Presence of nickel in dental bone replacement grafts and barrier membranes. A review of the literature and analysis of commercially available biomaterials

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ABSTRACT

Aim: To review the potential risks associated with the use of bone grafting materials in relation to their nickel content and analyze the elemental makeup of commonly used bone grafts and barrier membranes in dentistry, specifically the concentration of nickel within these materials. Materials and methods: Analysis of the individual dental bone grafting materials was performed with a high-resolution energy dispersive x-ray fluorescence spectrometer. Eight different varieties of bone grafts and variety of barrier membranes were analyzed.

Results: All bone grafts and barrier membranes analyzed contained varying levels of nickels. The nickel content of barrier membranes ranged from 4.49 ppm to 9.34 ppm while the nickel content of bone grafts ranged from 6.60 ppm to 38.15 ppm.

Conclusion: The presence of nickel in bone grafts and barrier membranes presents a potentially emerging issue of clinical significance. Nickel's toxic, carcinogenic, and allergenic properties have been exhibited experimentally. The evidence suggests that these properties of nickel may play a role in long-term implant success and may possibly contribute to unintended systemic outcomes.

Keywords. Bone augmentation; nickel; spectrophotometry; toxicity

Introduction

More than five million dental implants are placed each year in the United States (Misch, 2014), an indication that the implant market is substantial, and likely growing. It is expected that about half of the implants placed will require a bone grafting procedure prior to insertion (Cha *et al.*, 2016). It is well-documented that dental implants placed in the grafted sites have a high long-term survival rate (De Angelis *et al.*, 2017; Lee *et al.*, 2016). However, considering the varying resorption rates of various bone replacement grafts, it is essential for clinicians to understand the possible unintended ramifications that may occur as a result of the bone grafting procedure. A bone graft is typically inserted as a semi-viscous powdery substance into the native bone and is thus easily able to migrate from its origin. Thus, a systemic, whole-body, perspective of the possible side effects of bone grafts must be taken.

Our findings suggest the presence of metal alloys in the dental bone grafts which we analyzed. A troublesome element that has been found in these bone grafts is nickel. Nickel is a metallic element that has been widely used in different osseous applications in the form of the NiTi, or nitinol nickel-titanium alloy (Shayesteh Moghaddam *et al.*, 2016). A reason for alarm is that nickel compounds are a Group 1 carcinogen according to the International Association for Research on Cancer (IARC) (International Agency for Research on Cancer), meaning that there is convincing evidence that nickel causes cancer in humans. In addition, metallic nickel is a group 2B carcinogen, meaning there is some evidence that it is carcinogenic to humans. The NIH echoes this sentiment in their "Report on Carcinogens, Fourteenth Edition", which reported that workers who were exposed to various different nickel compounds had a higher risk of death from lung or nasal cancer (National Institutes of Health). This report also states

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that injection of nickel compounds in rodents caused dose-dependent increase in tumors. It was found that subcutaneous, intramuscular, intraperitoneal, subperiosteal, intra-femoral, intra-pleural, intracerebral, intrarenal, intra-testicular, and intraocular injections of nickel compounds were all reported to have caused cancer, typically sarcomas, at the site of injection, including some liver tumors from certain strains of mice (National Institutes of Health). The NIH report confirms that the evidence strongly propounds the high risk of carcinogenicity of metallic nickel. This is due to the dissolution of nickel in the body, and it's release of ionic nickel which is genotoxic and carcinogenic. Studies in animal and human cells have shown that soluble and insoluble forms of nickel were proponents of genetic damage which resulted in DNA strand breaks, mutations, chromosomal damage, cell transformation, and disrupted DNA repair (Chen *et al.*, 2010; Dumala *et al.*, 2017; Morales *et al.*, 2016; Scanlon *et al.*, 2017; Sen and Costa, 1985). Perhaps, the most concerning statement found in the NIH report was that chromosomal aberrations have been found in humans with occupational exposure to nickel. The WHO similarly reports that metallic nickel dust induced tumors in hamster following intra-tracheal installation (World Health Organization, 2000). Moreover, nickel dermatitis and sensitivity to nickel is a widespread irritant. In North America it has been reported that 17.5% of the population is sensitive to nickel, a figure that has increased in the past 20 years (Warshaw *et al.*, 2019).

It is important to note that there is evidence of osteoclastic biocorrosion of metals, such as nickel, in which metal ions are released from their origin and cause an inflammatory response associated with increased levels of IL-1β, IL-6, and TNF-α (Cadosch *et al.*, 2009). Moreover, the corrosion of these metals by osteoclasts results in the concurrent release of metal ions (Cadosch *et al.*, 2009). The release of these metal ions, in turn, also enhances the activity of the osteoclasts in breaking down bone structure due to the increase in pro-inflammatory cytokines (Cadosch *et al.*, 2009).

