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# Nicotine's Influence on Musculoskeletal Healing: A Review Featuring nAChRS and miRNA

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## Abstract

Nicotine is the main ingredient of smoking cessation therapies and electronic cigarettes. New to the market, electronic cigarettes, which are not regulated by Food and Drug Administration (FDA), have been marketed as the safe and alternative approach to cigarette smoking. Although containing significantly fewer amounts of toxic chemicals, electronic cigarettes, as well as other nicotine replacement therapies, still present additional health hazards due to significant nicotine exposure. The effects of nicotine exposure on musculoskeletal health have been extensively studied, but the mechanisms behind these effects are still unknown. Current research, however, suggests that these effects are mediated by the nicotinic acetylcholine receptors (nAChRs) of the musculoskeletal system. These receptors, which are activated in the presence of nicotine, undergo conformational changes that eventually alter the ionic permeability of their respective membranes. The results of these actions are linked to changes in cell proliferation, differentiation and microRNA expression.

***Index terms***— cigarette smoking, electronic cigarette, nicotine replacement therapies, nicotine, nicotine acetylcholine receptor, wound healing, bone healing, muscu

## 1 Introduction

ccording to recent statistics from the Centers for Disease Control and Protection (CDC), the prevalence of tobacco use among Americans is, as of 2011, around 19% (CDC, 2011). Cigarette smoking, which kills nearly 440,000 Americans each year (CDC, 2011), is the leading cause of preventable death worldwide. Awareness of the diseases associated with cigarette smoking was initiated with the release of the 1964 Surgeon General's Report, which celebrates its 50th anniversary this year. In addition to increasing the susceptibility to various cancers, cigarette smoking also adversely affects the musculoskeletal system; increasing the risk of progressive bone diseases (Porter & Hanley, 2001) and delaying wound (Sopori, 2002), fracture (Alemdaro?lu et al., 2009), and bone healing (Krannitz et al., 2009) following traumatic injury.

The extent of these effects, however, is believed to be dose dependent and also reversible, to a certain degree, following smoking cessation (Sloan et al., 2010;Fusby et al., 2010). Although these health hazards associated with cigarette smoking are well known, additive chemicals, such as nicotine, make it extremely difficult for chronic users to quit.

## 2 II.

## 3 Nicotine

Nicotine is the quintessential compound responsible for an individual's addiction to cigarettes and/or other tobacco-containing substances (Benowitz, Hukkanen & Jacob, 2009). The most widely used source of nicotine

comes from the tobacco plant, which is processed to manufacture cigarettes as well as numerous nicotine replacement therapies. Although nicotine is included in all, the specific concentration used within each product varies from company to company. Table **??** displays the nicotine levels for an average cigarette and the most common nicotinecontaining products used for nicotine replacement therapies. Individual products also contain unique methods for nicotine deployment. The most common method for the release of nicotine in the human body is through the burning (combustion) of tobacco, such as seen in smoking cigarettes. In smoking cessation products, such as nicotine gum, transdermal patches and inhalers, nicotine is released through alkalinebuffered diffusion mechanisms designed for targeted areas of absorption (skin, mouth, lungs, etc.). Electronic cigarettes, which recently burst into the scene as the "safer" alternative to cigarettes, use vaporization to release nicotine from a liquid solution.

