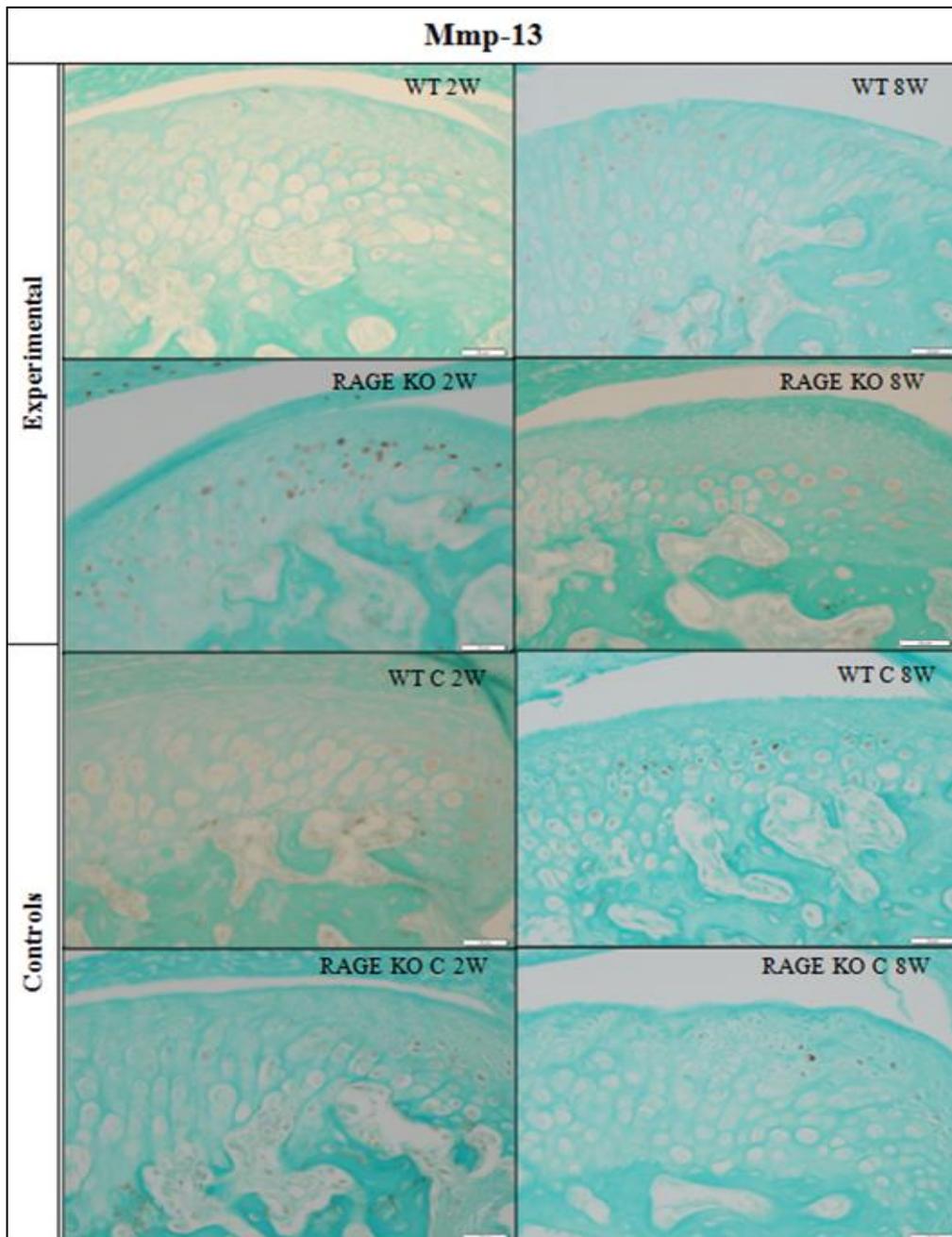


Figure 3.4: IHC Staining - Mmp-13.

At 2 week and 8 week WT experimental mice, show the highest levels of positive Mmp-13 staining. RAGE KO experimental mice show reduced staining, indicating less Mmp-13 activation, and reduced extracellular matrix (ECM) degradation in comparison to WT mice.