In addition to the activity of osteoclasts, there are other mechanisms of concern by which metal ions may be released from their origin. A well-known pathway by which metal ions enter the blood after implantation is through electrochemical corrosion, a phenomenon, which is well studied and understood (Sansone *et al.*, 2013). Typically, this process involves the exchange of electrons and cations between the metal and the surrounding solution (Sansone *et al.*, 2013). The main products of this interaction are metal oxides, hydroxides and phosphates. The result of this corrosion is the release of nanoparticles, which enter the bloodstream (Sansone *et al.*, 2013). The particularly challenging and difficult issue associated with the release of nanoparticles is that their smaller size, and larger total surface area compared to larger particles, makes them much more bioactive (Sansone *et al.*, 2013). Metallic nanoparticles have been found in tissues of patients with dental implants (Sansone *et al.*, 2013; Schmalz *et al.*, 2018). These particular nanoparticles were found in the jawbone marrow 60-700 μm from the dental implants, and they were about 0.4-40 μm (Schmalz *et al.*, 2018). The most concerning aspect regarding this finding is that these nanoparticles were released after insertion of the implant, meaning that they were able to migrate a considerable distance from the site of implant location. In the present study we assessed the elemental composition of a variety of bone grafts including allografts, porcine and bovine grafts, as well as synthetic bone replacements and membranes. Both particulate bone grafts and block bone grafts were analyzed.

Nickel carcinogenicity and toxicity

According to the International Agency for Research on Cancer (IARC), National Institute of Health (NIH) and the World Health Organization (WHO), it is well known that nickel is a potent carcinogenic agent (International Agency for Research on Cancer; National Institutes of Health; World Health Organization, 2000). Although very few, if any, human studies have been performed, there have been animal studies that have studied how nickel induces cancerous growths (Dunnick *et al.*, 1995; Jun *et al.*, 2011). Their findings are of concern and show us the great risk this element poses if not accounted for when used in industry and healthcare.

Nickel compounds are known human carcinogens (Group 1 carcinogens) that specifically target the lung, nasal cavity, and paranasal sinuses (Tokar *et al.*, 2011). The IARC further reports that inhalation of nickel sub-sulfide or nickel oxide caused development of lung tumors in rats, as well as producing adrenal tumors, which are significant as those tumors developed at a site distant to the initial port of entry (Tokar *et al.*, 2011). The IARC also reports evidence of trans-placental carcinogenicity of nickel, as nickel exposure in adult rats caused kidney tumors and rare malignant pituitary tumors (Tokar *et al.*, 2011).

Another study assessed how inhalation exposure, direct injection and direct installation of nickel metal powder affected mice, rats, guinea-pigs and hamsters (McGregor *et al.*, 2000). The study found that intra-tracheal instillation of nickel in rats caused a significant number of squamous-cell carcinomas and adenocarcinomas of the lungs (McGregor *et al.*, 2000). Intra-pleural injections of nickel caused sarcoma development in rats as well (McGregor *et al.*, 2000), while intramuscular injection of nickel powder caused sarcomas in rats and hamsters (McGregor *et al.*, 2000). Intraperitoneal injections caused local carcinoma,

mesothelioma, and sarcoma growths in rats (McGregor *et al.*, 2000). Moreover, subcutaneous delivery of nickel metal pellets induced sarcomas in rats (McGregor *et al.*, 2000). Similar findings were reported in other experimental animals, where the nickel compounds caused tumor development in all sites of application (Denkhaus and Salnikow, 2002; Dumala *et al.*, 2017).

A study by Dumala and colleagues (2017) evaluated the genotoxicity of orally administered nickel oxide nanoparticles, a variety of nickel that is becoming increasingly widespread in numerous applications, also reported alarming findings (Dumala *et al.*, 2017). This study assessed DNA damage that occurred to female Wistar rats after oral exposure to nickel oxide nanoparticles at varying concentrations of 125, 250, and 500 mg/kg body weight. It was found that a significant amount of DNA damage was caused in the peripheral blood lymphocytes, liver, and kidney as a result of the nickel oxide nanoparticle exposure (Dumala *et al.*, 2017). A greater frequency of micronuclei was found in nickel oxide treated rats (Dumala *et al.*, 2017), indicating higher levels of chromosome damage in these rats.

The effects of implanted nickel in rats both as nanoparticles and bulk material have also been investigated (Hansen *et al.*, 2006; Tokar *et al.*, 2011). It was found that all animals developed large growths at both sites of implantation, these growths were later determined to be rhabdomyosarcomas (Hansen *et al.*, 2006).

Metallic nickel nanoparticles and nickel oxide nanoparticles have exhibited a carcinogenic effect on human lung NCI-H460 epithelial cells (Pietruska *et al.*, 2011). The nickel nanoparticles were observed to activate the HIF-1α pathway in the cells (Pietruska *et al.*, 2011). This is significant as HIF-1α is commonly found in solid human cancer.

Molecular mechanism of nickel carcinogenicity and toxicity

It is at the molecular level that nickel may interact with DNA, cell receptors, and various organelles to carry out its effects. Accordingly, the specific molecular mechanisms by which nickel induces its carcinogenic effects have been under investigation for a long time.

One mechanism by which nickel induces its carcinogenic effects is through the disruption and modification of DNA events in the cell. Nickel is able to bind to DNA and nuclear proteins while also interfering with nucleotide and base excision repair (Cameron *et al.*, 2011). Long-term exposure of HL-60 human leukemia cells to nickel cations resulted in DNA fragmentation, cell death, and the production of reactive oxygen species (Cameron *et al.*, 2011). Nickel (II) has been shown to exhibit genotoxic effects when phagocytosed into cells, which are compounded by its inhibition of DNA repair and its production of reactive oxygen species (Kasprzak *et al.*, 2003). DNA has been found to be epigenetically active in modifying gene expression through varying DNA methylation and histone acetylation (Kasprzak *et al.*, 2003). Nickel is especially active in regard to promoting and inhibiting genes and transcription factors involved with hypoxia (Kasprzak *et al.*, 2003).

