Catalyst, Volume 2, Issue 1, 2017

A central role for reactive oxygen species (ROS) in the pathogenesis of temporomandibular joint disorders: All roads lead to ROS

Adam Kevin Wade-Vallance*, Maxwell Ng*, Katherine Kuo*, Candice Luo*, Seana Adams*, Gurleen Dhaliwal* & Caberry Yu*

*Authors contributed to equally to this work.

Abstract

Temporomandibular joint disease (TMD) is a musculoskeletal pain disorder occurring at the temporomandibular joint (TMJ), the interface of the skull's temporal bone and the mandible[1]. This literature review examines the anatomy, epidemiology, biochemistry and cellular biology of TMD in order to frame underlying biochemical and cellular events within the human context of the disease. This review identifies several key elements pertaining to TMD pathogenesis, including mechanical stress-induced hypoxia-reperfusion, disruption of mitochondrial function, arachidonic acid catabolism (through prostaglandins and leukotrienes), cartilage degradation, bone resorption, and breakdown of joint lubrication. Based on a thorough analysis of established correlations and causations we propose an overarching mechanism that provides a holistic representation of TMD, something noticeably absent in the literature to-date. This mechanism clearly highlights the central role of reactive oxygen species (ROS) in the pathogenesis of TMD, a conclusion holding significant implications for both treatment and our understanding of the disease.

Introduction

The TMJ is a synovial joint located bilaterally at the jaw[2]. It is directly involved in the opening and closing of the mouth, mastication, lateral excursions, and protrusion[2]. Primary joint structures include the mandibular fossa, articular tubercle, and mandible head between which an articular disc is situated[2]. The articular disc, composed of fibrous connective tissue, mitigates friction by separating surrounding bones[2]. TMD typically arises from chronic compression of the TMJ, which induces a number of detrimental effects including the production of ROS and anterior disc displacement, a painful TMD symptom clinically known to limit jaw mobility[3].

ROS, such as superoxide anion ($O^{2-}\cdot$) and hydroxyl radicals ($OH\cdot$), serve diverse cellular functions in signal transmission, cellular activation, and regulation[3]. Despite these essential roles, ROS levels must be regulated by antioxidants to prevent deleterious off-target reactivity[3]. If this balance is compromised and ROS accumulate, serious consequences including DNA damage, protein denaturation and lipid peroxidation can occur[3]. This phenomenon, termed oxidative stress, acts to propagate TMD[3].

Currently, few review articles discuss the effects of oxidative stress on TMD pathology[3-5]. Milam et al. propose a pathway for oxidative stress-induced TMJ damage. They describe mechanical stress as the primary instigator of ROS synthesis with subsequent regulatory pathways involving ROS[5]. Although recent studies continue to investigate the effects of mechanical stress on the TMJ, they fail to properly discuss the full implications of mechanical stress on the downstream ROS pathways[6]. Several primary research studies have investigated specific cellular mechanisms disrupted by ROS in TMD, such as the lysing of hyaluronic acid, but rarely do they reference TMD's primary cause - mechanical stress[4,6-8]. Inadequate contextual framing is also observed in a study exploring the role of iron in $OH\cdot$ formation via the Fenton reaction, which does not consider potential sources of iron[7]. By synthesizing the limited scopes of individual papers such as these, we have created a more complete view of the role of ROS in TMD (Figure 1). Temporomandibular joint disease (TMD) is a musculoskeletal pain disorder occurring at the temporomandibular joint (TMJ), the interface of the skull's temporal bone and mandible[1]. This literature review examines the anatomy, epidemiology, biochemistry and cellular biology of TMD in order to frame underlying biochemical and cellular events within the human context of the disease. This review identifies several key elements pertaining to TMD pathogenesis, including mechanical stress-induced hypoxia-reperfusion, disruption of mitochondrial function, arachidonic acid catabolism (through prostaglandins and leukotrienes), cartilage degradation, bone resorption, and breakdown of joint lubrication. Based on a thorough analysis of established correlations and causations we propose an overarching mechanism that provides a holistic representation of TMD, something noticeably absent in the literature to-date. This mechanism clearly highlights the central role of reactive oxygen species (ROS) in pathogenesis of TMD, a conclusion holding significant implications for both treatment and our understanding of the disease.