# 4 a) Absorption and Metabolism

Nicotine is a weak base (pKa = 8.0) and its rate of absorption is primarily dependent on the pH and surface area of the environment. In acidic environments with smaller surface areas, nicotine does not rapidly cross cell membranes, whereas in alkaline environments with larger surface areas, it is readily absorbed. As a consequence of this, nicotine from cigarette smoke is not readily absorbed in the mouth, but is readily absorbed in the lungs through the alveoli. As a result, about 2.3-3.5 mg of nicotine, which accounts for approximately 80 to 90% of inhaled nicotine, is absorbed during smoking (Benowitz, Jacob & Denaro, 1991). Average blood-nicotine levels in chronic smokers have been shown to reach 19.0 ± 11.3 ng/ml after the first cigarette and 22.9 ± 11.2 ng/ml after the second cigarette, correlating to 0.117 ± 0.070 ? M and 0.141 ± 0.069 ? M, respectively **??**Herning et al., 2009). The various forms of nicotine replacement therapies, such as nicotine gum, transdermal patches and inhalers, are buffered to a more alkaline pH to facilitate the absorption of nicotine through cell membranes. As a result, nicotine absorption is slower when compared to smoking cigarettes and the increase in nicotine blood levels is more gradual.

The most common pathway for the metabolism of nicotine is the cotinine pathway, which accounts for 70 to 80% of the nicotine metabolized by the human body (Hukkanen, Jacob & Benowitz, 2005). The remaining amount is exposed to the bodily tissues and the highest affinity for nicotine is seen in the liver, kidney, spleen, and lung, whereas the lowest affinity is seen in adipose tissue (Urakawa et al., 1994). Nicotine also binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Perry et al., 1999). Cigarette smoking itself influences the rate of metabolism of nicotine. Research has found that the clearance of nicotine is significantly slower in cigarette smokers compared with nonsmokers (Benowitz & Jacob, 1993). In support of this observation are two crossover studies comparing the clearance of nicotine in the same subjects when smoking compared with not smoking. After 4 days of smoking abstinence, nicotine clearance was increased by 14% (Benowitz & Jacob, 2000), and after 7 days of abstinence, nicotine clearance was 36% higher (Lee, Benowitz & Jacob, 1987) when compared with overnight abstinence from cigarettes. Because the same enzyme metabolizes nicotine and cotinine, it has been postulated that cotinine might be responsible for the slowed metabolism of nicotine in smokers. In a study in which nonsmokers received an intravenous infusion of nicotine with and without pretreatment with high doses of cotinine, there was no effect of cotinine on the clearance of nicotine (Zevin, Jacob, **??**enowitz, 1997). Also carbon monoxide at levels and in a pattern similar to those experienced during smoking had no effect on nicotine and cotinine clearance (Benowitz & Jacob, 2000). Further studies must be performed in order to understand the biological mechanisms that control the rate at which nicotine is metabolized by the human body.

# 5 III.

# 6 The Nicotinic Effect on Musculoskeletal Healing

Nicotine is quickly dispersed throughout the body via cardiac circulation, where it is subsequently exposed to a majority of the internal tissues. The effects of nicotine metabolization throughout the body have been studied extensively; however, its implications in regards to musculoskeletal health and repair are still H being investigated. The subsequent sections, therefore, aim to summarize the findings of recent scientific experiments investigating the effect of nicotine on the wound and skeletal healing processes. Healing, in general, is a complex process orchestrated by several role players whose ultimate goal is to efficiently restore damaged tissue to its original state. The basic mechanisms behind wound and skeletal healing and the effects of nicotine on these processes have previously been reviewed (Misery, 2004;Martin et al., 2009;Kallala et al., 2013); however, our aim herein is to present recent and human-only-based research.

In order to do so, the following filters and search titles were used when gathering potential publications on the PubMed database (http://www.ncbi.nl-m.nih.gov/ pubmed): Publication Dates: 5 Years; Species: Human; Title: (Wound Healing OR Skin OR Soft Tissue OR Blood Vessels AND Nicotine) OR (Bone OR Fracture Fixation OR Fracture Healing OR Osteoblast OR Osteoclast AND Nicotine). a) Wound Healing Two of the major role players involved in the wound healing process are fibroblasts and endothelial progenitor cells. Fibroblasts, which produce extracellular matrix as well as collagen, and endothelial progenitor cells, which give rise to the endothelial cells that help form new capillaries, are simultaneously recruited by activated macrophages and cell mediators to the site of injury in order to replace damaged tissues (reviewed in Martin et al., 2009). Although efficient, these cells

can become ineffective when exposed to outside factors such as nicotine. Therefore, current therapies, which aim to facilitate regeneration, use chemical agents and growth factors to enhance the number and function of fibroblasts and endothelial progenitor cells.

