The Effects of Mechanical Vibration on Human Chondrocytes In Vitro

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THE EFFECTS OF MECHANICAL VIBRATION ON HUMAN CHONDROCYTES *IN VITRO*

by

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ABSTRACT
THE EFFECTS OF MECHANICAL VIBRATION OF HUMAN CHONDROCYTES *IN VITRO*

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Introduction: Inflammation is the biological basis of temporomandibular joint disorders (TMD), when severe it can lead to osteoarthritis. One of the physical therapies used to manage this condition is mechanical vibration, as has been used in medicine for many years as a non-pharmacological therapy. Recently an FDA approved dental device called AcceleDent has been introduced to increase the rate of tooth movement and decrease pain. As the device-generated vibration transmits to the TMJ, it is important for us to investigate whether mechanical vibration influences TMJ on its biologic basis – chondrocytes under normal and inflammatory conditions.

Materials and Methods: Human chondrocyte cell line C-28/I2 cells were maintained in DMEM supplemented with 10% FBS. The cells were plated at a density of $5x10^5$/well to 24-well plates for differentiation or 6-well plates for gene expression studies. For differentiation study, the cells were assigned to 4 subgroups i.e. osteogenic, normal, osteogenic + IL-1β (1ng/mL), and normal + IL-1β (1ng/mL) and subjected to vibration of 0 Hz (control) or 0.3g/30Hz. The vibration was applied for 1 hour per day for 21 consecutive days with medium refreshed every 3 days, followed by Alizarin Red staining. For gene expression study, the cells were subjected to vibration of 0 Hz (control) or 0.3g/30Hz with or without IL-1β (10ng/mL) for 1 hour, followed by quantitative Polymerized Chain Reaction (qPCR) to evaluate gene expression levels of SOX9 and MMP13 genes. One-way ANOVA was used to statistically test the difference between experimental groups ($P \leq 0.05$ considered significant).

Results: 0.3g/30Hz vibration exhibited a strong positive influence on the chondrocyte differentiation, while IL-1β showed a minimal effect. Vibration increased SOX9 mRNA expression by 1.28 fold when compared to controls. IL-1β decreased SOX9 mRNA expression by 0.65 fold which was partially recovered by the vibration to 0.83 fold. Vibration decreased MMP13 mRNA expression by 0.89 fold when compared to controls. IL-1β increased MMP13 mRNA expression by 1.44 fold, which was slightly recovered by the vibration to 1.30 fold.

Conclusions: Mechanical vibration (0.3g/30Hz) is able to increase differentiation of human chondrocytes under normal and inflammatory conditions. Mechanical vibration anabolically regulates gene expressions of SOX9 (upregulated) and MMP13 (downregulated), and can partially recover the catabolic changes of SOX9 and MMP13 induced by inflammation. Mechanical vibration (0.3g/30Hz) does not appear to harm human chondrocytes *in vitro* and may help control or reduce inflammation.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Inflammation is a common protective response by the body. However, when inflammation is chronic in nature it becomes pathologic. In dentistry some patients present with chronic or acute inflammation of the temporomandibular joint (TMJ). The temporomandibular joint is located bilaterally between the temporal bone and mandible. It is unique in that it allows for both hinge and gliding movements, and is therefore considered a ginglymoarthrodial joint. Some dental patients have complaints of pain, clicking/popping, limited functioning, headaches, or a combination of these. Clinicians must perform thorough examination along with proper diagnostic imaging to make the correct diagnosis. Treatment is very complex because of the varied etiology of temporomandibular disorders (TMD). One of the more common etiologies of TMD is inflammation, which is seen in degenerative joint disease, or osteoarthritis (OA). Symptoms of OA include pain, stiffness, and swelling (arthritis.org). Currently there is no cure for OA. OA primarily affects joints of the knees and hips in the elderly population. This disease is not only limited to these specific locations in the elderly but also other areas of the body including the TMJ region in people of all ages. Treatment methods for OA of the TMJs seek to alleviate pain and allow for improved functioning. Some treatments include medications, physical therapy, occlusal appliances, stress reduction, and surgery. Since inflammation appears to be one of the main pathogenic mechanisms of articular cartilage breakdown, anti-inflammatory medications are often prescribed as a way to help slow the progression of OA. Medications must be evaluated not only

regarding their ability to slow the progression of disease but also their effect on the metabolism and reparative nature of the cell involved. Greco et. al found that Naproxen, a non-steroidal anti-inflammatory drug (NSAID) decreased IL-1β induced catabolic activity on chondrocytes. They also found that Naproxen reduced the expression of genes involved in matrix production such as collagen 2A1 in resting conditions. Physical therapy is also used often in combination with medications to treat OA. Dentists often prescribe occlusal appliances, such as splints in an attempt to reduce proprioception and help patients find a more stable joint position, centric relation. This approach also helps reduce the wear and tear of the dentition from parafunction. Stress is also considered a contributing factor to the progression of temporomandibular joint disorders and thus stress reduction may help patients with symptoms. Surgery is typically the last approach taken to treat TMD when all other methods have failed. Although these methods prove useful in some patients, they may only provide temporary relief for others. Research in the future should be directed at determining the molecular mechanism at which OA is initiated and progresses to help provide better options for treating OA. At the base of the molecular mechanism is the chondrocyte, a specialized cell type found in articular cartilage tissues. Chondrocytes are unique cells that are required for articular cartilage formation. These cells are derived from mesenchymal stem cells and are regulated by multiple cytokines and transcription factors (Lin Z, et. al, 2006). They do not contain blood vessels and nerves and are therefore not well equipped to repair or regenerate. Identifying molecules for chondroprotection is currently a priority in modern medicine (Greco K, 2011). Chondrocytes are found throughout the entire body including the temporomandibular joints. These joints are of particular importance to dental

professionals and the population who suffer from TMD. The National Institutes of Health estimated that in 1996, in the United States more than 10 million people suffered from TMD (NIH, 1996). Due to the increase in the aging and general population that number is likely getting even higher today. It is critical for practitioners to develop a proper classification of the varied types of TMD as well as to target on the etiological factors contributing to the disorder.

Mechanical vibration has been studied and used as treatment for muscle and bone deficiencies especially in post-menopausal women suffering from osteoporosis. Mechanical vibration has been shown to enhance bone mineral density in astronauts who have been in space for an extended period of time (LeBlanc A, et. al, 2000). Being in space vastly reduces the amount of peripheral loading that can be done and the mechanical vibration serves as a way to load tissues. Vibration does not only affect muscle and bone but a multitude of tissues in the body. Mechanical vibration has been shown to enhance chondrocyte proliferation (Kaupp JA, Waldman SD, 2008). Mechanoreceptors located within the chondrocytes seem to elicit a cellular response; the exact mechanism of this response is not fully understood. A better understanding of this mechanism may gain insight into the functioning of chondrocytes and provide clinicians with better options to treat diseases of articular cartilage damage. Whole body vibration (WBV) continues to be a well-researched area in medicine and may prove to be promising for treating a variety of diseases in both medical and dental fields.

Mechanical vibration has recently been introduced to the field of orthodontics. A commercially available device called AcceleDent (by OrthoAccel Inc.) claims to increase the rate of orthodontic tooth movement and decrease pain by applying cyclical vibrations

to the dentition (www.acceledent.com). There is however conflicting evidence about the effectiveness of applying vibration to both increase the rate of orthodontic tooth movement and decrease pain experienced during orthodontic treatment. Besides focusing on the effectiveness of this product it is also critical to review the safety of it. The device is FDA-cleared but little evidence exists regarding its effects on TMJ. This device is currently used by orthodontists and dentists who commonly see patients who could suffer from TMD. When applied, the vibration from AccleDent device is not only localized to the dentition but also transmitted to the surrounding tissues including the TMJ (Liu D, 2012). It would be doing an injustice to patients being treated with this device to not further evaluate the effects of mechanical vibration on the temporomandibular joints. Since mechanical vibration has been shown to have a positive influence on chondrocyte proliferation (Kaupp, J.A., Waldman, S.D. 2008), we would like to evaluate the effects of mechanical vibration applied at the same frequency used by AcceleDent, to determine whether this vibration has a positive, negative, or no influence on these cells, *in vitro*.