It has been found that nickel is able to activate the extracellular Ca^{2+}-sensing receptor (CaSR) (Cameron *et al.*, 2011; Cortijo *et al.*, 2010), this in turn activates signaling events which cause the cell to turn on calcium and hypoxia-inducing factor pathway (Cameron *et al.*, 2011). Turning on the hypoxia-inducible factors are particularly problematic since they allow cells to be viable in anaerobic environments, thus allowing cancerous cells to thrive and become malignant and metastatic (Cameron *et al.*, 2011). Other studies have shown nickel's involvement in the hypoxia-inducible signaling pathway, which affects cellular iron levels by competing with iron transporters and iron-regulated enzymes (Cameron *et al.*, 2011). There is strong evidence that nickel upregulates HIF-1 and several genes which are induced by HIF-1, such as genes for glycolytic enzymes and glucose transporters (Cameron *et al.*, 2011).

Nickel has also been found to cause DNA damage in cultured HeLa cells (Kawanishi *et al.*, 2002). Specifically, the Ni(3)S(2) compound was seen to cause a significant increase in 8-hydroxydeoxyguanosine formation (Kawanishi *et al.*, 2002). This is particularly troubling as 8-hydroxydeoxyguanosine is a biomarker for oxidative DNA damage (Fenga *et al.*, 2017; Kawanishi *et al.*, 2002). Meaning a greater formation rate of 8-hydroxydeoxyguanosine indicates a greater degree of DNA damage. This is additional evidence that nickel is an active genetic modifier that poses a severe risk to the oncogenic state of the cells it is exposed to. Moreover, another study examined nickel's effects on the p53 tumor suppressor gene in human kidney epithelial cells (Maehle *et al.*, 1992). Cells treated with nickel were found to have altered p53 protein expression and a mutation of thymine to cytosine at codon 238 (Maehle *et al.*, 1992).

Nickel is also well known in epigenetics as an agent that silences genes through DNA methylation (Arita and Costa, 2009). A study exploring the mechanisms by which nickel- caused oxidative stress induced tumors has looked specifically at the p16 gene and MAP kinase pathway (Govindarajan *et al.*, 2002). It was observed that nickel sulfide-induced oxidative stress appeared to result in the silencing of the p16 tumor suppressor gene and the activation of the MAP kinase pathway (Govindarajan *et al.*, 2002). Notably, all the tumors exhibited hypermethylation of the p16 gene. Nickel has been implicated in various mechanisms of epigenetic changes, including inducing alterations to chromatin structure, specifically by hetero-chromatization, DNA methylation, and histone modifications (Sun *et al.*, 2013).

In the context of methylation nickel is able to inhibit the activity of the jmjc-domain containing demethylases, which activate genes by demethylating them (Sun *et al.*, 2013). The jmjc domain of these demethylases have a catalytic site that binds iron; however, in the presence of nickel(II) they are more likely to bind to nickel since their affinity constant of nickel(II) is about three times greater than that of iron (Sun *et al.*, 2013). This causes permanent inhibition of the demethylase activity.

Past and ongoing research has studied molecular mechanisms by which nickel is able to induce carcinogenicity. Several genetic, epigenetic, and signaling mechanisms have been identified in which nickel acts as a modifier and agent of change. This aspect of nickel's carcinogenicity is still under investigation as more aspects of its molecular and cellular effects are being uncovered.

Nickel hypersensitivity and dermatitis

Nickel is recognized as a skin irritant, that causes dermatitis. In fact, nickel is the leading contact allergen in most industrialized countries as it is the most frequent cause of contact allergy worldwide (Ahlström *et al.*, 2019). In Europe, the prevalence of nickel allergy is approximately 8% to 19% in adults (Ahlström *et al.*, 2019). Irritation and vascular hand eczema have been reported in cases wherein metallic implants have been inserted into a patient (Ahlström *et al.*, 2019). This is indicative of the metal ions that are released from these implants, and the health risks they may pose. Moreover, a link was found between metal allergic contact dermatitis and dental alloys in a European study (Ahlström *et al.*, 2019). Dental materials, such as brackets, may be especially susceptible to corrosion due to the inherent nature of the oral environment, which is thought to increase the release of nickel (Ahlström *et al.*, 2019).