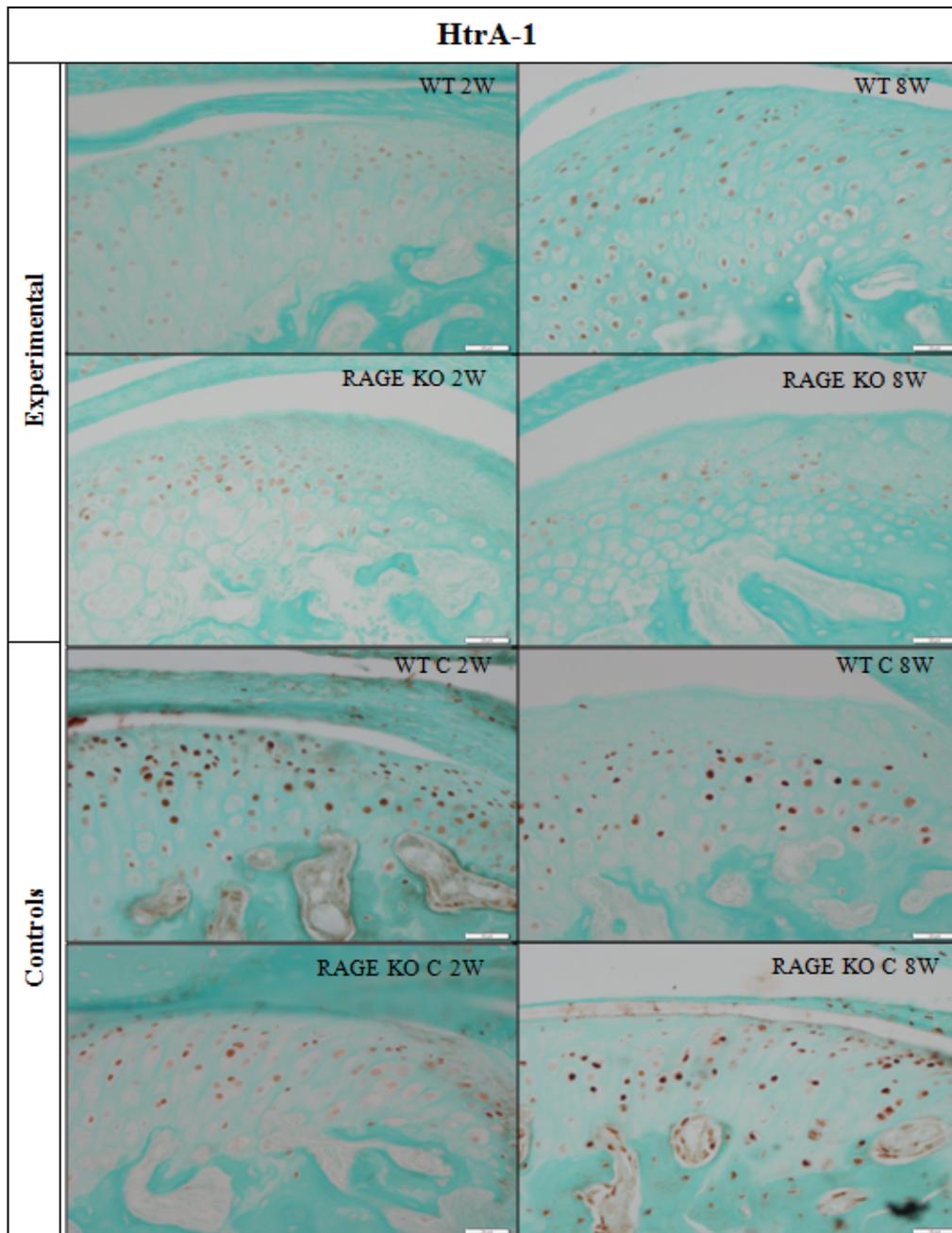


Figure 3.5: IHC Staining - HtrA-1.

At 2 week and 8 week WT experimental mice show higher levels of positive HtrA-1 staining than RAGE KO mice, demonstrating the misalignment's direct effects on pericellular matrix (PCM) and ECM degradation.

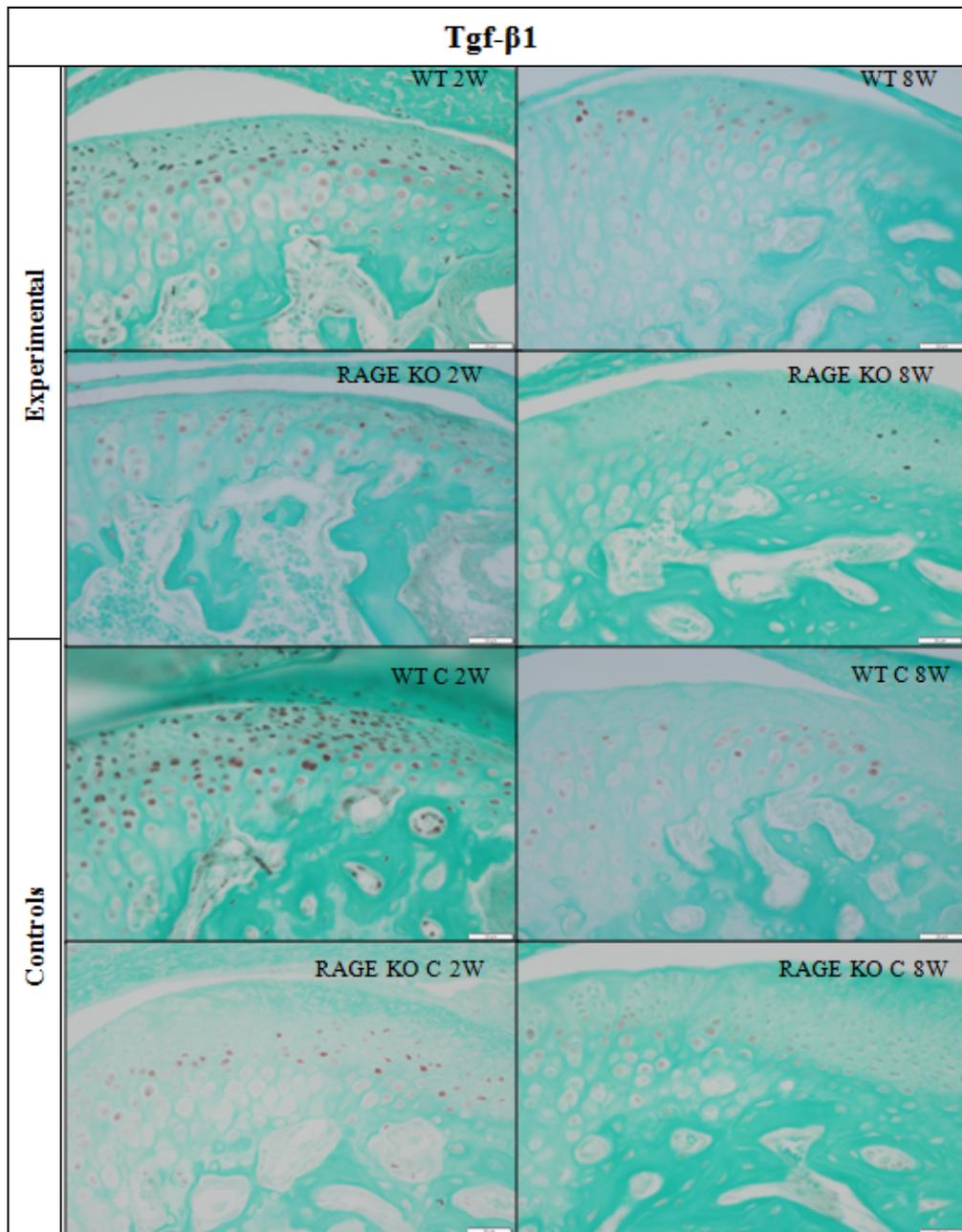


Figure 3.6: IHC Staining - Tgf- β 1.
 At 2 week and 8 weeks, WT and RAGE KO experimental mice show higher levels of positive Tgf- β 1 staining. Misalignment of the TMJ induces high and constant Tgf- β 1 expression over time.