Based on current knowledge, we hypothesize that mechanical vibration when applied under certain circumstances can possibly be used as a therapy for treating patients with TMD. The proposed mechanism under investigation is that vibration reduces inflammation within the temporomandibular joint. Our aim is to investigate whether there is a response at the cellular level to mechanical vibration of human chondrocytes *in vitro*.

CHAPTER 2
LITERATURE REVIEW

Inflammatory process

Inflammation is famously known for its four cardinal signs as described by Celsus in 30 A.D.: rubor, calor, dolor, and tumor. These are also known as redness, heat, pain, and swelling, respectively. The goal of the body is to achieve tissue integrity and homeostasis (Martin 1997; Singer and Clark, 1999). The process to reach this homeostasis includes inflammation, tissue formation, and tissue remodeling. Cells interact with one another as well as cell matrices to perform this task (Eming, Krieg, 2007). People have been dealing with inflammation since the beginning of time. In an attempt to treat this condition people would use extracts from willow leaves, which contain salicilin. Salicylic acid was chemically synthesized in 1860 in Germany and 39 years later Bayer's research director Dr. Heinrich Dresser introduced it in 1899 as Aspirin. Today there are multiple drugs used to treat inflammation, most notably a group known as NSAIDs, or non-steroidal anti-inflammatory drugs. Inflammation is caused by a release of chemical mediators from tissues and migrating cells. Some of these mediators include prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor, and interleukin-1 (Vane J, and Botting R, 1987). NSAIDs work primarily by inhibiting cyclooxygenase (COX), which is an enzyme that catalyzes prostaglandins. There are two main COX isoenzymes: COX-1 and COX-2. COX-1 is responsible for catalyzing prostaglandins that protect the stomach and kidney. COX-2 is responsible for responding to inflammatory stimuli (Vane J, and Botting R, 1998).

Osteoarthritis (OA) is the most common type of arthritis. It is also known as degenerative joint disease and occurs when cartilage breaks down. This disease is typically slowly progressing and seen most often in the elderly population. Patients with OA have a breakdown of cartilage between the bones in the joint. Also, the affected bones tend to slowly get bigger. Symptoms of OA include joint pain, stiffness, decreased function, and swelling (rheumatology.org). Etiologies include those of both a biochemical and biomechanical nature. When mechanical loading of a joint is well below or above the normal physiologic range cartilage destruction tends to occur. Genetic, dietary, estrogen levels, bone mineral density, muscle weakness, obesity, and joint laxity have all been reported as risk factors relating to osteoarthritis (Ann Intern Med. 2000). There are many factors that lead to degradation of cartilage including direct or indirect modulation of anabolic and catabolic factors. Matrix metalloprotease (MMP) 13 is one of the key catabolic factors in OA because of its ability to degrade collagen and other matrix components (Liang Z, et. al, 2012). MMP13 is one of the most abundant proteinases and has been found to regulate cell migration, alterations in the ECM, and apoptosis in growth plate cartilage (Malemud CJ, 2006). Daily knee loading has been shown to suppress cartilage destruction in mice with surgically induced osteoarthritis. MMP13 levels were found to be elevated in mice with OA. Knee loading reduced MMP13 activity and thus reduced cartilage destruction (Hamamura K, et. al, 2013). Alcaraz M, et. al recommended selective inhibitors of matrix metalloproteinases, aggrecanases, and other proteinases as a therapeutic approach in treating OA. They also recommended promoting anabolic factors by using growth factors and other regulatory molecules.

OA is typically thought of affecting the knee and hip joints. However, in the field of dentistry OA has been studied in regard to the temporomandibular joints. Some findings of OA associated with the TMJ include mandibular condyle flattening, deformity, sclerosis, degeneration of the disc, and areas of erosion of the articular cartilage of both the condyle and temporal bone (Gidarakou K, et. al, 2003). Dental professionals are evaluating and treating these joints everyday. One common sign is disc displacement, which often presents as a clicking sound on opening or closing of the mandible. This displacement may progress to OA of the TMJ over time (Westesson P, et. al, 1984). Kubota et. al reported that IL-1β levels in synovial fluid of the TMJ have a positive correlation with OA change. Their findings suggest that IL-1β levels in the TMJ may be an important marker for early detection of bone deterioration that are not detectable by radiographs. Another study by Alstergren et. al showed that IL-1β in the synovial fluid is associated with pain and hyperalgesia of the TMJ region. They concluded that IL-1β seems to be a warning signal of tissue destruction. IL-1β is often used in experiments to create an environment that mimics inflammation. Some treatment options for OA of the TMJ include medications such as Ibuprofen and Glucosamine sulfate. One study comparing these two medications showed that both have a positive influence with regard to pain suffered from OA (Thie N, et. al, 2001). This indicates that anti-inflammatory medications are one of the best options when the TMD is inflammatory in nature. It is very difficult to make a definitive diagnosis of TMD because there are many different presentations and etiologies of the pain and dysfunction. In a meta-analysis by Kim MR, et. al, they stated that a reliable and valid diagnostic classification system for TMD is needed to conduct better research in the future. In

general, TMD falls under two categories in which the disorder is believed to have originated: joint and muscular. Joint problems arise from within the joint while muscular problems often involve the muscles of mastication. It is extremely difficult to diagnose the type of TMD a patient is suffering from, and may be even more difficult to treat. As mentioned previously medications are one form of treatment. Other common types of treatment involve occlusal appliances such as splints, stress reduction, physical therapy, and surgery. For patients undergoing orthodontic or dental treatment it is necessary to perform a thorough exam prior to initiating treatment, this must include the temporomandibular joint regions. It should also be of interest to the clinician whether the prescribed treatment plan and appliances used have an influence on the TMJs.

Chondrocytes

Chondrocytes are a special type of cell found in articular cartilage tissue. They are crucial for cartilage formation and functionality. Chondrocytes are derived from pluripotent mesenchymal stem cells and regulated by various cytokines and transcription factors (Lin Z et. al, 2006). Human chondrocytes are typically round cells, they represent about 5-10% of the total cartilage volume (Hunziker EB, et. al, 2002). Adult cartilage does not contain blood vessels or nerves and is therefore not well equipped for wound healing and regeneration. Constant mechanical loading is necessary for normal functioning of the joints where cartilage covers the surfaces of bones (Liu J, et. al, 2001). When the constant mechanical loading is diminished the cartilage will breakdown, as is the case with osteoarthritis. Many studies have looked at mechanical vibration as a means

to produce constant loading to cartilage. Several of these studies have shown beneficial results but are difficult to make generalizations because of differences in experimental design (i.e. frequency, magnitude, subjects, etc.). The exact mechanism where an external mechanical stimulus leads to changes in internal cell signaling is not fully understood. Mechanical stresses have been shown to directly alter cellular processes including gene expression, signal transduction, growth, and differentiation *in vitro* (Chen CS, Ingber DE, 1999). There are a number of potential mechanoreceptors that respond to vibration within chondrocytes such as integrins, connexins, stretch-activated ion channels, and cilia (Lee, HS and Salter, DM). These receptors help communicate the cells extracellular environment to the internal environment.

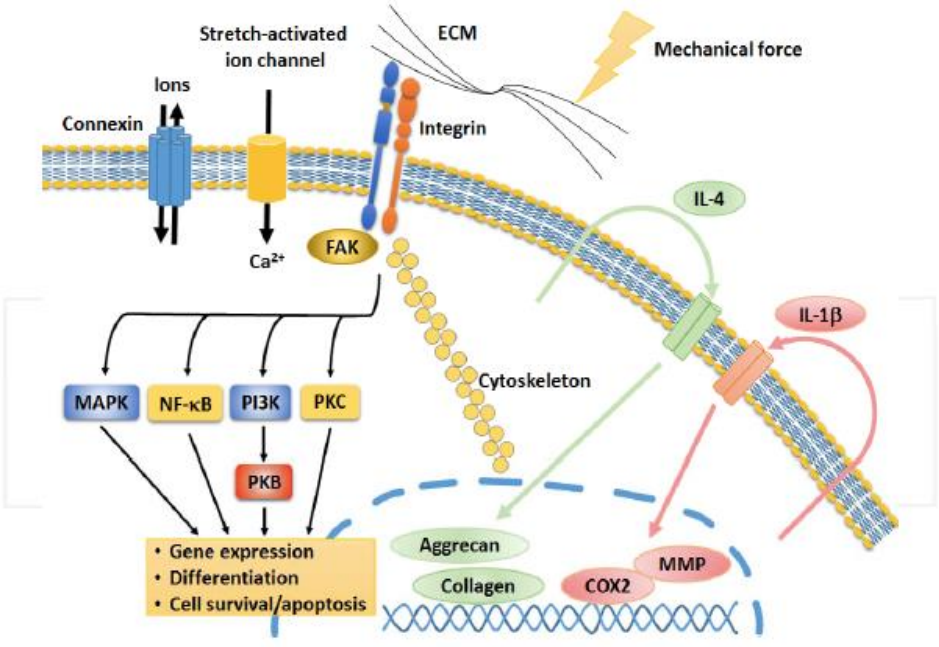

Figure 1: The major mechanotransduction pathways in chondrocytes (Lee, HS and Salter, DM).