# 7 b) Fibroblast-Based Studies

In 2010, Choi et al. (2010) observed that nicotine increased the expression of early growth response-1 (EGR-1) in cultured human skin dermal fibroblasts (HSDFs) (Choi et al., 2010). The increased expression of EGR-1, which encodes a protein involved in collagen production and skin wound repair, is suggested by Choi et al. (2010) to improve the function of HSDFs, which, in turn, will facilitate the wound healing process. In a later study, Silva et al. (2012) investigated the effects of nicotine on the viability and migration potential of human gingival fibroblasts (HGFs) (Silva et al., 2012). The researchers observed that nicotine had little to no effect on cell viability and cell death, but did stimulate cell migration. Ultimately, however, Silva et al. (2012) concluded that the effect of nicotine on human gingival fibroblasts was not enough to significantly affect the healing potential of these cells. Tinti & Soory (2012), investigating the oxidative effects of nicotine on HGFs and human periosteal fibroblasts (HPFs), determined that the detrimental effects of nicotine oxidation on **??**011) investigated the acute and chronic effects of nicotine on the proangiogenic activity of HUVECs (H.S. Park et al., 2011). The group looked at the effect of nicotine on several factors including: production of nitric oxide (NO), expression of endothelial nitric oxide synthase (eNOS), cell viability, migration potential and morphology and the results from these experiments can be summarized into two relatable conclusions. The first conclusion is that nicotine, regardless of exposure time, has an affect on the angiogenic activity of HUVECs. This result was supported by the variation in values between nonexposed and exposed groups for all factors. The second conclusion is that the degree of this nicotinic effect is dependent on exposure time. H.S. Park et al. (2011) showed that the production levels of NO and eNOS were significantly higher in acute vs. chronic exposed HUVECs. The migratory function and tubular formation (number and length of circles) of acutely exposed HUVECs was also significantly better when compared to the chronic exposed groups.

# 8 d) Combined Studies

In 2011, Laytragoon-Lewin et al. (2011) investigated the effects of pure nicotine on humanderived fibroblasts and endothelial cells (Laytragoon-Lewin et al., 2011). The researchers showed that, compared to the control, nicotine exposure increased the proliferative capacity and altered the morphology of both cell types. In addition, the researchers evaluated nicotine's effect on the expression of 96 well-defined genes common to both cell types, which were grouped into 5 categories: Cell Cycle and DNA Damage, Apoptosis and Cell Senescence, Signal Transduction and Adhesion, Angiogenesis, and Invasion and Metastasis. Surprisingly, nicotine caused a differential expression in 80% of endothelial and 73% of fibroblast genes investigated within an hour of exposure.

# 9 e) Skeletal Healing

The dose dependent effect of nicotine is well known and has been recently demonstrated in many of the cells that comprise the skeletal tissues. The process of bone fracture healing is very similar to the process of wound healing. It can be divided into three phases: reactive phase, reparative phase and remodeling phase. During the reactive phase, blood vessels surrounding the fracture site constrict to prevent further bleeding. At the same time, extravascular blood cells form a clot, known as a hematoma, in the fracture site. All the cells within the clot undergo apoptosis, allowing for the migration and proliferation of fibroblast cells within the clot, forming granulation tissue. The fibroblasts create a provisional extracellular matrix for the migration and proliferation of cells necessary for the formation of new bone. Once this phase is complete, the reparative phase begins with the migration, differentiation and proliferation of precursor cells from the periosteum, a connective tissue membrane covering the bone. These precursor cells include mesenchymal stem cells, which differentiate into chondrocytes and osteoblasts, which are responsible for the formation of new cartilage and new bone, respectively. During this phase, various preliminary bone structures are formed by chondrocytes and replaced by osteoblasts (Ham & Harris, 1971). Finally, during the remodeling phase, the preliminary bone structure is reinforced with compact bone. It can take anywhere from 3 to 5 years for the newly formed bone to achieve its original strength (Ham & Harris, 1971). The time frame in which wound healing and bone fracture healing take place depends on a patient's age and general condition, which includes a patient's exposure to nicotine. **??**012) observed significant enhancements of both qualities at lower nicotine doses (1.0?M), but significant impairments at higher doses of (10?M). In addition, Ying et al. also investigated the effect of nicotine on the expression/ production of aggrecan; however, no significant changes were noted. that fall outside of the normal. This approach further of nicotine (6.17?M [1000ng/ml]) significantly inhibited cell-mediated calcium deposition, osteocalcin (OCN) expression, and bone morphogenetic protein-2 (BMP-2) expression.