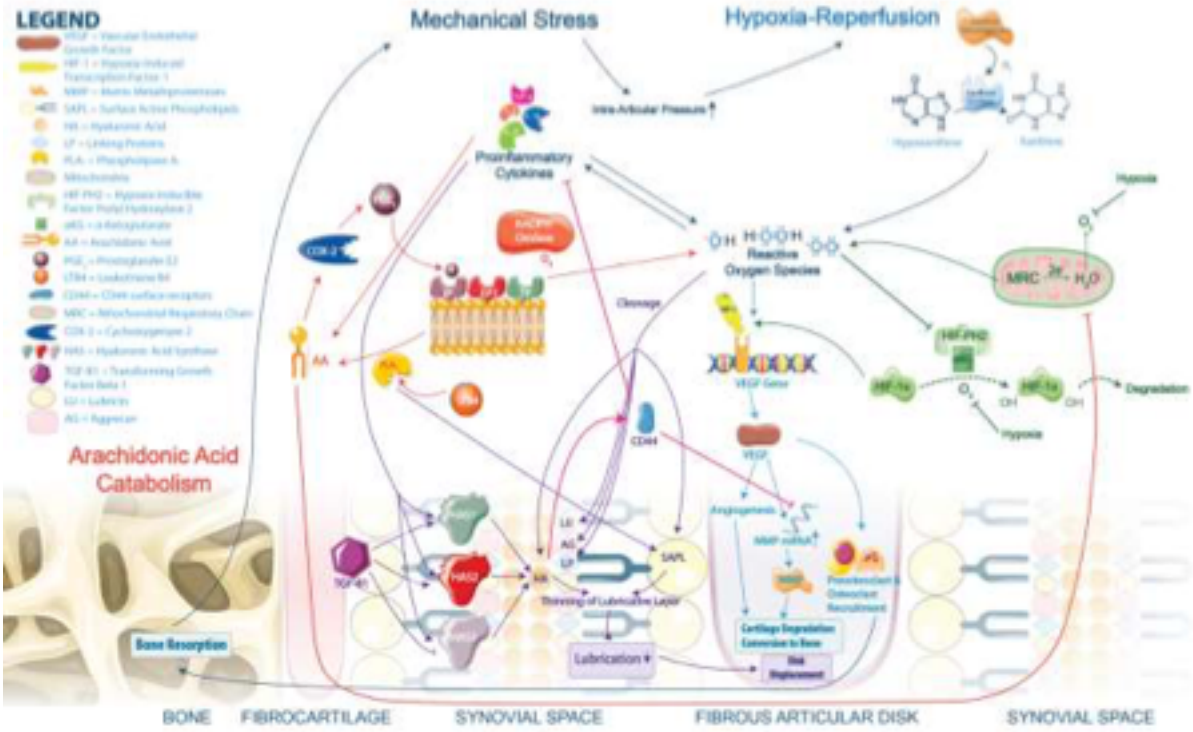

Figure 1: Overview of central pathways involved in the pathogenesis of temporomandibular joint disorder highlighting the central role of reactive oxygen species. Illustrated here are the hypoxia reperfusion, mitochondrial dysfunction, arachidonic acid catabolism, disc displacement via impaired lubrication, bone resorption, and cartilage degradation pathways. Although emphasis is placed on the left side of the image, these processes would in reality be mirrored across the fibrous articular disc. This figure was developed using Adobe Illustrator, please see the legend for all abbreviations.

Xanthine Oxidase-Mediated ROS generation

ROS have expansive involvement in the pathogenesis of TMD[8-18]. One ROS-producing mechanism is the xanthine oxidase pathway (Figure 1)[9-10]. First, some form of mechanical stress at the TMJ, such as tight clenching or prolonged maximal mouth opening during a dental procedure, increases intra-articular pressure[9]. Once joint pressure exceeds 40 mmHg, peripheral arteriolar blood pressure is overpowered, resulting in transient hypoxia of TMJ tissues[9]. Hypoxia induces a metabolic shift in affected tissues that produces ROS following TMJ relaxation and tissue reperfusion[14-15]. This shift is mediated by xanthine oxidase, which generates $O^{2-}\cdot$ during reoxygenation by reacting in the presence of oxygen with hypoxanthine that accumulates in the hypoxic state[8, 14-18].