A primary function of chondrocytes is to maintain the extracellular matrix (ECM) through catabolic and anabolic processes. There are two main components to the ECM, type II collagen and aggrecan. Type II collagen gives cartilage its tensile strength while aggrecan provides osmotic resistance to resist compressive loads (Knudson CB and Knudson W, 2001). Severe compressive loading can damage the ability of the chondrocyte to repair the ECM. Chondrogenesis occurs during embryo development. The process of chondrogenesis is regulated by multiple transcription factors, of which SOX9 is considered to be the main regulator of chondrocyte differentiation. This transcription factor is required to maintain the chondrocytic phenotype (Bi W, et. al, 1999). An experiment by Papadopoulou et. al involved animals who were subjected to either a hard (normal loading) or soft (unloading) diet. They postulated that reduced levels of SOX9 immunoexpression could be associated with increased loading conditions. In yet another study, Xiong et. al found enhanced SOX9 expression in condylar cartilage of rats that underwent mandibular advancement (unloaded condition). SOX9 promotes cell survival and activates the genes for many cartilage specific components and regulatory factors (Lefebvre V, 2016). In articular cartilage, chondrocyte maturation is arrested which prevents further differentiation towards a terminal hypertrophic state. In OA articular chondrocyte homeostasis is disrupted and chondrocytes begin to undergo endochondral ossification. MMPs also act as an initiating factor leading to endochondral ossification, vascular invasion, and altered bioavailability of growth factors and chondrocyte apoptosis (Borzi et. al, 2010). MMP13 has been shown to have direct angiogenic activities that are associated with OA severity and clinical disease activity (Bonnet CS et. al 2005; Walsh DA et. al, 2007). A potential

therapy to treat OA could be to target MMP13 in hopes to maintain homeostasis and prevent unwanted vascularization of mature articular cartilage.

Studies using human primary articular chondrocytes are limited because they are difficult to obtain, the cartilage cannot be controlled, and the number of cells is not adequate. After the primary cells are isolated they show very little proliferative capacity (Sabatini, 2004). If they do show proliferative activity it usually indicates a loss of differentiated phenotype. The loss of phenotype includes a change in cell shape and also a change in the pattern of gene expression (Stokes DG, et. al, 2002). To avoid these limitations most experimental designs utilize immortalized chondrocyte cell lines. Thus a good model for studying human cartilage is to use immortalized human chondrocytes. Viruses SV40-TAg and HPV-16 have been used to immortalize these cells. One of the negative effects of using these viruses to create study models is the stable integration of immortalizing genes disrupts the normal cell-cycle control but does not stabilize expression of the type II collagen gene (COL2A1). This is the most sensitive marker of the differentiated chondrocyte phenotype (Sabatini, 2004). Three immortalized cell lines are commonly used: T/C-28a2, C-28/I2, and T/C-28a4. The latter two are derived from the first. These cells are said to retain their morphology and maintain continuous proliferation in monolayer culture (Finger F, et. al, 2003). C-28/I2 cells display the highest levels of matrix anabolic and catabolic genes and are therefore preferred to be used for investigating anabolic and catabolic activity and regulation. Of the three above mentioned cell lines, C-28/I2 showed the highest SOX9 levels, indicating that this cell line most closely resembles primary chondrocytes. However, Finger F. et. al also states that none of these cell lines appear to be a direct substitute for primary chondrocytes.

Temporomandibular Joint

Temporomandibular joint (TMJ) is where mandible articulates with cranium, specifically temporal bone. This joint is present on both the right and left sides and functions as a single unit. TMJ is very complex as it allows for both hinge and gliding movements, therefore considered as a ginglymoarthrodial joint. Located between the condyle of mandible and glenoid fossa of temporal bone is an articular disc. This disc is composed of dense fibrous connective tissue. Attached to the posterior aspect of the disc is an area known as retrodiscal tissues. This is a region of loose connective tissue that is highly vascularized and innervated. Capsular ligaments are attached both anterior-superiorly and anterior-inferiorly to the disc. These anterior attachments are composed primarily of collagen fibers. Another anterior attachment to the disc is made with the superior head of the lateral pterygoid muscle. The capsular ligament is also attached medially and laterally to the disc, which separates the TMJ into a superior joint space and inferior joint space. These spaces are composed of highly specialized endothelial cells forming a synovial lining. The synovial lining produces synovial fluid, which acts as a lubricant as well as a facilitator for providing metabolic requirements (Okeson, 2008).

The surfaces of condyle and temporal fossa are composed of four layers or zones. The most intimate layer with the bony articulations is termed the articular zone. This is the functional surface. It is unique from other joints because it is composed of dense fibrous connective tissue and not hyaline cartilage. Some benefits to this are the dense fibrous connective tissue is less susceptible to the effects of aging and it has a better ability to repair itself. The second zone is the proliferative zone. This area contains

undifferentiated mesenchymal tissue. This area responds to the functional loads placed on the joints. The third zone, or the fibrocartilaginous zone, allows resistance to compressive and lateral forces. The fourth and last zone is known as the calcified cartilage zone. In this zone there are chondrocytes and chondroblasts. When these cells die their contents are evacuated and replaced by bone cells (Okeson, 2008).

The articular cartilage is composed of chondrocytes and intercellular matrix. The chondrocytes are responsible for producing collagen, proteoglycans, glycoproteins, and enzymes that help form the matrix. The chondrocytes play a critical role for proper functioning and maintenance of the TMJs (Okeson, 2008).

Clinical applications of mechanical vibration

Significant bone loss is often seen in astronauts who have been in space for extended periods of time, due primarily to a lack of peripheral loading (LeBlanc A, et. al, 2000). Vibration therapy is used in medicine as a non-pharmacological analogue of physical activity. Its purpose is to promote bone and muscle strength in individuals who are frail (Thompson W, et. al, 2014). With a growing number of the aging population come many challenges to the healthcare field. One such challenge is treating osteoporosis. Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Consensus Development Conference, 1993). Typically as people age their mobility and muscle function decreases. An ideal treatment would be to increase the loading of bones by increasing the amount of

exercise an individual does. Exercise and other types of physical activity however become extremely difficult for frail individuals. The theory of vibration therapy is to target the musculoskeletal system in ways that mimic physical activity. This type of therapy is very beneficial since it does not involve the use of any pharmacological agents. Many elderly patients are taking multiple medications and would not be subjected to additional drugs, which may have adverse effects. One study evaluated the effects of low-intensity whole body vibration (WBV) on bone mineral density (BMD) in humans (Rubin C, et. al, 2004). The study specifically looked at postmenopausal women subjected to 2x10 minutes of low intensity (0.2g, 30Hz) and a control group (placebo plate). The control group was found to have lost 2% femoral neck BMD while the treatment group gained 0.04% BMD. Another study looked at the ability of WBV to augment the anabolic effects of dynamic exercise. They found that the vibration group had an enhanced effect of training to increase lumbar BMD (Gomez-Cabello A, et. al, 2014). The experimental group was also found to suffer less falls than the control group. Studies have shown that children with immobility-associated disabilities may also benefit from low intensity vibration therapy (Ward K, et. al, 2004). There appear to be many indications for mechanical vibration in medicine. The mechanotransduction pathways need to be further studied to gain a better understanding of which patients specifically would benefit the most from mechanical vibration.