# 10 g) Periodontal Ligament Cell-Based Studies

The increased incidences of alveolar bone degenerating diseases, such as periodontitis, have been well documented in smokers and tobacco users alike (S.I. Lee et al., 2012; **??**ergstrom, 2004;Ojima et al., 2006). The oral cavity is the initial site of toxic exposure for all tobacco-containing products and many nicotinecontaining products

(e-cigarettes, nicotine gums, and nicotine lozenges). During their use, nicotine remains in the oral cavity for extended periods of time causing a rapid increase in concentration. As a result, the tissues of the oral cavity are extremely susceptible to the effects of nicotine exposure.

A 2009 study by H. Lee et al. (2009), investigating the effects of nicotine on periodontal ligament (PDL) cells, showed that nicotine downregulated the expression of osteoblastic differentiation markers ALP, OCN, and osteopontin (OPN) (H. Lee et al., 2009). In order to prevent additional cytotoxic effects, nicotine decreased the expression of osteoprotegerin (OPG) while simultaneously increasing the expression of receptor activator of nuclear factorkappa B ligand (RANKL) and the production of transcription factor NF-E2-related factor-2 (Nrf2) and heme oxygenase-1 (HO-1).

A study by S.I. Lee et al. (2012) demonstrated that nicotine exposure promotes endoplasmic reticulum (ER) stress and facilitates extracellular matrix degradation via downregulation of extracellular matrix molecules, such: as collagen type I, elastin, and fibronectin; and upregulation of matrix metalloproteinases (MMPs), including: MMP-1, MMP-2, MMP-8 and MMP-9 (S.I. Lee et al., 2012). Interestingly though, S.I. Lee et al. (2012) demonstrated that these negative effects could be attenuated through the use of the experimental drug Salubrinal and small interfering RNA.

# 11 h) Adult Stem Cell-Based Studies

Currently, a majority of the research in this field has shifted its focus towards the effect of nicotine on adult stem cells. This shift is especially important because these cells are the progenitors for many of the bone remodeling cells. Presently, the mesenchymal stem cells (MSCs) derived from the human bone marrow are most investigated population of these cells.

A study by Ruiz et al. (2012) investigated the dose dependent effects of nicotine on the mechanical properties of human bone marrow -derived MSCs (h MSCs) (Ruiz et al., 2012). At 0.5 and 1.0 ?M concentrations, nicotine significantly increased the stiffness of the h MSC cytoplasm and nucleus. The authors suggest that this increase in stem cell stiffness reduces the ability to respond to mechanical stimuli and therefore hinders mechano-induction. A stiffer stem cell would also experience retardation in locomotion seeing as it would be less compliant and consequently more likely to encounter difficulties when traveling out of the bone marrow.