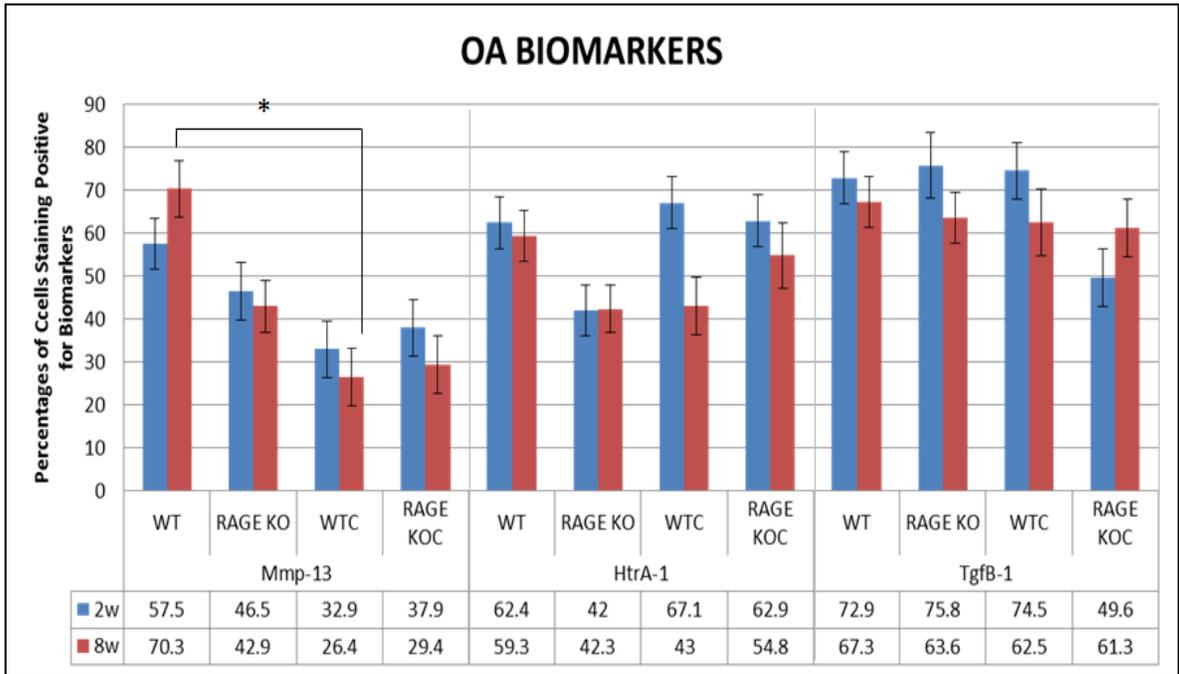


Figure 3.7: Osteoarthritis (OA) Biomarkers: Mmp-13, HtrA-1, and Tgf-β1.

This figure shows percentages of positively stained chondrocytes expressing Mmp-13, HtrA-1, and Tgfβ-1. WT experimental mice had overall higher percentages of Mmp-13 and HtrA-1 staining than RAGE KO experimental mice, demonstrating the protective effect of knocking out RAGE receptors. Additionally, there was a statistical difference between 8 week WT experimental and control mice for Mmp-13 ($p < .0091$), showing the effectiveness of the misalignment in inducing OA in the TMJ. There were no significant differences in percentages of Tgf-β1 in all groups.

RAGE in the expression of Mmp-13 and subsequent OA development. Knocking out RAGE did not attenuate all Mmp-13 expression, and we attribute this to the fact that TMJ misalignment activates other pathways that induce expression of Mmp-13. Some of these pathways are HtrA-1, Ddr-2, Mmp-13, and Toll-like Receptor 4 pathways (31, 44, 61, 71).

HtrA-1 has been shown to increase as OA progresses in the knee joints and TMJs of humans and mice (37, 72, 73). Its gradual increase in levels contributes to cartilage degradation, final chondrocyte differentiation, and inhibition of growth factor activity (34). In our study, we did not observe a gradual increase of HtrA-1 over time; however, we found that HtrA-1 expression in WT mice was 20% higher than in RAGE KO mice (Figure 3.5 and 3.7). We cannot explain why there was not an observable up-regulation of this protease over time, but we hypothesize that this event may be related to the steady and high Tgf- β 1 expression that may be triggered by the cartilage's self-repair mechanism as a result of induced stress (Figure 3.6 and 3.7). Thus, we hypothesize that high Tgf- β 1 expression may be counteracting HtrA-1 activity by inducing chondrogenesis, ECM synthesis, inhibition of the terminal chondrocyte differentiation, and blocking self-inhibition (74).

Another study on the knee joint shows that there is an inverse expression pattern of Tgf- β 1 and HtrA-1 in early and late OA (30). Although HtrA-1 expression in experimental group was higher in WT (62%-59%) than in RAGE KO (42%-42.3%) (Figure 3.7), there was not a statistically significant difference. However, this result suggests that there might be a link between RAGE and HtrA-1 pathways that is blocked by the absence of RAGE signaling. Further studies need to be conducted in order to understand this possible link. Genetically altered and surgical TMJ OA mouse models have shown HtrA-1 expression in 3 months old mice and 4 weeks post surgical OA induction. In contrast, in our model, HtrA-1 expression appears at 2

weeks (2.5 month of age) after OA induction (44). This shows that our model is effective in inducing early OA biomarker expression.