The molecular mechanisms by which nickel exerts its irritative effects have been elucidated through a number of human and animal studies (Bechara *et al.*, 2017; Guo *et al.*, 2019; Saito *et al.*, 2016). First, nickel is able to migrate through the skin to activate keratinocytes which leads to the release of cytokines, including IL-1β and TNF-α (Saito *et al.*, 2016). Thereafter nickel attaches itself to the major histocompatibility complex molecules on Langerhans cells and dendritic cells which have been upregulated from the aforementioned cytokines (Saito *et al.*, 2016). Specifically, in dendritic cells, the TLR4 pathway is activated (Saito *et al.*, 2016). The cytokines are able to control and modify the expression of E-cadherin and chemokines, including matrix metalloproteinase-9, secondary lymphoid tissue chemokine, and macrophage inflammatory protein-3β which are produced by antigen presenting cells (Saito *et al.*, 2016). These antigen presenting cells travel to draining lymph nodes where they present the haptens to T cells, and this is where the hypersensitive reaction is produced at the site of exposure (Saito *et al.*, 2016). This is because re-exposure to the same hapten will cause the hypersensitive reaction again at the site of exposure (Saito *et al.*, 2016). This process occurs because the haptenated-peptide presentation causes hapten-specific T cells to be activated, proliferated, and differentiated (Saito *et al.*, 2016). These T cells are then able to travel directly to the skin and produce the inflammatory cytokines and chemokines at the exposure site which cause the allergic reaction and skin lesions commonly seen due to nickel (Saito *et al.*, 2016). Importantly, the macrophages and pro-inflammatory molecules that are activated promote the differentiation of osteoclast precursors into mature osteoclasts (Hallab and Jacobs, 2009). This subsequently leads to greater resorption rates and potentially higher implant failure (Hallab and Jacobs, 2009).

In an experiment to determine the dose-dependent relationship between nickel and dermatitis, 40 nickel-sensitive individuals were exposed to oral doses of nickel in the form of nickel sulfate hexahydrate and were examined for reactions one day after exposure (Jensen *et al.*, 2003). The minimal dose of nickel hexahydrate, which contained 0.3 mg of nickel elicited a clinically cutaneous reaction in four out of ten tested subjects who were nickel-sensitive (Jensen *et al.*, 2003). Another study estimated that about 30-50% of nickel taken orally was excreted a day later, however it should be noted that subjects in this study had fasted overnight and were given a dose of 10 μg of Ni/kg body weight (Patriarca *et al.*, 1997).

The effect of nickel as an implant material on outcomes of dental implant surgery

Allergies to metal implants have also increasingly been implicated as major causes of implant failure (Schalock *et al.*, 2012). Nickel is among the most common metals, which may cause implant failure due to metal sensitization (Pacheo, 2015). Other than implant failure, which is likely the worst outcome of any acute effect exerted by nickel, other complications that may occur include several varying diseases from dermatitis to implant loosening (Haddad *et al.*, 2019). Reports exist of patients suffering from eczema reaction due to nickel-containing pure titanium osteosynthesis and a cerclage with steel wire (Thomas, 2014). Moreover, it has been reported that the prevalence of metal sensitivity among patients with failed or poorly functioning implants is approximately 60% (Hallab and Jacobs, 2009). Among patients with implants that have failed or are failing, the prevalence of metal sensitivity is six times the prevalence of the general population, and approximately two to three times that of all patients with metal implants (Hallab and Jacobs, 2009). The minimum risk levels and the lowest observed adverse effect levels (LOAEL's) for nickel are presented in Table 1 (Agency for Toxic Substances and Disease Registry, 2005).

Table 1. LOAEL and effects of nickel based on various routes of exposure and species

Species	Route of Exposure	Dose (single dose or Minimal Risk Level/LOAEL)	Observed Effects
Rats	Acute Duration Inhalation (6 hours/ day for 12 days in a 16-day period - for nickel sulfate)	LOAEL Nickel Sulfate: 0.7 mg Ni/m^3 Soluble Nickel Compound: 0.25 mg Ni/m^3	Respiratory and Body Weight Effects,
Rats	Intermediate Duration Inhalation (13 weeks, 6 hours/day, 5 days/ week)	LOAEL Nickel Sulfate: 0.11 Ni/m^3 Nickel Subsulfide: 0.22 Ni/m^3 Nickel Oxide: 3.9 Ni/m^3	Chronic lung inflammation, atrophy of the nasal olfactory epithelium (for nickel sulfate and nickel subsulfide)
Rats	Chronic Exposure Inhalation (2 years, 6 hours/day 5 days/ week)	LOAEL Nickel Sulfate: 0.06 mg Ni/m^3 Nickel sub-sulfide: 0.11 mg Ni/m^3 Nickel Oxide: 0.5 mg Ni/m^3	Chronic lung inflammation and bronchiolization at 0.06 mg Ni/m^3 and atrophy of the olfactory epithelium at 0.11 mg Ni/m^3 for nickel sulfate. Chronic lung inflammation, alveolar epithelial hyperplasia, fibrosis, and rapid and shallow breathing at 0.11 mg Ni/m^3, and atrophy of the nasal olfactory epithelium at 0.73 mg Ni/m^3 for nickel sub-sulfide. Chronic lung inflammation and alveolar epithelial hyperplasia for nickel oxide
Humans	Oral Single Challenge Dose	0.01 mg Ni/kg as nickel sulfate	Allergic dermatitis
Mice	Oral single gavage Dose	23 mg Ni/kg as nickel nitrate	Increases in sperm head abnormalities
Rats	Intermediate duration oral exposure	8.6 mg Ni/kg/day as nickel chloride, nickel acetate, or nickel sulfate	Significant decreases in body weight and organ weight (liver, kidney, pituitary)