In 2012, a study by B. showed that nicotine had dose dependent effects on human alveolar bone marrow-derived mesenchymal stem cells (hABMMSCs) (B. . The researchers investigated the effect of nicotine (1?M -5mM) on the proliferation of hABMMSCs and observed no changes at low concentrations (1?M-100?M), significant increases at moderate concentrations (1-2mM), and significant decreases at high concentrations (5mM). High concentrations of nicotine also caused significant detrimental effects to cell morphology, ALP activity, calcium accumulation, and osteogenic gene expression. A majority of these effects, including: reduced ALP activity, reduced calcium deposition, and reduced expression of OCN, bone sialoprotein (BSP), collagen type I ? 1 (COL1A1), and runt-related transcription factor 2 (Runx2), were observed at the 2mM concentration. These results confirm the dual effects of nicotine and, although not explicitly stated, suggests that the threshold value for positive to negative effects in hABMMSCs exists somewhere in the mM range.

Ng. et al. ( **??**013) also investigated the effects of nicotine on h MSCs as well as PDL-derived stem cells (PDLSC) (Ng et al., 2013). At 1?M, nicotine significantly reduced the proliferation and migration potential of both adult stem cell populations. The osteogenic differentiation potential of h MSCs and PDLSCs was also inhibited by nicotine as made evident by reductions in alkaline phosphatase activity and calcium deposition. Nicotine also significantly downregulated the expression of protein tyrosine kinase 2 (PTK2), a gene associated with cell migration, and also downregulated the osteogenic genes RUNX2, alkaline phosphatase (ALPL), osteocalcin (BGLAP), COL1A1 and collagen type I ? 2 (COL1A2). Ng et al. ( **??**013) also were the first to demonstrate that nicotine had a dose dependent effect on the microRNA (miRNA) expression profiles of PDLSCs. Moreover, the authors noted that half of the top 10 differentially expressed miRNAs were related to osteogenesis.

These recent studies continue to demonstrate the potent effects of nicotine on musculoskeletal tissue regeneration. Whether direct or indirect, the effects of nicotine exposure appear to be beneficial at low concentrations, but detrimental once concentrations exceed a certain threshold. Most studies aim to investigate the effects of nicotine at physiological concentrations with hopes of identifying these cellspecific threshold values; however, a majority of these studies tend to investigate vast concentration ranges demonstrates the lingering uncertainty surrounding the exact effects of nicotine exposure in the musculoskeletal system and throughout the body. Although numerous studies detail the general effects of nicotine on certain cells, few detail the specific mechanisms behind nicotine's action. Current research, however, points to nicotinic acetylcholine receptors as the main potential mediator of the nicotinic effect.

# 12 IV. Nicotinic Acetylcholine Receptors

Once internalized and in the blood stream, nicotine is free to complex with a subset of cholinergic receptors known as nicotinic acetylcholine receptors (nAChRs). These specific receptors, believed to be the main mediators behind nicotine's cellular effects, have been identified on numerous cellular populations including, but not limited to: epithelial cells, keratinocytes, vascular endothelial cells, osteoblasts, embryonic stem cells and mesenchymal stem

cells (Picciotto et al., 2001) and serve to regulate the flow of specific ions across these membranes (Albuquerque et al., 2009). Although all nAChRs serve the same basic purpose, the downstream implications initiated by receptor activation vary from location to location (Boulter et al., 1987;Papke et al., 1989;Papke & Heinemann, 1991;Portugal & Gould, 2008); this variation is partly due to the different interactions that occur with different tissue components, but mostly to the specific combination of subunits that are used to build each nACh R.