Our results show that WT mice at 8 weeks have cartilage degradation that is reflected by high summed Modified Mankin scores and elevated levels of catabolic biomarker expression. We expect that even higher scores due to severe fibrillations would appear if mice were monitored after 8 weeks post misalignment. We hypothesize that these scores would be the result of prolonged HtrA-1 and Mmp-13 expression. This is the first study to demonstrate that absence of RAGE in the TMJ provides a protective effect against the development of progression of TMJ OA in a non-invasive mouse model. Therefore, our findings, in conjunction with our previous study focusing on knee joints, concludes that RAGE plays a key role in OA progression [30]. Thus, RAGE deactivation or aggressive reduction of inflammation could be potential targets to prevent the progression of TMJ OA.

Authors' Contributions

DLK, EMCM designed the experiment. EMCM performed all experimental procedures. PRR provided the mice. DLK, EMCM, and DKM contributed to writing the manuscript. All authors assisted in carrying out experimental procedures and interpreting the results.

Funding

This work was supported by a Mentoring Environments Grant from the Brigham Young University Office of Research and Creative Activities.

Ethical Approval

All animal work described in this paper was approved by the University Institutional Animal Care and Use Committee (IACUC Protocol 12-0801; Kooyman and Chavez PI).

Acknowledgments

The authors thank Dr. Dennis Eggett (Department of Statistics, Brigham Young University) for conducting statistical analyses, Dr. Craig Hollis for kindly donating a curing light.

References

1. Van Holten T, Roest M, Riphagen J, Jansen C, Naarding P, Adriaansen H, et al. Citalopram is a more potent platelet function inhibitor than paroxetine in a case-control study. *J Thromb Haemost*. 2012 Mar 19. PubMed PMID: 22429872. Epub 2012/03/21. Eng.
2. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. *Instructional course lectures*. 1998;47:477-86. PubMed PMID: 9571449. Epub 1998/05/08. eng.
3. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2013 Jan;21(1):16-21. PubMed PMID: 23194896. Epub 2012/12/01. eng.
4. Loeser RF, Yammani RR, Carlson CS, Chen H, Cole A, Im HJ, et al. Articular chondrocytes express the receptor for advanced glycation end products: Potential role in osteoarthritis. *Arthritis and rheumatism*. 2005 Aug;52(8):2376-85. PubMed PMID: 16052547. Pubmed Central PMCID: PMC1488730. Epub 2005/07/30. eng.
5. Nepper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem*. 1992 Jul 25;267(21):14998-5004. PubMed PMID: 1378843. Epub 1992/07/25. eng.
6. Hori O, Brett J, Slattery T, Cao R, Zhang J, Chen JX, et al. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphotericin. Mediation of neurite outgrowth and co-expression of RAGE and amphotericin in the developing nervous system. *J Biol Chem*. 1995 Oct 27;270(43):25752-61. PubMed PMID: 7592757. Epub 1995/10/27. eng.
7. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annual review of immunology*. 2010;28:367-88. PubMed PMID: 20192808. Epub 2010/03/03. eng.
8. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell*. 1999 Jun 25;97(7):889-901. PubMed PMID: 10399917. Epub 1999/07/10. eng.
9. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, et al. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem*. 1992 Jul 25;267(21):14987-97. PubMed PMID: 1321822. Epub 1992/07/25. eng.
10. Buckley ST, Ehrhardt C. The receptor for advanced glycation end products (RAGE) and the lung. *Journal of biomedicine & biotechnology*. 2010;2010:917108. PubMed PMID: 20145712. Pubmed Central PMCID: PMC2817378. Epub 2010/02/11. eng.
11. Toure F, Zahm JM, Garnotel R, Lambert E, Bonnet N, Schmidt AM, et al. Receptor for advanced glycation end-products (RAGE) modulates neutrophil adhesion and migration on glycoxidated extracellular matrix. *Biochem J*. 2008 Dec 1;416(2):255-61. PubMed PMID: 18643777. Epub 2008/07/23. eng.
12. Hudson BI, Kalea AZ, Del Mar Arriero M, Harja E, Boulanger E, D'Agati V, et al. Interaction of the RAGE cytoplasmic domain with diaphanous-1 is required for ligand-stimulated cellular migration through activation of Rac1 and Cdc42. *J Biol Chem*. 2008 pub 1996/08/22. eng.

18. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, et al. Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. *Proceedings of the National Academy of Sciences of the United States of America*. 1994 Sep 13;91(19):8807-11. PubMed PMID: 8090728. Pubmed Central PMCID: PMC44695. Epub 1994/09/13. eng.
19. Reynolds PR, Wasley KM, Allison CH. Diesel particulate matter induces receptor for advanced glycation end-products (RAGE) expression in pulmonary epithelial cells, and RAGE signaling influences NF-kappaB-mediated inflammation. *Environ Health Perspect*. 2011 Mar;119(3):332-6. PubMed PMID: 21087909. Pubmed Central PMCID: PMC3059995. Epub 2010/11/Dec 5;283(49):34457-68. PubMed PMID: 18922799. Pubmed Central PMCID: PMC2590709. Epub 2008/10/17. eng.
13. Kim JY, Park HK, Yoon JS, Kim SJ, Kim ES, Ahn KS, et al. Advanced glycation end product (AGE)-induced proliferation of HEL cells via receptor for AGE-related signal pathways. *Int J Oncol*. 2008 Sep;33(3):493-501. PubMed PMID: 18695878. Epub 2008/08/13. eng.
14. Reddy MA, Li SL, Sahar S, Kim YS, Xu ZG, Lanting L, et al. Key role of Src kinase in S100B-induced activation of the receptor for advanced glycation end products in vascular smooth muscle cells. *J Biol Chem*. 2006 May 12;281(19):13685-93. PubMed PMID: 16551628. Epub 2006/03/23. eng.
15. Lin L, Park S, Lakatta EG. RAGE signaling in inflammation and arterial aging. *Frontiers in bioscience : a journal and virtual library*. 2009;14:1403-13. PubMed PMID: 19273137. Pubmed Central PMCID: PMC2661616. Epub 2009/03/11. eng.
16. Bianchi R, Giambanco I, Donato R. S100B/RAGE-dependent activation of microglia via NF-kappaB and AP-1 Co-regulation of COX-2 expression by S100B, IL-1beta and TNF-alpha. *Neurobiology of aging*. 2010 Apr;31(4):665-77. PubMed PMID: 18599158. Epub 2008/07/05. eng.
17. Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, et al. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature*. 1996 Aug 22;382(6593):685-91. PubMed PMID: 8751438. E20. eng.
20. Ruan BH, Li X, Winkler AR, Cunningham KM, Kuai J, Greco RM, et al. Complement C3a, CpG oligos, and DNA/C3a complex stimulate IFN-alpha production in a receptor for advanced glycation end product-dependent manner. *J Immunol*. 2010 Oct 1;185(7):4213-22. PubMed PMID: 20817881. Epub 2010/09/08. eng.
21. Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, et al. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immun*. 2002 May;3(3):123-35. PubMed PMID: 12070776. Epub 2002/06/19. eng.
22. Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, et al. Understanding RAGE, the receptor for advanced glycation end products. *Journal of molecular medicine (Berlin, Germany)*. 2005 Nov;83(11):876-86. PubMed PMID: 16133426. Epub 2005/09/01. eng.
23. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochimica et biophysica acta*. 2000 Dec 20;1498(2-3):99-111. PubMed PMID: 11108954. Epub 2000/12/08. eng.
24. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and

- inflammation. *Journal of translational medicine*. 2009;7:17. PubMed PMID: 19292913. Pubmed Central PMCID: PMC2666642. Epub 2009/03/19. eng.
25. Morbini P, Villa C, Campo I, Zorzetto M, Inghilleri S, Luisetti M. The receptor for advanced glycation end products and its ligands: a new inflammatory pathway in lung disease? *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* 2006 Nov;19(11):1437-45. PubMed PMID: 16941014. Epub 2006/08/31. eng.
 26. Bohlender JM, Franke S, Stein G, Wolf G. Advanced glycation end products and the kidney. *American journal of physiology Renal physiology*. 2005 Oct;289(4):F645-59. PubMed PMID: 16159899. Epub 2005/09/15. eng.
 27. He M, Kubo H, Ishizawa K, Hegab AE, Yamamoto Y, Yamamoto H, et al. The role of the receptor for advanced glycation end-products in lung fibrosis. *American journal of physiology Lung cellular and molecular physiology*. 2007 Dec;293(6):L1427-36. PubMed PMID: 17951314. Epub 2007/10/24. eng.
 28. Park EY, Seo MJ, Park JH. Effects of specific genes activating RAGE on polycystic kidney disease. *American journal of nephrology*. 2010;32(2):169-78. PubMed PMID: 20606421. Epub 2010/07/08. eng.
 29. Yammani RR, Carlson CS, Bresnick AR, Loeser RF. Increase in production of matrix metalloproteinase 13 by human articular chondrocytes due to stimulation with S100A4: Role of the receptor for advanced glycation end products. *Arthritis and rheumatism*. 2006 Sep;54(9):2901-11. PubMed PMID: 16948116. Epub 2006/09/02. eng.
 30. Larkin DJ, Kartchner JZ, Doxey AS, Hollis WR, Rees JL, Wilhelm SK, et al. Inflammatory markers associated with osteoarthritis after destabilization surgery in young mice with and without Receptor for Advanced Glycation End-products (RAGE). *Frontiers in Physiology*. 2013;4.
 31. Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2009 Jul;17(7):917-22. PubMed PMID: 19230720. Pubmed Central PMCID: 2941347.
 32. Reboul P, Pelletier JP, Tardif G, Cloutier JM, Martel-Pelletier J. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. *The Journal of clinical investigation*. 1996 May 1;97(9):2011-9. PubMed PMID: 8621789. Pubmed Central PMCID: PMC507274. Epub 1996/05/01. eng.
 33. Wang Y-L, Li X-J, Qin R-F, Lei D-L, Liu Y-P, Wu G-Y, et al. Matrix metalloproteinase and its inhibitor in temporomandibular joint osteoarthrosis after indirect trauma in young goats. *British Journal of Oral & Maxillofacial Surgery*. 2008 Apr;46(3):192-7. PubMed PMID: WOS:000255070200005.
 34. Oka C, Tsujimoto R, Kajikawa M, Koshiba-Takeuchi K, Ina J, Yano M, et al. HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins. *Development (Cambridge, England)*. 2004 Mar;131(5):1041-53. PubMed PMID: 14973287. Epub 2004/02/20. eng.
 35. Hu SI, Carozza M, Klein M, Nantermet P, Luk D, Crowl RM. Human HtrA, an evolutionarily conserved serine protease identified as a differentially expressed gene product in osteoarthritic cartilage. *J Biol Chem*. 1998 Dec 18;273(51):34406-12. PubMed PMID: 9852107. Epub 1998/12/16. eng.