Material and methods

We analyzed the presence of Nickel in several bone replacement grafts and barrier membranes. To analyze the individual dental bone grafting materials, we used a high-resolution energy dispersive x-ray fluorescence (EDXRF, Nex De Rigaku, Applied Rigaku Technologies, Austin, TX, USA) spectrometer. This machine is able to perform elemental analysis of solids, liquids, powders, alloys, and thin films. Data were obtained through the machine's QuantEZ software which displayed the metal alloy composition of each material in parts per million (ppm). The machine was calibrated to baseline prior to use to ensure accuracy of the measurements. In addition, calibration was performed based on the known quantity of pure metals, such as titanium, zinc, zirconium, nickel and magnesium tested with the machine. We used also expired materials (n=10) to get more pilot information about the elemental analysis of the biomaterials before we started the present spectrometric analysis. We directed the software to take measurements in ppm. We then selected three different regions of analysis, low z, mid z, and high z, corresponding to elements of low to high mass. An analysis time of 60 seconds was used. The material was placed into a helium environment in the machine. Prior to placing the samples of each material in the chamber of the machine for analysis each sample was placed on top of a polyethylene plastic wrap. Afterwards, the sample was placed in the chamber for 60 seconds, until the software indicated that the analysis was complete.

The following brands of dental bone grafts, barrier membranes and other biomaterials for reconstruction of soft tissues, such as Helitape Absorbable Collagen (Integra Miltex), OSSIX Bone Grafting Material (Glymatrix technology), ZCORE Porcine Xenograft (Osteogenics), PepGen P-15 Putty Bovine (Dentsply Sirona), Perio-System Bio-Oss Collagen (Geistlich), Bone Ceramic Bone Graft Substitute (Straumann), Symbios MTF Human Allograft Tissue (MTF Biologics), Mucograft Collagen Matrix Membrane (Geistlich), MatrixDerm Regenerative Collagen Dental Membrane (CollagenMatrix), Mucograft Resorbable Collagen Matrix (Geistlich), BioMend Extend Absorbable Collagen Membrane (Zimmer-Biomet), CurV Pre-Shaped Collagen Membrane (Zimmer-Biomet) were analyzed. Five samples were randomly selected for each biomaterial (n=5) and measurements were repeated twice for each sample, except for Helitape Absorbable Collagen (Integra Miltex) and Bone Ceramic Bone Graft Substitute (Straumann) for which one measurement was taken.

Results

The average levels of nickel in various types of dental bone replacement grafts and barrier membranes are shown in the Tables 2 and 3. In addition, characteristic spectrometric analysis of different bone grafting materials and barriers is presented in the Figures 1-4 demonstrating the peaks of Nickel ion presence.

Discussion

The data from this study demonstrate the presence of nickel in dental bone replacement grafts. It is very important to understand how nickel may have made its way into bone grafts. Previous studies showed that nickel has been found in the livers of cattle, indicating that there is some uptake of this element by bovine (Counotte *et al.*, 2019) which may explain its presence in bovine-derived bone grafts for dental applications. Moreover, it has been estimated that mean nickel intake for adults in the United States has been estimated to be between 69 and 162 μg per day. It has been shown that dietary nickel is retained by the body, it may be possible that the retained nickel is deposited in certain tissues, such as the liver and kidneys (Patriarca *et al.*, 1997). In

Table 2. Mean levels (ppm) of nickel measured in various commonly used bone grafts based on spectrometric analysis

Bone Graft	Mean (ppm)	Standard Deviation (ppm)
Helitape Absorbable Collagen (Integra Miltex)	13.10	N/A
OSSIX Bone Grafting Material (Glymatrix technology)	38.15	29.20
ZCORE Porcine Xenograft (Osteogenics)	32.25	30.33
PepGen P-15 Putty Bovine (Dentsply)	29.90	13.86
Perio-System Bio-Oss Collagen (Geistlich)	6.60	0.88
Bone Ceramic Bone Graft Substitute (Straumann)	12.00	N/A
Symbios MTF Human Allograft Tissue (MTF Biologics)	30.80	0.28

Table 3. Mean levels (ppm) of nickel measured in various commonly used Membranes based on spectrometric analysis based on spectrometric analysis, mean is based on two measurements of each material

Membrane	Mean (ppm)	Standard Deviation (ppm)
Mucograft Collagen Matrix Membrane (Geistlich)	9.34	7.01
MatrixDerm Regenerative Collagen Dental Membrane (CollagenMatrix)	4.49	2.37
BioMend Extend Absorbable Collagen Membrane (Zimmer-Biomet)	6.51	4.39
CurV Pre-Shaped Collagen Membrane (Zimmer-Biomet)	7.18	6.68