To date, 16 unique subunit varieties have been identified in the mammalian species (Dani & Bertrand, 2007; **??**ukas et al., 1999). Functional nAChRs are created from a specific combination of 5 of these subunits. This combination is dependent on the location of the cell in the body and receptors on these cells are arranged in one of two conformations, homopentameric or heteropentameric (Hurst, Rollema & Bertrand, 2013). In the former arrangement, commonly only seen in neuronal tissues, nAChRs are created using only one subunit type. On the other hand, heteropentameric nAChRs, which exist in a wider variety of tissues, are created using a mix of subunit varieties. Although slightly different in function, all subunits used to form functional nAChRs share the same basic structure. Each subunit is divided into three major domains: an extracellular amino acid domain, a transmembrane domain containing 4 individual units (labeled TM1-TM4), and a cytoplasmic domain composed of an amino acid loop (Albuquerque et al., 2009). Although almost entirely consistent amongst subunit varieties, the amino acid sequences of these domains are unique to each subunit. Variations in only a few amino acids are enough to influence receptor features such as agonist binding and ionic preference (Wallace & Bertrand, 2013;Albuquerque et al., 2009;Galzi et al., 1992;Corringer et al., 1999).

Subunits are typically classified a s either ?-or non-? subtype depending on their amino acid sequence (Albuquerque et al., 2009). To date, 9 nAChR ?-subunits have been identified in the mammalian species: ?1, ?2, ?3, ?4, ?5, ?6, ?7, ?9 and ?10 (Albuquerque et al., 2009). ?-subunits contain a characteristic cysteinecysteine bond proximal to TM1 in their extracellular domain, which is critical for agonist binding (Albuquerque et al., 2009). In heteropentameric nAChRs, ?-subunits contribute to the "positive" side of the ligand-binding channel and influence the ligand affinity of the receptor (Albuquerque et al., 2009); however, there are two exceptions, the ?5 and ?10 subunit. Although both subunits are classified as ?, neither contributes to the "positive" side of the ligandbinding channel (Albuquerque et al., 2009). On the other hand, the non-? subunits, as well as the ?10 subunit, contribute to the "negative" face of the ligandbinding channel and influence the ligand selectivity of the receptor. To date, only 7 different nAChR non ?subunits have been identified in the mammalian species: ?1-?4, ?, ?, and ?. Together the ?-and the non ?-subunits (in the heteropentameric case) align to create a ligand-binding site. When present in sufficient quantities, receptor agonists, such as nicotine, bind to this region and activate the receptor. If closed, receptor activation leads to the opening of the transmembrane ionic channel (reviewed in Albuquerque et al., 2009;Dani & Bertrand, 2007). In this conformation, extracellular ions are free to flow into the intracellular domain. The physiological implications arising from the increase in ionic permeability across the membrane following nAChR activation vary from tissue to tissue (S.Y. Huang and Winzer-Serhan, 2006; **??**ia et al., 1997;Villablanca, 1998;Sharma & Vijayaraghavan, 2002); however, for the purposes of this review we will only mention; albeit brief due to the lack of research, the nicotinic receptors of the musculoskeletal system and the potential cellular effects that may arise following receptor activation due to nicotine. a) Musculoskeletal nAChRs i.

# 13 Muscle Tissue

Compared to the rest of the body, muscular nAChR expression is relatively basic/straightforward. Muscular nAChRs exist in only one of two heteropentameric conformations, 2?1/?1/?/? and 2?1/?1/?/? (Albuquerque et al., 2009); however, ?4, ?5, ?7 and ?4 subunit transcripts have been identified in early skeletal development (Corriveau et al., 1995).

# 14 H

Differing by only one subunit, the two muscular nicotinic receptors have unique sites of expression and characteristic functions. ?-containing receptors are typically found on immature, non-innervated muscle and are known to have ionic channels that remain open for longer periods of time after receptor activation (Albuquerque et al., 2009). As the muscle begins to develop, the subunit composition of the nAChR will gradual change by replacing the ? subunit with the ? subunit. This process is critical for successful muscle development (Hurst, Rollema & Bertrand, 2013). These new receptors, which aggregate proximal to the axon terminals (Corriveau et al., 1995), are different from their immature counterparts in that they are more susceptible to activation by receptor agonists (Conti et al., 1994;Lindstrom, 1997;Missias et al., 1996). As a result, ?containing nAChRs can be activated more rapidly and with lower concentrations of receptor agonists. The receptors inherently gate both Na+ and Ca2+ ions; however, the higher permeability lies with Na+ (Albuquerque et al., 2009). In the muscular case, the activation of nAChRs typically causes an inward flux of Na+, which depolarizes the membrane (Fagerlund & Eriksson, 2009) and releases intracellular Ca2+.

ii.