36. Grau S, Richards PJ, Kerr B, Hughes C, Caterson B, Williams AS, et al. The role of human HtrA1 in arthritic disease. *J Biol Chem*. 2006 Mar 10;281(10):6124-9. PubMed PMID: 16377621. Epub 2005/12/27. eng.
37. Wu J, Liu W, Bemis A, Wang E, Qiu Y, Morris EA, et al. Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis. *Arthritis and rheumatism*. 2007 Nov;56(11):3675-84. PubMed PMID: 17968891. Epub 2007/10/31. eng.
38. Weger M, Renner W, Steinbrugger I, Kofer K, Wedrich A, Groselj-Strele A, et al. Association of the HTRA1 -625G>A promoter gene polymorphism with exudative age-related macular degeneration in a Central European population. *Molecular vision*. 2007;13:1274-9. PubMed PMID: 17679948. Epub 2007/08/08. eng.
39. Mullany SA, Moslemi-Kebria M, Rattan R, Khurana A, Clayton A, Ota T, et al. Expression and functional significance of HtrA1 loss in endometrial cancer. *Clin Cancer Res*. 2011 Feb 1;17(3):427-36. PubMed PMID: 21098697. Pubmed Central PMCID: PMC3057564. Epub 2010/11/26. eng.
40. Bakay M, Zhao P, Chen J, Hoffman EP. A web-accessible complete transcriptome of normal human and DMD muscle. *Neuromuscular disorders : NMD*. 2002 Oct;12 Suppl 1:S125-41. PubMed PMID: 12206807. Epub 2002/09/11. eng.
41. Holtzer R, Wang C, Verghese J. The relationship between attention and gait in aging: facts and fallacies. *Motor Control*. 2012 Jan;16(1):64-80. PubMed PMID: 22402221. Epub 2012/03/10. eng.
42. Holt RF, Lalonde K. Assessing toddlers' speech-sound discrimination. *Int J Pediatr Otorhinolaryngol*. 2012 Mar 6. PubMed PMID: 22402014. Epub 2012/03/10. Eng.
43. McHedlishvili N, Wieser S, Holtackers R, Mouysset J, Belwal M, Amaro AC, et al. Kinetochores accelerate centrosome separation to ensure faithful chromosome segregation. *J Cell Sci*. 2012 Mar 7. PubMed PMID: 22399803. Epub 2012/03/09. Eng.
44. Polur I, Lee PL, Servais JM, Xu L, Li Y. Role of HTRA1, a serine protease, in the progression of articular cartilage degeneration. *Histol Histopathol*. 2010 May;25(5):599-608. PubMed PMID: 20238298. Pubmed Central PMCID: 2894561. Epub 2010/03/20. eng.
45. Johansen ML, Holtedahl KA, Davidsen AS, Rudebeck CE. 'I Deal With the Small Things': The Doctor-Patient Relationship and Professional Identity in GPs' Stories of Cancer Care. *Health (London)*. 2012 Mar 7. PubMed PMID: 22397893. Epub 2012/03/09. Eng.
46. Xu L, Polur I, Servais JM, Hsieh S, Lee PL, Goldring MB, et al. Intact pericellular matrix of articular cartilage is required for unactivated discoidin domain receptor 2 in the mouse model. *Am J Pathol*. 2011 Sep;179(3):1338-46. PubMed PMID: 21855682. Pubmed Central PMCID: 3157196.
47. Roberts AB, Sporn MB. Transforming growth factor beta. *Advances in cancer research*. 1988;51:107-45. PubMed PMID: 2906217. Epub 1988/01/01. eng.
48. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB. Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. *Laboratory investigation; a journal of technical methods and pathology*. 1994 Aug;71(2):279-90. PubMed PMID: 8078307. Epub 1994/08/01. eng.

49. Thorp BH, Anderson I, Jakowlew SB. Transforming growth factor-beta 1, -beta 2 and -beta 3 in cartilage and bone cells during endochondral ossification in the chick. *Development (Cambridge, England)*. 1992 Apr;114(4):907-11. PubMed PMID: 1618152. Epub 1992/04/01. eng.
50. Itayem R, Mengarelli-Widholm S, Hulth A, Reinholt FP. Ultrastructural studies on the effect of transforming growth factor-beta 1 on rat articular cartilage. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*. 1997 Mar;105(3):221-8. PubMed PMID: 9137518. Epub 1997/03/01. eng.
51. Redini F, Mauviel A, Pronost S, Loyau G, Pujol JP. Transforming growth factor beta exerts opposite effects from interleukin-1 beta on cultured rabbit articular chondrocytes through reduction of interleukin-1 receptor expression. *Arthritis and rheumatism*. 1993 Jan;36(1):44-50. PubMed PMID: 8424835. Epub 1993/01/01. eng.
52. Embree MC, Kilts TM, Ono M, Inkson CA, Syed-Picard F, Karsdal MA, et al. Biglycan and Fibromodulin Have Essential Roles in Regulating Chondrogenesis and Extracellular Matrix Turnover in Temporomandibular Joint Osteoarthritis. *Am J Pathol*. 2010 Feb;176(2):812-26. PubMed PMID: WOS:000274111400030. English.
53. Hyytiainen M, Penttinen C, Keski-Oja J. Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. *Critical reviews in clinical laboratory sciences*. 2004;41(3):233-64. PubMed PMID: 15307633. Epub 2004/08/17. eng.
54. Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol*. 2002 Feb;118(2):211-5. PubMed PMID: 11841535. Epub 2002/02/14. eng.
55. Blaney Davidson EN, Vitters EL, van der Kraan PM, van den Berg WB. Expression of transforming growth factor-beta (TGFbeta) and the TGFbeta signalling molecule SMAD-2P in spontaneous and instability-induced osteoarthritis: role in cartilage degradation, chondrogenesis and osteophyte formation. *Ann Rheum Dis*. 2006 Nov;65(11):1414-21. PubMed PMID: 16439443. Pubmed Central PMCID: PMC1798346. Epub 2006/01/28. eng.
56. Bakker AC, van de Loo FA, van Beuningen HM, Sime P, van Lent PL, van der Kraan PM, et al. Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2001 Feb;9(2):128-36. PubMed PMID: 11237660. Epub 2001/03/10. eng.
57. Takahashi K, Hashimoto K, Fujiyama A, Yamada J, Kobayashi N, Morisaki M, et al. Stereochemistry of reduction of the C-24,25 double bond in the conversion of desmosterol into cholesterol. *Tetrahedron Lett*. 2003 Jan 6;44(2):341-4. PubMed PMID: WOS:000180321000031. English.
58. Pelletier JP, Roughley PJ, DiBattista JA, McCollum R, Martel-Pelletier J. Are cytokines involved in osteoarthritic pathophysiology? *Semin Arthritis Rheum*. 1991 Jun;20(6 Suppl 2):12-25. PubMed PMID: 1866626. Epub 1991/06/01. eng.
59. Ballock RT, Heydemann A, Wakefield LM, Flanders KC, Roberts AB, Sporn MB. TGF-beta 1 prevents hypertrophy of epiphyseal chondrocytes: regulation of gene expression for cartilage matrix proteins and metalloproteases. *Developmental biology*. 1993 Aug;158(2):414-29. PubMed PMID: 8344460. Epub 1993/08/01. eng.
60. Liu YD, Liao LF, Zhang HY, Lu L, Jiao K, Zhang M, et al. Reducing dietary loading decreases mouse temporomandibular joint degradation induced by anterior crossbite