In the field of orthodontics vibration has recently been introduced as an adjunctive to decrease treatment times and reduce the pain experienced from orthodontic treatment. Conflicting evidence exists regarding the effectiveness of using mechanical vibration to decrease treatment times. A study by Leethanakul C, et. al concluded that in combination

with orthodontic force, vibratory stimuli increased the secretion of IL-1β in gingival crevicular fluid and also appeared to increase the bone resorption and accelerate orthodontic tooth movement. The vibrational source for their study was an electric toothbrush vibrating at 125Hz and patients were instructed to apply the vibrational source to specific teeth for a minimum of 5 minutes, 3 times per day for 2 months. There are other sources that can be used to apply vibrational force to the dentition. AcceleDent is an FDA cleared, class II medical device that applies mechanical vibration to the dentition with the goal of speeding up orthodontic tooth movement and decreasing the amount of pain with treatment. In a study by Pavlin D, et. al, they found that vibrational forces applied at 0.25N (25g) and a frequency of 30Hz to the dentition led to an increase in the speed of orthodontic tooth movement. The study involved space closure rates in maxillary first premolar extraction cases. They concluded a significant difference in tooth movement between the vibration and non-vibration groups (1.16 mm/month vs. 0.79 mm/month). Kau CH, et. al reported seeing 2-3 mm/month of tooth movement with mechanical vibration. Their study did not include control groups but they stated that "conventional wisdom regarding normal rates of tooth movement are about 1 mm of movement per month." Bowman SJ, et. al concluded that AcceleDent reduced the time needed for dental alignment and leveling. He also stated that there was a 30% increase in the rate of tooth movement during leveling of the mandibular arch. However, in a prospective randomized clinical trial Woodhouse NR, et. al found no evidence showing that vibrational force can significantly increase the rate of tooth movement during orthodontic alignment. They conducted their study using three groups: AcceleDent group, a sham group with a non-vibrating replica of the AcceleDent device, and fixed appliances

only. All cases were first premolar extraction cases. They evaluated the rates of orthodontic teeth for alignment in the mandibular arch with fixed appliances and found no statistical difference between the three groups. Albeit there is conflicting evidence regarding the effectiveness of mechanical vibration as a supplement to orthodontic treatment, seemingly more orthodontists and dentists are using these devices to treat patients. Along with the efficacy of mechanical vibration treatment in orthodontics, it is also important to consider the safety of using such devices. It is very appealing to clinicians to be able to decrease the length of treatment and achieve the same results. However, one of the rules orthodontists should follow is to do no harm. Our study is attempt to evaluate if any deleterious effects are placed on human chondrocytes *in vitro* from mechanical vibration. Another objective of the study is to evaluate whether there is a reduction of inflammation when cells are subjected to mechanical vibration at a frequency similar to that of AcceleDent.

With its increasing use, it is crucial to critically evaluate any possible side effects of AcceleDent. One area that has not been thoroughly looked at is the temporomandibular joint responses to mechanical vibration. The mechanical vibration force from AcceleDent is transmitted not only to the dentition but also the TMJ region. In a study using a scanning laser Doppler vibrometer, it has been shown on a dry skull that the vibrations from AcceleDent can be transmitted to the TMJ area (Liu D, 2013). The overall aim of our study is to evaluate the effects of mechanical vibration on the cultured human chondrocytes *in vitro.*

CHAPTER 3
MATERIALS AND METHODS

This study was conducted using the human chondrocyte cell line C-28/I2 (Finger F, et. al, 2003), a gift from Dr. Mary Goldring (Hospital for Special Surgery, NY). The mechanism by which vibration exerts its effect on human chondrocytes was evaluated by looking at cell differentiation and gene expression of different chondrocyte markers involved in cell regulation. The effect of vibration on human chondrocytes was evaluated by comparing differentiation between chondrocytes at 0 and 30Hz, with or without presence of IL-1β. Gene expressions of MMP13 and SOX9 were also analyzed before and after vibration and with or without presence of IL-1β.

Human chondrocyte cell line C-28/I2

Human chondrocyte cell line C-28/I2 was used because it has been suggested that these cells may be the best among the cell lines to investigate matrix anabolism and catabolism in chondrocytic cells (Finger F, et. al, 2003). These cells do not exactly replicate those of primary chondrocytes but are commonly used in experiments because of the difficulty in attaining large quantities of primary chondrocytes. The chondrocytes were routinely maintained in DMEM with 10% FBS and 1% Penicillin/Streptomycin.

Mechanical vibration setup

When ready, the C-28/I2 cells were plated and placed onto a rigid platform that was custom made to fit a standard multi-well tissue culture plate. The base (ThorLabs Max Series Modular Flexure Stage, with a DRV120 actuator) was capable of delivering measured amounts of vertical vibration generated by a modular piezoelectric device. Vibration (30Hz) was delivered by a function generator (Instek: Model FG 8015G). A current amplifier (Advanced Motion controls, Camarillo CA, Model Brush Type PWM Servo Amplifier) delivered 0.3g of acceleration to the vibration plate. An accelerometer was used to confirm the correct amount of vibration was being transmitted to the plate of the cells (Endevco). The setup was housed in a thermos box of 37^{o}C. Pictured below is a similar device, used by Kulkarni et al, 2013.

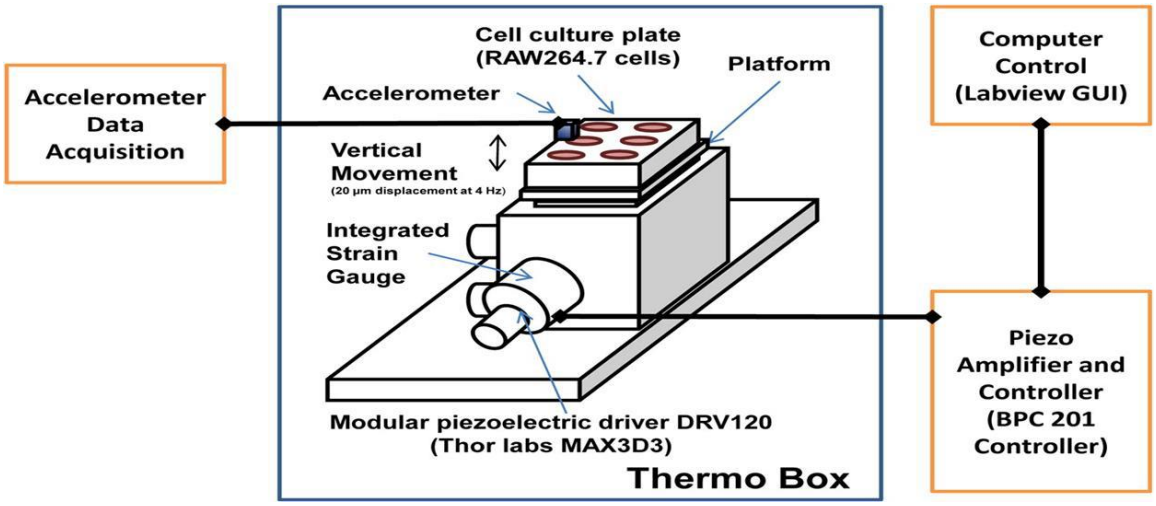

Figure 2: Mechanical vibration system composed of 1) Vibration generator, 2) Modulator, and 3) Accelerometer.

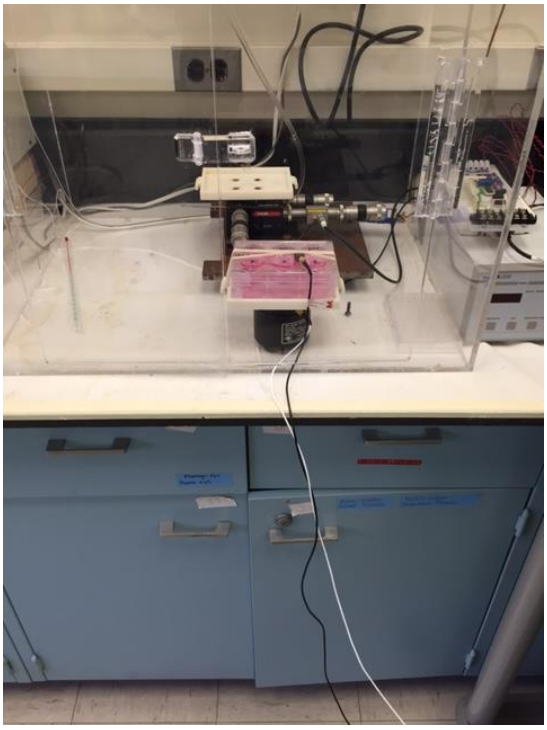

Figure 3: Vibration plate with accelerometer attached for measuring the delivery of mechanical vibration.