Bone Tissue H MSCs play an integral role in maintaining and repairing many tissues of the musculoskeletal system. Research within the last decade has revealed that, like many other tissues, h MSCs exhibit various nAChR subunits and are therefore susceptible to the nicotinic effect. In a 2009 study, Hoogduijn et al. (2009) screened

MSC cells collected from the femoral head for the presence of nAChR subunits. Out of the 7 subunits investigated via RT-PCR (?3, ?5, ?7, ?9, ?10, ?2 and ?4), 3 (?3, ?5, and ?7) were identified (Hoogduijn, Cheng & Genever, 2009). In addition, Hoogduijn et al. (2009) showed that intracellular calcium stores increased following in vitro treatment with 10?M nicotine. Schraufstatter et al. (2009) obtained similar results when treating hMSCs with a 2?M concentration of nicotine, but further showed that the intracellular calcium flux occur directly through ?7 homopentameric nAChR channels (Schraufstatter, DiScipio & Khaldoyanidi, 2009). Contrary to the Hoogduijn et al. (2009) report, Schraufstatter et al. (2009) conducted RT-PCR for 13 nAChR subunits: ?1, ?2, ?3, ?4, ?5, ?6, ?7, ?9, ?10, ?1, ?2, ?3 and ? 4 and identified levels for all except ?6, ?10, and ?1. More importantly, however, protein levels for ?7, ?2, and ?4 were identified in these cells, indicating that subunits capable of interacting with nicotine were in fact translated from mRNA transcripts.

Excluding the hMSC population, only four nAChR subunits have been identified within the human bone tissue. It is possible that the typical 5 subunitbased nicotinic receptors do not exist in these tissues, however this cannot be said with certainty seeing as the research in this field is relatively new and thus much has yet to be discovered. In 1997, Romano et al. (1997) identified the ?7 n AChR subunit in the periosteum of human bone samples (Romano et al., 1997). This finding is particularly interesting because in general, the ?7 subunit is capable of forming homopentameric nAChRs like those seen in neuronal tissues. Shortly after Romano's discovery, Walker et al. (2001) identified the presence of the ?4 nAChR subunit within the core of the human bone and in osteoblast cells (Walker et al., 2001). Moreover, Walker et al. (2001) observed that osteoblast proliferation was improved following low doses of nicotine, but unaffected once D-tubocurarine (a known nAChR antagonist) was introduced, suggesting that the ?4 nAChR subunit could be a mediator of this process. Most recently, En-Nosse et al. ( **??**009), also working with human osteoblasts, identified both ?3 and ?5 subunits in human bone tissues (En-Nosse et al., 2009), bringing the total nAChR subunit expression in bone to only 4 ? subunits. As previously mentioned, heteropentameric nAChRs require non-? subunits in order to create functional ligand-binding sites. Therefore, in their absence, the effects of nicotine on bone cells would only be possible via homopentameric nAChRs.

iii.

# 15 Ligament Tissues

To date, the only nAChR subunits identified in human ligament tissues are ?7 and ?4. Wang et al. (2010) was the first to identify the expression of any nAChR on human ligament tissues when they identified the ?7 subunit on cultured periodontal ligament cells (PDLs) (Wang et al., 2010). In addition, Wang et al. (2010) observed that nicotine treatment caused an increase in receptor subunit expression, whereas treatment with alpha-bungarotoxin, a specific ?7 receptor antagonist, reversed these effects. In 2012, S.Y. later confirmed the expression of ?7 nAChRs in human ligament tissue and also identified the presence ?4 nAChR subunit, while investigating the apoptotic effect of nicotine on periodontal ligament derived stem cells (S.Y. . In addition to identifying these subunits, S.Y. showed that the gene expression of both subunits was upregulated in the presence of nicotine. Moreover, the apoptotic effect observed in the presence of nicotine was reversed once nAChR antagonists were introduced. This research hints at the importance of nAChRs in the ligament and further supports the overarching notion that nicotine can influence cellular physiology via nicotinic receptors. iv.