- prosthesis. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2014 Feb;22(2):302-12. PubMed PMID: 24316289. Epub 2013/12/10. eng.
61. Holt DW, Henderson ML, Stockdale CE, Farrell JT, Kooyman DL, Bridgewater LC, et al. Osteoarthritis-like changes in the heterozygous sedc mouse associated with the HtrA1-Ddr2-Mmp-13 degradative pathway: a new model of osteoarthritis. *Osteoarthritis Cartilage*. 2012 May;20(5):430-9. PubMed PMID: WOS:000303297100013. English.
 62. Walker CG, Ito Y, Dangaria S, Luan X, Diekwisch TG. RANKL, osteopontin, and osteoclast homeostasis in a hyperocclusion mouse model. *European journal of oral sciences*. 2008 Aug;116(4):312-8. PubMed PMID: 18705798. Pubmed Central PMCID: PMC2597431. Epub 2008/08/19. eng.
 63. Holt DW, Henderson ML, Stockdale CE, Farrell JT, Kooyman DL, Bridgewater LC, et al. Osteoarthritis-like changes in the heterozygous sedc mouse associated with the HtrA1-Ddr2-Mmp-13 degradative pathway: a new model of osteoarthritis. *Osteoarthritis Cartilage*. 2012 May;20(5):430-9. PubMed PMID: 22155431. Epub 2011/12/14. eng.
 64. Bertram S, Rudisch A, Innerhofer K, Pumpel E, Grubwieser G, Emshoff R. Diagnosing TMJ internal derangement and osteoarthritis with magnetic resonance imaging. *J Am Dent Assoc*. 2001 Jun;132(6):753-61. PubMed PMID: 11433854. Epub 2001/07/04. eng.
 65. Sunk IG, Bobacz K, Hofstaetter JG, Amoyo L, Soleiman A, Smolen J, et al. Increased expression of discoidin domain receptor 2 is linked to the degree of cartilage damage in human knee joints: a potential role in osteoarthritis pathogenesis. *Arthritis and rheumatism*. 2007 Nov;56(11):3685-92. PubMed PMID: 17968949. Epub 2007/10/31. eng.
 66. Lippiello L, Hall D, Mankin HJ. Collagen synthesis in normal and osteoarthritic human cartilage. *The Journal of clinical investigation*. 1977 Apr;59(4):593-600. PubMed PMID: 845251. Pubmed Central PMCID: PMC372262. Epub 1977/04/01. eng.
 67. Bomsta BD, Bridgewater LC, Seegmiller RE. Premature osteoarthritis in the Disproportionate micromelia (Dmm) mouse. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2006 May;14(5):477-85. PubMed PMID: 16431140. Epub 2006/01/25. eng.
 68. Xu L, Flahiff CM, Waldman BA, Wu D, Olsen BR, Setton LA, et al. Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho). *Arthritis and rheumatism*. 2003 Sep;48(9):2509-18. PubMed PMID: 13130470. Epub 2003/09/18. eng.
 69. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *The Journal of bone and joint surgery American volume*. 1971 Apr;53(3):523-37. PubMed PMID: 5580011. Epub 1971/04/01. eng.
 70. Xu L, Polur I, Servais JM, Hsieh S, Lee PL, Goldring MB, et al. Intact Pericellular Matrix of Articular Cartilage Is Required for Unactivated Discoidin Domain Receptor 2 in the Mouse Model. *Am J Pathol*. 2011 Sep;179(3):1338-46. PubMed PMID: WOS:000298307300028. English.
 71. Salazar A, Polur I, Servais JM, Li Y, Xu L. Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular joints of osteoarthritic mouse models. *Journal of Oral Pathology & Medicine*. 2013:n/a-n/a.

72. Hinton RJ, Carlson DS. Response of the mandibular joint to loss of incisal function in the rat. *Acta Anat (Basel)*. 1986;125(3):145-51. PubMed PMID: 3962576. Epub 1986/01/01. eng.
73. Chen J, Sorensen KP, Gupta T, Kilts T, Young M, Wadhwa S. Altered functional loading causes differential effects in the subchondral bone and condylar cartilage in the temporomandibular joint from young mice. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2009 Mar;17(3):354-61. PubMed PMID: 18789726. Pubmed Central PMCID: PMC2646810. Epub 2008/09/16. eng.
74. Fernandez-Criado C, Martos-Rodriguez A, Santos-Alvarez I, Garcia-Ruiz JP, Delgado-Baeza E. The fate of chondrocyte in osteoarthritic cartilage of transgenic mice expressing bovine GH. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2004 Jul;12(7):543-51. PubMed PMID: 15219569. Epub 2004/06/29. eng.