Mitochondria

Mitochondria represent another source of ROS[10]. Under physiological conditions, mitochondria release $O^{2-}\cdot$: this release is exacerbated under hypoxic conditions due to mitochondrial inhibition, as follows and shown in Figure 1[10-11]. Generally, mitochondria attempt to pass off electrons through the mitochondrial respiratory chain to synthesize water10,12. Oxygen depletion stymies this pathway, causing electron leakage12. This leakage engenders O2-·, which leads to the oxidative decarboxylation of α- Ketoglutarate and the subsequent inhibition of its cofactor, prolyl hydroxylase domain-2 (PHD2)[11]. The role of PHD2 is to hydroxylate hypoxia- inducible factor 1α (HIF-1α) in order to target it for proteasomal degradation. Therefore its inhibition causes HIF-1α accumulation[10,11,19]. Amassed HIF-1α acts as a subunit to complete the HIF-1 transcription factor[10,11,19]. Increased HIF-1 leads to certain detrimental effects implicated in TMD, as discussed in 'Cartilage degradation and bone resorption'.

Arachidonic Acid Catabolism: Prostaglandins & Leukotrienes

Prostaglandins (PGs) are first synthesized from arachidonic acid (AA) by most human cells[20]. In the context of TMD, PGs increase ROS synthesis[21]. Mechanical stress, cytokines, and growth factors can release AA from the phospholipid membrane[20]. ROS stimulate cytokine production, causing upregulation of PG synthesis[22]. PG synthesis occurs via the enzyme cyclooxygenase-2 (COX- 2), which produces ROS as a byproduct (Figure 1). This results in greater levels of ROS in the joint[22].

Once released, AA is metabolized by COX-1 and COX-2 to form PGH2[20]. PGH2 is subsequently converted into PGE2, a process known to generate ROS as a byproduct[23]. In fact, Kerins et al. demonstrated a reduction in orofacial pain in a rat TMD model following COX-2 inhibition by measuring

mealtime patterns[24]. Untreated rats had longer mealtimes (indicating more pain) than treated rats, suggesting COX-2 is integral to TMJ inflammation and pain. Further studies showed COX-2 inhibition downregulates NADPH activity, thus reducing ROS levels[25]. This is potentially because AA can synthesize ROS through NADPH independent of COX-1 and COX-2[26-28]. As an alternative to conversion into PGs, Cocco et al. demonstrated that AA produces ROS by interfering with the mitochondrial electron transport chain, as AA addition to respiring cell increased hydrogen peroxide (H_2O_2) levels compared to basal conditions[29].

Leukotrienes (LTs) may also stimulate inflammation and subsequent ROS production in the TMJ[3]. LT synthesis begins when 5-lipoxygenase-activating protein translocates AA to 5-lipoxygenase, which converts the AA to LTA4[20]. LTA4 then undergoes one of several conversion pathways, the most pertinent of which forms LTB4[20]. LTB4 produces ROS through a rac-dependent pathway which mediates AA-induced ROS generation through a PLA2 linked cascade[30]. Woo et al. explored LTB4 as a crucial mediator in tumour necrosis factor-α (TNF-α) induced ROS generation via NADPH[30]. Results revealed dose-dependent ROS upregulation by LTB4, the effects of which were inhibited by an LTB4 receptor (BLT) antagonist[30]. Furthermore, blocking of the specific G-protein coupled to BLT receptors decreased ROS production in a dose- dependent manner[30]. Hence, these studies thus delineate the vital role of PGs and LTs on the production of ROS through arachidonic acid catabolism.

Once ROS accumulate via the aforementioned pathways, including the xanthine oxidase, mitochondria pathway, and arachidonic acid pathways, they damage collagen and the lubricative layer by reacting with key molecules in the joint and compromising their functional integrity through processes which will forthwith be explored.

Cartilage degradation and bone resorption

The TMJ is composed of bone, a fibrous articular disc, and fibrocartilage (Figure 1), which contains fibroblasts, chondrocytes, and type I and II collagen[31-33]. Cartilage plays an essential role in TMJ function by reducing joint loading and providing a surface for the articulation of the disc[31,34]. ROS indirectly degrade the type I collagen found in the articular cartilage, as discussed in detail below[7].