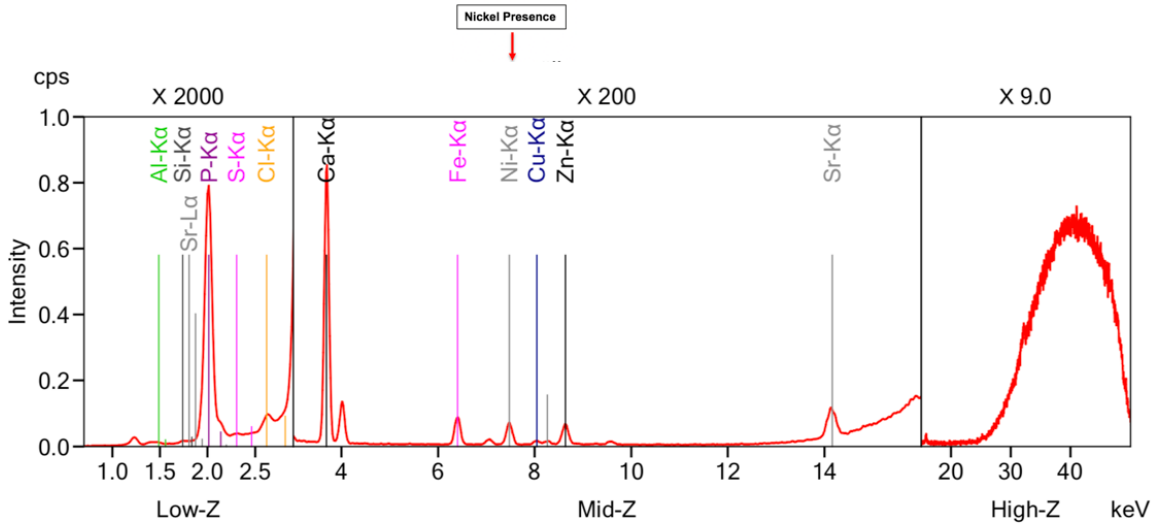

Figure 1: Spectrometric Analysis of large particles of a bovine mineral presenting nickel as an element (17.5ppm)

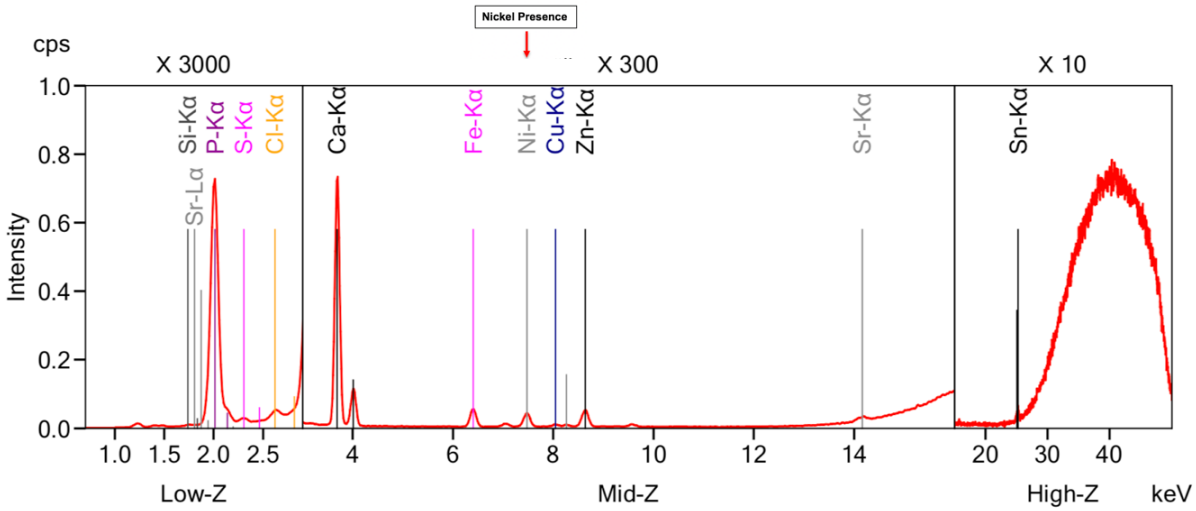

Figure 2: Spectrometric Analysis of large particles of a particulate allograft (0.25-1mm) presenting nickel as an element (20.2ppm)

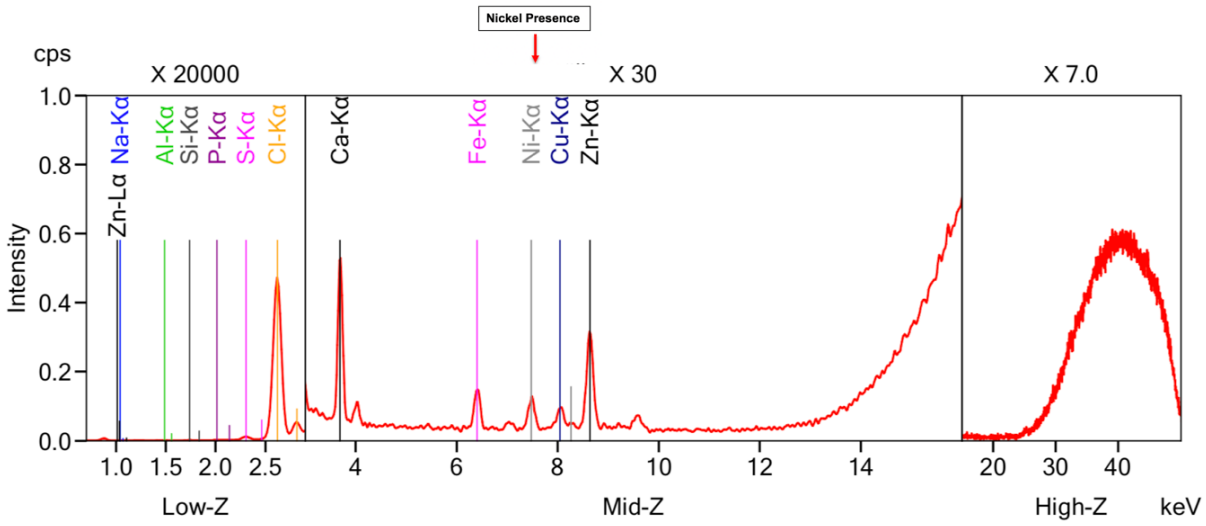

Figure 3: Spectrometric analysis of a biomaterial for improvement of soft tissues (4.38ppm)