1) Cell differentiation Assay

Human chondrocyte cell line C-28/I2 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin, while the differentiation medium contained additionally 1% Liquid Media Supplement (ITS) and ascorbic acid (50µg/ml).

For differentiation study, the cells were seeded on 24-well cell culture plates at a density of $5x10^5$/well, and assigned within the plate to the study groups shown below (Table 1). After confluence, the cells were subjected to 0.3g/30Hz mechanical vibration or treated as sham control, 1 hour per day for consecutive 21 days. Culture medium was refreshed every 3 days.

Osteogenic medium	Osteogenic medium + IL-1β
Normal medium	Normal medium + IL-1β

Table 1: Representation of how 24-well plates were assigned for the study of cell differentiation.

To examine the levels of differentiation of C-28/I2 cells, Alizarin Red staining method was used: the cells were rinsed with PBS once. Then 1mL of 10% formalin was added to each well and fixed for 15 minutes at room temperature. Each well was washed with double distilled water (dH2O).

The staining protocol was as follows: add 500µL 40mM ARS (pH 4.1) per well, incubate the plates at room temperature for 20 min with gentle shaking, wash wells 4 times, 5 minutes each time, with 1mL dH2O. The results of staining were observed under a reverse microscope and scanned into .tiff files by using Photoshop software (version 5.0).

2) Gene Expression

Human chondrocyte cell line C-28/I2 cells were cultured and seeded onto 6-well culture plates. The chondrocytes were routinely maintained in complete DMEM medium (Sigma) with 10% FBS and 1% Penicillin/Streptomycin.

Prior to gene expression experiment, viability test was done using Trypan blue exclusion assay to rule out the possibility of cell death before and after vibration, which can bias the interpretation of gene expression results.

Four groups were evaluated in the study. The experiment was designed as follows:

(-)IL-1β, (-) Vibration (control)	(-)IL-1β, (+) Vibration
(+)IL-1β, (-) Vibration	(+)IL-1β, (+) Vibration

Table 2: Experimental design for evaluation gene expression.

The experimental groups were placed onto the vibration apparatus described above and subjected to 0.3g/30Hz for 1 hour. IL-1β was added to the designated group right at the start of vibration at a concentration of 10ng/mL. The control group was placed onto the vibration apparatus but subjected to 0g/0Hz for 1 hour. The cells were lysed immediately after 1 hour of vibration.

RNA extraction and Reverse transcription reaction.

Total RNA was extracted from the chondrocytes using TRIzol, following the protocol from the manufacturer (Bio-RAD). Next, the RNA was treated with DNase (Life Technologies Corporation) to remove any genomic DNA. A spectrophotometer was used to determine the concentration of the total RNA using the formula:

Concentration of RNA= OD260 x 40 (constant) x dilution factor (ug/ml)

After DNase treatment, reverse transcription was performed using Oligo (dT) 20 primers and SuperScript II as instructed by the manufacturer (Life Technologies Corporation).

End-point PCR

End-point PCR was performed to validate the primers used in the qPCR. The end-point PCR reaction was run at 94°C for 2 minutes; followed by 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds. 32 cycles were performed. Then the reaction was run at 72°C for 10 minutes and finally 4°C. Taq polymerase was used for the reaction.

Real-time PCR (qPCR)

All qPCR was run using the StepOne real-time PCR system (Applied Biosystem). For analysis, reactions containing SYBR Green Mix with ROX (MIDSCI), 20µM of forward and reverse primers, and cDNA were loaded into the wells of a 48-well plate on ice. The cycling parameters were 95°C for 10 minutes for enzyme activation followed by 40 cycles of 95°C (15s) and 60°C (60s), and then a melting curve to ensure amplification of a single product. All reactions were performed in triplicate and the mean threshold cycle (ct) for each gene product for each sample was used for analysis. The mRNA levels of target genes were normalized to that of a housekeeping gene GAPDH.

Primer sequences were as follows:

MMP13 (F)	5'TTACCAGACTTCACGATGGCATT3'
MMP13 (R)	5'TCGCCATGCTCCTTAATTCC3'
SOX9 (F)	5'CGCCATCTTCAAGGCGCTGC3'
SOX9 (R)	5'CCTGGGATTGCCCCGAGTGC3'
GAPDH (F)	5'GAAGGTGAAGGTCGGAGTC3'
GAPDH (R)	5'GAGATGGTGATGGGATTTC3'

Table 3: Sequences of primers used for human chondrocytes.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA. A p-value less than or equal to 0.05 was considered statistically significant. Tukey post hoc was performed to find the significant differences between groups. Descriptive statistics were also presented for all experimental groups (SPSS version 23).

CHAPTER 4
RESULTS

Cell Differentiation

Cell differentiation is seen when a cell becomes more specialized. Cell differentiation of each cell type is controlled by a specific program of gene expression. This is the result of gene expression being either up-regulated or down-regulated. This in turn will affect how the cell can function. In the case of TMJ chondrocytes, this differentiation may allow for the cell to better repair itself from injury as well as maintain its ability to function properly. In addition, the cells showed more differentiation in osteogenic medium as expected (Fig. 4, 5). The results of our study indicate that mechanical vibration (0.3g/30Hz) increases cell differentiation of human chondrocyte cell line C-28/I2 cells *in vitro* when compared with controls (Fig. 4, 5). The presence of IL-1β (1ng/ml) seemed to have minimal impact on differentiation of the cells. A large difference in the amount of differentiation occurred when comparing the sham group (no vibration) with the mechanical vibration group and also between the two types of growth medium. Overall, our study shows that cultured human chondrocytes in osteogenic medium are able to differentiate and enhanced by vibrational stimulation. There was no significant effect of IL-1β on the differentiation of the C-28/I2 cells.

Sham Group (No vibration)

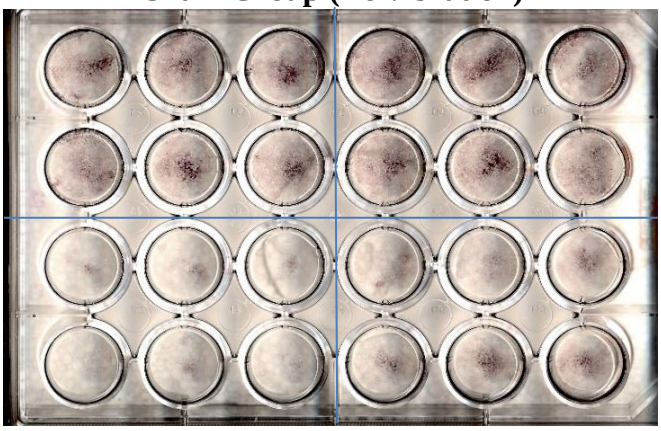

Figure 4: Differentiation of human chondrocytes in the sham (control) subjected to no mechanical vibration.

30 Hz Vibration

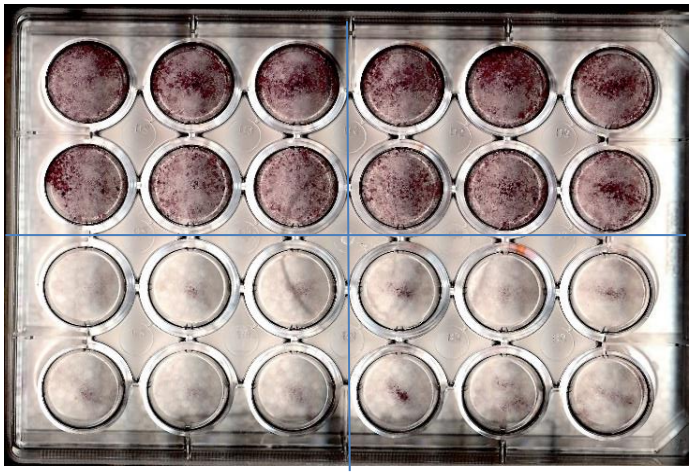

Figure 5: Differentiation of human chondrocytes in the sham (control) subjected to 0.3g/30Hz mechanical vibration.

Osteogenic medium	Osteogenic medium + IL-1β
Normal medium	Normal medium + IL-1β

Table 4: Layout of the subgroups of 24-well plates for study of cell differentiation.