# 16 Cartilage Tissues

To date, the only human cartilage tissue investigated for the presence of nAChRs is that of the human growth plate chondrocytes. A study preformed by Kawakita et al. (2008) revealed that chondrocytes Volume XIV Issue I Version I Year ( ) 2014 the presence of nicotine, these chondrocytes experienced diminished matrix production and inefficient hypertrophic differentiation; an affect that was prevented in the complementary murine models when using the ?7 nAChR specific antagonist methyllycaconitine. However, until the "preventative" effect of MLA is translated into the human samples of this study, it cannot be definitively stated that the negative effects of nicotine were mediated via the nAChRs of the chondrocytes.

V.

# 17 Conclusion

Nicotine accumulation can occur via chronic smoking and/or the overuse of nicotine replacement therapies. Furthermore, due to its chemical nature, nicotine readily accumulates in some tissues more than others and therefore blood serum concentrations are usually not indicative of the true bodily concentrations (Department of Health and Human Services, 1988). The dose dependent effects of nicotine on human cellular physiology have been, and continue to be, extensively studied. Nicotine's effects, which are typically beneficial at low doses and detrimental at higher doses, are believed to affect numerous cellular processes, including wound and skeletal healing mechanisms (Ma et al., 2011), via ligand-gated nAChRs. In the presence of nicotine, these receptors undergo a conformation change and open their transmembrane ion channels, allowing for ion flow across the membrane. The intracellular flow of ions is believed to influence several secondary messenger signaling pathways (Kihara et al., 2001; **??**est et al., 2003;Brunzell, Russell & Picciotto, 2003;Miñana et al. 1998;Meyer, Gahring & Rogers 2002); however, relationships between these pathways and their effect on the musculoskeletal system have yet to be established.

Nicotine exposure has also been shown to affect miRNA expression (Ng et al., 2013). miRNA are small, non-coding RNAs (~22 nucleotides), which can alter gene expression by forming complimentary base pairs with mRNA strands (Bartel, 2004). These miRNA are expressed throughout the body, including in muscular and skeletal tissues, and have been shown to affect cell viability, cell differentiation and even organ development by downregulating the genes associated with these biological processes (Callis, Chen & Wang, 2007). Each miRNA can target several genes, and therefore upregulation of a single strand can affect various biological processes. A link between the nicotinic effect and the miRNA expression has yet to be fully determined; however, there does appear to be a correlation between the two. In addition, it would also be interesting to see if miRNA expression was also altered as a consequence of nAChR activation. If so, a variety of therapeutic approaches, such as anti-sense miRNA or nAChR antagonists, could be devised to reverse and combat the negative effects of nicotine exposure on biological processes, such as wound and skeletal healing. Year 2014

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The detrimental health effects associated with cigarette smoking are well known. Although many people are aware of these consequences, millions continue to use tobacco-based products on a daily basis. Individuals who try to quit smoking, however, usually do so with the assistance of nicotine replacement therapies that help them gradually overcome their addictions to nicotine. Although not labeled as such, the electronic cigarette is quickly becoming the most popular of the nicotine replacement therapies. These devices simulate regular cigarettes, but use only vapor to deliver nicotine doses. New to the market, ecigarettes, which are not regulated by the Food and Drug Administration, have been marketed as "a safe alternative" to cigarette smoking. Although containing significantly fewer amounts of toxic chemicals, ecigarettes, as well as other nicotine replacement therapies, still present additional health hazards due to significant nicotine exposure. [1]


Figure 1: H

Figure 2:

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