As explained previously, hypoxia induces ROS and subsequent HIF-1 accumulation by stabilizing the HIF-1α subunit[19]. HIF-1 binds the hypoxia response element on the gene promoter of vascular endothelial growth factor (VEGF), consequently upregulating VEGF expression under hypoxic conditions (Figure 1)[35,36]. Experiments in cardiac myocytes demonstrate direct HIF-1α stabilization by mechanical stress, reinforcing the critical role ROS generation plays in TMD[37,38]. Pufe et al. used bovine cartilage discs to imply that HIF-1α induced by mechanical stress increased VEGF expression[39].

ROS-induced VEGF upregulation causes multiple degenerative consequences contributing to TMD such as cartilage degradation and bone resorption (Figure 1)[6,31]. Matrix metalloproteinases (MMPs), specifically MMP-1 and MMP-13, are known to degrade fibrocartilage collagen, which forms the cartilaginous extracellular matrix that is essential to the health of the TMJ[40]. Pufe et al. observed upregulation of MMP-1, MMP-3, and MMP-13 and downregulation of tissue inhibitors of matrix metalloproteinase-1 (TIMP-1) and TIMP-2 in articular discs following VEGF induction by mechanical overload[39]. Similarly, another experiment observed increased MMP-1, MMP-3, MMP-9 and TIMP-1 mRNA levels in chondrocytes subjected to mechanical stress[41]. Further experiments ratified these results of MMP mRNA upregulation following tensile stress[42], illustrating how ROS can affect several downstream proteins that act detrimentally to TMJ integrity.

As previously described in this article, mechanical stress can produce ROS in the TMJ. Regarding condylar bone resorption, Tanaka et al. observed increased VEGF immunopositive chondrocytes following mechanical stress in rat TMJs[43]. Furthermore, the area subjacent to the deep hypertrophic cartilage demonstrated signs of significant increase in osteoclast presence[43]. VEGF chemo-attracts osteoclasts; inhibition of VEGF function via antagonist chimeric proteins or anti-VEGF antibodies impairs osteoclast invasion[44,45]. Thus, the stimulation, differentiation, and migration of osteoclasts into cartilage are all induced by VEGF production[31,43,44]. This may result in cartilage destruction through vascular invasion, which converts cartilage into bone[31]. Therefore, the production of ROS that leads to VEGF accumulation causes physiological degradation of the TMJ.

TMJ lubrication, hyaluronic acid, and oxidative stress

The lubricative layer expedites the articulation of the various bony components of the TMJ and is thus essential to normal function[1,46]. Surface-active phospholipids (SAPLs) are the central molecules responsible for this phenomenon[1,47-49]. Their polar heads bind the articular surfaces while their dual hydrophobic moieties extend into the joint space forming hydrogen bonds[1,47]. The resulting hydrophobic interface enables low friction coefficients even under high loads[1,47]. Hills observed that washing away SAPLs and lamellar body depositions caused a 150% friction increase, confirming the importance of SAPLs in TMJ lubrication[47].

Hyaluronic acid (HA) is widely accepted to be protective in joint lubrication[1,3,50]. HA was initially presumed to directly lubricate the TMJ, but this hypothesis was overturned when hyaluronidases were observed to negligibly affect joint lubrication[51]. It was later established that polymeric HA fulfills a protective, body-guarding function for SAPLs through

dose-dependent inhibition of phospholipase A2 (PLA2)[1,49]. PLA2 degrades SAPLs and is constitutively secreted into the TMJ synovial fluid, making HA's bodyguarding function critical[1,49].