Gene Expression

Validation of PCR primers:

End-point PCR reactions were done for quality assurance that the primers (GAPDH, SOX9, and MMP13) were working properly (Fig. 6, 7). The end-point PCR reaction measures the amount of accumulated PCR product at the end of the PCR cycles. Superscript II enzyme in the reaction system is for reverse transcripting mRNA to cDNA which is further amplified through PCR. Without Superscript II enzyme the observed gel bands represented contamination of genomic DNA, based on which DNAse was used to purify the RNA samples before RT-PCR reaction. With Superscript II enzyme, the single gel bands represented the signals of the targeting genes. Once the primers' validity was proved by the end-point PCR, qPCR reactions were used because of their higher precision, sensitivity, and quantitative results. The data from the end-point PCR testing are qualitative and presented below.

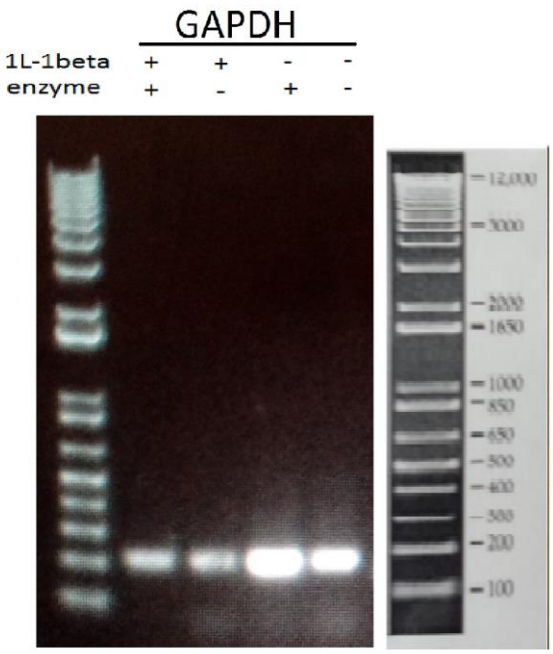

Figure 6: End-point PCR testing of housekeeping gene, GAPDH.

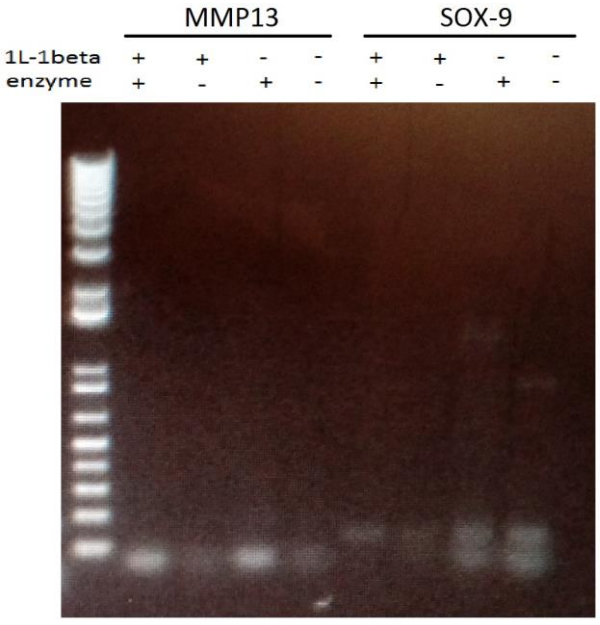

Figure 7: End-point PCR testing of primers MMP13 and SOX9.

Relative expression of SOX9 and MMP13:

Raw data calculated from qPCR machine were expressed in cycle threshold (ct) values. These values represent an area near the beginning of the exponential phase of the reaction. The ct values indicate how many cycles were needed to detect an indicative signal from the samples. The ct values were then used to calculate relative fold difference with controls using the equation: $2^{-\Delta\Delta CT}$.

SOX9 expression (ct value)								
gene	**SOX9 expression (ct value)**				**GAPDH(ct value)**			
group	**control**	**IL-1β**	**30HZ**	**IL-1β+30 HZ**	**control**	**IL-1β**	**30HZ**	**IL-1β+30 HZ**
Exp 1	20.5328045	21.2894325 3	19.3510417 9	19.7957592	12.8309946	12.2347937	12.9766902 9	12.0514497 8
	20.5441399	20.6605262 8	19.1927623 7	19.7891883 9	12.8095007	12.0801859	12.7390794 8	12.0992155 1
	20.5231533	20.5846614 8	19.1123104 1	19.6625919 3	12.6688004	12.0592222	12.5271959 3	12.2139654 2
Exp 2	15.4700317	15.7888040 5	15.8891954 4	15.5283470 2	14.5096979	13.9686432	14.1053361 9	13.9315385 8
	15.2507124	16.4003143 3	16.2890300 8	15.3431043 6	14.3530769	13.9190626	13.8963031 8	13.9475002 3
	15.051898	15.8334941 9	16.3664913 2	15.5521001 8	14.3139286	13.6542721	13.8315219 9	14.2096433 6

Exp 3	19.9958019	20.46 00563	20.01 89743	20.34 64202 9	13.34 1119 8	13.28 6344 5	12.17 72613 5	12.83 16240 3
	20.0295467	19.90 028	20.03 09639	20.34 50050 4	12.67 2685 6	13.30 0914 8	12.55 28049 5	12.51 35860 4
	20.0123425	19.84 20009 6	19.92 65937 8	20.22 98240 7	12.40 8614 2	13.14 1422 3	12.87 21838	12.71 69256 2

Table 5: SOX9 expression ct values from qPCR analysis.

gene	**SOX9 expression**			
group	**control**	**IL-1β**	**30HZ**	**IL-1β+30HZ**
Exp 1	1	0.515291691	2.449608326	1.098827124
Exp 2	1	0.129035592	0.540064692	0.627398729
Exp 3	1	1.301827788	0.839147627	0.75022018
mean	**1**	**0.648718357**	**1.276273549**	**0.825482011**
SD	**0**	**0.597672478**	**1.027082531**	**0.244559686**

Table 6: qPCR findings, results indicate fold difference when compared with controls. The formula $2^{-\Delta\Delta CT}$ was used to calculate results.

SOX9 Expression

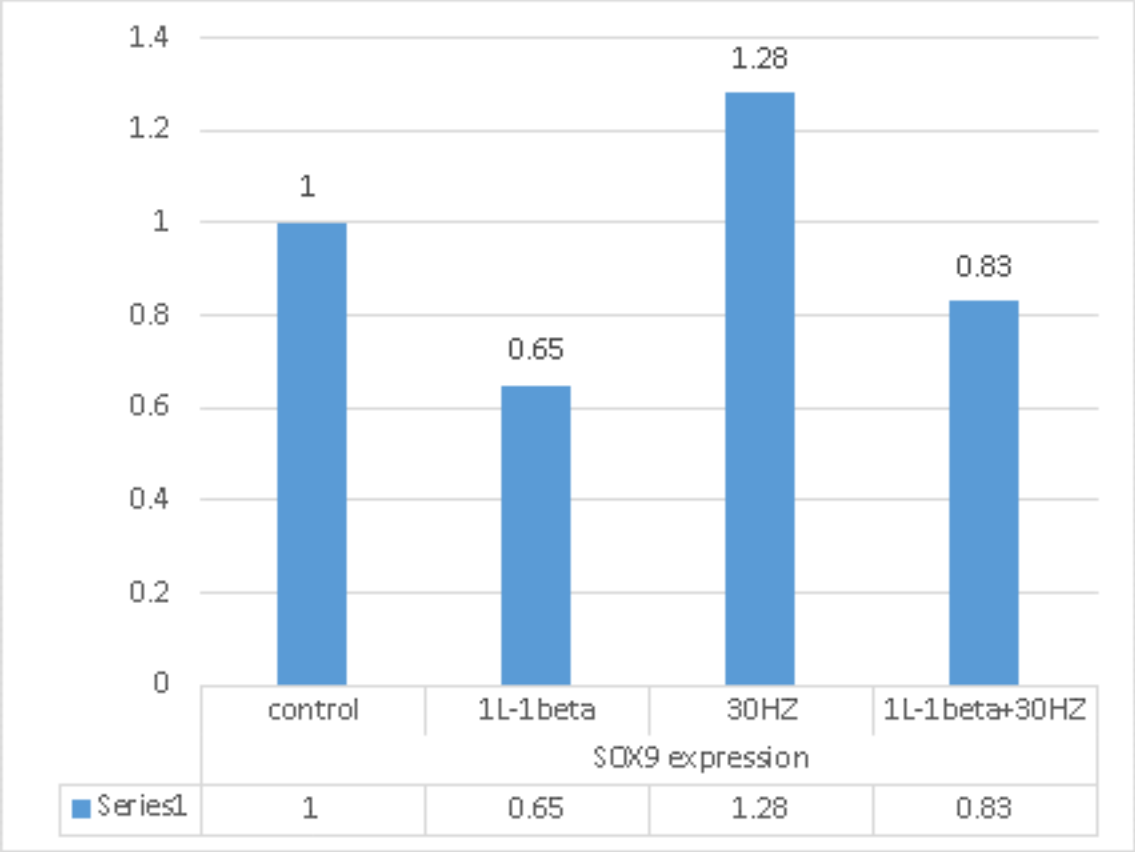

Figure 8: Graph representing relative fold difference in SOX9 expression when compared with controls.