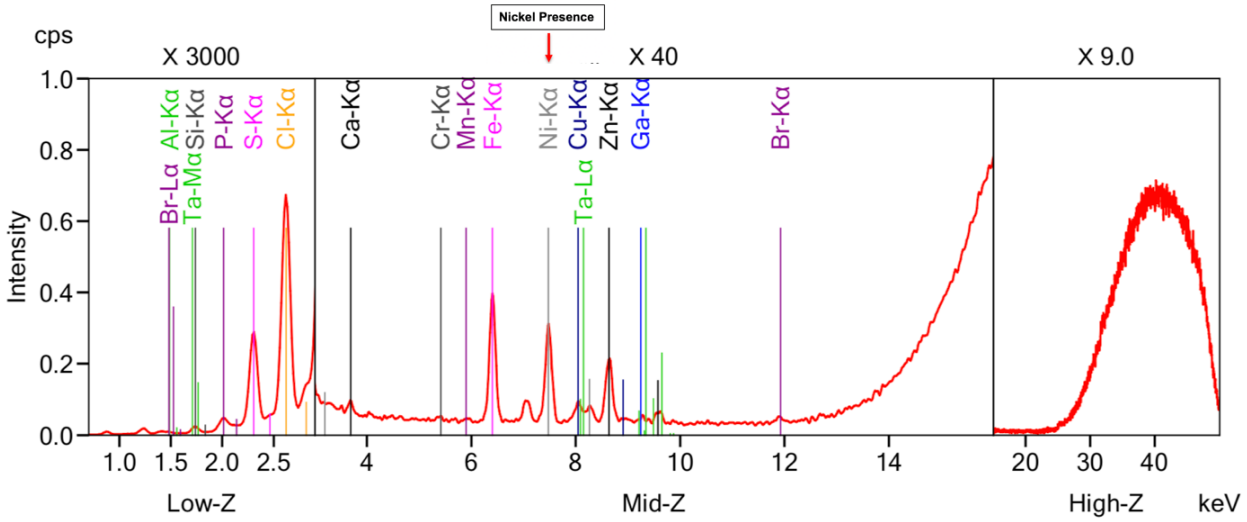

Figure 4: Spectrometric analysis of a collagen membrane for alveolar ridge reconstruction (11.9ppm)

addition, various concentrations of Nickel have been found in human bone samples (Brodziak-DopieraŁa *et al.*, 2011; Łanocha-Arendarczyk *et al.*, 2016; Povarova *et al.*, 2007), which may explain the presence of nickel in human allografts (Agency for Toxic Substances and Disease Registry, 2005). It has been suggested that the concentration of nickel in the human bone is associated with several factors such as environmental exposure, diet, geographical range, occupational exposure and health condition (Cameron *et al.*, 2011; Łanocha-Arendarczyk *et al.*, 2016).

Although dietary nickel may be the cause of nickel's presence in allografts and xenografts, there may be another explanation for this. When bone grafts are processed they are typically cut into pieces with a handsaw, further broken down by another saw, then ground into fine particles using a milling machine (Lee *et al.*, 2012). These metallic, stainless steel devices used to process the bone graft contain about 8-15% nickel (Kamerud *et al.*, 2013). Nickel can easily be corroded off the stainless-steel devices due to friction against the bone and nickel particles will end up in the bone graft as a result during the manufacturing process.

The present data show the presence of nickel in all evaluated bone replacement grafts and barrier membranes. The presence of nickel in these biomaterials may potentially cause significant complications when used in clinical settings. The presence of nickel in many major brands of dental bone grafts and barrier membranes should prompt more investigation into how this element may potentially cause implant failure among patients with nickel sensitivity.

While nickel does enhance the performance of certain prosthetics in orthopedic and dental applications, it is a known carcinogen and an obvious danger to health. While some claim that the nickel in these materials and products is not harmful due to being in a biologically inert form it is our perspective that the research has demonstrated a contrary viewpoint. Bone, especially, is a dynamic tissue and is constantly undergoing change. This change typically involves the breakdown of any material that is in and around its structure. When bone grafts are used to form bone, they too will be broken down in the resorptive process. This puts nickel in the position of being freed as an ion, and migrating through the body. In fact, we discussed how this may occur, and how nanoparticles migrating through the body from bone grafts are a concern.