Several nuances can be applied to this general understanding of HA function. Firstly, HA can be subdivided into high-molecular weight (HMW) and low-molecular weight (LMW) varieties which are produced by three separate HA synthases (HAS)[52-57]. HAS-1 and HAS-2 polymerize HMW-HA at ~2000 kilodaltons (kDa) and are upregulated by pro-inflammatory cytokines and transforming growth factor β-1 (TGFβ-1)[56,57]. HAS-3 polymerizes LMW-HA at only 200 kDa and is upregulated by pro-inflammatory cytokines but downregulated by TGFβ-1[53-57]. HMW-HA scavenges ROS at the expense of molecular integrity more effectively and broadly than LMW-HA[58]. HMW-HA also inhibits PLA2 activity, whereas LMW-HA exacerbates it[53,58-60]. Wang et al. examined the effects of HMW-HA on the gene expression of 16 osteoarthritis-associated factors in fibroblast-like synoviocytes (FLS) from human osteoarthritic knees[61]. They found HMW-HA significantly downregulates interleukin-8, inducible nitric- oxide synthase, aggrecanase-2 and TNF-α mRNA[61]. Down-regulatory effects against all 16 tested factors were observed, yet insufficient sample size (15) hindered the significance of these results[61]. Wang et al. further demonstrated that inhibition of CD44 (HA's major receptor) with monoclonal antibodies impeded these down- regulatory effects, invoking CD44 as an intermediate in HMW-HA's gene regulatory effects (Figure 1)[61].

HA filaments aggregate non-covalently with the proteoglycans aggrecan and lubricin, this interaction is facilitated by the glycoprotein link protein (LP) [1,46,48,60,62-63]. Aggrecan is a macromolecule (~1-4MDa) which contributes to joint loading capacity, binding both HA and LP63. Lubricin acts as a water-soluble carrier of SAPLs to enable their deposition on articular surfaces1,46. Finally, LP associates with HA and both proteoglycans through separate binding domains to facilitate aggregation[60,63].

ROS interrupt this tightly-knit system in diverse ways[1,3,58,60,63,64]. For example, Roberts et al. found OH· mediates LP peptide bond cleavage[60]. They postulate OH· radicals are produced from H_2O_2 by a Fenton reaction localized at a free iron ion chelated by histidine residues within LP itself[60]. This hypothesis represents a useful microcosm illustrating how free transition metal ions (like iron) can exacerbate the negative effects of ROS. Importantly, HA suffers limited cleavage by H2O2, but more vigorous depolymerisation via OH· attack[58,60,63]. These destructive effects compromise protein integrity and cumulatively abolish all aforementioned intermolecular associations (save for that between HA and LP), thereby exposing the SAPLs to PLA2-mediated lysis[1,3,49]. Loss of SAPLs results in a thinner lubricative layer, increasing joint friction[63]. Increased friction can stress joint tendons, culminating in progressive anterior disc displacement[1,3,60,63].

Cumulatively, these studies demonstrate a significant protective, anti-inflammatory role for HMW-HA in the TMJ. This is supported by in-vivo animal studies, such as Lemos et al.'s study of osteoarthritic rat RMJs that found a decrease in adverse morphological changes and MMP activity following HMW-HA administration[65]. Manfredini et al. reviewed HMW-HA injection in humans and found a consistent and lasting reduction in pain[66]. Despite these promising results and a clear biochemical basis, HMW-HA therapy must be thoroughly compared to corticosteroids in future trials to elucidate the superior treatment[66].

Conclusion

ROS are central to the pathogenesis of TMD, yet very few articles holistically discuss the effects of oxidative stress on the TMJ[1,3-18]. To our knowledge, no review article has aimed to create a single picture broadly summarizing all molecular pathways involved in TMD- pathogenesis. Following a comprehensive review of the literature, we found that mechanical stress- induced hypoxia-reperfusion disrupts the respiratory chain reaction of mitochondria, contributing to ROS generation[10,12]. AA also plays a critical role in ROS production through the metabolism of AA into PGE2 by COX enzymes[20-21,23]. Furthermore, AA is an important leukotriene precursor[30]. Leukotrienes such as LTB4 are crucial mediators in TNF-α-induced, AA-induced, and rac-dependent ROS generation pathways[3,55]. The upregulated ROS production can increase HIF-1 and VEGF. VEGF's subsequent upregulation of vascular invasion, and MMP and osteoclast activity can degrade the cartilaginous components of the TMJ, thus compromising its structural integrity. As ROS accumulate in the TMJ they degrade HA, exposing SAPLs to PLA2- mediated lysis. This thins the lubricative layer, increasing joint friction and thereby engendering anterior disc displacement[1,3,58,60,62-64]. Based on information presented in this review it is not surprising that HMW-HA has shown therapeutic potential in both animals and humans[65-66]. Indeed, this review supports a central role for ROS in TMD pathogenesis as observed in Figure 1, revealing its importance as a target for TMD treatments.