Relative SOX9 expressions when compared with controls were as follows: 1 (control), 0.65 (IL-1β), 1.28 (30Hz vibration), 0.83 (30Hz vibration + IL-1β).

MMP13 expression (ct value)								
gene	**MMP13 expression (ct value)**				**GAPDH(ct value)**			
group	**control**	**IL-1β**	**30HZ**	**IL-1β+30HZ**	**control**	**IL-1β**	**30HZ**	**IL-1β+30HZ**
Exp 1	26.9076347	26.06450272	26.69604111	26.06713486	14.5096979	13.96864319	14.10533619	13.93153858
	26.9816875	25.78997803	26.75273323	26.16218376	14.3530769	13.91906261	13.89630318	13.94750023
	27.1171246	25.89294243	26.65904045	26.1843338	14.3139286	13.65427208	13.83152199	14.20964336
Exp 2	27.6872158	27.34939194	27.36285782	26.81067467	13.3411198	13.28634453	12.17726135	12.83162403
	27.3990784	27.32592583	27.24290657	26.75548935	12.6726856	13.30091476	12.55280495	12.51358604
	27.1416492	27.30974007	27.3378849	26.84295845	12.4086142	13.14142227	12.8721838	12.71692562

Table 7: MMP13 expression ct values from qPCR analysis.

gene	**MMP13 expression**			
group	**control**	**IL-1β**	**30HZ**	**IL-1β+30HZ**
Exp 1	1	1.455417156	0.902310967	1.415774107
Exp 2	1	1.430364251	0.883544624	1.200730133
mean	**1**	**1.442890704**	**0.892927796**	**1.30825212**
SD	0	0.017715079	0.013269809	0.152059052

Table 8: qPCR findings, results indicate fold difference when compared with controls. The formula $2^{-\Delta\Delta CT}$ was used to calculate results.

MMP13 expression

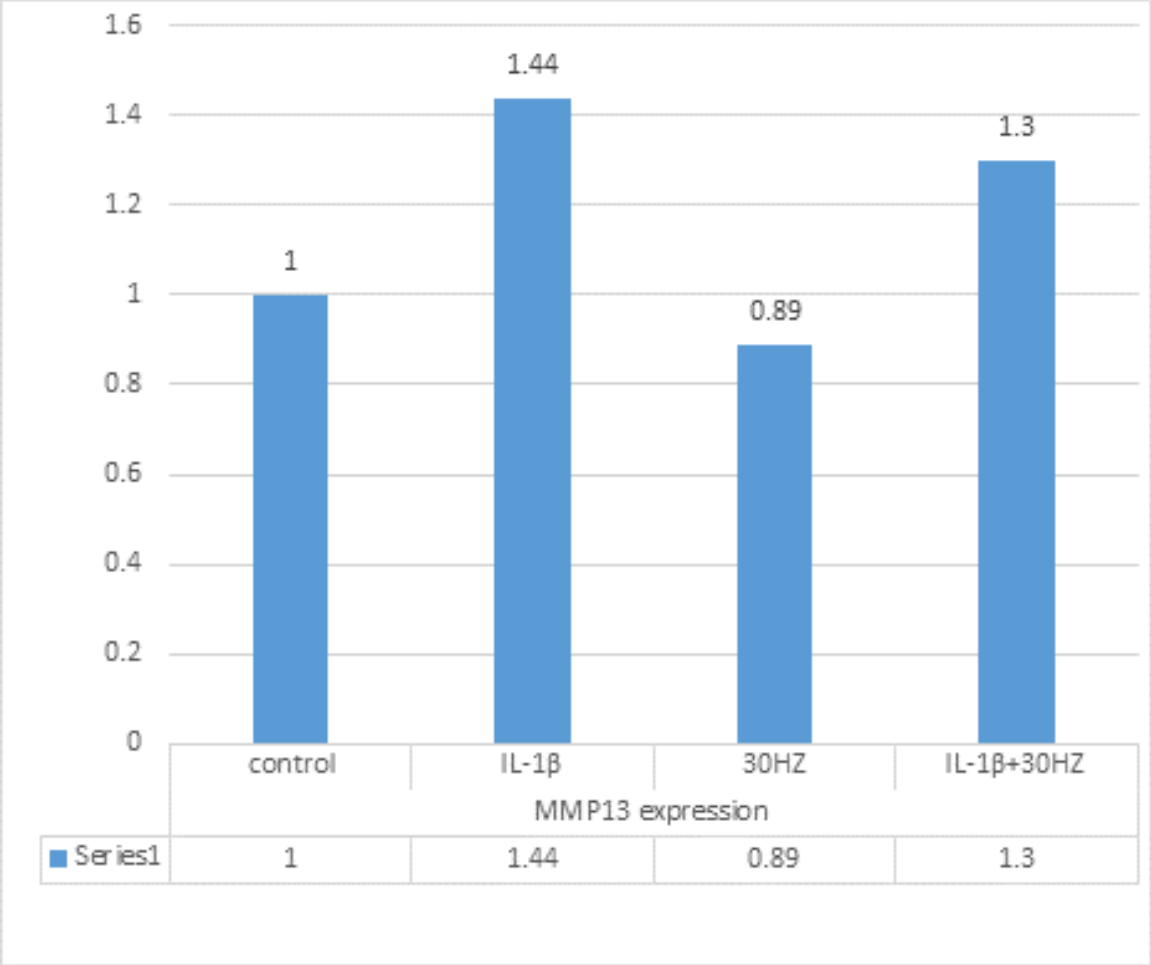

Figure 9: Graph representing relative fold difference in MMP13 expression when compared with controls.

Relative MMP13 expression when compared with controls was as follows: 1 (control), 1.44 (IL-1β), 0.89 (30Hz vibration + IL-1β), 1.30 (30Hz vibration + IL-1β).

A general trend was noticeable with both gene expressions of SOX9 and MMP13. IL-1β decreased the gene expression of SOX9. Mechanical vibration increased the expression of SOX9. When mechanical vibration was combined with IL-1β the effects of IL-1β seemed to be countered to a small degree. With MMP13, IL-1β increased gene

expression. Mechanical vibration decreased expression of MMP13. When combined (mechanical vibration + IL-1β) the effects of IL-1β were countered to some degree.

Statistical Analysis

Descriptives for SOX9

SOX9

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.0000	3	1.000000	.0000000	.0000000	1.000000	1.000000	1.0000	1.0000
2.0000	3	.648718	.5976725	.3450664	-.835982	2.133419	.1290	1.3018
3.0000	3	1.276274	1.0270825	.5929864	-1.275141	3.827688	.5401	2.4496
4.0000	3	.825482	.2445597	.1411966	.217962	1.433002	.6274	1.0988
Total	12	.937618	.5710987	.1648620	.574760	1.300477	.1290	2.4496

Table 9: Descriptive statistics for SOX9 gene expression.

ANOVA

SOX9

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.644	3	.215	.583	.643
Within Groups	2.944	8	.368		
Total	3.588	11			

Table 10: One-way ANOVA for SOX9 analysis. Results are not statistically significant ($P \leq 0.05$ considered statistically significant).