Another important angle to consider is the interaction of human plasma with implants and bone grafts bone grafts. A key factor in determining the corrosion rate of metal ions from implants and bone grafts is the pH of the bio-environment in which the materials are found. When implants are placed the disruption of blood supply which typically follows the procedure usually results in a bacterial infection around the implant (Eliaz, 2019). As a result, the pH in that area can drop to as low as 4.0, making the implant and bone graft more susceptible to corrosion (Eliaz, 2019). Furthermore, other interactions between blood, implants, and bone grafts occur which have an influence upon the corrosion rate of the implanted materials. For instance, the blood which passes through the area could have a high oxygen content, or a higher bicarbonate content, causing increases or decreases in pH which could lead to a greater degree of corrosion (Eliaz, 2019).

Our study has shown that commercially available and widely used bone grafting materials and barrier membranes contain metal compounds that are proven to cause genetic damage in the cells that they interact with. Furthermore, these compounds, along with the bone graft and barrier membranes, are implanted into the tissue of patients with the intention that they become fully integrated and bioactive with the patient's body. It is unlikely that these conditions would not produce adverse effects given the evidence we have presented regarding nickel's oncogenic potential. However, it should be noted that the level of nickel present is not consistent with every type of bone graft and barrier membrane. Furthermore, the amount of nickel that causes toxic and carcinogenic effects may vary by cell, location, and exposure to the body. Although time may be a factor as well as patients are constantly exposed to nickel if it is directly implanted into their alveolar bone. Therefore, the contents of the bone graft, i.e. the nickel, which is dispersed throughout the material, will eventually be released into the surrounding tissues and circulatory system. Given the research on the effect nickel exerts on cancer-related genes in cells any amount of nickel exposure to the body should be of great concern.

Besides the carcinogenic and genotoxic risks and dangers of the metal alloys included in the bone grafts and barrier membranes, other risks exist which may endanger the success of the implant. Metal ion nanoparticles, specifically those of nickel, leach and migrate from the grafted site, promoting production of macrophages and pro-inflammatory molecules which cause osteoclast precursors to become fully functioning osteoclasts. Due to the increase in the number of osteoclasts the resorption rate of bone at the implant site might be greatly increased, and thus the chance of graft resorption is increased as well.

To further demonstrate the relationship between nickel and its effect on the epithelium, specifically on the oral mucosa, we can investigate the interaction between nickel-containing dentures and pathologies of the oral mucosa. A study looking at the effects of nickel-based alloy dentures found that these dentures caused the oral mucosa of subjects to develop papillomatosis lesions (Scrieciu *et al.*, 2015). In another case report a

patient with a positive patch test for nickel, and negative for other tested metals, reported reddened mucosal hyperplasia on the hard palate in addition to a burning sensation, pruritus, and bleeding within two weeks of placing an upper denture containing nickel (Özkaya and Babuna, 2011). When the patient ceased use of the nickel-containing dentures the mucosal changes completely regressed (Özkaya and Babuna, 2011). We can draw a parallel between oral use of nickel and topical use by looking at a study which explored the relationship between piercings, nickel allergy, dental braces and hand eczema specifically in adolescents. The study reported that girls were more likely to be allergic to nickel if they had their first piercing during a specific period of time during adolescence, moreover a significant relationship was observed between hand eczema and nickel allergy (Mortz *et al*., 2002). However, the investigators also reported that application of dental braces before any piercings were applied significantly reduced the likelihood of developing a nickel allergy (Mortz *et al*., 2002). A possible connection we can make here is that when an individual's primary exposure to nickel is cutaneous, the body is likely to become sensitized and respond antagonistically to future exposures to nickel whether they occur cutaneously or orally. Furthermore, an initial oral exposure to nickel will have protective effects against future sensitization to nickel. This could also mean that nickel affects the body through different pathways depending on where the exposure occurs. The other implication of these studies is that there is a different, but similar response to nickel when sensitized patients are exposed either orally or cutaneously. The cutaneous response would result in eczema while the oral response would result in pathological lesions in the mucosa accompanied by a burning and irritative sensation. These studies could help us understand the underlying mechanism by which nickel might cause pathologies in the oral mucosa, and whether these pathologies are similar in nature to cutaneous pathologies.

It should also be noted that our procedure to examine the bone grafts does have certain limitations. Although nickel was consistently found in each measurement of most bone graft materials and barrier membranes, there were fluctuations in the concentrations of nickel. This could be due to the specific area of the bone graft that was analyzed as the spectrometer did not analyze the whole sample of bone graft or barrier membrane, rather a specific section of sample was measured as well as from different lot numbers of materials. Moreover, it should be noted that three different charges of the bone graft were used. This is significant as each package of bone graft comes from different sources.

Considering the significance of the presence of Nickel in the bone replacement grafts and other biomaterials, more research is required to understand more about potential risks from metals such as nickel in bone grafts, and how they might affect public health in the long-term. In addition, future studies are needed to evaluate the manufacturing process of these biomaterials in order to alter this process to minimize the element nickel from these biomaterials. It should be also noted that the clinical significance of the presence of nickel in bone replacement grafts and barrier membrane still is unknown.

Conclusion

Our findings suggest a need for further studies to be performed exploring how the nickel content of bone grafts and barrier membranes may affect clinical outcomes for patients. Given nickel's widespread use in these materials, it is essential that clinicians understand its possible effects on patients.

Disclosure

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