References:

1. Nitzan, D. W. The process of lubrication impairment and its involvement in temporomandibular joint disc displacement: A theoretical concept. J. Oral Maxillofac. Surg. 59, 36-45 (2001).
2. Drake, R., Tibbitts, R., & Horn, A. Gray's anatomy for students. Philadelphia: Churchill Livingstone, Elsevier (2015).

3. Kawai, Y., & Kubota, E. Oxidative stress and temporomandibular joint disorders. Jpn. Dent. Sci. Rev. 44, 145-150 (2008).
4. Matsuo, W. et al. The scavenging effects of TMJ synovial fluids on active oxygen generation. J. Jpn. Soc. TMJ. 5, 347-355 (1993).
5. Milam, S. B., Zardeneta, G., & Schmitz, J. P. Oxidative stress and degenerative temporomandibular joint disease: A proposed hypothesis. J. Oral Maxillofac. Surg. 56, 214-223 (1998).
6. Tanne, K. Degenerative changes of articular cartilage in association with mechanical stimuli. Jpn. Dent. Sci. Rev. 44, 38-47 (2008).
7. Zardeneta, G., Milam, S. B., & Schmitz, J. P. Iron-dependent generation of free radicals: plausible mechanisms in the progressive deterioration of the temporomandibular joint. J. Oral Maxillofac. Surg. 58, 302-308 (2000).
8. Kawai, Y., Kubota, E., & Okabe, E. Reactive oxygen species participation in experimentally induced arthritis of the temporomandibular joint in rats. J. Dent. Res. 79, 1489-1495 (2000).
9. Nitzan, D. W. Intraarticular pressure in the functioning human temporomandibular joint and its alteration by uniform elevation of the occlusal plane. J. Oral Maxillofac. Surg. 52, 671-679 (1994).
10. Jaeschke, H., & Mitchell, J. R. Mitochondria and xanthine oxidase both generate reactive oxygen species in isolated perfused rat liver after hypoxic injury. Biochem. Biophys. Res. Commun. 160, 140-147 (1989).
11. Semenza, G. L. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. Biochim. Biophys. Acta. 1813, 1263-1268 (2011).
12. Lenaz, G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. IUBMB life 52, 159-164 (2001).
13. Chandel, N. S. et al. Reactive Oxygen Species Generated at Mitochondrial Complex III Stabilize Hypoxia-inducible Factor-1α during Hypoxia A MECHANISM OF O2 SENSING. J. Biol. Chem. 275, 25130-25138 (2000).
14. Kayyali, U. S., Donaldson, C., Huang, H., Abdelnour, R., & Hassoun, P. M. Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. J. Biol. Chem. 276, 14359-14365 (2001).
15. Poss, W. B., Huecksteadt, T. P., Panus, P. C., Freeman, B. A., & Hoidal, J. R. Regulation of xanthine dehydrogenase and xanthine oxidase activity by hypoxia. Am. J. Physiol. Lung Cell Mol. Physiol. 270, L941-:946 (1996).
16. Fridovich, I. Superoxide radical: an endogenous toxicant. Annu. Rev. Pharmacol. Toxicol. 23, 239-257 (1983).
17. Lee, M. C. et al. Evidence of reactive oxygen species generation in synovial fluid from patients with temporomandibular disease by electron spin resonance spectroscopy. Redox Rep. 9,331-336 (2004).
18. Tomida, M. et al. Intra-articular oxidative state correlated with the pathogenesis of disorders of the temporomandibular joint. Br. J. Oral Maxillofac. Surg. 42, 405-409 (2004).
19. Nathan, C., Cunningham-Bussel, A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat. Rev. Immunol. 13, 349-361 (2013).
20. Funk, C. Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology. Science. 294, 1871-1875 (2001).
21. Kidd B. Mechanisms of inflammatory pain. Br. J. Anaesth. 87, 3-11 (2001).
22. Feng, L. I., Xia, Y. I., Garcia, G. E., Hwang, D., & Wilson, C. B. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. J. Clin. Invest. 4, 1669-1675 (1995).
23. Tabatabaie, T., Vasquez-Weldon, A., Moore, D. R., & Kotake, Y. Free Radicals and the pathogenesis of type 1 diabetes β-cell cytokine- mediated free radical generation via cyclooxygenase-2. Diabetes. 52, 1994-1999 (2003).
24. Kerins, C., Carlson, D., McIntosh, J., & Bellinger, L. A role for cyclooxygenase II inhibitors in modulating temporomandibular joint inflammation from a meal pattern analysis perspective. J. Oral Maxillofac. Surg. 62, 989- 995 (2004).
25. Martínez-Revelles, S. et al. Reciprocal relationship between reactive oxygen species and cyclooxygenase-2 and vascular dysfunction in hypertension. Antioxid. Redox Signal. 18, 51- 65 (2013).
26. Hernanz, R., Briones, A., Salaices, M., & Alonso, M. New roles for old pathways? A circuitous relationship between reactive oxygen species and cyclo-oxygenase in hypertension. Clin. Sci. 126, 111-121 (2014).
27. Paravicini, T., & Touyz, R. NADPH Oxidases, Reactive Oxygen Species, and Hypertension: Clinical implications and therapeutic possibilities. Diabetes Care. 31, S170-S180 (2008).
28. Burke, J., & Dennis, E. Phospholipase A2 Biochemistry. Cardiovasc. Drug. Ther. 23, 49-59 (2009).
29. Cocco, T., Di, M., Papa, P., & Lorusso, M. Arachidonic acid interaction with the mitochondrial electron transport chain promotes reactive oxygen species generation. Free Radic. Biol. Med. 27, 51-59 (1999).
30. Woo, C. et al. Leukotriene B4 Stimulates Rac-ERK Cascade to Generate Reactive Oxygen Species That Mediates Chemotaxis. J. Biol. Chem. 277, 8572-8578 (2001).