Multiple Comparisons

Dependent Variable: SOX9

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0000	2.0000	.3512816	.4952980	.891	-1.234837	1.937400
	3.0000	-.2762735	.4952980	.942	-1.862392	1.309845
	4.0000	.1745180	.4952980	.984	-1.411600	1.760636
2.0000	1.0000	-.3512816	.4952980	.891	-1.937400	1.234837
	3.0000	-.6275552	.4952980	.606	-2.213674	.958563
	4.0000	-.1767637	.4952980	.983	-1.762882	1.409355
3.0000	1.0000	.2762735	.4952980	.942	-1.309845	1.862392
	2.0000	.6275552	.4952980	.606	-.958563	2.213674
	4.0000	.4507915	.4952980	.800	-1.135327	2.036910
4.0000	1.0000	-.1745180	.4952980	.984	-1.760636	1.411600
	2.0000	.1767637	.4952980	.983	-1.409355	1.762882
	3.0000	-.4507915	.4952980	.800	-2.036910	1.135327

Table 11: Tukey HSD analysis of SOX9 gene expression. No significance found.

Descriptives for MMP13

MMP-13

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.0000	2	1.000000	.0000000	.0000000	1.000000	1.000000	1.0000	1.0000
2.0000	2	1.442891	.0177151	.0125265	1.283727	1.602054	1.4304	1.4554
3.0000	2	.892928	.0132698	.0093832	.773703	1.012152	.8835	.9023
4.0000	2	1.308252	.1520591	.1075220	-.057944	2.674449	1.2007	1.4158
Total	8	1.161018	.2453774	.0867540	.955877	1.366158	.8835	1.4554

Table 12: Descriptive statistics for MMP13 gene expression.

ANOVA

MMP13

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.398	3	.133	22.467	.006
Within Groups	.024	4	.006		
Total	.421	7			

Table 13: One-way ANOVA for MMP13 analysis ($P \leq 0.05$ considered statistically significant). P=0.006 and is thus considered significant.

Multiple Comparisons

Dependent Variable: MMP13

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0000	2.0000	-.4428907*	.0768308	.015	-.755658	-.130124
	3.0000	.1070722	.0768308	.563	-.205695	.419839
	4.0000	-.3082521	.0768308	.052	-.621019	.004515
2.0000	1.0000	.4428907*	.0768308	.015	.130124	.755658
	3.0000	.5499629*	.0768308	.007	.237196	.862730
	4.0000	.1346386	.0768308	.407	-.178128	.447406
3.0000	1.0000	-.1070722	.0768308	.563	-.419839	.205695
	2.0000	-.5499629*	.0768308	.007	-.862730	-.237196
	4.0000	-.4153243*	.0768308	.019	-.728091	-.102557
4.0000	1.0000	.3082521	.0768308	.052	-.004515	.621019
	2.0000	-.1346386	.0768308	.407	-.447406	.178128
	3.0000	.4153243*	.0768308	.019	.102557	.728091

*. The mean difference is significant at the 0.05 level.

Table 14: Tukey HSD analysis of MMP13 gene expression. Statistical significant differences are seen between groups 1 and 2, 2 and 3, and 3 and 4 (marked with an *).

The results according to the one-way ANOVA showed no statistically significant difference between the SOX9 experimental groups (Table 10). There was significance observed with the MMP13 experimental groups (Table 13). Tukey HSD (Table 14) showed there were significant differences between: groups 1 and 2 (control vs. IL-1β), 2 and 3 (IL-1β vs. vibration), and 3 and 4 (vibration vs. IL-1β + vibration).

CHAPTER 5
DISCUSSION

Cell differentiation

Our data show that mechanical vibration like that exerted by AcceleDent (0.3g/30Hz) does not pose a negative influence on chondrocytes, rather enhance them to be differentiated *in vitro*. This process of differentiation allows for cells to become further specialized than they were previously. Although the results are qualitative it is clear that differentiation increased significantly by mechanical vibration. IL-1β seemingly did not cause a significant change in the amount of differentiation. It is interesting that IL-1β – the potent inflammatory mediator was not shown to be a significant inhibitor of differentiation. This can be due to the used concentration (1ng/mL) was not high enough to affect the C-28/I2 chondrocytes. Aside from being of qualitative measure, this study did have other limitations. The chondrocyte cell line C-28/I2 was used in place of primary chondrocytes. These cells were utilized because primary chondrocytes are difficult to obtain, cannot be controlled, and the number of cells is often inadequate. Due to the complexity of the human body and cells within it there may be a difference in the way the cells respond to mechanical vibration than that of our study. Further evaluation should be done regarding the effects of mechanical vibration on cell differentiation.

Gene expression

SOX9

IL-1β was used to mimic an inflammatory environment. SOX9, an important transcription factor, promotes cell survival (Lefebvre V, 2016). When cells were stimulated with IL-1β, the expression of SOX9 decreased. When mechanical vibration of 0.3g/30Hz was applied to the group with IL-1β, the level of SOX9 expression was increased (Fig. 8). Up-regulation of SOX9 indicates that the effect of mechanical vibration are acting to counter the effect of IL-1β. Experiments using primary chondrocytes from the TMJ region should be used to verify if the same relationship exists. If mechanical vibration can decrease levels of inflammation, it may prove to be a valuable therapy in treating patients who suffer from TMD that is of an inflammatory nature. Although our data do not show a statistical significance, it is suggestive of a pattern that mechanical vibration anabolically up-regulates SOX9 expression in both normal and inflammatory conditions.

MMP13

MMP13 is a proteinase that breaks down collagen and other matrix components. It is known for its catabolic activity (Liang Z, et. al, 2012). Our data showed an increase in MMP13 expression with the addition of IL-1β (Fig. 9), which is in agreement with previous work from Lee and Salter. They showed that IL-1β causes downstream events leading to the up-regulation of MMPs. The effects of mechanical vibration were shown to down-regulate expression of MMP13. Our data suggest that mechanical vibration counters the effect of IL-1β. (Fig. 9). This model may be more representative of how mechanical vibration can alter the cellular pathways present in an inflammatory state

because of the direct relationship between IL-1β and MMP13. Due to the complexity of the human body and the multitude of biological pathways, it should not be assumed that this exact relationship exists in an *in vivo* model. Further study should be done to better understand the molecular pathways of inflammation and how cells respond to therapies targeting on inflammation.

Overall, mechanical vibration does not appear to have a negative effect on human chondrocytes *in vitro.* Our data suggest it may be beneficial to patients who suffer from osteoarthritis of an inflammatory nature. We tested the hypothesis that mechanical vibration would help reduce inflammation within the TMJ. Our data indicate that inflammation can be reduced to some degree in an *in vitro* model (Fig. 8, 9). Studies should be done using primary chondrocytes from the temporomandibular joints to evaluate whether the same relationships exist. Due to time constraints the expression of MMP13 was only run through two trials. Further testing will be done to further evaluate this relationship. Therapies used to treat OA should aim to target the inflammatory nature. Mechanical vibration may work well to help alleviate inflammation and slow the progression of OA.

Although the preliminary data are not statistically significant, it indicates a general trend that mechanical vibration counters the effects of IL-1β. Since increased IL-1β levels seems to be an early indicator of progression of OA and also that of inflammation (Alstergren et. al, 2003), it makes sense that decreasing this interleukin would help halt this pathological progression. Again, further studies are needed using an *in vivo* model to gain a better understanding of the relationship that exists between mechanical vibration and pathologies involving inflammation.

CHAPTER 6
CONCLUSION

Mechanical vibration (0.3g/30Hz) is able to increase differentiation of human chondrocytes C-28/I2 with and without treatment of IL-1β (1ng/mL), while IL-1β (1ng/mL) itself shows minimal effect on the differentiation of human chondrocytes C-28/I2. Mechanical vibration anabolically regulates gene expressions of SOX9 (upregulated, $p > 0.05$) and MMP13 (downregulated, $p<0.05$), and can partially counter the catabolic changes of SOX9 and MMP13 induced by the treatment of IL-1β (10ng/mL). Under the specific experimental conditions and limitations of this study, it can be concluded that mechanical vibration (0.3g/30Hz) does not appear to harm human chondrocytes C-28/I2 *in vitro* and may help enhance the normal function of chondrocytes and potentially reduce inflammation. Further studies should be done to gain a better understanding of the effects of mechanical vibration and the chondrocytes (cartilage) of TMJ, in order to safely use vibration in orthodontics as well as potentially help manage TMD.

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