CURRICULUM VITAE

Elizabeth Murayama Chávez Matías

sud_beth@hotmail.com
801- 400-9852

- 2014 Master of Science in Physiology and Developmental Biology, Brigham Young University, Provo, UT
2004 Dental Surgeon, Universidad Nacional Mayor de San Marcos, Lima, Peru
2003 Bachelor Degree in Dentistry, Universidad Nacional Mayor de San Marcos, Lima, Peru

RESEARCH EXPERIENCE

2012—2014 Master Research Assistant, Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT (research advisor David L. Kooyman)

TEACHING EXPERIENCE

2012—2014 Teaching Assistant, Brigham Young University

Courses: Human Physiology Lab (PDBIO 305)
Human Physiology Lecture (PDBIO 305)

PUBLICATIONS

Author:

E.M. Chavez M., D.K. Mecham, C.S. Black, J.W. Graf, S.K. Wilhelm, K.M. Andersen, J.A. Mitchell, J.R. Macdonald, E.S. Finlay, P.R. Reynolds, D.L. Kooyman. “A Novel Mouse Model of Temporomandibular Joint Osteoarthritis” (Manuscript in revision)

E.M. Chavez M., D.K. Mecham, J.W. Graf, R. Wood, J.A. Mitchell, C.S. Black, S.K. Wilhelm, K.M. Andersen, J.R. Macdonald, P.R. Reynolds, D.L. Kooyman. “Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and without Receptor for Advanced Glycation End Products (RAGE)” (Manuscript in revision)

Co-Author:

Larkin DJ, Kartchner JZ, Doxey AS, Hollis WR, Rees JL, Wilhelm SK, Draper CS, Peterson DM, Jackson GG, Ingersoll C, Haynie SS, Chavez E, Reynolds PR, Kooyman DL. “Inflammatory markers associated with osteoarthritis after destabilization surgery in young mice with and without Receptor for Advanced Glycation End-products (RAGE)” *Frontiers in Physiology*, 2013 May 28; 4:121. doi: 10.3389/fphys.2013.00121.

POSTER PRESENTATIONS

Author:

E.M. Chavez M., D.K. Mecham, E.S. Finlay, J.W. Graf, R. Wood, C.S. Black, S.K. Wilhelm, K.M. Andersen, J.A. Mitchell, J.R. Macdonald, P.R. Reynolds, and D.L. Kooyman. “Expression

of Osteoarthritis Biomarkers in Condylar Cartilage of Mice that lack of Receptor for Advanced Glycation End Products (RAGE)”

Co-Author:

K.M. Andersen, C.S. Black, P.K. Crepeau, J.Z. Kartchner, D.K. Mecham, M.R. Fabrizio, S.K. Wilhelm, E.M. Chavez M., P.R. Reynolds, and D.L. Kooyman “TGF- β 1 and HtrA1 Interactive Pathway in the Molecular Progression of Osteoarthritis”

S.K. Wilhelm, D.K. Mecham, E.M. Chavez, J.E. Vanderuff, I.T. Christenson, M.R. Fabrizio, N.J. Strobel, M.J. Siebert, K.M. Andersen, P.K. Crepeau, E.S. Finlay, J.Z. Kartchner, P.R. Reynolds, and D.L. Kooyman. “Effects of TLR-4 blockers on the severity of OA in mice lacking the receptor for advanced glycation end products (RAGE)”

D.J. Larkin , J.Z. Kartchner , A.S. Doxey , J.L. Rees , W.R. Hollis , S.K. Wilhelm, D.M. Peterson, G. Jackson, S.S. Haynie, E. Chavez, P.R. Reynolds, and D.L. Kooyman. “Inflammatory Markers Associated with Osteoarthritis after destabilization surgery in Mice with and without Receptor for Advanced Glycantion End Products (RAGE)”

S.K. Wilhelm, A.S. Doxey, J.Z. Kartchner, J.E. Vanderuff, D.M. Peterson, D.J. Larkin, K. Andersen, C.S. Draper, E. Chavez, P.R. Reynolds, and D.L. Kooyman. “Apoptosis Associated with Osteoarthritis is Attenuated in Mice Lacking the Receptor for Advanced Glycation End Products (RAGE)”

AWARDS

2012-2014 Teaching Assistantship and Research Assistantship, Physiology and Developmental Biology, Brigham Young University, Provo, UT

2012-2014 Graduate Student Funding Scholarship by the department of Physiology and Developmental Biology, Brigham Young University, Provo, UT.

GRANTS

Research was supported by Mentoring Environments Grant from Brigham Young University Office of Research and Creative Activities.

WORK EXPERIENCE

2004—2010 Dentist private practice

2005—2006 Dentist Dental Clinic “Sonrisas y Salud”

2001—2002 Hospital Internship “Nacional Hipolito Unanue”

VOLUNTEER WORK

2011- Present. Dental assistant volunteer at Mountainlands Family Health Center, Provo, UT

2013- Present. Shadowing - Dental Clinic of Primary Children Medical Center, Salt Lake City, UT

2012- Fall Kid’s crew volunteer at Primary Children Medical Center, Salt Lake City-Utah

2011- 2012 Spanish-English interpreter volunteers at University Hospital of Utah
2012- 2012 Emergency Room volunteer at Utah Primary Children Medical Center
2002- 2003 Internship at Hospital Nacional Hipolito Unanue, Lima, Peru
2001- 2004 As a Dentist, I participated in many Humanitarian Dental Projects in my country led
by the Academy of LDS Dentistry.

LANGUAGES

English (fluent)
Spanish (native)