31. Kuroda, S. et al. Biomechanical and biochemical characteristics of the mandibular condylar cartilage. Osteoarthr. Cartil. 17, 1408- 1415 (2009).
32. Wang, L., Lazebnik, M., & Detamore, M. S. Hyaline cartilage cells outperform mandibular condylar cartilage cells in a TMJ fibrocartilage tissue engineering application. Osteoarthr. Cartil. 17, 346-353 (2009).
33. Klinge, R. F. The structure of the mandibular condyle in the monkey (Macaca mulatta). Micron. 27, 381-387 (1996).
34. Singh, M., & Detamore, M. S. Tensile Properties of the Mandibular Condylar Cartilage. J. Biomech. Eng. 130, 011009-1 – 011009-7 (2008).
35. Forsythe, J. A. et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol. Cell. Biol. 16, 4604-4613 (1996).
36. Lin, C., McGough, R., Aswad, B., Block, J. A., & Terek, R. Hypoxia induces HIF-1α and VEGF expression in chondrosarcoma cells and chondrocytes. J. Orthop. Res. 22, 1175-1181 (2004).
37. Kim, C-H., Cho, Y-S., Chun, Y-S., Park, J- W., & Kim, M-S.. Early Expression of Myocardial HIF-1α in Response to Mechanical Stresses Regulation by Stretch-Activated Channels and the Phosphatidylinositol 3-Kinase Signaling Pathway. Circ. Res. 90, E25-E33 (2002).
38. Chun, Y-S., Kim, M-S., & Park, J-W. Oxygen-dependent and -independent regulation of HIF-1alpha. J. Korean Med. Sci. 17, 581-588 (2002).
39. Pufe, T. et al. Mechanical Overload Induces VEGF in Cartilage Discs via Hypoxia-Inducible Factor. Am. J. Pathol. 164, 185-192 (2004).
40. Nagase, H., Visse, R., & Murphy, G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc. Res. 69, 562-573 (2006).
41. Honda, K. et al. The effects of high magnitude cyclic tensile load on cartilage matrix metabolism in cultured chondrocytes. Eur. J. Cell Biol. 79, 601-609 (2000).
42. Wong, M., Siegrist, M., & Goodwin, K. Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes. Bone. 33, 685-693 (2003).
43. Tanaka, E. et al. Vascular endothelial growth factor plays an important autocrine/paracrine role in the progression of osteoarthritis. Histochem. Cell Biol. 123, 275-281 (2005).
44. Engsig, M. T. et al. Matrix Metalloproteinase 9 and Vascular Endothelial Growth Factor Are Essential for Osteoclast Recruitment into Developing Long Bones. J. Cell. Biol. 4, 879-890 (2000).
45. Niida, S. et al. Vascular Endothelial Growth Factor Can Substitute for Macrophage Colony-Stimulating Factor in the Support of Osteoclastic Bone Resorption. J. Exp. Med.. 190, 293-298 (1999).
46. Das, S. et al. Synergistic interactions between grafted hyaluronic acid and lubricin provide enhanced wear protection and lubrication. Biomacromolecules. 14, 1669-1677 (2013).
47. Hills, B. A. Oligolamellar lubrication of joints by surface active phospholipid. J. Rheumatol. 16, 82-91 (1989).
48. Schwarz, I. M., Hills, B. A. Surface-active phospholipid as the lubricating component of lubricin. Rheumatology. 37, 21-26 (1998).
49. Nitzan, D. W., Nitzan, U., Dan, P., & Yedgar, S. The role of hyaluronic acid in protecting surface−active phospholipids from lysis by exogenous phospholipase A2. Rheumatology. 40, 336-340 (2001).
50. Swann, D. A. et al. Role of hyaluronic acid in joint lubrication. Ann. Rheum. Dis. Title. 33, 318- 326 (1974).
51. Linn, F. C. Lubrication of animal joints: II the mechanism. J. biomechanics. 1, 193-205 (1968).
52. Necas, J., Bartosikova, L., Brauner, P., & Kolar, J. Hyaluronic acid (hyaluronan): a review. Vet Med (Praha). 53, 397-411 (2008).
53. Campo, G. M. et al. Effect of cytokines on hyaluronan synthase activity and response to oxidative stress by fibroblasts. Br. J. Biom. Sci. 66, 28-36 (2009).
54. Li, J. et al. Regulation of HAS expression in human synovial lining cells of TMJ by IL-1β. Arch. Oral Biol. 53, 60-65 (2008).
55. Tanimoto, K. et al. Proinflammatory cytokines regulate the gene expression of hyaluronic acid synthetase in cultured rabbit synovial membrane cells. Connect. Tissue Res. 42, 187-195 (2001).
56. Tanimoto, K. et al. Effects of TGF-β on Hyaluronan Anabolism in Fibroblasts Derived from the Synovial Membrane of the Rabbit Temporomandibular Joint. J. Dent. Res. 83, 40- 44 (2004).
57. Itano, N. et al. Three Isoforms of Mammalian Hyaluronan Synthases Have Distinct Enzymatic Properties. J. Biol. Chem. 274, 25085-25092 (1999).
58. Presti, D., Scott, J. E. Hyaluronan−mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH·) radicals is dependent on hyaluronan molecular mass. Cell Biochem. Funct. 12, 281-288 (1994).
59. Moseley, R. et al. Comparison of the antioxidant properties of HYAFF®-11p75, AQUACEL® and hyaluronan towards reactive oxygen species in vitro. Biomaterials. 23, 2255- 2264 (2002).
60. Roberts, C. R., Roughley, P. J., & Mort, J. S. Degradation of human proteoglycan aggregate induced by hydrogen peroxide. Biochem. J. 259, 805-811 (1989).
61. Wang, C. T., Lin, Y. T., Chiang, B. L., Lin, Y. H., Hou, S. M. High molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early

osteoarthritis. Osteoarth. Cartil. 14, 1237- 1247 (2006).
62. Koyama, E. et al. Lubricin is required for the structural integrity and post-natal maintenance of TMJ. J. Dent. Res. 93, 663-670 (2014).
63. Waddington, R. J., Moseley, R., & Embery, G. Periodontal Disease Mechanisms: Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis. 6, 138-151 (2000).
64. Henrotin, Y. E., Bruckner, P., & Pujol, J. P. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarth. Cart. 11, 747-755 (2003).
65. Lemos, G. A., Rissi, R., Pimentel, E. R., & Palomari, E. T. Effects of high molecular weight hyaluronic acid on induced arthritis of the temporomandibular joint in rats. Acta Histochem. 117, 566-575 (2015).
66. Manfredini, D., Piccotti, F., & Guarda- Nardini, L. Hyaluronic acid in the treatment of TMJ disorders: a systematic review of the literature. Cranio. 28, 166-176 (2010).